THE EFFECTS OF CONJUGATED LINOLEIC ACID (CLA) SUPPLEMENTATION ON BODY COMPOSITION, STRENGTH AND ENERGY EXPENDITURE IN HUMANS WHEN COMBINED WITH RESISTANCE TRAINING

A Thesis Submitted to the College of Graduate Studies and Research in Partial Fulfillment of the Requirements for the Degree of Master of Science in the College of Kinesiology University of Saskatchewan Saskatoon

By
Craig Pinkoski

© Craig Pinkoski, September 2003. All rights reserved.
PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a Master of Science degree in Kinesiology from the University of Saskatchewan I, Craig Pinkoski, agree to make it freely available for inspection at the libraries of this university. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professor who supervised my thesis work or, in their absence, by the Dean of the College of Kinesiology. It is understood that any copying or publication or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any materials in my thesis.

Requests for permission to copy or make other use of material in this thesis in whole or in part should be addressed to:

Dean of the College of Kinesiology
University of Saskatchewan
87 Campus Drive
Saskatoon, Saskatchewan
S7N 5B2
ABSTRACT

Animal studies have demonstrated that conjugated linoleic acid (CLA) increases resting metabolic rate, enhances fatty acid oxidation, reduces body fat and increases lean tissue mass. The purpose of this study was to investigate if these beneficial effects were observed in human subjects while resistance training (3x/wk). Men and women (n=37; 18-34 yrs) were randomly assigned in a double blind fashion to supplement CLA (5.0 g/d) or placebo concurrently with seven weeks of resistance training. The following were measured before and after training: body composition (air displacement plethysmography), elbow flexors and knee extensors muscle thickness (ultrasound), strength (chest and leg press 1-RM), knee extensors and flexors isokinetic torque (isokinetic dynamometer), resting metabolic rate and respiratory exchange ratio (open circuit indirect calorimetry). The CLA group gained significantly more lean mass (2.4 kg vs. 0.8 kg, p=0.03) and combined chest and leg press strength (178 kg vs. 54 kg, p=0.05) than the placebo group. Time main effects (p<0.05) were observed for reduced fat mass, and increased muscle thickness of elbow flexors and knee extensors with no differences between groups. Neither group had significant changes in resting metabolic rate, energy substrate utilization or respiratory exchange ratio. It was concluded that CLA supplementation was effective at enhancing gains in lean mass and strength when combined with regular resistance training.
ACKNOWLEDGEMENTS

No thesis is ever completed without the help of others. I am grateful for Dr. Philip Chilibeck’s tireless support and enthusiasm throughout the development, implementation and completion of my project and accompanying dissertation. To Dr. Karen Chad and Dr. Keith Russell, my committee members, I thank them both for their continued input and confidence in my abilities as a researcher. They provided an environment where I was afforded as much independence as I was able to demonstrate. For assistance during those early morning sessions and comedy relief I thank the ‘coffee pushers’ and soon to be doctors, Darren Candow and Jon Farthing; to think I got through my masters without coffee. Doug Jacobson was invaluable in the physiology lab dispensing know-how, humour and uncut opinions on pretty much everything; especially about the orange jugs. To the group of training supervisors I owe my gratitude as insanity would certainly have set in without their help. I also feel compelled to include thanks to the participants of my investigation as their dedication to the study was beyond any researcher’s expectations. To Dr. Patrick Krone and Dr. Cristofre Martin, I thank for giving me my ‘big break’ in the field of research. I thank Bioriginal for donating the supplements. Without their generous gift this study would not have been possible.

Lastly, I thank my wife Tarra for believing in me throughout the hardships of my academic career. Without her compassion and support I would not have come to the University of Saskatchewan to pursue, and recently attain, my academic aspirations. I’m finally going to be a real doctor.
DEDICATION

I dedicate this thesis to my departed Godfather and uncle, Frank Murphy. A man who was selflessly committed to the mystery of magic and how much pleasure it brought to his nephew. I also dedicate this to my wife, Tarra, having endured throughout the completion of my thesis and other endeavors I have chosen to pursue.

"Nothing in the world can take the place of persistence. Talent will not; nothing is more common than unsuccessful men with talent. Genius will not; unrewarded genius is almost a proverb. Education will not; the world is full of educated derelicts. Persistence and determination alone are omnipotent."

Calvin Coolidge
TABLE OF CONTENTS

PERMISSION TO USE................................................................. i
ABSTRACT..................................................................................... ii
ACKNOWLEDGEMENTS............................................................. iii
DEDICATION................................................................................ iv
TABLE OF CONTENTS................................................................... v
LIST OF TABLES........................................................................ viii
LIST OF APPENDICES.................................................................... ix
LIST OF ABBREVIATIONS.......................................................... x

CHAPTER

1. THEORETICAL FRAMEWORK................................................... 1
   1.1 Introduction........................................................................ 1
   1.2 Review of Literature.......................................................... 3
      1.2.1 Conjugated Linoleic Acid............................................. 3
      1.2.2 Conjugated Linoleic Acid and Body Composition............. 6
      1.2.3 Conjugated Linoleic Acid and Resting Metabolic Rate...... 15
      1.2.4 Conjugated Linoleic Acid: Proposed Mechanisms of Action.... 19
      1.2.5 Conjugated Linoleic Acid: Effective Dosages and Toxicity..... 36
   1.3 Statement of Problem and Hypotheses................................. 41
      1.3.1 Statement of Problem................................................... 41
      1.3.2 Hypotheses................................................................ 42
LIST OF TABLES

TABLE 1.1  Mode of measurements for evaluation of dependent variables .......... 44

TABLE 2.1  Compositional list of Conjugated Linoleic Acid supplement .......... 47

TABLE 3.1  Physical characteristics (mean ± standard error) of subjects at baseline 60

TABLE 3.2  Baseline values (mean ± standard error) for measured dependent variables
            of both groups ........................................................................... 61

TABLE 3.3  Values (mean ± standard error) for dependent variables as recorded at pre
            and post evaluation ..................................................................... 66
## LIST OF APPENDICES

<p>| APPENDIX A | Certificate of Ethics Approval | 122 |
| APPENDIX B | Participant Consent Form | 124 |
| APPENDIX C | Physical Activity Readiness Questionnaire | 129 |
| APPENDIX D | Respiratory Exchange Ratio Values for Major Fuels | 131 |
| APPENDIX E | 3-Day Food Diary Booklet | 133 |</p>
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>Arachidonic acid</td>
</tr>
<tr>
<td>ADG</td>
<td>Average daily gain</td>
</tr>
<tr>
<td>AP</td>
<td>Air displacement plethysmography</td>
</tr>
<tr>
<td>AREE</td>
<td>Absolute resting energy expenditure</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>BAT</td>
<td>Brown adipose tissue</td>
</tr>
<tr>
<td>BFM</td>
<td>Body fat mass</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>BMT</td>
<td>Biceps muscle thickness</td>
</tr>
<tr>
<td>BP</td>
<td>Bench/chest press</td>
</tr>
<tr>
<td>BW</td>
<td>Body weight</td>
</tr>
<tr>
<td>c9,t11</td>
<td>cis-9,trans-11</td>
</tr>
<tr>
<td>CI</td>
<td>Caloric intake</td>
</tr>
<tr>
<td>CP</td>
<td>Combined press</td>
</tr>
<tr>
<td>CLA</td>
<td>Conjugated linoleic acid</td>
</tr>
<tr>
<td>CMT</td>
<td>Combined muscle thickness</td>
</tr>
<tr>
<td>COX</td>
<td>Cyclooxygenase</td>
</tr>
<tr>
<td>CPT</td>
<td>Carnitine palmitoyl transferase</td>
</tr>
<tr>
<td>CT</td>
<td>Combined torque</td>
</tr>
<tr>
<td>DEXA</td>
<td>Dual energy x-ray absorptiometry</td>
</tr>
<tr>
<td>EE</td>
<td>Energy expenditure</td>
</tr>
<tr>
<td>FA</td>
<td>Fatty acid</td>
</tr>
<tr>
<td>HDL</td>
<td>High density lipoprotein</td>
</tr>
<tr>
<td>iNOS</td>
<td>Inducible-nitric oxide synthase</td>
</tr>
<tr>
<td>KET</td>
<td>Knee extension torque</td>
</tr>
<tr>
<td>KFT</td>
<td>Knee flexion torque</td>
</tr>
<tr>
<td>LA</td>
<td>Linoleic acid</td>
</tr>
<tr>
<td>LBM</td>
<td>Lean body mass</td>
</tr>
<tr>
<td>LOX</td>
<td>Lipoxygenase</td>
</tr>
<tr>
<td>LP</td>
<td>Leg press</td>
</tr>
<tr>
<td>LPL</td>
<td>Lipoprotein lipase</td>
</tr>
<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger ribonucleic acid</td>
</tr>
<tr>
<td>MT</td>
<td>Muscle thickness</td>
</tr>
<tr>
<td>PC</td>
<td>Phosphatidylcholine</td>
</tr>
<tr>
<td>PE</td>
<td>Phosphatidylethanolamine</td>
</tr>
<tr>
<td>PGE₂</td>
<td>Prostaglandin E₂</td>
</tr>
<tr>
<td>PGF₂α</td>
<td>Prostaglandin F₂α</td>
</tr>
<tr>
<td>PGHS</td>
<td>Prostaglandin H synthase</td>
</tr>
<tr>
<td>PI</td>
<td>Phosphatidylinositol</td>
</tr>
<tr>
<td>PL</td>
<td>Phospholipid</td>
</tr>
<tr>
<td>PPAR</td>
<td>Peroxisome proliferator-activated receptor</td>
</tr>
<tr>
<td>PS</td>
<td>Phosphatidylserine</td>
</tr>
</tbody>
</table>
PUFA - Polyunsaturated fatty acid
RER - Respiratory exchange ratio
RM - Repetition maximum
RMR - Resting metabolic rate
RQ - Respiratory quotient
RREE - Relative resting energy expenditure
SAD - Sagittal abdominal diameter
SCD - Stearoyl-CoA desaturase
tt10,c12 - trans-10,cis-12
TG - Triacylglyceride
TV - Training volume

UCP - Uncoupling protein
Vb - Body volume
V'b - Corrected body volume
VTG - Thoracic gas volume
VLMT - Vastus lateralis muscle thickness
WHR - Waist-to-hip ratio
1-RM - One repetition maximum
15kd PGF2α - 15-keto-dihydro-prostaglandin F2α
%BF - Percent body fat
CHAPTER 1
THEORETICAL FRAMEWORK

1.1 Introduction

Conjugated linoleic acid (CLA) encompasses a group of 18-carbon polyunsaturated fatty acid isomers derived from the essential lipid, linoleic acid (LA). Of the group of 28 possible isomers cis-9,trans-11 (c9,t11) and trans-10,cis-12 (t10,c12) CLA are considered the two most biologically active. These and other CLA isomers are naturally occurring and found in such common foods as beef and lamb and the dairy products of these animals.

Interest in CLA intensified after Ha et al. (1987) detected the presence of an anticarcinogenic agent in the isolate of grilled beef. Since that time numerous studies have been conducted to uncover an abundance of biological activities attributed to CLA. Of particular interest are the purported effects of CLA on body composition in animals and humans and if these changes are a consequence of altered metabolic rate and substrate utilization. A number of animal studies have demonstrated that supplementing CLA to mice, rats, hamsters, chicken and pigs reduced body fat (Akahoshi et al., 2002; Aletor et al., 2001; Azain et al., 2000; Bee, 2000; DeLany et al., 1999; Dugan et al., 1997; Dugan et al., 1999; Koba et al., 2002; Nakanishi et al., 2001; O’Quinn et al., 1998a; O’Quinn et al., 1998b; Ostrowska et al., 2003; Pariza, 1999; Park et al., 1997; Park et al., 1999a; Poulos et al., 2001; Sher et al., 2003; Strangl, 2000; Swan et al., 2001; Takahashi et al., 2003; Terpstra et al., 2002; Thiel-Cooper et al., 2001; West et al., 1998; West et al., 2000; Wiegand et al., 2001; Wiegand et al.,
enhanced gains in lean body mass (Dugan et al., 1997; Dugan et al., 1999; Kramer et al., 1998a; Park et al., 1999a; Strangl et al., 2000), increased daily energy expenditure (Nagao et al., 2003; Terpstra et al., 2002; West et al., 1998; West et al., 2000), and increased fatty acid oxidation (Nagao et al., 2003; Ohnuki et al., 2001a; Ohnuki et al., 2001b; West et al., 1998). Moreover, the influence CLA appears to exert on body composition is not realized through normal dietary levels; supplementation is required.

The potential utility of CLA to provide individuals with a non-pharmacological means for reducing body fat and enhancing gains in lean muscle is vast, but premature. Nevertheless, CLA is currently marketed as a potential ergogenic aid purported to reduce body fat and enhance gains in lean mass. Human studies have provided more equivocal results than animal studies concerning the effects of CLA supplementation on body composition. To date, human studies have provided research lacking power due to low subject numbers and have not provided sufficient control or description of exercise regimes employed (Atkinson et al., 1999; Belury et al., 2003; Blankson et al., 2000; Kreider et al., 2002; Lowery et al., 1998; Thom et al., 2001; Zambell et al., 2000). Additionally, biological activities of CLA have been largely assigned to c9,t11 and t10,c12 CLA isomers. Previous human studies have tended to administer mixtures of CLA with relatively low concentrations of the two noted active isomers (Benito et al., 2001a; Benito et al., 2001b; Kelley et al., 2000; Kreider et al., 2002; Medina et al., 2000; Zambell et al., 2000; Zambell et al., 2001). It is the intention of the present study to address such weaknesses and provide practical knowledge concerning the utility of CLA to enhance the reductions in body fat and increase gains in lean tissue.
mass realized from regular resistance training. The present study offers greater power, an effective exercise regime previously revealed to induce gains in lean body mass, and the administration of a CLA mixture predominantly composed of the two active CLA isomers. The overall purpose of this study was to determine if CLA alters body composition and strength when supplemented to individuals participating in a prescribed resistance training regime.

1.2 Review of Literature

1.2.1 Conjugated Linoleic Acid

Conjugated linoleic acid (CLA) refers to a group of 18-carbon (octadecadienoic acid) positional (position of double bonds) and geometric (cis or trans) isomers of the omega-6 essential fatty acid, linoleic acid (LA). Unlike LA, the two double bonds of CLA isomers are not separated by a methylene carbon defining the point of conjugation. To date, CLA isomers have been identified with conjugated double bonds at carbon numbers 7 and 9, 8 and 10, 9 and 11, 10 and 12, 11 and 13, and 12 and 14 (Banni 2002; Destaillats et al., 2003). The double bond at each of these carbons can assume either a cis (c or Z) or trans (t or E) configuration.

Naturally occurring, CLA is found in the meats and dairy products of ruminant animals such as cattle, sheep, goats and lamb. Ruminant animals possess Butyrovibrio fibrisolvens bacteria that contain linoleate cis-12,trans-11- isomerase (Kepler et al., 1967). This enzyme isomerizes LA into c9,t11 CLA, also referred to as rumenic acid (RA) originally coined by Parodi’s group in 1998 (Kramer et al., 1998a). Bacterial
conversion is only one of several possible means by which CLA isomers are formed in vivo. For example, CLA is formed when Δ-9 desaturase introduces a cis double bond between carbons 9 and 10 of t11-octadecenoic acid (C18:1); the second intermediate of LA biohydrogenation (Corl et al., 2001; Parodi, 2002; Pollard et al., 1980). There is also limited support that RA is formed from humans fed t11-octadecenoic acid via Δ-9 desaturase activity (Adlof et al., 2000). Based on this and earlier studies (Emken et al., 1985; Emken et al., 1986; Emken et al., 1988; Emken et al., 1990; Emken et al., 1992) it seems plausible that some CLA isomers may be formed naturally in the human body when certain polyunsaturated fatty acids (PUFAs) are present in the diet. CLA can also be produced through industrial processing of foods such as frying fats and oils (Destailhats et al., 2002; Sébédio et al., 1988), partial hydrogenation of vegetable oils (Jung et al., 1999), alkali isomerization of linoleic acid (Banni, 2002; Eulitz et al., 1999) and through selective sigmatropic (a type of pericyclic reaction) and geometrical isomerization reactions (Destailhats et al., 2003).

Research interest in CLA greatly intensified after Ha and colleagues (1987) identified CLA as the anticarcinogenic isolate from grilled beef that inhibited chemically-induced skin neoplasia in mice. Since that time, numerous studies have applied CLA mixtures and specific isomers to confirm CLA’s anticarcinogenic properties both in vitro (Cho et al., 2003; Ip et al., 1999; Majumder et al., 2002; Miller et al., 2002; Yamasaki et al., 2002) and in vivo (Banni et al., 1999; Cheng et al., 2003; Hubbard et al., 2003; Ip et al., 1996; Ip et al., 1997; Park, H. et al., 2001; Thompson et al., 1997; Yang et al., 2001) employing various cell culture lines and animal models. Furthermore, CLA has been shown to exert such beneficial physiological effects as
improved serum lipid profiles (Gavino et al., 2000; Kritchevsky et al., 2000; Lee et al., 1994; Mougios et al., 2001; Noone et al., 2002; Sher et al., 2003), improved glucose tolerance (Hamura et al., 2001; Henricksen et al., 2003; Houseknecht et al., 1998; Ryder et al., 2001), immune system modulation (Bassaganya-Riera et al., 2001; Chew et al., 1997; Corino et al., 2002a; Hontecillas et al., 2002; Sugano et al., 1998; Takahashi, K., et al., 2002; Wong et al., 1997; Yang et al., 2000; Yu, Y. et al., 2002), attenuation of immune-induced catabolism (Cook et al., 1993; Miller et al., 1994; Yang et al., 2001), enhanced feed efficiency (O’Quinn et al., 2000; Ostrowska et al., 2003; Wiegand et al., 2001; Wiegand et al., 2002), increased metabolic rate (Nagao et al., 2003; Terpstra et al., 2002; West et al., 1998; West et al., 2000), reduced body fat (Akahoshi et al., 2002; Atkinson et al., 1999; Blankson et al., 2000; Mougios et al., 2001; Park et al., 1997; Park et al., 1999a; Risérus et al., 2001; Smedman et al., 2001; Takahashi et al., 2003; Terpstra et al., 2002; West et al., 1998; West et al., 2000; Thom et al., 2001), and increased lean body mass (Atkinson et al., 1999; Dugan et al., 1997; Dugan et al., 1999; Lowery et al., 1998; Park et al., 1999a; Strangl et al., 2000).

It is important to note that the biological activities, such as those mentioned above, are attributed to two specific isomers of CLA. These isomers, c9,t11 and t10,c12 CLA have received the designation as the active CLA isomers and are generally what researchers refer to when making reference to CLA. Recent advances in CLA research have provided isomer-specific biological activities differentiating c9,t11 and t10,c12 CLA. In light of the CLA mixture including both active isomers in near equal amounts employed in the present study and that both isomers account for essentially all biological activities it is not advantageous to further discuss individual
properties of the isomers [reviewed in Belury, 2002a; Evans et al., 2002a; Martin et al., 2002]. It is noteworthy that t10,c12 appears to be responsible for reducing body fat and that c9,t11 appears to be responsible for enhancing growth (Pariza et al., 2001). Moreover, c9,t11 is a more potent peroxisome proliferator-activated receptor-α ligand (PPARα) which is a nuclear receptor involved in regulating genes influencing energy metabolism, cell differentiation, proliferation and apoptosis (Moya-Camarena et al., 1999). Advantages to mixtures or purified doses of either active isomer are just starting to be revealed.

1.2.2 Conjugated Linoleic Acid and Body Composition

Despite the myriad of physiological effects CLA has been reported to induce, the influence on changes in body composition is the most thoroughly investigated which is likely a result of the potential clinical significance for treating obesity and promoting a healthy body composition. Studies investigating the effects of CLA on body composition have been completed using cell cultures, animals, and humans; however, the majority of the studies have employed growing animals. Most of these studies have concluded that CLA affects body composition by reducing body fat or enhancing gains in lean mass or total growth.

Animal Studies. CLA reduces body fat and increases lean mass in various species including mice (Akahoshi et al., 2002; DeLany et al., 1999; Nakanishi et al., 2001; Park et al., 1999a; Park et al., 1999b; Takahashi, Y., et al., 2002; Takahashi et al., 2003; Terpstra et al., 2002; West et al.,1998; West et al., 2000), rats (Azain et al., 2000;
Koba et al., 2002; Park et al., 1997; Poulos et al., 2001; Strangl, 2000), rabbits (Corino et al., 2002b), chicken (Aletor et al., 2001; Du et al., 2002; Szymczyk et al., 2001), hamsters (Sher et al., 2003) and pigs (Bee, 2000; Dugan et al., 1997; Dugan et al., 1999; Dugan et al., 2001; Kramer et al., 1998b; O'Quinn et al., 1998a; O’Quinn et al., 1998b; Ostrowska et al., 1999; Ostrowska et al., 2003; Pariza, 1999; Swan et al., 2001; Thiel-Cooper et al., 2001; Wiegand et al., 2001; Wiegand et al., 2002). In 1994, Chin and colleagues made a noteworthy finding demonstrating that feeding a CLA mixture to rats resulted in enhanced feed efficiency (ratio of weight gain to amount of feed ingested) and weight gain. The same group later discovered feeding CLA to mice resulted in repartitioning of body fat to lean mass (Park et al., 1997; Park et al., 1999a). CLA-fed mice experienced reductions in fat mass of up to 88% (West et al., 1998), however, average reported values are around 50% for mice (Akahoshi et al., 2002; DeLany et al., 1999; Nakanishi et al., 2001; Park et al., 1999a; Takahashi et al., 2003; Terpstra et al., 2002; West et al., 2000) and 15% for rats (Azain et al., 2000; Koba et al., 2002; Poulos et al., 2001; Strangl, 2000). Interestingly, Park et al. (1999a) revealed that CLA-induced repartitioning of fat to lean mass was more complex than simply reducing fat accumulation in growing animals. Gains in whole body protein seemed to precede the reduction in body fat mass (Park et al., 1999a). It appears that CLA not only reduced body fat, but also enhanced gains in lean tissue thought to stem from diminished protein catabolism. Support for this was provided by Miller et al. (1994) reporting feeding CLA to mice partially attenuated the catabolic response to an endotoxin injection resulting in a reduction in the loss of muscle mass. Yang and associates (2000) offered additional support demonstrating CLA supplementation
attenuated lean mass reductions in immuno-compromised mice. The mice were susceptible to disease-related muscle protein loss and CLA was found to slow this process. Strangl (2000) completed a two stage study clearly differentiating the effects of CLA on fat deposition and lean mass accretion. The first phase comprised feeding CLA or sunflower oil (control) to rats for three weeks. In the second phase rats were calorie-restricted and continued to supplement CLA or sunflower oil. CLA-fed rats gained less fat in the first phase and gained more lean mass in phase two than the control group. These results strongly suggest CLA supplementation attenuated gains in fat mass and enhanced lean mass gains when the mice had limited caloric intake. An earlier study completed by West et al. (1998) also found feeding CLA to mice resulted in reduced body fat however, no differences were apparent between animals fed high or low fat diets.

Due to recent innovations in methods for synthetically producing CLA large animal studies have become more feasible. Consequently, CLA studies employing pigs became more popular making these animals the closest model to humans that have currently been investigated. A group in Lacombe, Alberta has become one of the leading research centers investigating the effects of CLA on body composition in pigs. Feeding CLA to pigs tended to decrease feed intake and enhance feed conversion efficiency without influencing average daily gains or total weight gains (Dugan et al., 1997; Dugan et al., 1999; Kramer et al., 1998b). Although CLA had no effect on total carcass weight, repartitioning of fat to lean mass was evident as subcutaneous fat was reduced and lean mass increased. Similarly, Cook and colleagues (as cited in Pariza, 1999) reported feeding CLA to pigs resulted in a marked reduction in back fat.
thickness and gains in lean mass without affecting total carcass weight or feed intake suggesting enhanced feed conversion efficiency. A recent study completed in Australia corroborated these results. Ostrowska et al. (2003) fed pigs varying concentrations of CLA for eight weeks and measured body composition by dual-energy X-ray absorptiometry (DEXA). The CLA-fed animals had enhanced gains in lean tissue and reduced fat mass with a concomitant linear decrease in caloric intake with increased CLA concentration. Similar reports on fat to lean repartition in pigs have been demonstrated by groups in Iowa (Swan et al., 2001; Thiel-Cooper et al., 2001; Wiegand et al., 2001; Wiegand et al., 2002) Kansas (O’Quinn et al., 1998a; O’Quinn et al., 1998b) and Switzerland (Bee, 2000).

Not all studies are in agreement concerning the effects of CLA on feed conversion efficiency and feed intake. Several supplementation studies with pigs have reported CLA had no influence on feed conversion efficiency or feed intake (Dunshea et al., 2002; Eggert et al., 2001; Ostrowska et al., 1999; Ramsay et al., 2001; Weber et al., 2001). Dugan and colleagues (2001) demonstrated that varying the concentration of total oil and CLA content in the pigs’ diet tended to differentially affect feed intake. Three levels of CLA, consisting of 0%, 0.25% or 0.5% of the total diet and two levels of total oil, at 2% or 6%, were evaluated. Overall, CLA did not affect feed intake or average daily gains with modest effects on lean mass gains, however 0.25% CLA at 2% total oil diet tended (p=0.06) to decrease feed intake compared to the 0% CLA group (Dugan et al., 2001). Avarette-Gatlin and others (2002) conducted a similar study feeding lean genotype pigs diets varying the amount of CLA and total fat provided. Average daily gains, feed efficiency, back fat thickness and fat-free mass
were not influenced by any amount of CLA supplementation. On the other hand, Bee (2000) demonstrated feeding CLA to sows increased total carcass weight and feed intake of their piglets compared to LA-fed controls. Gross morphological inspection suggested the CLA-fed animals had greater lean mass. The apparent disagreement on how CLA influences body composition of pigs is likely the result of methodological differences. The amount and isomeric composition of CLA and control diets, duration of supplementation, age and type of pigs, and methods for measuring composition are not equivalent in each study. Some evidence exists suggesting that the two active isomers have different physiological effects therefore the concentrations of these isomers could potentially affect the outcome. Growing animals respond better to CLA supplementation and male pigs eat more food and are thus exposed to higher doses of CLA if given ad libitum in normal chow. These factors must be taken into consideration when comparing studies. Nevertheless, most studies demonstrated that feeding CLA to pigs markedly enhanced fat to lean tissue repartitioning.

**Human Studies.** In light of the effects on body compositional changes reported using pigs, among other species, interest in the research community peaked in the late 1990’s concerning the potential benefits CLA may provide in human subjects. Despite the accumulating studies completed on humans, however, very few have investigated the effects of CLA on body composition. Only three of these studies have investigated the concurrent effects of CLA supplementation with strenuous exercise as described in full text peer-reviewed journals (Blankson et al., 2000; Kreider et al., 2002; Thom et al.,
2001). Before these studies are discussed it is first important to mention the studies that laid much of the groundwork on CLA and body compositional changes in humans.

Mougios and others (2001) evaluated the effects of different doses of CLA over two separate phases. 24 healthy subjects (male and female) were assigned to receive a CLA mixture in a double blind fashion over two 4 week periods. In the first phase, subjects received either 0.7 g/d of CLA or soybean oil (placebo). Dosages were doubled to 1.4 g/d for the final 4 weeks. Body fat was determined by the sum of the thickness of ten skinfolds at baseline, 4 and 8 weeks. The CLA group had greater reductions in body fat compared to controls when given 1.4 g/d but not 0.7 g/d. These results suggested a minimal dose of CLA may be required to obtain the sought benefits on body composition.

In Sweden, 53 healthy subjects (men and women) received either 4.2 g/d of a CLA mixture or olive oil for a total of 12 weeks. Body composition was determined using anthropometric measurements and bioelectrical impedance. Body weight, body mass index (BMI), sagittal abdominal diameter (SAD) and waist-to-hip ratio (WHR) were not significantly different between the two groups. However, the CLA group did lose a significantly higher percentage of body fat than the control group further supporting the notion that CLA may reduce body fat (Smedman et al., 2001). The same group completed a similar study with 24 abdominally obese middle-aged (39-64 yrs) men supplemented with either 4.2 g/d of a CLA mixture or olive oil for a 4 week period. BMI, waist circumference and WHR all significantly decreased in the CLA group, but no differences were indicated between groups. In contrast, SAD decreased significantly more in the CLA group than the control group suggesting some,
but not all, indicators of obesity may be influenced by short-term supplementation with CLA (Risérus et al., 2001). It is important to note that not all studies have reported favourable results concerning the effects of CLA on body composition.

One such study investigated the effects of supplementing CLA (3 g/d) on 17 healthy females (20-41 yrs) confined to a metabolic suite for 94 days (Zambell et al., 2000). The first 30 days was an accommodation period during which no supplements were given. Body composition was determined using total body electrical conductivity and DEXA. The results indicated that the CLA mixture had no effect on fat mass, fat-free mass, total body weight or percent body fat when compared to the placebo group who received sunflower oil. Additionally, BMI was unaffected by supplementing with CLA (Medina et al., 2000). The absence of any evident physiological effects imparted by CLA may be a result of the compositional makeup of the CLA mixture. In the studies reporting more favourable results the composition of the CLA is primarily c9,t11 and t10,c12 and these isomers are in near equal proportions. The studies by Zambell et al. (2000) and Medina et al. (2000) used CLA with lower amounts of the two active isomers and higher amounts of other isomers. As mentioned above (Section 1.2.1), it is these two isomers that have demonstrated biological activity. Furthermore, interactions between isomers may occur potentially impeding the effects observed on body composition. Additionally, CLA may promote lean tissue growth while reducing or preventing the accumulation of body fat which may not be appropriately addressed by the study designs employed above (Atkinson, 1999). It may therefore, be more advantageous to supplement CLA while subjected to periods of enhanced catabolism and/or anabolism as is the case with resistance training. Studies employing CLA
supplementation during periods of exercise training are discussed in the following paragraphs.

Atkinson et al. (1999) evaluated the efficacy of CLA to reduce body fat when administered to obese subjects. Eighty obese individuals received either CLA (2.7 g/d) or an equal amount of an unspecified placebo for a six month period. A standardized diet and unspecified exercise regime were prescribed for the duration of the study. With the use of underwater weighing body composition was determined at the beginning and the conclusion of the experiment. Initial analyses of the data indicated that CLA had no effect on body composition or total weight. Post hoc analyses did indicate that subpopulations in the CLA group tended to lose fat and gain lean mass while the placebo subpopulation tended to increase overall body fat. Due to the ambiguity of the methodology and lack of details provided such as the type of exercise regime, composition of CLA, age and gender of subjects, these results are of limited practical use.

In one of the few studies that investigated dose effects, Blankson et al. (2000) investigated the effects of supplementing a range of doses (1.7 g/d, 3.4 g/d, 5.1 g/d, 6.8 g/d) of a CLA mixture to 47 overweight or obese men and women for 12 weeks. Body composition was determined using DEXA. Nearly all doses, including 1.7 g/d, resulted in a marked reduction in body fat mass. Doses above 3.4 g/d did not further enhance this effect. There was a trend toward increased lean body mass at the highest dosage however significance was not achieved. Additionally, the authors stated that subjects were offered to follow a standard training program however no information on the type of training or subject compliance were given.
Thom et al. (2001) conducted the first study to combine exercise training and CLA supplementation in healthy non-obese subjects. Twenty healthy subjects (10 male, 10 female) from 18-30 years of age were recruited to participate in a regular exercise regime while receiving supplementation for a period of 12 weeks. Subjects were randomly assigned in a double blind fashion to receive 1.8 g/d of a CLA mixture consisting primarily of equal amounts of c9,t11 and t10,c12 isomers or hydrogel capsules. Body composition was determined with near infrared light at the midpoint of the biceps at baseline, 4, 8 and 12 weeks. BMI and total body weight were not significantly different between groups at any time period. In contrast, body fat percentage was significantly lower in the CLA group than the placebo group at 4 weeks and remained as such throughout the duration of the study. Interpreting the results from this study is problematic as very little information concerning the exercise regime and the subjects’ training experience are given. It does, however, seem apparent that a lower dose (1.8 g/d) of CLA may have the potential to reduce body fat, further supporting the results reported by Blankson et al. (2000).

In contrast, a more recent and well defined study conducted by Kreider and colleagues (2002) demonstrated that supplementing higher doses of CLA to resistance trained individuals did not influence body composition. Twenty three experienced resistance trained men (>1 year; 3x/wk) received a CLA mixture (6 g/d) with fatty acids (3 g/d) or an olive oil placebo (9 g/d) while continuing with their training regimes for a total of 4 weeks. The subjects were matched by body weight and training volume then randomly assigned to either group. Body composition was determined by DEXA at baseline and at 4 weeks. CLA was reported to have no influence on total body
weight, percent body fat, fat mass or lean mass. Similar to the studies by Zambell et al. (2000) and Medina et al. (2000) reporting that CLA had no effect on body composition, Kreider's study also utilized a similar CLA isomeric composition. Only approximately 40% of the total CLA content consisted of the c9,t11 and t10,c12 isomers therefore serving as a limitation of the study as these two isomers are of greatest importance with respect to biological activity. Phenotypic changes may not have been realized as higher concentrations of the two active isomers may be required. Interactions between the active and inert isomers may result in deactivation of the biological activities normally observed; however, further research is needed to elucidate the viability of such possibilities.

1.2.3 Conjugated Linoleic Acid and Resting Metabolic Rate

The potential effects CLA may exert on energy expenditure offers a potential mechanism by which reductions in body fat could be realized. For this reason, details concerning this mechanism will be further discussed in Section 1.2.4. Nevertheless, having investigated the effects of CLA on metabolic rate as a dependent variable in the present study it is imperative a separate section supporting the employment of this variable be included.

In 1998, West and colleagues investigated the effects of a dietary CLA mixture on energy expenditure, among other variables, in mice. In addition to CLA supplementation, the animals were fed either a high-fat (45% of total calories) or low-fat (15% of total calories) diet for the entire 6 weeks of the study. Energy expenditure was increased by supplementing CLA irrespective of the level of fat intake.
Interestingly, total energy intake was significantly reduced in both CLA groups. Overall 24-hour respiratory quotient (RQ) values were not affected by CLA intake. Nighttime RQ values were markedly reduced (indicating increased fat oxidation) in CLA-fed mice compared to controls in the low-fat group with little effect in the high-fat group. A more recent study by the same group confirmed the above results indicating five weeks of feeding CLA to mice increased energy expenditure (West et al., 2000).

Ohnuki and others (2001b) administered a single dose of CLA to mice to investigate the acute effects of CLA on energy expenditure. After 90 minutes oxygen consumption was significantly greater with CLA than mice receiving LA. The CLA group had slightly lower RQ values (p<0.1) and higher body temperatures suggesting more fat and total energy, respectively, were being expended with the consumption of CLA. The same group also reported that longer term administration of lower concentrations of CLA to mice resulted in enhanced oxygen consumption (Ohnuki et al., 2001a).

A study completed by Terpstra et al. (2002) utilized the formula below to determine the effects of CLA supplementation on energy expenditure in young mice.


All rats were fed high-fat diets, however one group was calorie-restricted and the other was not. Both CLA groups were found to have increased energy expended as heat, increased energy lost in the excreta and reduced stored energy (calculated and measured). CLA significantly increased total energy expenditure in the calorie-restricted mice yet both CLA groups were in a state of negative energy balance.
A recent study investigated CLA isomer-specific effects on energy metabolism in rats (Nagao et al., 2003). Two experiments were conducted. The first experiment involved supplementing rats with or without a CLA mixture (primarily consisting of c9t11 and t10,c12 isomers) for four weeks. In the second experiment, rats were fed diets supplemented with c9,t11 or t10,c12 CLA for 10 days. The CLA group in experiment 1 significantly increased energy expenditure and oxygen consumption in comparison to the control group. In experiment 2, mice fed t10,c12 CLA had higher oxygen consumption and energy expenditure than those receiving the c9,t11 isomer. From these results the authors suggested the t10,c12 isomer is responsible for any effects CLA may have on energy metabolism. Due to the absence of a control group in experiment 2 it is assumptive to interpret anything further than a relative effect on energy metabolism between the two isomers.

In contrast to most of the results previously discussed, a study was conducted on rats suggesting CLA had little effect on energy metabolism (Azain et al., 2000). Rats were fed a CLA mixture for 7 weeks. To measure energy metabolism rats were placed in respiration chambers for 48 hours and 24 hour energy expenditure was measured at baseline, 1, 4 and 7 weeks. CLA had no effect on oxygen consumption, energy expenditure or heat production. RQ was significantly lower in CLA-fed rats suggesting a higher degree of fats were being utilized as an energy source compared to control animals. These results may differ from other studies as rats are less responsive to CLA than mice. Total CLA content in the diet was also lower than most studies supplying a maximal dose of 0.5g /100g diet. Furthermore, the content of t10,c12 CLA (45.6%) was substantially lower than that found in Nagao et al.’s (2003) study.
(89%), which also employed rats. Nagao and colleagues (2003) suggested that t10,c12 CLA was significantly more potent at enhancing oxygen consumption and energy expenditure than c9,t11 CLA.

Very few studies have investigated the effects of CLA on energy metabolism in humans. One well controlled study involved the confinement of seventeen healthy females (20-41 yrs) to a metabolic suite for a total of 94 days (Zambell et al., 2000). After 30 days of accommodation subjects were randomly assigned in a double blind fashion to receive a CLA mixture (3 g/d) or sunflower oil (3 g/d). Indices of energy metabolism were measured at baseline, 4 and 8 weeks. CLA had no influence on energy expenditure or respiratory exchange ratio (RER) at rest or during light exercise. Importantly, the CLA mixture used was composed of only ~40% of the active isomers, c9,t11 and t10,c12. In light of the results by Azain et al. (2000) discussed above and other studies reporting the biological activities of c9,t11 and t10,c12 CLA, it is possible that these isomers may have interacted with other isomers potentially negating any biological activity or were at too low a concentration to adequately exert any physiological changes. Another explanation for the variable results reported on the effects CLA may exert on energy expenditure has been offered by Terpstra (2001). Terpstra (2001) suggested that metabolic differences between animals and humans may contribute to the disparity between studies. More specifically, Terpstra (2001) emphasized that relative metabolic rate, referred to as metabolic weight, greatly differs between animals based largely on body weight. The smaller an animal is the higher its metabolic rate is relative to its weight. Thus, the overall reduction in body fat in response to enhanced energy expenditure will be much higher in the smaller animal
with the already higher metabolic rate compared to larger animals including humans. Importantly, Terpstra (2001) offers an explanation pertaining to the influence of altered energy expenditure on body fat, but does not explain the differential responses of energy expenditure to CLA; however it does suggest pre-existing metabolic differences may manifest in different metabolic responses to CLA supplementation. It therefore possible that the efficacy of CLA to alter metabolism may be, in part, determined by the different metabolic weights of the various animal models studied.

1.2.4 Conjugated Linoleic Acid: Proposed Mechanisms of Action

CLA has numerous purported physiological effects serving as an anticarcinogenic, antidiabetic, antiatherosclerotic, immunomodulatory, and antiadipogenic mediator [reviewed in Bassaganya-Riera et al., 2002; Belury, 2002a; Belury, 2002b; Kelly, 2001; Kritchevsky, 2000; Parodi, 2002; Wahle et al., 2002]. Despite CLA having many proposed biological activities a definitive mechanism has yet to be proven. A variable multitude of mechanisms are currently under investigation, however, only those pertaining to body compositional changes will be discussed in detail. To achieve reductions in body fat and enhanced gains in lean mass CLA may influence the type of energy utilized, energy consumption and expenditure, enzymes involved in lipogenic and lipolytic pathways or expression of these enzymes, fatty acid metabolism, and the activity and/or expression of pro-inflammatory cytokines. There is also some evidence suggesting that CLA influences the differentiation, proliferation and programmed cell death of adipocytes. Consideration of the myriad of biological effects CLA has and the emerging evidence supporting
various biochemical properties it is becoming evident that one biological mechanism
does not adequately explain all of these effects. In the context of body composition
alone there is support that CLA exerts its influence by multiple mechanisms (Belury,
2002a; Calder, 2002; Ostrowska et al., 2003; Pariza et al., 2000; Park et al., 1999a;
Parodi, 2002; Risérus et al., 2003; Takahashi et al., 2003).

CLA, Lipogenesis and Lipolysis. One means by which CLA may reduce body fat is by
modulating the activity of lipolytic and lipogenic enzymes. Park and colleagues (1997)
investigated the activity of various enzymes in response to CLA fed to mice or applied
to adipocyte cultures. They found CLA markedly enhanced activity of carnitine
palmitoyl transferase (CPT), also called carnitine acyl transferase, and reduced heparin-
releasable lipoprotein lipase (LPL) activity. CPT is an enzyme involved in the
transporting of fatty acid moieties across the outer (CPT I) and inner (CPT II)
mitochondrial membranes. Upon arrival into the mitochondrial matrix the fatty acid
moiety is oxidized via β-oxidation ultimately serving as an energy source. The more
active CPT becomes the more fat that can be oxidized resulting in an overall reduction
in available fatty acids for storage. On the other hand, LPL is an enzyme involved in
liberating fatty acids from triacylglycerides, which in turn can be shunted to the
mitochondria to be oxidized for energy or more likely transported to adipose tissue to
be stored. It is interesting to note that increased LPL activity is associated with the
development of obesity (Nilsson-Ehle, 1981). Increased activity of CPT indicates
more fat is being utilized as a source of energy and reduced LPL activity indicates less
fat is being stored. Furthermore, the activity of lipolytic and lipogenic enzymes often
work in synchrony such that increasing the activity of one pathway indicates a concomitant decrease in the activity of the other pathway.

The same group later supported their earlier findings this time utilizing c9,t11 CLA, t10,c12 CLA or an equal mixture of the two isomers (Park et al., 1999b). The CLA mixture and t10,c12 CLA, but not c9,t11 CLA, reduced LPL activity in cultured adipocytes, serving as a potential mechanism for the reduced percent body fat also reported (Park et al., 1999b). Further studies by this group have provided more support suggesting CLA, and more specifically, t10,c12 CLA, effectively reduces LPL activity in mice and adipocyte cell culture (Park, Y., et al., 2001a; Park, Y., et al., 2001b; Xu et al., 2003). Their results also suggest that suppression of LPL activity may precede the reduction in body fat as short-term (4 days) supplementation of CLA reduced LPL activity in the absence of any changes in body composition. It may be that CLA is far more efficacious than initially believed as biochemical events such as altered LPL activity are not immediately evident when observing changes in body composition.

Evans and others (2002b) demonstrated t10,c12 but not c9,t11 CLA enhanced fatty acid oxidation in mouse adipose tissue, however LPL activity was not assessed. A group in the Netherlands reported that both active isomers suppressed LPL activity with equal efficacy. Interestingly, c9,t11 CLA suppression was abolished by the addition of insulin suggesting t10,c12 CLA may more effectively inhibit LPL activity under physiological conditions (Lin et al., 2001). A comprehensive study completed in Japan investigated the effects of supplementing mice with a CLA mixture on the expression and activity of several enzymes involved in lipid metabolism (Takahashi et al., 2003). Enzymatic assays revealed significant increases in the activity and mRNA
expression of several oxidative (including CPT) and lipogenic enzymes in the liver. The physiological basis by which CLA both increases hepatic fatty acid synthesis and oxidation is unclear at present. The author offers a probable explanation stating that increased activity of lipogenic enzymes may be a consequence of large reductions in fat mass serving as a potential mechanism whereby CLA may reduce body fat. Support for this theory has been provided suggesting enhanced lipogenic enzyme activity and expression may be a compensatory hepatic mechanism when faced with accumulating levels of TGs often accompanying considerable fatty acid oxidation (Moitra et al., 1998). More recently Koba et al. (2002) reported supplementing rats with CLA significantly increased hepatic TG concentration likely in response to the enhanced reductions in adipose tissue weight.

Not all studies conclude that CLA inadvertently upregulates the expression and activity of lipogenic enzymes in response to enhanced lipolysis and consequent liberation of FAs. There is increasing evidence that CLA has a marked influence on the activity of stearoyl-CoA desaturase (SCD) [reviewed in Ntambi et al., 2002]. SCD is a microsomal enzyme involved in catalyzing a necessary step of monounsaturated fatty acid synthesis. It may therefore serve as another mechanism whereby CLA influences body composition by reducing SCD activity and consequent synthesis of various fatty acids. The details surrounding how CLA may influence SCD activity remain to be elucidated. Studies have consistently reported CLA reduced SCD activity (Choi et al., 2000; Choi et al., 2001; Choi et al., 2002; Lee et al, 1998; Park et al., 2000; Smith et al., 2002) and mRNA or protein expression (Lee et al., 1998, Choi et al., 2000; Choi et al., 2002) supporting the idea that CLA may influence body
composition through lipogenic enzymatic regulation. There is less agreement on isomer-specific effects of CLA on SCD. Lee and others (1998) demonstrated feeding a CLA mixture to mice for a 2 week period effectively reduced SCD expression (as measured by mRNA levels) and activity. They further demonstrated in cell culture that c9,t11 CLA alone did not influence SCD expression. When the c9,t11 isomer was combined with other CLA isomers SCD expression was reduced. Similar results have since been demonstrated in mice (Park et al., 2000) and cultured adipocytes (Choi et al., 2000) suggesting another CLA isomer or combination of CLA isomers are responsible for reducing SCD expression. In contrast, Choi et al. (2002) reported c9,t11 and t10,c12 CLA individually reduced SCD activity in human breast cancer cell lines. Interestingly, the cell lines responded differently as the two CLA isomers decreased SCD protein expression in one cell line (MDA-MB-231) but not the other (MCF-7) suggesting that the effects of CLA on SCD activity may be cell specific. The details surrounding the relationship between CLA and SCD have yet to be resolved. It does seem clear that CLA exerts some influence, whether direct or indirect, on this integral lipogenic enzyme. It is also becoming more apparent that CLA may reduce fat mass, at least in part, by influencing SCD, LPL and/or CPT activity.

CLA and Energy Balance. Another putative mechanism explaining the body compositional changes observed with CLA supplementation is through pathways regulating energy expenditure. Unequivocal evidence has indicated CLA may reduce energy intake (Ostrowska et al., 2003; Ryder et al., 2001; Takahashi, Y., et al., 2002; Terpstra et al., 2002; West et al., 1998), enhance gain-to-feed ratio (O’Quinn et al.,
increase energy expenditure (EE) (West et al., 1998; West et al., 2000; Terpstra et al., 2002), alter substrate metabolism (Azain et al., 2000; Ohnuki et al., 2001b; West et al., 1998), and influence the expression of key metabolic proteins (Peters et al., 2001; Rodriguez et al., 2002; Ryder et al., 2001; Takahashi, Y., et al., 2002; Tsuboyama-Kasaoka et al., 2000; West et al., 2000).

In the late 1990’s evidence surrounding the fat reducing effects CLA exerted in animal models was accumulating and preliminary results had suggested that CLA affected key enzymes involved in lipid metabolism (Park et al., 1997). Nevertheless, accounts of energy intake and output were not available prompting West and others (1998) to investigate such indices in mice. CLA was found to reduce total energy intake over a six week period offering yet another viable mechanism for researchers to pursue. Further support for this mechanism has since been provided in some studies (Ostrowska et al., 2003; Ryder et al., 2001; Takahashi, Y., et al., 2002; Terpstra et al., 2002; West et al., 1998) but not all subsequent studies (DeLany et al., 1999; Dunshea et al., 2002; Eggert et al., 2001; O’Quinn et al., 2000; Weber et al., 2001; Wiegand et al., 2001; Wiegand et al., 2002; West et al., 2000). Interestingly, Ryder et al. (2001) reported isomer-specific effects of CLA on energy intake. Rats fed a 50:50 mixture of c9,t11 and t10,c12 CLA for 14 days significantly reduced the amount of ingested feed. When rats were instead fed a nearly pure dose of c9,t11 their feed intake increased. Isomer-specific effects of CLA remain largely unexplored. Unfortunately, most studies completed in humans accounting for energy intake utilized a standard mixture of CLA isomers with substantially lower concentrations of c9,t11 and t10,c12 CLA,
thus serving as a potential explanation for the contrasting results concerning caloric intake.

All human studies reporting energy intake, to date, have demonstrated that CLA does not influence energy intake. Researchers in California reported no difference in total dietary intake between those supplementing CLA and those taking sunflower oil (Benito et al., 2001a; Benito et al., 2001b; Kelley et al., 2000; Zambell et al., 2000; Zambell et al., 2001). It is important to note that dietary intake was controlled in all of these studies based on the American Heart Association’s Step II Diet or the USDA Handbook 8 (as cited in Kelley et al., 2000). Further independent studies not limiting energy intake have since provided similar results strongly suggesting CLA does not impact body composition at moderate dosages (3.0-6.0 g/d) through changes in energy intake in humans (Mougios et al., 2001; Risérus et al., 2002; Smedman et al., 2001). Kreider and others (2002) investigated the effects of supplementing CLA during a resistance training regime. With resistance training there is an increased demand to supply energy through dietary means providing an excellent condition for observing the influence of CLA on energy intake. As previously demonstrated, supplementing CLA did not influence the amount of total energy the subjects ingested (Kreider et al., 2002).

The effects of CLA on feed efficiency (ratio of weight gains to amount of food ingested) have also been reported in an attempt to explain, in part, the source of body compositional changes. There are two possible means to enhancing feed efficiency: realizing the same weight gains while reducing total caloric intake; and increasing total weight gains in the absence of a relative increase in energy consumption. In the latter
case, increased weight gain has been shown while body fat declined suggesting marked lean mass deposition. Accordingly, enhanced lean tissue gains were demonstrated in pigs in the absence of increased energy consumption (O’Quinn et al., 2000; Wiegand et al., 2001; Wiegand et al., 2002). No human studies have formally accounted for feed efficiency however accurate inferences can be formed. In most humans studies no differences between CLA and placebo groups have been observed when accounting for total energy intake and body weight (Benito et al., 2001a; Benito et al., 2001b; Kelley et al., 2000; Kreider et al., 2002; Mougios et al., 2001; Risérus et al., 2002; Smedman et al., 2001; Zambell et al., 2000; Zambell et al., 2001). Unfortunately, those studies reporting positive effects of CLA on body composition have not accounted for energy consumption (Blankson et al., 2000; Thom et al., 2001). Contrasting information has recently been provided by a study reporting CLA supplementation (8.0 g/d) which induced a reduction in body weight with no change in energy intake (Belury et al., 2003). As changes in body composition were not accounted for, interpretation of the results is severely limited in terms of energy balance and fat to lean partitioning. Nevertheless, these findings suggest that a high dose of CLA may influence feed efficiency in humans.

In addition to energy intake a few studies also accounted for total energy expenditure (EE) and/or respiratory exchange ratio (RER) or respiratory quotient (RQ). In mice, CLA has been shown to increase EE, when compared to control animals, in some (Nagao et al., 2003; Terpstra et al., 2002; West et al., 1998), but not all (Azain et al., 2000) studies. Although Azain et al. (2000) reported CLA had no effect on EE, they did report a significant reduction in RQ. This suggests that substrate utilization
simply changed to more fatty acids being oxidized to provide the same energy output as before supplementation. West et al. (1998), on the other hand, reported a concomitant increase in EE with no change in 24-hour RQ values. When split up into day and night, nighttime RQ was significantly lower in the CLA group. Zambell et al. (2001) supplemented a CLA mixture (3.0 g/d) for 9 weeks to healthy women and reported no changes in EE or substrate utilization (respiratory exchange ratio). Not surprisingly, CLA did not influence fat mass, fat-free mass or percentage body fat.

Despite the equivocal results reported in human subjects, researchers continue to investigate the effects of CLA on energy balance as a potential mechanism for reduced fat mass. Researchers have suggested that CLA may influence energy balance by regulating the activity and/or expression of important proteins involved in energy production. One such protein is uncoupling protein (UCP), an inner mitochondrial membrane (IMM) protein. UCP permits the dissipation of energy as heat by uncoupling the production of adenosine triphosphate (ATP) from the proton gradient (which drives the addition of a phosphate to adenosine diphosphate forming ATP) across the IMM [see Paulo et al., 1998 for further details]. Upregulation of UCP activity thus serves as a potential channel whereby EE can be influenced. If energy is being converted to heat rather than a usable energy source (such as ATP) then necessarily less energy will be stored as fat and/or more energy substrate (fats, carbohydrates) will be shuttled into the mitochondria. It appears this may be a mechanism whereby CLA exerts some influence.

Tsuboyama-Kasaoka and colleagues (2000) demonstrated CLA upregulated UCP-2 mRNA (a UCP isoform) in mice adipose tissue after 11 days of
supplementation. Similarly, Ryder and others (2001) reported enhanced UCP-2 expression in skeletal muscle and adipose tissue of rats fed a CLA mixture or CLA comprised predominantly (91%) of c9,t11. These studies suggest CLA may enhance EE through upregulation of UCP expression.

West and others (2000) reported CLA treatment in mice increased UCP-2 mRNA expression in brown adipose tissue (BAT) and had no effect on UCP-1 expression. Further studies have indicated that CLA appears to upregulate the expression of UCP-2 in BAT of rodents (Ealey et al., 2002; Peters et al., 2001; Roche et al., 2002; Takahashi, Y., et al., 2002). Interestingly, Rodriguez et al. (2002) found differential effects of CLA isomers on UCP-1 expression in mouse BAT. c9,t11 CLA enhanced UCP-1 expression whereas t10,c12 CLA had the opposite effect while reducing adipogenesis and also reduced UCP-2 expression. In light of the results the authors suggested that reduced adipogenesis with t10,c12 CLA and enhanced thermogenic abilities of BAT with c9,t11 CLA may, in part, explain the effects of CLA on body composition and EE in animal models. In addition, the authors note that combined effects of c9,t11 and t10,c12 CLA may have profound nutritional significance on adiposity and EE. Two recent studies have demonstrated the upregulation of protein or mRNA expression of UCP-3 in skeletal muscle further suggesting enhanced energy expenditure via heat production (Ealey et al., 2002; Roche et al., 2002). Despite the controversy that continues to surround the potential regulation of UCP gene expression it is likely CLA influences energy balance by more than one mechanism. Due to the novelty of UCP upregulation by CLA human research has yet to shed light on the issue.
CLA, Cell Differentiation, Proliferation and Apoptosis. Another mechanism offering an explanation for the ability of CLA to reduce adipose tissue is through the induction of cellular differentiation, proliferation and programmed cell death (apoptosis). CLA has been documented to attenuate the differentiation and proliferation of numerous cancer cell lines (Chen et al., 2003; Cho et al., 2003; Igarashi et al., 2001; Ip et al., 1999; Liu et al., 1999; Miller et al., 2002; Palombo et al., 2002) as well as adipocytes (Brodie et al., 1999; Brown et al., 2003; Evans et al., 2000; Kang et al., 2003; Satory et al., 1999). The molecular mechanisms involved in regulating cellular differentiation and proliferation are complex and beyond the scope of this discussion, however some details are pertinent [reviewed in Belury, 2002b; Rudolph et al., 2001; Tang et al., 2002; Wahle et al., 2002]. It appears a link between the activity of peroxisome proliferator-activated receptors (PPAR), regulated by CLA, influences cell differentiation and proliferation of adipocytes (among other cells). PPARs are part of a superfamily of nuclear receptors involved in regulating genes involved in energy metabolism, cell differentiation, proliferation and apoptosis (Bishop-Bailey et al., 2003; Houseknecht et al., 2002). As CLA has been shown to be a potent PPAR ligand, it is possible the effects observed on cellular development are through PPAR-induced events. A number of studies have investigated the effects of CLA on PPAR activity however results remain mixed. Increased PPAR activity is associated with a resultant increase in LPL activity indicating CLA should in some manner interfere with this relationship. Some (Brown et al., 2003; Houseknecht et al., 1998; Kang et al., 2003; Rodriguez et al., 2002; Ryder et al., 2001; Takahashi, Y., et al., 2002), but not all (Choi
et al., 2000; Hontecillas et al., 2002; McNeel et al., 2003; Meadus et al., 2002; Meadus et al., 2003; Moya-Camarena et al., 1999; Peters et al., 2001) studies have indicated that CLA reduced the expression (mRNA) or protein levels of PPAR (α or γ isoforms) suggesting a more complex interaction is at work. The apparent discrepancy between studies is in part a result of tissue-specific expression patterns of PPAR isoforms, molecular differences between various cell lines and animal models, duration of exposure to CLA, and CLA isomer-specific effects on PPARs. For example, PPARγ2 mRNA expression in cultured (3T3-L1) preadipocytes was enhanced after 2 days of exposure to t10,c12 CLA. After 6 days of exposure to the same isomer PPARγ2 expression markedly decreased to below baseline levels. As expected, t10,c12 CLA reduced triglyceride (TG) content while c9,t11 CLA increased triglyceride content (Evans et al., 2001). The results suggest t10,c12 enhances adipocyte proliferation with acute (2 days) exposure but decreases proliferation with more chronic (6 days) exposure through PPARγ2-mediated action. Despite the lack of clarity surrounding CLA and PPAR activity a great deal of agreement suggests CLA reduces cellular proliferation in adipocytes. Additional support has been provided by prevailing evidence suggesting CLA may not only reduce cell proliferation but enhance apoptosis (Brown, J., et al., 2001; Chen et al., 2003; Cho et al., 2003; Evans et al., 2000; Hargrave et al., 2002; Ip et al., 1999; Ip et al., 2000; Kim et al., 2002; Liu et al., 2002; Miller et al., 2002; Park, H., et al., 2001; Palombo et al., 2002; Tsuboyama-Kasaoka et al., 2000). By increasing the number of cells programmed to death, CLA was originally demonstrated to reduce cancer growth and later found to have similar effects in normal (pre)adipocytes (Evans et al., 2000; Kang et al., 2003). A few studies have
suggested that CLA is able to attenuate the expression of various anti-apoptotic genes and increase the expression of pro-apoptotic genes, although it is not yet known if this is a PPAR-mediated mechanism (Ip, et al., 2000; Liu et al., 2002; Majumder et al., 2002; Miller et al., 2002). Interestingly, increased UCP-2 expression has also been associated with apoptosis establishing yet another link between mechanisms (Mills et al., 2002; Tsuboyama-Kasaoka et al., 2000). Again the molecular mechanism(s) explaining the ability of CLA to induce apoptosis are complex and largely unexplained.

CLA, Eicosanoids and Immunomodulation. CLA may also influence body composition by modulation of events surrounding immunity through eicosanoid formation. Eicosanoids are a group of lipids with hormone-like properties comprising the prostaglandins, leukotrienes and thromboxanes and are derived through cyclooxygenase (COX) and lipoxygenase pathways of most immune cells. The eicosanoids are involved in modulating inflammation and the immune response in part by regulating cytokine synthesis (Calder et al., 2002; Robinson, 1987). Given that CLA has been implicated to some degree in all of these events researchers began to focus on the probable link between CLA and eicosanoids (Belury, 2003; Bulgarella et al., 2001; Iwakiri et al., 2002; Sugano et al., 1998; Whigham et al., 2002; Yang et al., 2003). Moreover, the primary origin of eicosanoids is from the C20 polyunsaturated fatty acid, arachidonic acid (AA) which itself is derived from linoleic acid (LA) the parent compound of CLA. Not surprisingly it has been demonstrated that CLA exerts its influence on the immune system, at least in part, through modulation of eicosanoids.
by altering the metabolism of LA and AA (Banni et al., 2001; Cantwell et al., 1999; Liu et al., 1998; Miller et al., 2001).

It is plausible that CLA may reduce the availability of AA by different means. Firstly, CLA has been reported to alter the distribution of AA into the different lipid fractions. Cantwell and colleagues (1999) demonstrated a reduction of AA in phospholipid (PL) fractions of rat hepatocytes. More details were provided by Miller and colleagues (2001) showing that the c9,t11 CLA isomer reduced the incorporation of AA into phosphatidylcholine (PC) fractions while increasing its presence into phosphatidylethanolamine (PE) of two breast cancer cell lines (MCF-7 and SW480 cells). Miller's group also reported reduced conversion of AA to the prostaglandin, PGE2, supporting the notion that CLA may regulate eicosanoids by changes in AA distribution among the phospholipid fractions. Interestingly, some evidence has suggested PC is the primary source of AA for eicosanoid synthesis (Hanel et al., 1993) via phospholipase A2 hydrolysis adding further support that CLA effectively reduces the availability of AA and consequent production of certain eicosanoids. In contrast, Ip and colleagues (1996) reported that CLA did not alter the distribution of FAs in PL fractions of rat mammary tissue. Another group found the c9,t11 CLA isomer increased AA incorporation into membrane PC in human saphenous vein endothelial cells (Urquhart et al., 2002). They did however report an overall reduction of AA incorporation into membrane PLs already incorporating CLA. Additionally, CLA treatment at lower concentrations resulted in a decrease of both constitutive and stimulated (calcium ionophore) eicosanoid production.
Even though there is little agreement concerning the suspected effects of CLA on the composition of PL fractions it does seem more apparent that CLA does influence neutral lipid FA composition. Studies have demonstrated that CLA is preferentially incorporated into neutral lipids in hepatocytes (Cantwell et al., 1999; Belury et al., 1997), skin cells (Kavanaugh et al., 1999) and mammary tissues (Banni et al., 1999). Banni and colleagues (2001) demonstrated CLA (c9,t11) supplementation had no apparent effect on the distribution of LA but did decrease AA levels in the mammary tissue of rats. The distribution of CLA and its metabolites were found predominantly in neutral lipids and to a lesser extent phosphatidylserine (PS) and phosphatidylinositol (PI). Although eicosanoid levels were not accounted for this study does offer support of a potential mechanism for tissue-specific effects of CLA.

A second means by which CLA may effectively reduce the AA pool for conversion to eicosanoids is through inhibition or direct competition of metabolic enzymes upstream of AA. Evidence exists supporting CLA’s involvement with the desaturase and elongase enzymes in normal PUFAs metabolism. Chuang et al. (2001b) indicated a marked decrease in the metabolic products of LA undergoing desaturation by Δ6-desaturase when in the presence of CLA. The same study further indicated no changes to Δ6-desaturase enzyme mRNA. A follow-up study by the same group confirmed these earlier findings and further reported that CLA inhibited the elongation of LA by human elongase (Chuang et al., 2001a). Δ9-desaturase activity and gene expression have been shown to be inhibited by CLA (Bee, 2000; Bretillon et al., 1999; Lee et al., 1998) and in particular the t10,c12 isomer (Baumgard et al., 2001; Lee et al., 1998; Sébédio et al., 2001). Interestingly, these results not only correspond to reduced
eicosanoid production but also studies revealing reductions in fat mass as CLA appears to interfere with lipogenesis.

Not only has CLA been implicated in regulating or interacting with enzymes upstream of AA synthesis there is evidence suggesting CLA may regulate enzymes downstream as well. CLA may be regulating the production of eicosanoids through inhibitory actions on cyclooxygenase (COX) and lipoxygenase (LOX) enzymes. Cyclooxygenation and lipoxygenation are cytosolic processes which involve the oxygenation and reduction of PUFAs such as AA to produce prostaglandins or thromboxanes and leukotrienes, respectively. As early as 1970, researchers have recognized the inhibitory effects of PUFA metabolites on COX activity (Nugteren, 1970). Bulgarella and others (2001) found CLA inhibited the oxidation of AA by the COX enzyme, prostaglandin H synthase (PGHS). It was further elucidated that CLA did not serve as a substrate for PGHS, but did act as a competitive inhibitor to this COX enzyme. Isomer-specific properties were also noted, reporting the c9,t11 CLA isomer had the strongest inhibition of PGHS activity in the presence of AA. Similarly, Iwakiri et al. (2002) demonstrated that CLA decreased prostaglandin E₂ (PGE₂) synthesis as a consequence, in part, of reduced COX-2 and inducible-nitric oxide synthase (iNOS) mRNA expression. More specifically, CLA was only effective at reducing the highly inducible forms of COX (COX-2) and NOS (iNOS) when these enzymes were previously induced by the bacterial endotoxin, lipopolysaccharide (LPS). In agreement, Whigham and colleagues (2002) found CLA-fed guinea pigs had reduced prostanoid and leukotriene production in comparison to controls only when COX and LOX activity was induced. It appears that CLA may modulate eicosanoid
production at the level of gene expression or by direct competitive inhibition of COX enzymes. Miller et al. (2001) found CLA decreased PGE$_2$ synthesis in the absence of reduced AA release suggesting CLA modulates eicosanoids by regulating downstream enzymes (i.e. COX or isomerase/reductase). Interestingly, Basu and others (2000a, 2000b) reported an increase in enzymatic lipid peroxidation in humans in response to 4 weeks and 12 weeks of CLA supplementation, respectively. The metabolite 15-keto-dihydro-PGF$_{2\alpha}$ (15kd PGF$_{2\alpha}$) had significantly higher urine and plasma levels in the CLA group as compared to the placebo group. As 15kdPGF2$\alpha$ is a major metabolite of PGF2$\alpha$ these results suggest CLA increases at least one pro-inflammatory eicosanoid undermining the proposed inhibitory actions of CLA on eicosanoid synthesis.

The majority of studies have reported that CLA markedly reduces eicosanoid synthesis in numerous cell lines and animal models (Iwakiri et al., 2002; Kavanaugh et al., 1999; Li et al., 1998; Li et al., 1999; Liu et al., 1998; Miller et al., 2001; Nakanishi et al., 2003; Torres-Duarte, 2003; Urquhart et al., 2002; Whigham et al., 2001; Whigham et al., 2002; Yu, Y., et al., 2002). Eicosanoids, such as PGE$_2$, enhance the inflammatory response by inducing cytokine synthesis. It is therefore hypothesized that inhibition of these eicosanoids will effectively reduce inflammatory-induced catabolism. Some evidence suggests CLA reduces the catabolic effects of immune stimulation (Cook et al., 1993; Miller et al., 1994; Yang et al., 2000). Miller et al. (1994) fed CLA to chicks, rats and mice prior to injection of a known catabolic agent (LPS) for 7, 14 and 15 days, respectively. They reported that CLA attenuated the reduction in body weight for all tested animals. Recently, a study was conducted
investigating the influence of acute CLA administration and variable dietary protein levels on dexamethasone-induced immunodepleted rats (Turini et al., 2003). Rats were injected with a pro-catabolic glucocorticoid and assigned to one of four dietary groups varying protein level (high or low) and the presence or absence of CLA for a total of four days. CLA and the high-protein diet (20% protein) resulted in enhanced fractional protein synthesis (spleen) compared to CLA-free and low-protein (10% protein) diets, respectively. CLA did not influence protein-turnover as would be expected from an anti-catabolic agent, which would attenuate the higher catabolic rate induced by the glucocorticoid. The author explained that dietary CLA may not be an effective form of acute treatment for immune-induce catabolism due to the apparent inability (of CLA) to inhibit catabolism regardless of protein intake. Whether or not longer term consumption of CLA may serve as a preventative measure for bouts of immune stress remains to be resolved.

1.2.5 Conjugated Linoleic Acid: Effective Dosages and Toxicity

CLA is a fatty acid found in normal foods most people ingest from day to day such as meat from cows, lamb, pork, chicken, and cheeses, milks and other dairy products derived from these animals (Banni et al., 1996; Chin et al., 1992; Fritsche et al., 1998; Ha et al., 1989; Lin et al., 1995; Parodi, 1977; Shantha et al., 1994; Shantha et al., 1995). Estimates of average CLA (all isomers) intake have been conducted and the earliest estimates ranging from 500 to 1000 mg/d (Ip et al., 1994; Parodi, 1994) appear to have overestimated actual intake. More recent studies in Germany and the United States have estimated rumenic acid (c9,t11 CLA), the most abundant isomer,
daily consumption to range from 52 to 430 mg/d (Fritsche et al., 1998; Herbel et al., 1998; Ritzenthaler et al., 1998). The variability of CLA consumption can be attributed at least in part to gender, as males consumed more than females, and the accuracy of the data collection methodology employed. For example, a 3-day food record has been demonstrated to be a more accurate estimate of nutritional intake than a 24-hour recall or food frequency questionnaires (Crawford et al., 1994; Mullenbach et al., 1992). In any case, the level of CLA ingested through a normal diet does not appear to exert any significant physiological benefits in animals or humans.

Previous studies have indicated that minimal doses of CLA are required to attain the sought biological effects and that the magnitude of the effects are dependent upon the dose utilized (Blankson et al., 2000; Mougios et al., 2001; Ostrowska et al., 2003). Some studies have reported linear decreases in fat deposition, fat content and increased average daily gains (ADG), lean deposition and protein content with increased consumption of CLA (Azain et al., 2000; DeLany et al., 1999; Dugan et al., 2001; Ostrowska et al., 1999; Ostrowska et al., 2003; Thiel-Cooper et al., 2001). In a few studies, minimal doses of CLA were required to significantly change body composition. DeLany and others (1999) reported a minimum dose of 0.5% CLA (percent of total diet by weight) was necessary to significantly reduce body fat and 1.0% CLA was necessary to increase percent protein content. Similarly, another group indicated lean tissue content was maximized at 5.0g of CLA/kg feed (Ostrowska et al., 1999; Ostrowska et al., 2003). Interestingly, it may be the presence of t10,c12 CLA that is responsible for altering body composition. Park and others (1999b) demonstrated feeding either a mixture of CLA containing equal amounts of c9,t11 and
t10,c12 or a t10,c12-enriched diet resulted in greater reductions in percent body fat and gains in protein content than mice fed the c9,t11-enriched diet. In most cases, the two active isomers are given in equal amounts and are the predominant isomers present in CLA mixtures. Animal studies are however of limited use in extrapolating to humans as relative intake of foodstuffs differ greatly. For example, a pig fed 5g of standard mixture of CLA per kg of feed will ingest from 20-25g of the CLA (Atkinson, 1999). For a 65 kg pig, these animals are ingesting from ~310-385mg/kg of body weight every day. Using the highest estimated average human daily intake of CLA (1000 mg/day), a 70kg person will ingest merely 14mg/kg of body weight daily. The question concerning what dosages are effective in humans thus remains.

Studies conducted by a group in California had subjects ingest 3.0-3.9g CLA/day. In all of these studies, CLA had no physiological effects on such factors as body composition (Medina et al., 2000; Zambell et al., 2000), fatty acid metabolism (Zambell et al., 2000; Zambell et al., 2001), blood lipid profiles (Benito et al., 2001a; Benito et al., 2001b), immune indices (Kelley et al., 2000) or eicosanoid secretions (Kelley et al., 2001). Another group of researchers had subjects supplement 4.2g CLA/day reporting significant reductions in percent body fat (Smedman et al., 2001) and sagittal abdominal diameter (Risérus et al., 2001) in as few as 4 weeks.

Other studies demonstrated the biological activities of CLA supplementation were realized at much lower doses. Blankson and others (2001) reported reductions in fat mass with as low as 1.7g CLA/day. Additionally, a trend toward enhanced gains in lean body mass was realized when supplementing the highest dose of 6.8 g/d. In
accordance with these results, Thom et al. (2001) demonstrated supplementing 1.8g of CLA daily significantly reduced percent body fat.

Mougios et al. (2001) conducted a study demonstrating a minimal dose of CLA was required to alter body composition. Four weeks of CLA supplementation at 0.7 g/day had no effect on body composition. When the dose was doubled (1.4 g/day) for an additional four weeks subjects had significantly lower percentage body fat and fat mass suggesting the lower dosage was not sufficient to induce phenotypical changes. It is not known whether the influence on body composition was the result of an increase in CLA ingested or a simple time effect from administering CLA for a total of 8 weeks.

Recently, Kreider et al. (2002) revealed supplementing higher doses of CLA had no effect on body composition among many other measures. Experienced resistance-trained subjects were administered 7.0g of CLA daily for a 4 week period with no significant changes in body composition. Clearly, the disagreement on the efficacy of CLA from study to study could be attributed, in part, to variability in methodology and the subjects’ profile. As previously discussed (see Section 1.2.2) the subjects in Kreider et al.’s study (2002) were allowed to continue with their own resistance training program and consisted of men with a minimum of one year of regular (at least 3 times per week) resistance training experience; whereas those in Thom et al.’s study (2001) performed 90 minutes of strenuous exercise followed by a standardized program (not described) and consisted of men and women with an unknown amount of training experience. It is plausible that subjects in Kreider’s study had more training experience than those in Thom’s study and therefore less likely to
achieve the considerable gains often observed in those with less training experience. Presently, it appears the isomeric composition of the CLA may be of some importance. The majority of human studies in which CLA did not induce changes in body composition utilized a CLA mixture comprised of ~40% or less of the biologically active isomers, c9,t11 and t10,c12 (Kreider et al., 2002; Medina et al., 2000; Risérus et al., 2001; Zambell et al., 2000). On the other hand, studies indicating that CLA does impact body composition utilized CLA with nearly twice the concentration or more, of the same two isomers (Belury et al., 2003; Blankson et al., 2000; Mougios et al., 2001; Smedman et al., 2001). A minimal dose does appear to exist as normal dietary intake is insufficient to induce physiological changes; however the most important factor seems to be the compositional makeup of CLA. The possibly remains that the presence of non-active CLA isomers may interact with and ultimately deactivate the active isomers in some manner preventing any physiological changes, however, research is severely lacking on this matter.

In all human studies completed no serious side effects have been reported. Blankson et al. (2001) described the drop-out of eight subjects out of a group of sixty as a result of adverse effects, seven of which may have been a consequence of supplementing CLA or placebo. The authors reported the majority of complaints were mild to moderate gastrointestinal discomfort and that no (gastrointestinal) differences were noted between the CLA and placebo group. Nevertheless, CLA has been demonstrated to be safely administered to humans in dosages as high as 8.0 g/d for 8 weeks and 6.8 g/d for up to 12 weeks.
There is limited evidence suggesting CLA may have a negative effect on insulin sensitivity. Risérus et al. (2002) had 60 middle-aged (35-65 yrs) men with signs of the metabolic syndrome (abdominal obesity, dyslipidemia, hypertension and insulin resistance) supplement 3.4g of a CLA mixture, t10,c12 CLA or a placebo for a period of 12 weeks. The t10,c12 CLA isomer alone was credited with decreasing insulin sensitivity. Also, the CLA mixture and t10,c12 resulted in reduced serum high density lipoprotein (HDL) levels. An earlier study using a CLA mixture reported that insulin levels tended to increase (Medina et al., 2000) further suggesting CLA may have diabetogenic effects. In contrast, antidiabetic effects have been reported in CLA-fed rats (Houseknecht et al., 1998; Ryder et al., 2001). In most cases no side effects, with the exception of the above, were reported.

1.3 Statement of Problem and Hypotheses

1.3.1 Statement of Problem

To date, no human data regarding metabolic rate, body composition, strength and indicators of catabolism is available with non-athlete resistance-trained individuals supplemented with CLA under controlled training conditions. It was the goal of this research to examine the effects of chronic supplementation of a consumer-available CLA mixture on body compositional changes, substrate utilization, metabolic rate and muscular strength in recreationally active (resistance-trained) and healthy men and women.
1.3.2 Hypotheses

The hypotheses of this research suggest that CLA will significantly reduce skeletal muscle catabolism as evidence by increased overall muscle mass and strength. To optimize the efficacy of CLA it is proposed that a moderately high dose of CLA (5.0 g/d) combined with resistance training will greatly enhance lean mass gains balanced with decreased fat mass. These results should be realized in the absence of any changes in gain to feed (caloric intake) ratio. It is also hypothesized that CLA will result in greater reductions (than placebo) in fat mass as a consequence, in part, from enhanced fatty acid utilization. Lastly, it is hypothesized that CLA will not effect relative resting energy expenditure. In summary, it is hypothesized that supplementation with CLA, versus sunflower oil (placebo), in combination with seven weeks of resistance training will produce the following outcomes:

1) Enhanced gains in muscle thickness
2) Enhanced gains in strength and torque
3) No effect on resting metabolic rate (relative and absolute)
4) Enhanced fatty acid oxidation (\( \downarrow \) respiratory exchange ratio)
5) No effect on gain to feed ratio
6) Reduced fat mass
7) Increased gains in lean mass

1.3.3 Limitations

Subjects in this study are free-living and were exposed to stresses and physiological demands which were not controlled. Dietary habits were not completely
controlled and analysis was based on a 3-day food record. Another potential limitation of this study was motivation which is a subjective factor that influences subjects' training performance and strength. Subjects also performed resistance training under supervised conditions making it unpredictable if similar results would be produced under normal (non-experimental) conditions. Lastly, any physiological changes induced through nutritional intervention can only be attributed to the specific isomeric mixture of CLA administered in the present study.

1.3.4 Delimitations

Result from the present investigation can be generalized only to males and females of similar age (18-34 yrs), training status, health and CLA intake.
CHAPTER 2
METHODS

2.1 Research Design

Healthy men and women were stratified according to gender and randomly assigned to the CLA or control group in a double-blind fashion. Subjects concurrently participated in a periodized total-body resistance-training program for a seven week period. The effects of CLA supplementation on the variables listed in Table 1.1 were measured immediately before and after the seven week period. Included in Table 1.1 are the respective methods employed for evaluating each variable.

Table 1.1 Mode of measurements for evaluation of dependent variables.

<table>
<thead>
<tr>
<th>Measured Variable</th>
<th>Mode of Measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Composition</td>
<td>Air displacement plethysmography</td>
</tr>
<tr>
<td>Muscle Thickness</td>
<td>Ultrasound</td>
</tr>
<tr>
<td>Muscle Strength</td>
<td>1-repetition maximum</td>
</tr>
<tr>
<td>Maximal Torque</td>
<td>Isokinetic dynamometer</td>
</tr>
<tr>
<td>Resting Metabolic Rate</td>
<td>Open circuit indirect calorimetry</td>
</tr>
<tr>
<td>Respiratory Exchange Ratio</td>
<td>Open circuit indirect calorimetry</td>
</tr>
<tr>
<td>Caloric Intake</td>
<td>3-Day food record</td>
</tr>
</tbody>
</table>

A double blind repeated measures design was employed in which all participants were matched by gender and body mass, and randomly assigned to either the CLA or placebo group (sunflower oil). All subjects trained concurrently with 7-
weeks of supervised strength training. The participants were measured before and shortly (within 72 hours) after the completion of the study. Measurements included: total body weight, percent body fat, lean body mass, fat mass, strength (leg and chest press), peak torque for knee extension and flexion, muscle thickness (elbow flexors and knee extensors), resting metabolic rate, and respiratory exchange ratio (RER).

In accordance with college and university policy the study proposal was submitted to and approved by the Biomedical Ethics Review Board at the University of Saskatchewan in Saskatoon, Saskatchewan (See Appendix A for certificate of approval) and each subject signed a consent form for participation (Appendix B).

2.2 Participant Characteristics

Forty (N=17 females and N=23 males) participants, ranging in age from 18-34 years volunteered for the study. All selected participants had some strength training experience within the past 4 months (at least twice a week for a minimum of three months) to minimize neuromuscular adaptations often observed in newly trained individuals (Mayhew et al., 1995; Narici et al., 1995). The majority however, had more than 2 years experience prior to involvement in the study. Participants were healthy, non-smoking individuals free from taking any ergogenic supplements (i.e. creatine monohydrate, steroids, and ephedrine) at least 8 weeks prior to testing and did not have any known pre-existing diseases as assessed by the Physical Activity Readiness Questionnaire (Appendix C) and initial interviews. All participants were asked not to change their diets or aerobic exercise regimes throughout the duration of the investigation.
2.3 Randomization and Supplementation

An intermediary was responsible for randomizing the participants and coding the supplements to ensure all participants and investigators remained blinded throughout the study. The participants were matched by gender and body mass, then randomly assigned by a coin toss to either receive CLA or the placebo (5 grams per day; see Table 2.1 for composition of CLA supplement) in identical capsules for a seven week duration. Capsules were placed in sealed and labeled (participant’s name) envelopes with one weeks supply and delivered to the investigator to distribute.

The decision to supplement 5 grams of CLA per day was based on previous research demonstrating that higher doses may be necessary to effectively alter body composition in human subjects (Blankson et al., 2000; deDeckere et al., 1999; Kelley et al., 2000; Risérus et al., 2001; Smedman et al., 2001; Thom et al., 2001).

The length of the study was chosen primarily to ensure sufficient time for muscular adaptation and to minimize non-compliance and drop-out rates which have been previously demonstrated to be less than 10% under similar conditions (Chilibeck et al., 1998; Chrusch et al., 2001). Twelve weeks of supplementation with a high dose (6.8 g/d) and 8.0 g/d of CLA for eight weeks have been well tolerated in both male and female subjects (Belury et al., 2003; Blankson et al., 2000). Changes in body composition have been evident in humans supplemented with 4.2g/CLA/d in as few as four weeks (Risérus et al., 2001) while six weeks was found to sufficiently induce muscle hypertrophy with similar resistance training (Farthing et al., 2003). Lastly, other nutritional intervention studies in our own and others’ labs have used a similar duration allowing us to make comparisons to these supplements. For example, our lab
Table 2.1 Compositional List of Conjugated Linoleic Acid Supplement

<table>
<thead>
<tr>
<th>Carbons:Double Bond</th>
<th>Common Name</th>
<th>Percent Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>Myristic acid</td>
<td>0.1%</td>
</tr>
<tr>
<td>16:0</td>
<td>Palmitic acid</td>
<td>6.4%</td>
</tr>
<tr>
<td>16:1</td>
<td>Palmitoleic acid</td>
<td>0.1%</td>
</tr>
<tr>
<td>18:0</td>
<td>Stearic acid</td>
<td>2.3%</td>
</tr>
<tr>
<td>18:1</td>
<td>Oleic acid</td>
<td>13.4%</td>
</tr>
<tr>
<td>18:2</td>
<td>Linoleic acid (c9,c12)</td>
<td>2.5%</td>
</tr>
<tr>
<td>18:2</td>
<td>c9,t11 CLA isomer</td>
<td>33.4%</td>
</tr>
<tr>
<td>18:2</td>
<td>t10,c12 CLA isomer</td>
<td>33.6%</td>
</tr>
<tr>
<td>20:0</td>
<td>Arachidic acid</td>
<td>0.3%</td>
</tr>
<tr>
<td>20:1</td>
<td>Eicosenoic acid</td>
<td>0.2%</td>
</tr>
<tr>
<td>24:0</td>
<td>Lignoceric acid</td>
<td>0.1%</td>
</tr>
<tr>
<td>24:1</td>
<td>Nervonic acid</td>
<td>0.1%</td>
</tr>
<tr>
<td>*Other</td>
<td></td>
<td>7.5%</td>
</tr>
</tbody>
</table>

* includes: gelatin, glycerin, purified water and caramel colour.

has previously assessed the efficacy of whey protein and glutamine combined with resistance training (Burke et al., 2001; Candow et al., 2001) while other researchers have completed similar studies with resistance training and creatine monohydrate (Becque et al., 2000; Syrotuik et al., 2001).

2.4 Procedures

2.4.1 Test Procedures

Cessation of any strength training a minimum of 3 days prior to testing was required of each participant. Two sessions of measures were necessary both before and after the training protocol. The first session included measuring resting metabolic rate,
RER, peak torque, body composition and muscle thickness. In the second session maximal strength was measured. There was a minimum of 2 days rest between the first and second sessions to offset any fatigue or muscle soreness caused by peak torque measurements. Detailed descriptions of all instruments used for each measurement are described in the sections to follow.

2.4.2 Training Protocol

All subjects were required to follow the same high volume, heavy load resistance-training program in a periodized fashion. The resistance training program involved twelve exercises chosen to include all major muscle groups. These exercises included: chest press, shoulder press, bicep curl, latissimus pulldown, back extension, leg press, knee extension, knee flexion, hip adduction, abduction, flexion and extension. Resistance training coincided with supplementation for the entire seven week duration. Each exercise was performed three times per week consisting of 3-4 sets of 4-10 repetitions at 75-90% of 1-RM. Training sessions were supervised as previous research has demonstrated greater gains compared to unsupervised training (Mazzetti, et al., 2000). Training sessions were completed at the convenience of each subject. There was no prescribed order to completing their weekly sessions, however each successive session was separated by at least one rest day to reduce the chance of injury and minimize fatigue. To ensure maximal gains the seven weeks of training were periodized on roughly a 2 1/3 -week cycle. The periodized program was therefore broken up into three blocks consisting of seven complete workouts each. Block one (sessions 1-7) consisted of 4 sets of 8-10 repetitions, with a 1-minute rest
between sets. Block two (sessions 8-14) consisted of 4 sets of 6-8 repetitions, with 1.5-minute rest between sets. Block three (sessions 15-21) consisted of 3 sets of 4-5 repetitions, with 2-minute rest between sets. For block one, intensity for the chest press and leg press were initially set at 60% of the subject’s 1-RM, while other intensities for additional exercises were set at approximately the subject’s 10 RM (i.e. maximal weight the subject could lift for 10 repetitions). For blocks two and three, initial intensities were set at subjects’ 8 RM and 5 RM, respectively. Intensities were progressively increased in 2-5 kg increments when a subject was able to complete the maximum number of repetitions required in a given block with good form. Subjects recorded their progress for each session (number of completed repetitions for all exercises) in a personal log book supplied by the investigator. The log books were regularly examined (after each session) by the investigator to ensure the subjects progressed (increased weight) and attended the required number of sessions.

2.4.3 Muscle and Fat Mass

Utilizing air displacement plethysmography (AP) precise measures of muscle and fat mass were determined. Similar to the principle of measuring body composition by hydrostatic weighing, AP was employed with the Bod Pod (Bod Pod S/L, Life Measurement Inc., Concord, CA.) which utilized the displacement of air instead of water. In a study comparing AP and hydrostatic weighing (HW) estimates of percent body fat and lean mass reported no significant differences in their coefficients of variation (1.7 ± 1.1% and 2.3 ± 1.9%, respectively) (McCrory et al., 1995). The high correlation (r = .93) for estimates of percentage body fat and lean mass between the
two methods provides excellent support for using AP as a reliable and valid method for evaluating body composition. In another study involving nearly 4500 men and women (total) the coefficient of variation for repeated measurements of body fat was 2.27% (Miyatake et al., 1999). The same study also reported a high correlation ($r = .91$) with dual energy x-ray absorptiometry (DEXA) for estimates of percent body fat. These results have been recently corroborated further attesting to the validity of AP as an effective method of measuring body composition (Weyers et al., 2002).

Prior to measurements all subjects were instructed to refrain from performing any exercise for 6 hours, refrain from eating for 3 hours, remove all jewelry including body piercings and shave or wax all excess hair that is a part of their normal routine. None of the subjects had beards and only one subject had a goatee and was asked to maintain it for the duration of the study. Subjects were weighed and measured (height) wearing a bathing suit and a swim cap after voiding. The females were asked to wear the same bathing suit for pre and post measurements and spandex shorts were provided for the males. Breathing tubes and nose clips were utilized to prevent any expired air from entering the chamber. Subjects were instructed to breath normally for 30-40s while body volume ($V_b$) was measured. After this the subjects are connected to the breathing tube and thoracic gas volume ($V_{TG}$) was measured which was subtracted from the original value ($V_b$) to give the corrected volume, denoted $V'_b$. The $V_{TG}$ was measured by having the subjects repeatedly contracting and relaxing their diaphragm while connected to the breathing tube and nose clip inside the Bod Pod. Repeated trials were completed until consistent results were achieved as determined by the Bod Pod software (Life Measurement Instruments, Software Version 1.69). Density was
then calculated by dividing the individual’s mass by the corrected $V_b$. Percent body fat was then estimated by the equation derived by Siri (1961):

\[
\text{Percent Fat} = \frac{495}{\text{density}} - 450
\]

All calculations and output scores were produced with Life Measurement Instruments, Software Version 1.69 provided with the Bod Pod.

**2.4.4 Muscle Thickness**

Muscle thickness for elbow flexors and knee extensors was assessed using B-Mode ultrasound (Aloka SSD-500, Tokyo, Japan). Prior to the initial measurements the subjects were instructed to refrain from exercising for 3 days thus minimizing exercise-induce water retention. Measurements were taken three to five days after their last training session to prevent any swelling in response to the last training session. Muscle thickness measures were performed prior to any strength measurements. Measurements were taken on the right limb only.

*Elbow Flexors.* A mark was made 60% of the distance down from the lateral border of the acromion process to the olecranon process, serving as the mid-point. A tape measure was then wrapped around the circumference of the arm at the mid-point mark and a second mark was made at the most anterior part. The second mark served as the reference point where the middle of the ultrasound probe was placed.

Once the reference points were taken each subject laid their arm extended in front of them flat on a tabletop with their bicep and palm facing upward. A transparency was placed over their arm tracing all obvious permanent landmarks such
as moles, scars and the elbow joint. Great care was taken using overhead transparency film and markings on the skin to ensure that identical sites were measured on each occasion.

A water-soluble transmission gel was applied to the measurement site and a 5-MHz ultrasound probe rested on the biceps. Once satisfied with the quality of the image the researcher captured the picture on the monitor as a still image. Once the image was captured the researcher was able to measure the thickness of the elbow flexors (mm) at three sites; the proximal site, the mid site, and the distal site, as determined by the segments (1 cm) on the monitor. The distal and proximal sites on the monitor were six centimeters apart with the mid site located equidistant between them. The mid site corresponded to the location of the second reference mark.

The muscle thickness measure was extracted from the still image using a similar method as described by Abe et al. (2000), with the distance from the inner border of the subcutaneous adipose layer to the surface of the humerus taken as elbow flexor muscle thickness. Three separate measurements were taken at each of the three sites. The closest two values were then averaged to achieve a final muscle thickness value for that site. If two values could not be selected from the previous three measures (i.e. high and low values were the same distance from the middle value), then a fourth measurement was performed.

*Knee Extensors.* A mark was made 60% of the distance down from the top of the Greater trochanter of the femur to the middle of the knee joint (between lateral condyle of the femur and the lateral condyle of the tibia), serving as the temporary mid-point.
A second mark was made 3.0 cm anteromedially from the mid-point mark. The second mark served as the reference point where the middle of the ultrasound probe was placed. Subjects were then seated on a table with their leg extended directly in front of them. The procedures for measuring knee extensors muscle thickness were identical to those employed for elbow flexor measures with the following exceptions. Thickness was determined by the distance from the inner layer of the subcutaneous adipose tissue to the femur and the monitor magnification was reduced to allow full visualization of the knee extensors.

Reproducibility of measurements of muscle thickness was determined on two separate days for 10 subjects (6 males and 4 females). For the distal, mid and proximal sites of the elbow flexors the coefficients of variation were 1.8%, 1.8%, and 2.6%, respectively. For the distal, mid, and proximal sites of the knee extensors the coefficients of variation were 1.1%, 1.5% and 3.0%, respectively.

2.4.5 Muscular Strength

Assessment of strength was the single maximal lift (1 repetition maximum) for the chest press and leg press using standard testing protocol (Chrusch et al., 2001). Chilibeck et al. (1998) previously demonstrated that the coefficient of variation for the chest press ranged from 2-6 %.

Chest Press. For the chest press, subjects warmed-up by performing 15-20 slow and controlled push-ups; 2-3 static stretches of the pectoral (chest) and deltoid (shoulder) muscles lasting 20-30 seconds; and 10 repetitions on the chest press with weight
deemed comfortable by the subject (Candow et al., 2001). Each subject was positioned with their back flat against the bench, feet flat on the floor with the handles of the chest press line up with the center of their chest. One repetition consisted of lowering the bar from the straight-arm position down to the chest and back to the straight-arm position. Subjects then selected a weight they felt they could press no more than three times and completed these repetitions to conclude the warm-up. After the initial weight selection subjects were to complete only one repetition. Subjects then selected a weight they felt they could complete only once. Weight was thereafter progressively increased 2kg or more for each successful repetition with a three minute rest between each attempt until their 1-RM was achieved.

*Leg Press.* Warm-up consisted of five minutes of moderate (50-60 rpm) stationary cycling; 3 static stretches of the quadriceps muscle group, hamstring and gluteal muscles; and 10 repetitions with the leg press using a comfortable weight chosen by the subject. The same progressive procedure employed for the chest press was utilized for the leg press. Subjects were seated in the leg press such that their upper torso formed a 105° to their thighs. One repetition consisted of lowering the press from a straight-leg position so their legs formed a 90° angle at the knee, then back to the starting position. Increments were 5kg or more to minimize the number of sets required to reach their 1-RM.
2.4.6 Maximal Torque

Changes in knee extension and flexion strength were assessed using an isokinetic dynamometer (Biodex System 3, Biodex Medical Systems, Inc., Shirley N.Y.). Coefficients of variation for extension and flexion have been previously demonstrated in our department to be 2.43% and 6.16%, respectively (Candow et al., 2001). The biodex was set in the concentric mode at an angular velocity of 60° per second. Before beginning any trials subjects warmed-up by riding a stationary bicycle at moderate intensity (50-60 rpm) for 5-7 minutes followed by any static stretches they deemed necessary. Subjects were seated with an angle of 85° of hip flexion and 90° of knee flexion. Stabilization belts were fastened across their chest, lap and upper leg to minimize upper body movement while testing. The rotational axis of the biodex arm was positioned in line with the center of the right knee joint. Once seated and fastened into the chair, the Biodex arm was strapped to the subject’s leg just above the ankle joint. To familiarize subjects with the biodex before commencing the testing they performed 5 repetitions at minimal exertion and one repetition at moderate and one near maximal exertion. One repetition consisted of knee extension to 170° followed by knee flexion back to the starting position of 90°. Knee extension and flexion were interrupted by a 2 second interval to negate any benefits produced by the myotatic stretch reflex. Arms were crossed across their chest for each trial. Four trials were completed at maximal effort with a 60 second rest between each trial. Encouragement was provided by the researcher. The highest recorded value (Newton·meter) of any trial served as their maximal output. Maximal values for knee extension and flexion were selected independently.
2.4.7 Resting Metabolic Rate and Respiratory Exchange Ratio

RER was assessed by open circuit indirect calorimetry using a ventilated hood connected to a metabolic cart (Sensor Medics Vmax29 Series, Anaheim, CA). The metabolic cart analyzed oxygen consumption and expired carbon dioxide levels to estimate resting metabolic rate and respiratory exchange ratio (Weir, 1949). Reproducibility of the metabolic cart was completed employing 9 individuals being measured two times with a 7 day interval between measurements. The coefficient of variation for metabolic rate (volume of oxygen consumed) was 13.2% and 1.9% for RER.

Prior to RER measurements each participant was asked to refrain from any exercise for 48 hours, omit any caffeine intake for 24 hours and fast for 12 hours. Upon arrival the participants were weighed and then rested in the supine position on a hospital bed for 30 minutes to promote whole-body relaxation (Broeder et al., 1992; Thomas et al., 1994; Vermeij et al., 1991). While remaining in a supine position after the initial 30-minute rest interval the ventilated hood was placed over the subject’s head. Outside air was then blown inside the hood at a fixed rate maintaining the carbon dioxide concentration to less than 1%. Constant measurements of flow rate, expired oxygen and carbon dioxide were taken for the duration of procedure. The procedure was completed when carbon dioxide and oxygen levels did not fluctuated more than 5% for five consecutive minutes indicated as steady state by the computer software or when 15 minutes had expired. To exclude inadvertent elevations in RER due to body movement(s) and account for subjects that did not achieve a steady state the lowest fifteen (totaling 5 minutes) RER readings (preset to 20 second intervals)
were averaged and recorded as the RER for that session. Measurements were taken in
the morning between 5:30-9:00 am and the pre and post testing for each subject was
completed at the same time of day. Estimates of daily energy expenditure (absolute)
and RER were provided by the software (Vmax / Sensor Medics Vision Software
Version 4.3) accompanying the metabolic cart based on gender, age and weight.
Relative energy expenditure was calculated from the absolute value divided by the
subjects’ weight. RER is the ratio of the volume of carbon dioxide produced divided
by the volume of oxygen consumed. From RER the major type of fuel source being
utilized for energy production can be determined. Due to differences in the chemical
composition of the major fuel sources (carbohydrates, proteins and fats) the volume of
oxygen consumed and carbon dioxide produced also vary. Consequently, researchers
are able to distinguish the primary fuel source being utilized based on the RER value
(see Appendix D for examples).

2.4.8 Dietary Assessment

To determine the effects of CLA on caloric intake average daily caloric intake
was assessed with a 3-day food record. Three-day food records have been found to
offer a more accurate estimate of caloric intake than other dietary assessments such as
24-hour food recalls and food frequency questionnaires (Crawford et al., 1994;
Mullenbach et al., 1992). A booklet was provided (Appendix E) for each participant to
record the amount and type of food and beverage consumed for two weekdays and one
weekend day. Fuel 2.1 software (Nutrition Software, Logiform Nutrition Sport, 1999)
was employed to estimate average daily energy consumption.
2.4.9 Training Volume

Training volume was calculated to determine if this was a contributing factor to observed changes in body composition, metabolic rate and caloric intake. Training volume was determined by summing the weight (kg) completed for a given exercise multiplied by the number of repetitions completed throughout the study. The values for each exercise were then added together to give their total training volume.

2.4.10 Statistical Analyses

Data was analyzed by a 2 x 2 (group x time) factorial analysis of variance (ANOVA) with repeated measures on the second factor. Dependent variables included: percent body fat, fat mass, muscle mass, total body weight, chest and leg press strength, combined muscular strength, muscle torque, resting metabolic rate, and respiratory exchange ratio, caloric intake, and training volume. Each analysis was followed with Tukey’s post hoc tests in the case of any significant interactions due to the stringent assessment of significance. Statistical significance was set at an alpha level of 0.05. Alpha levels ranging from 0.06 to 0.10 were considered a tendency toward statistical significance.
3.1 Results

3.1.1 Participant Characteristics

The CLA (N=20; 12 males, 8 females) and placebo (N=20; 11 males, 9 females) groups consisted of participants with some strength training experience within the past 4 months (at least twice a week for a minimum of three months). One subject did not comply with pre-testing protocol and another subject was unable to refrain from smoking and both were thus dropped from the study. Another subject was unable to complete the training due to injuries sustained through activities outside of the study leaving a total of 37 participants (17 females, 20 males) successfully completing the training program and supplementation. Of the 37 participants one was excluded from all analyses as aerobic activities were substantially increased during the study. Two other participants were unable to complete some of the post-measures (all strength measures) due to injury and were therefore excluded from the analysis of those measures only. Only one subject (in the placebo group) complained of transient abdominal discomfort, however this individual has a medical history of occasional gastrointestinal problems. None of the subjects dropped out of the study due to adverse events from supplementation. Of the 37 subjects that completed the study all subjects reported having ingested all their supplements with the exception of one subject who missed 2 pills (2.0 g of CLA).
No significant differences were identified (independent sample t-test) prior to supplementation and training between the CLA and placebo groups for any physical characteristics (Table 3.1) or measured dependent variables (Table 3.2).

Table 3.1 Physical characteristics (mean ± standard error) of subjects at baseline.

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (y)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLA</td>
<td>23.5 ± 1.0</td>
<td>172 ± 2.7</td>
<td>74.7 ± 3.8</td>
</tr>
<tr>
<td>Placebo</td>
<td>21.4 ± 0.6</td>
<td>172 ± 2.4</td>
<td>69.7 ± 3.3</td>
</tr>
</tbody>
</table>

No significant differences between groups.
Table 3.2 Baseline values (mean ± standard error) for dependent variables.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Group</th>
<th>Mean</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>LBM (kg)</td>
<td>CLA</td>
<td>56.6 ± 2.9</td>
<td>p=0.69</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>51.3 ± 2.7</td>
<td>p=0.69</td>
</tr>
<tr>
<td>BFM (kg)</td>
<td>CLA</td>
<td>18.3 ± 1.9</td>
<td>p=0.76</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>15.7 ± 1.4</td>
<td>p=0.76</td>
</tr>
<tr>
<td>%BF</td>
<td>CLA</td>
<td>24.1 ± 1.8</td>
<td>p=0.98</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>22.8 ± 1.9</td>
<td>p=0.98</td>
</tr>
<tr>
<td>MT (cm)</td>
<td>CLA</td>
<td>12.27 ± 0.11</td>
<td>p=0.24</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>12.80 ± 0.16</td>
<td>p=0.94</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.41 ± 0.20</td>
<td>p=0.94</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.69 ± 0.23</td>
<td>p=0.94</td>
</tr>
<tr>
<td>1-RM (Kg)</td>
<td>CLA</td>
<td>4220 ± 25</td>
<td>p=0.76</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>4188 ± 24</td>
<td>p=0.87</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5447 ± 35</td>
<td>p=0.87</td>
</tr>
<tr>
<td></td>
<td></td>
<td>649 ± 52</td>
<td>p=0.87</td>
</tr>
<tr>
<td>5-Torque (N·m)</td>
<td>CLA</td>
<td>7209 ± 14</td>
<td>p=0.59</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>7197 ± 12</td>
<td>p=0.89</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8112 ± 6</td>
<td>p=0.89</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9321 ± 60</td>
<td>p=0.89</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8106 ± 6</td>
<td>p=0.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9305 ± 18</td>
<td>p=0.54</td>
</tr>
<tr>
<td>AREE (kcal/d)</td>
<td>CLA</td>
<td>1652 ± 82</td>
<td>p=0.67</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>1561 ± 63</td>
<td>p=0.67</td>
</tr>
<tr>
<td>RREE (kcal/kg/d)</td>
<td>CLA</td>
<td>22.7 ± 0.5</td>
<td>p=0.16</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>23.9 ± 0.6</td>
<td>p=0.16</td>
</tr>
<tr>
<td>RER</td>
<td>CLA</td>
<td>0.81 ± 0.01</td>
<td>p=0.12</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.78 ± 0.01</td>
<td>p=0.12</td>
</tr>
<tr>
<td>CI (kcal/d)</td>
<td>CLA</td>
<td>2048 ± 148</td>
<td>p=0.71</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>2324 ± 198</td>
<td>p=0.71</td>
</tr>
</tbody>
</table>

LBM = lean body mass; BFM = body fat mass; %BF = percent body fat; MT = muscle thickness; 1-RM = one repetition maximum; AREE = absolute resting energy expenditure; RREE = relative resting energy expenditure; RER = respiratory exchange ratio; CI = caloric intake. 1 sum of biceps muscle thickness; 2 vastus lateralis muscle; 3 combined muscle thickness; 4 1-RM for chest press; 5 1-RM for leg press; 6 1-RM for combined chest and leg press; 7 knee extension torque; 8 knee flexion torque; 9 sum of knee extension and flexion torque.
3.1.2 Body Composition

Total Body Weight. No significant time effect (p=0.18) or group differences (p=0.49) were observed. The placebo and CLA groups had slight but insignificant increases in body weight when measured at the conclusion of the study (Table 3.3).

Lean Body Mass. An interaction between group and time (Table 3.3) was identified as those supplemented with CLA gained significantly more lean mass than those receiving the placebo (2.4 kg vs. 0.8 kg; p=0.03).

Body Fat Mass. Body fat mass was significantly reduced over the seven weeks of the study (-0.5 kg and -1.7 kg for placebo and CLA, respectively; time main effect, p=0.003). An interaction between group and time was not observed however, a trend (p=0.08) was evident indicating the CLA group tended to lose more body fat than the placebo group (Table 3.3).

Percent Body Fat. Percentage of body fat was significantly reduced over time (-0.8% and -2.3% for placebo and CLA, respectively; time main effect, p=0.002). There was also a tendency (p=0.09) for the CLA group to attain a greater reduction in percent body fat than the placebo group (Table 3.3).

3.1.3 Muscle Thickness

Biceps Muscle Thickness. A time main effect for biceps muscle thickness was observed in both groups (2.56 ± 0.11 cm to 2.68 ± 0.10 cm, p=0.02). Subjects in the
CLA group tended (0.23 mm vs. 0.04 mm; group x time, p=0.09) to have greater gains than subjects taking the placebo (Table 3.3).

*Vastus Lateralis Muscle Thickness.* The placebo and CLA groups had significantly increased Vastus lateralis muscle thickness from $4.55 \pm 0.14$ to $4.76 \pm 0.11$ cm (time main effect, p=0.01). There were no differences between groups over time (Table 3.3).

*Combined Muscle Thickness.* Combined muscle thickness (Vastus lateralis and biceps muscle thickness) was significantly increased in both groups from pre to post evaluation ($7.11 \pm 0.19$ cm to $7.44 \pm 0.17$ cm; time main effect, p=0.003). There were no differences between groups over time (Table 3.3).

### 3.1.4 Muscular Strength

*Chest Press 1-RM.* For chest press 1-RM there was a group by time (p=0.03) interaction (Table 3.3). Participants in the CLA group significantly increased muscle strength more than those in the placebo group (61 kg vs. 34 kg).

*Leg Press 1-RM.* Subjects from both groups significantly increased their leg press 1-RM over the duration of the study ($426 \pm 22$ kg to $534 \pm 29$ kg, p<0.001). No differences between groups were observed over time (92 kg vs. 123 kg, in the placebo and CLA groups, respectively; Table 3.3).
Combined 1-RM. Both groups significantly increased combined 1-RM (chest and leg press) over time (629 ± 34 kg to 751 ± 45 kg, p=0.001), however, those in the CLA group had significantly greater gains from pre to post analysis (group x time, p=0.05) than subjects in the placebo group (Table 3.3).

Knee Extension Torque. Knee extension torque significantly (p=0.002) increased from pre-training to post-training in both groups (203 ± 10 N-m to 213 ± 9 N-m; time main effect, p=0.002). No group differences were evident (Table 3.3).

Knee Flexion Torque. There were no changes over time for either group for knee flexion torque (p=0.52). No group differences were found (Table 3.3).

Combined Torque. There was a time main effect (314 ± 14 N-m to 326 ± 13 N-m p=0.02) for combined torque (knee extension and flexion torque) in both groups and no evident between group differences were observed (Table 3.3).

3.1.5 Energy Expenditure and Respiratory Exchange Ratio

Resting Energy Expenditure. There was a tendency for subjects in both groups to reduce their average daily resting energy expenditure (1612 ± 53 kcal/d to 1550 ± 58 kcal/d), however significance was not attained (p=0.08). There were no significant differences between groups over time (Table 3.3).
Relative Resting Energy Expenditure. Similar to energy expenditure, relative resting energy expenditure tended to decrease in both groups from $23.2 \pm 0.4 \text{kcal/kg/day}$ to $22.3 \pm 0.6 \text{kcal/kg/day}$ nearly reaching significance ($p=0.07$). There were no significant differences between groups over time (Table 3.3).

Respiratory Exchange Ratio. A trend for reduced respiratory exchange ratio (RER) over time was observed ($p=0.06$). RER tended to decrease in both groups from $.79 \pm .01$ to $.77 \pm .01$. There were no significant differences between groups over time (Table 3.3).

3.1.6 Caloric Intake and Training Volume

No differences in caloric intake were observed between the CLA and placebo group, respectively (2048 $\pm$ 148 kcal/d to 2175 $\pm$ 181 kcal/d and 2324 $\pm$ 198 to 2091 $\pm$ 155 kcal/d, $p=0.20$). Caloric intake did not change over time in either group ($p=.70$).

No differences were evident for training volume between the CLA and placebo group (558,487 $\pm$ 47,208 kg and 485,918 $\pm$ 30,905 kg, respectively; $p=0.72$).
Table 3.3. Values (mean ± standard error) for dependent variables as recorded at pre and post evaluation.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Pre</th>
<th>Post</th>
<th>Time x Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (kg)</td>
<td>CLA</td>
<td>74.7 ±3.8</td>
<td>75.3 ±3.9</td>
<td>p=0.56</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>69.7 ±3.3</td>
<td>70.0 ±3.5</td>
<td></td>
</tr>
<tr>
<td>LBM (kg)</td>
<td>CLA</td>
<td>56.6 ±2.9</td>
<td>59.0 ±3.9</td>
<td>*p=0.03</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>51.3 ±2.7</td>
<td>52.1 ±2.8</td>
<td></td>
</tr>
<tr>
<td>BFM (kg)</td>
<td>CLA</td>
<td>18.3 ±1.9</td>
<td>16.6 ±1.6</td>
<td>p=0.08</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>15.7 ±1.4</td>
<td>15.2 ±1.7</td>
<td></td>
</tr>
<tr>
<td>%BF</td>
<td>CLA</td>
<td>24.1 ±1.8</td>
<td>21.7 ±1.8</td>
<td>p=0.09</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>22.8 ±1.9</td>
<td>22.0 ±1.8</td>
<td></td>
</tr>
<tr>
<td>BMT (cm)</td>
<td>CLA</td>
<td>2.27 ±0.11</td>
<td>2.50 ±0.14</td>
<td>p=0.09</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>2.80 ±0.16</td>
<td>2.84 ±0.14</td>
<td></td>
</tr>
<tr>
<td>VLMT (cm)</td>
<td>CLA</td>
<td>4.41 ±0.20</td>
<td>4.69 ±0.13</td>
<td>p=0.94</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>4.66 ±0.19</td>
<td>4.82 ±0.16</td>
<td></td>
</tr>
<tr>
<td>CMT (cm)</td>
<td>CLA</td>
<td>6.69 ±0.23</td>
<td>7.19 ±0.21</td>
<td>p=0.50</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>7.47 ±0.27</td>
<td>7.66 ±0.25</td>
<td></td>
</tr>
<tr>
<td>BP (kg)</td>
<td>CLA</td>
<td>220 ±25</td>
<td>281 ±29</td>
<td>*p=0.03</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>188 ±24</td>
<td>222 ±24</td>
<td></td>
</tr>
<tr>
<td>LP (kg)</td>
<td>CLA</td>
<td>447 ±35</td>
<td>570 ±48</td>
<td>p=0.30</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>399 ±25</td>
<td>491 ±28</td>
<td></td>
</tr>
<tr>
<td>CP (kg)</td>
<td>CLA</td>
<td>649 ±52</td>
<td>827 ±62</td>
<td>*p=0.05</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>583 ±57</td>
<td>637 ±72</td>
<td></td>
</tr>
<tr>
<td>KET (N·m)</td>
<td>CLA</td>
<td>112 ±6</td>
<td>115 ±7</td>
<td>p=0.60</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>106 ±6</td>
<td>107 ±6</td>
<td></td>
</tr>
<tr>
<td>KFT (N·m)</td>
<td>CLA</td>
<td>209 ±14</td>
<td>220 ±14</td>
<td>p=0.83</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>197 ±12</td>
<td>207 ±12</td>
<td></td>
</tr>
<tr>
<td>CT (N·m)</td>
<td>CLA</td>
<td>321 ±20</td>
<td>335 ±20</td>
<td>p=0.67</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>305 ±18</td>
<td>315 ±18</td>
<td></td>
</tr>
</tbody>
</table>
### Table

<table>
<thead>
<tr>
<th></th>
<th>CLA</th>
<th>Placebo</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AREE (kcal/d)</td>
<td>1652 ±82</td>
<td>1595 ±79</td>
<td>0.89</td>
</tr>
<tr>
<td>RREE (kcal/kg/d)</td>
<td>22.7 ±0.50</td>
<td>21.9 ±0.74</td>
<td>0.78</td>
</tr>
<tr>
<td>RER</td>
<td>CLA</td>
<td>Placebo</td>
<td>0.19</td>
</tr>
<tr>
<td>CI (kcal/d)</td>
<td>2048 ±148</td>
<td>2175 ±181</td>
<td>0.20</td>
</tr>
<tr>
<td>TV (kg)</td>
<td>CLA</td>
<td>Placebo</td>
<td>0.72</td>
</tr>
</tbody>
</table>

* denotes a significant interaction (p < 0.05)

### 3.2 Discussion

Conjugated linoleic acid (CLA) has been purported to have several physiological effects and various mechanisms have been offered to explain these effects. Of particular interest was the effect of dietary CLA on body composition in the present and previous studies. Previous studies have suggested that CLA may influence body fat and attenuate skeletal muscle atrophy by modulating the immune response to stress (Cook et al., 1993; Lefkowith et al., 1990; Meydani et al., 1992; Miller et al., 1994; Sugano et al., 1998). In light of the potential anti-catabolic effects CLA may possess researchers have studied the effects of CLA in stress-induced animals and humans. Probable anti-catabolic effects of CLA allow for the potential of CLA to
maximize gains in lean muscle during regular bouts of high intensity activities that stimulate not only immune-induced catabolism, but skeletal muscle protein turnover (Biolo et al., 1995; MacDougall et al., 1995; Phillips et al., 1997). Studies have evaluated the effects of CLA on exercising humans, but few have provided detailed information on the exercise protocol. Moreover, most of these studies have not controlled the exercise program (type, frequency and intensity of exercises). The purpose of the present study was, therefore, to investigate the effects of CLA on body composition in humans performing regular vigorous resistance training.

Animal studies and to a lesser extent human studies, have suggested that CLA may reduce body fat mass by increasing energy expenditure (Nagao et al., 2003; Terpstra et al., 2002; West et al., 1998; West et al., 2000), enhancing gain to feed ratio (Dugan et al., 1997; Dugan et al., 1999; Kramer et al., 1998b; Ostrowska et al., 2003; Pariza, 1999), and increasing whole body fat oxidation (Nagao et al., 2003; Ohnuki et al., 2001; West et al., 1998). To test if these same outcomes are achieved in subjects while resistance training, the present study accounted for changes in percent body fat, caloric intake, resting energy expenditure and resting substrate utilization.

The current study has demonstrated that CLA increased lean body mass and combined strength (chest and leg press 1-RM) when supplemented concurrently with seven weeks of regular resistance training. The present study addressed the need to account and control for the exercise regime employing the same high-intensity resistance training program for every participant. All sessions were supervised by trained fitness consultants to ensure proper technique and appropriate weights were utilized thus achieving control not offered by previous studies. Variables that have
been previously evaluated with CLA supplementation such as resting energy expenditure, and respiratory exchange ratio were further evaluated at a higher daily CLA dose in the present study (5.0g/d vs. 3.0g/d) and with a higher total concentration of the active isomers, c9,t11 and t10,c12 CLA (~74% vs. ~40%). The implications of the results from the present study, such as increased strength (combined 1-RM) and lean body mass, add some scientific credence to many of the claims made by companies marketing CLA suggesting that this unique group of fatty acids may serve as an ergogenic agent.

As previously suggested CLA may enhance gains in lean tissue as a consequence of anti-catabolic effects (Cook et al., 1993; Miller et al., 1994; Yang et al., 2001). Miller et al. (1994) demonstrated that feeding CLA to mice partially attenuated the catabolic response to an endotoxin injection. The end result of feeding CLA was a reduction of the overall loss of muscle mass in the injected mice. Yang and others (2000) provided further support reporting CLA-fed mice, which were also immuno-compromised, had less disease-related loss of muscle protein. Results from the present study such as increased lean body mass and combined 1-RM strength merely suggest this may be a viable mechanism whereby CLA enhances gains in muscle mass. CLA has also been suggested to have anabolic effects (Lowery et al., 1998), however, evidence concerning this point has yet to be provided.

Supplementing CLA resulted in greater gains in lean body mass than those in the placebo group without a concomitantly significant change in caloric intake, training volume or body fat mass. Evidently, CLA has some physiological activity acting to enhance gains in lean tissue that is not moderated by changes in metabolic rate, caloric
intake or training volume. The absence of induced changes by CLA on metabolic rate, caloric intake and training volume in the present study reiterate previous results with human subjects.

In light of the influence CLA had on gains in lean mass it was expected that those in the CLA group would also have greater gains in strength as a consequence of increased muscle mass. Leg press 1-RMs were not significantly different between groups, however chest press alone and combined strength scores yielded a statistically sizeable difference. Participants in the CLA group increased their chest press and combined strength scores more than those in the placebo group. Alone, this result implies that CLA enhances gains in strength as a consequence of increased muscle mass. Nevertheless, there are a few important points to note concerning this claim. The participants trained with the chest press and leg press creating a circumstance by which neuromuscular adaptation is a possible explanation for increased scores. Moreover, knee flexion and extension torque did not differ between groups further suggesting strength may not have been influenced. Adaptation for the Biodex (measured knee torque) is negligible as subjects used the apparatus only twice separated by a seven week period allowing for minimal learning and time for detraining to occur. Nevertheless, knee flexion and extension exercises on different apparatuses were employed in the training protocol demonstrating further that increases in strength measures were a consequence, at least in part, from neuromuscular adaptation. It is unlikely that CLA influences neuromuscular learning. No evidence to date has lead researchers to believe this is a plausible mechanism of action for CLA,
suggesting something more than neuromuscular adaptation is responsible for the group differences in strength.

To account for the discrepancy between combined strength gains and knee torque in the present study it is possible that knee torque may have reached significance if a larger group had been used, however initial power calculations (power = 0.8) suggest otherwise. Large within subject variability is a limitation of the Biodex and may partially account for the absence of group differences. It is also probable CLA supplementation enhanced strength as muscular size was increased in both groups with a tendency for greater gains in the CLA group. This tendency may have presented as significantly different with greater participant numbers, however, this discrepancy remains a limitation of the results.

CLA has repeatedly been found to reduce body fat in animals (Akahoshi et al., 2002; Aletor et al., 2001; Azain et al., 2000; Bee, 2000; DeLany et al., 1999; Dugan et al., 1997; Dugan et al., 1999; Koba et al., 2002; Kramer et al., 1998b; Nakanishi et al., 2001; O’Quinn et al., 1998a; O’Quinn et al., 1998b; Ostrowska et al., 2003; Pariza, 1999; Park et al., 1997; Park et al., 1999a; Poulos et al., 2001; Sher et al., 2003; Strangl, 2000; Swan et al., 2001; Takahashi et al., 2003; Terpstra et al., 2002; Thiel-Cooper et al., 2001; West et al., 1998; West et al., 2000; Wiegand et al., 2001; Wiegand et al., 2002) and humans (Atkinson et al., 1999; Basu et al., 2000a; Basu et al., 2000b; Blankson et al., 2000; Mougios et al., 2001; Risérus et al., 2001; Smedman et al., 2001; Thom et al., 2001). In the present study, both groups had marked reductions in body fat mass and percent fat yet no group differences were observed. There was a tendency for participants in the CLA group to lose more fat than those in the placebo group. The
efficacy of the resistance training program that was implemented may have exceeded the ability of CLA to further enhance any loss in fat mass. Changes in body fat brought about by resistance training may have resulted in maximizing the biological processes responsible for such changes that further enhancement (and thus greater fat loss) through CLA supplementation was no longer attainable. That is, the physiological effects of the resistance training program and CLA supplementation on body fat may be redundant and not additive. In light of previous research, this explanation is challenged.

Thom et al. (2001) had subjects (men and women) supplement only 1.8g/d of CLA in combination with regular (90 min, 3x/wk) strenuous exercise and reported a marked reduction in percent body fat compared to the placebo group. Furthermore, significant group differences (in percent body fat) were observed in as few as 4 weeks and continued to conclusion (12 weeks) of the study. These results suggest CLA is able to reduce body fat while performing strenuous exercise which is in contrast to the results observed in the present study. The type of exercise in Thom et al.’s (2001) study was not described making it difficult to make more definitive judgments and comparisons. Kreider and others (2002) recruited experienced (>1yr, 3x/wk) resistance trained men to supplement CLA (6.0g/d) while performing their regular training program for a four week period. Despite the higher dosage, CLA was found to have no effect on fat or lean mass. Subject numbers were comparable to that of Thom et al.’s (2001) ensuring statistical power. Composition of the CLA may have played a factor in the variability between these studies as Thom et al. (2001) utilized a near 50:50 isomeric blend of c9,t11 and t10,c12 CLA, whereas Kreider et al. (2002) utilized a
mixture consisting of numerous CLA isomers. The isomeric composition of the present study consisted of a much higher percentage of the two active isomers than Kreider et al. (2002); however, several other isomers were also present. Additionally, both Thom et al. (2001) and the current study reported changes in body composition while Kreider et al. (2002) did not which supports that the isomeric composition is an unlikely basis for disagreement between studies. Absence of a detailed description of the exercise regimes for either Thom et al. (2001) or Kreider et al. (2002) makes it difficult to compare the results of the present study with these studies. In interpreting the data the possibility that the type and/or intensity of training may have a significant impact on the ability of CLA to influence body composition must be considered as we do not yet know if this is, in fact, the case.

In accordance with the hypothesis of the present investigation, and previous research, CLA did not influence resting energy expenditure. The present study further reports that a dosage of 5.0g/CLA/d appears to be as ineffective, as a lower dose (3.0g/d) previously demonstrated, at influencing resting energy expenditure. It is possible that a dosage relative to that given in animal studies (based on grams of CLA/kg body weight) is required to induce changes in energy expenditure. This would mean administering a daily dose of CLA equivalent to more than 20 times what was provided in the present study. With the exception of one investigation with human subjects researchers have demonstrated that CLA does not alter energy expenditure. The authors of the study indicating that CLA increased metabolic rate stated that the higher metabolic rate was a consequence of greater fat-free mass observed in the CLA group compared to the placebo group (Kamphuis et al., 2003). It is evident then that
supplementing CLA did not directly effect metabolic rate but rather increased the presence of metabolically active tissues (fat-free mass) resulting in an expected increase in metabolic rate.

Respiratory exchange ratio (RER), an indicator of substrate utilization, was not significantly influenced by dietary CLA in the current study. Previous studies in animals (Azain et al., 2000; West et al., 1998), but not humans (Zambell et al., 2000) have indicated that CLA may enhance lipid oxidation switching from carbohydrates to fatty acids as the primary energy source. Support that CLA induces a change in substrate utilization is limited however possibilities remain. A study conducted with mice demonstrated that nighttime fatty acid utilization was enhanced in the CLA group, but not in the placebo group (West et al., 1998). Moreover, 12 hour daytime and 24 hour measurements indicated no difference between the CLA and control group suggesting the effects of CLA on substrate utilization are time specific. It is possible that CLA may increase fat oxidation overnight, when carbohydrate oxidation is normally high, returning to normal values during the day when the current study performed this evaluation; however such a hypothesis remains to be explored.

Furthermore, measurements with the animals are assessed at the tissue level (directly) whereas human studies have thus far estimated substrate utilization through breath by breath (indirect) analysis serving as a methodological means for variable results. The indirect method is influenced by low acidity and hyperventilation (due to stress) both of which result in inadvertently high estimates of RER due to elevated carbon dioxide levels. In any case it seems apparent that a moderately high dose of CLA for seven
weeks in combination with regular resistance training does not alter substrate utilization when measured during daytime hours (morning).

Results from the current study are applicable to healthy males and females of similar ages and training status. Strength measures are influenced by the participants’ motivation serving as a potential limitation of the present study. Daily food consumption was not controlled and 3-day dietary records required accurate accounts of all calories ingested by the participants. Failure to record items properly or at all or substantially altering their dietary intake is another potential limitation as these factors would not be evident to the investigator. Lastly, the CLA mixture employed consisted of numerous isomers limiting the applicability of these results with isomer-specific studies as interactions between isomers remain a distinct possibility. Moreover, specific biological activities such as enhanced gains in lean mass cannot be attributed to specific isomers as multiple isomers were present.

It is evident that a commercially available mixture of CLA may serve as an ergogenic aid by enhancing gains in lean mass and strength realized from regular heavy load resistance training. Due to the trend observed for a reduction in body fat mass (CLA vs. placebo) it remains unclear if CLA influences lipolytic or lipogenic processes as previously suggested. The mechanism(s) responsible for the biological activities of CLA appear not to include modulation of energy expenditure, substrate utilization or caloric intake in humans. To further investigate the physiological efficacy of CLA isomers it is imperative that isomer-specific studies are conducted with humans. The safety of dietary chronic CLA supplementation is also an issue as long-term (>12 months) studies have yet to be completed.
CHAPTER 4
SUMMARY & CONCLUSIONS

4.1 Summary

The purpose of this study was to determine if CLA modifies the effects of a whole-body resistance training program on body composition, metabolic rate, strength, caloric intake and training volume. Healthy, recreationally active men and women (18-34 yrs) were randomly assigned in a double blind fashion to either sunflower oil placebo group or CLA (5.0g/d) for seven weeks concurrently with regular (3x/wk) resistance training.

Whole body resistance training was employed as the exercise model in light of previous research providing evidence that resistance training is effective for inducing gains in muscle mass. The training intensity was periodized throughout the duration of the study.

Body composition was found to be affected by supplementing CLA. Improvements in lean body mass, but not fat mass, were significantly greater in the CLA group than the placebo group. The CLA group also had a greater increase in combined strength (chest and leg press 1-RM) as compared to the placebo group.

Body fat mass, muscle thickness, knee extension/flexion torque, caloric intake, training volume, metabolic rate and respiratory exchange ratio did not significantly differ between groups.
4.2 Conclusions

Within the limitations of this study design it can be concluded that dietary CLA supplementation of 5.0g/d for seven weeks concurrently with resistance training enhanced gains in lean body mass and strength, but did not alter body fat mass, caloric intake, training volume, energy expenditure or respiratory exchange ratio.

4.3 Recommendations

There is some evidence suggesting that combining endurance and resistance training may more effectively induce a greater catabolic response than resistance training alone (Bell et al., 2000; Kraemer et al., 1995). The anti-catabolic effects that CLA may posses, and consequent impact on body composition, would likely be more apparent during periods of heightened protein catabolism. In an effort to further understand the involvement CLA may have on catabolism and consequently lean mass, supplementation during such exercise programs as combined endurance and resistance exercise should be investigated.

The dosage utilized in the present protocol was higher than most published studies to date. Nevertheless, higher dosages of CLA may be required to effectively enhance reductions in fat mass induced by regular resistance exercise. Higher doses will also provide vital information concerning the tolerability and toxicity of CLA in various populations. Longer experimental periods are also necessary to investigate any cumulative healthy benefits or side effects that are not obvious in the short-term. The propensity of supplementation companies to market CLA as an ergogenic aid necessitates completing long-term research for the safety of the public.
It is important that future studies, particularly with humans, employ specific isomers of CLA rather than mixtures to elucidate the nature of isomer-specific activities and interactions. Clearly, differences exist between the physiological responses of animal and human models to CLA which intensifies the importance of human research. Isomer-specific physiological effects are becoming more apparent and should be examined to better understand the biochemical and molecular mechanisms underlying observed phenotypic changes. Some studies currently provide isomer-specific effects of CLA, however data with humans and specifically when combined with exercise programs is lacking. Importantly, the results from the present investigation must therefore be applied judiciously. The results from the present study suggest CLA will serve as an effective ergogenic aid for individuals interested in maximizing gains in muscle mass through resistance training. Without further data concerning isomeric activities and interactions of CLA, application of the results apply only to the specific mixture of isomers employed in the present study.

Additionally, the mechanisms by which CLA acts remain largely uncovered. To add further understanding to the limits and precise physiological actions of CLA in humans it is imperative researchers focus on investigating probable mechanisms of action with human subjects. Of particular interest are the underlying mechanisms whereby CLA may induce changes in body composition including potential effects on the following: linoleic acid metabolism, arachidonic acid synthesis, and consequent eicosanoid synthesis; activity and gene expression of pro-inflammatory eicosanoids (primarily prostaglandins) and cytokines; activity and gene expression of lipolytic and
lipogenic enzymes; activity and expression of genes modulating energy metabolisms (such as uncoupling proteins); and modulation of energy substrate utilization.

Finally, most studies have employed healthy or obese subjects which are otherwise free of disease. In light of the current results and other studies demonstrating that CLA increased lean tissue mass it may be advantageous to utilize CLA as a potential form of treatment. If CLA is to possess anti-catabolic properties it may serve as a relatively inexpensive and easily implemented means for treating individuals with chronic muscle wasting, such as those with muscular dystrophy, amyotrophic lateral sclerosis or paralysis.
REFERENCES


Igarashi, M., & Miyazawa, T. (2001). The growth inhibitory effect of conjugated linoleic acid on human hepatoma cell line, HepG2, is induced by a change in fatty acid metabolism, but not the facilitation of lipid peroxidation in the cells. *Biochimica et Biophysica Acta, 1530,* 162-171.


APPENDICES
APPENDIX A

Certificate of Ethics Approval
Certificate of Approval

PRINCIPAL INVESTIGATOR
Philip D. Chilibeck

DEPARTMENT
Kinesiology

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT
Royal University Hospital
103 Hospital Drive
Saskatoon SK S7N 0W8

SPONSORING AGENCIES
BIORIGINAL FOOD AND SCIENCE CORPORATION

TITLE:
The Effects of Conjugated Linoleic Acid (CLA) Supplementation Combined with Resistance Training on Body Composition and Muscular Strength

ORIGINAL APPROVAL DATE
01-Aug-2002

CURRENT EXPIRY DATE
01-Aug-2003

APPROVAL OF
Protocol and Revised Consent Form

CERe11Fica110n
The University of Saskatchewan Biomedical Research Ethics Board has reviewed the above-named research project at a full-board meeting (any research classified as minimal risk is reviewed through the expedited review process). The proposal was found to be acceptable on ethical grounds. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to this research project, and for ensuring that the authorized research is carried out according to governing law. This Approval is valid for the above time period provided there is no change in experimental protocol or in the consent process.

ONGOING REVIEW REQUIREMENTS/REB ATTESTATION
In order to receive annual renewal, a status report must be submitted to the Chair for Committee consideration within one month of the current expiry date each year the study remains open, and upon study completion. Please refer to the following website for further instructions: http://www.usask.ca/research/ethics.shtml. In respect to clinical trials, the University of Saskatchewan Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations and carries out its functions in a manner consistent with Good Clinical Practices. This approval and the views of this REB have been documented in writing.

APPROVED.

Dr. Barry D. McLennan, Ph.D., Chair
University of Saskatchewan
Biomedical Research Ethics Board

Please send all correspondence to:
Office of Research Services
University of Saskatchewan
Room 210 Kirk Hall, 117 Science Place
Saskatoon, SK S7N 5C8
Phone: (306) 966-4053  Fax: (306) 966-8597

123
APPENDIX B

Participant Consent Form
CONSENT FORM

Title: The effects of conjugated linoleic acid (CLA) supplementation combined with resistance training on body composition, metabolic rate and muscular strength.

Researchers: Dr. Philip Chilibeck, Ph.D., Associate Professor (966-6469) and Craig Pinkoski, B.Sc., student researcher ( ), College of Kinesiology, University of Saskatchewan.

Sponsor: Bioriginal Food and Science Corp.

Purpose of the Study: Conjugated linoleic acid (CLA) is a type of fat found naturally in beef, lamb and dairy products. When ingested in higher than usual quantities, it is thought to alter the way our bodies utilize fat, in that it promotes the burning of fat. It is also thought to prevent muscle damage and may therefore help muscle to repair itself after exercise training. It therefore is thought to promote muscle gain. The purpose of the study is to determine the effectiveness of CLA combined with resistance training, for increased muscle mass, reducing body fat and improving muscular strength.

Possible benefits of the study: You may experience an increase in muscle mass and strength and a loss of fat mass with the CLA supplementation or the exercise program. These benefits, however, are not guaranteed.

Procedures: Initially, you will be given a questionnaire that asks you whether you have any health problems that will prevent you from participating in an exercise program (called the Physical Activity Readiness Questionnaire). You will be randomized (like flipping a coin) to one of two groups. One group will receive 5 grams of CLA pre day for a 7-week duration. The second group will receive placebo (a supplement that looks like CLA, but is really just ordinary sunflower oil). Neither you nor the investigators will know whether you are receiving CLA or placebo until the end of the experiment. Whether you receive CLA or placebo, you will be asked to participate in a strength-training program for the 7 weeks that you receive the supplement. This will involve performing 12 exercises in weight lifting machines designed for training all major muscle groups. Training will take place 3 times per week and will involve 1 hour to 1 ½ hours each training session. A qualified trainer will supervise each training session. At two different time points (before and after the 7 weeks), you will be asked to undergo the following measurements:

1) Body Composition (muscle and fat mass) will be assessed with air displacement plethysmography. This procedure takes about 15 minutes and involves sitting in a chamber which measures the amount of air that is displaced by you body. The amount of air displaced is proportional to you body volume, which along with your body mass is used to determine your body density. The amount of muscle and fat mass you have is then calculated based on your body density.
2) Muscular strength will be assessed on three different weight lifting machines. One of these tests will involve your upper body musculature and the other two tests will involve lower body musculature. These tests will take 45 minutes to complete.

3) Resting metabolic rate (the amount of energy you burn at rest) and the amount of fat you burn will be measured by having you breathe into a clear plexiglass hood that will be placed over your head while you are lying on a padded table. The amount of calories and fat you burn is determined indirectly by the amount of oxygen you consume and the amount of carbon dioxide you expire. The test takes 30 minutes to complete and requires that you not eat food and only drink water for 12 hours beforehand.

4) We will ask you to record all the food you eat in diary over a 3-day period.

Foreseeable risks and discomforts:
There have been six studies conducted with a total of 109 human subjects who took doses of CLA ranging from 1.4 to 6.8 grams per day for 4-12 weeks and no serious side effects have been reported. One study reported that some subjects had increased incidence of upset stomach. In this study, 11 subjects who ingested 5 g of CLA per day over 12 weeks reported occasions where they had an upset stomach. This was compared with 8 subjects in the placebo group who reported 3 occasions where they had an upset stomach. In another study, 3 out of 26 subjects receiving 4.2 g of CLA per day for 12 weeks reported mild diarrhea at some occasion.

There is a risk of muscle or joint injury during the exercise testing and training; however, an experienced individual will supervise all testing and training sessions and all sessions will be preceded by a 10-minute warm-up to minimize the risk of injury.

There is a risk that you may feel claustrophobic during the measurement of resting metabolic rate when the plexiglass hood is placed over your head. From previous studies in our lab, we have not had anyone experience this.

There may be unforeseen and unknown risks during the study or after the study is completed.

Because this is an experimental nutritional supplement, we would like you permission to inform your family doctor of your involvement in this study.

You are free to withdraw from the study at any time and this will not affect your academic status, if a student, or access to health care or other services.
Research-Related Injury: There will be no cost to you for participation in this study. You will not be charged for the supplement or any research procedures. In the event you become ill or injured as a result of participating in this study, necessary medical treatment will be made available at no additional cost to you. By signing this document you do not waive any of your legal rights.

Treatment alternatives: You do not have to participate in this study if you want to decrease your fat mass or increase your muscle mass and strength. To decrease your fat mass you could alter your diet by decreasing your intake of fats or calories or you could take part in an aerobic exercise program. To increase your muscle mass and strength, you could do an exercise program that is different from that of the current study. For example, you could do exercises that do not involve lifting weights on machines, but which involve lifting your own body weight (i.e. chin-ups or push-ups).

Confidentiality: Precautions will be taken to protect you anonymity. All data related to the study will be stored in a locked office at the College of Kinesiology at the University of Saskatchewan. The data collected from the study will be presented to Bioriginal and will be used in a thesis or publication articles, but in any publication only aggregate data will be reported and you will be unidentifiable.

If you have questions regarding the research project, you can call Dr. Philip Chilibeck at 966-6469 (work) or (home) or Craig Pinkoski (master student) at . If you have questions about your rights as a research subject, you can call the Office of Research Services at the University of Saskatchewan at 966-4053.

We will advise you of any new information that will have any bearing on your decision to continue in the study.

You will be told your individual results and the overall research outcome at the end of the study.
By Signing below, you acknowledge that the study and the information presented in the consent document have been explained to you and that you understand the contents, and that you have received a copy of this consent form for your own records.

Participant Signature: __________________________

Date: __________________________

Researcher Signature: __________________________

Witness: __________________________
APPENDIX C

Physical Activity Readiness Questionnaire
APPENDIX D

Respiratory Exchange Ratio Values for Major Fuel Sources
RER OF MAJOR FUEL SOURCES

Glucose (per mole):

\[ \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 \rightarrow 6\text{CO}_2 + 6\text{H}_2\text{O} + \text{energy} \]

\[ \text{RER} = \frac{6\text{CO}_2}{6\text{O}_2} = 1.0 \]

Protein (per mole of albumin):

\[ \text{C}_{72}\text{H}_{112}\text{N}_2\text{O}_{22}\text{S} + 77\text{O}_2 \rightarrow 63\text{CO}_2 + 38\text{H}_2\text{O} + \text{SO}_3 + 9\text{CO}(\text{NH}_2) \]

\[ \text{RER} = \frac{63\text{CO}_2}{77\text{O}_2} = 0.82 \]

Fatty acids (per mole of palmitic acid):

\[ \text{C}_{16}\text{H}_{32}\text{O}_2 + 23\text{O}_2 \rightarrow 16\text{CO}_2 + 15\text{H}_2\text{O} + \text{energy} \]

\[ \text{RER} = \frac{16\text{CO}_2}{23\text{O}_2} = 0.7 \]
APPENDIX E

3-Day Food Diary Booklet
3-DAY
FOOD INTAKE DIARY

INTRODUCTION

This booklet is used to record your detailed daily food intake. It is meant to give the researchers some idea of your usual dietary intake. Therefore, it is very important that you do not alter your eating habits while taking part in this study. In other words, do not let the fact that you are writing down what you eat influence your choice of foods. The names of the participants in this study will be kept confidential.

The utility of the results of this study depends on the accuracy with which you record your daily food intake. Please write down full details on all the food and drink that you consume each day.

INSTRUCTIONS

1) The purpose of this diary is to record all the food (including drinks) which you eat for a three day period. The three day period should include 2 weekdays and 1 weekend day.

2) Two pages are provided for each day of the three day period.

3) After each meal or snack that you eat, please write down in detail each separate food item you consumed – including the type of food (e.g. processed cheese) and the amount of food in household measurements (e.g. 1 cup of cooked spaghetti). A meal will have to be listed by its separate parts (e.g. fried steak – 8 oz., french fries – 1 cup, coleslaw – 3 tbsp.).

4) The best way to record the information is by carrying this diary around with you wherever you go. Before going to sleep, you should look over the diary to check that you have not missed anything and that nothing was reported incorrectly. Remember to include snacks!

5) If you eat fast food, you can just list the type of food you ate (e.g. 1 Big Mac, 1 large fries, 1 super-sized diet coke).

6) The following pages explain the use of household measures, and the description of foods. A sample day’s diet sheet is given. Please take the time to read these pages as it will help to make your diet record more accurate.
RECORDING IN THE DIARY

1) Please use household measures. For example:

- **cup**: vegetables, cereal, fruit, milk, beverages
- **tablespoon**: sauce, fats
- **teaspoon**: sugar, honey, drink, mix
- **slices**: bread, bacon

2) State the **type** of food eaten. For example:

- **Milk**: homo, 2%, 1%, skim, goat’s, soy
- **Cheese**: processed, Swiss, spread
- **Bread**: enriched, white, 60% whole wheat, sweet cinnamon bun, bran muffin
- **Cereal**: Sugar pops, Miniwheats, granola
- **Meat**: hamburger, fried chicken – breasts, all-beef wieners, scrambled eggs, Egg Beaters, cod fillets
- **Others**: raspberry jam, Bezel margarine, Caesar dressing, ketchup, oatmeal cookies.

3) State the **amount** of food eaten. For example:

- **Cheese**: 1" cube cheddar
  - 3 tbsp lite cream cheese
  - ½ cup 2% creamed cottage cheeses
- **Fruit**: ½ cup canned peaches (in heavy syrup)
  - 12 grapes (green)
  - 1 medium banana
- **Bread**: 2 slices of 100% whole wheat
  - 1 large kaiser
- **Cereal**: 1 ¾ cups Special K
  - 1 shredded wheat biscuit
- **Meat**: 1 cup baked beans with pork
  - 2 cups tuna casserole (tuna, cream of mushroom soup, noodles, peas)
  - 4 thin slices of roast beef
- **Vegetables**: 2 slices of cucumber
  - ½ cup boiled cabbage

4) Include manner of cooking: fried, boiled, raw.
-the meat sauce was made with:
  2 onions (med)
  1/2 lb. lean ground beef
  2 cans tomatoes (19oz/1)

-the garlic bread was made using French bread and 1 Tbsp. of butter on each slice.

Here is a sample:

<table>
<thead>
<tr>
<th>Time</th>
<th>Food Description</th>
<th>Amount</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:30am</td>
<td>Waffles-white flour</td>
<td>3, 8&quot;x4&quot; each</td>
<td></td>
</tr>
<tr>
<td></td>
<td>syrup - Aunt Jemima</td>
<td>1/2 cup</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yogurt(low fat) - peach</td>
<td>125 ml</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Coffee, 1 tsp. sugar</td>
<td>1 cup</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Milk (2%)</td>
<td>1/4 cup</td>
<td></td>
</tr>
<tr>
<td>10:30am</td>
<td>Chocolate chip cookies</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Coffee, 1 tsp. sugar</td>
<td>1 cup</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Milk (half &amp; half - 10%)</td>
<td>1/4 cup</td>
<td></td>
</tr>
<tr>
<td>12:30</td>
<td>Sandwich</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-2 slices whole wheat bread.</td>
<td>2 slices</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mozzarella cheese (3&quot;x1/4&quot;x2&quot;)</td>
<td>2 slices</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-salami</td>
<td>4 slices</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-lettuce</td>
<td>1 leaf</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-butter</td>
<td>1 tsp.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-mayonnaise</td>
<td>1 tsp.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-tomato</td>
<td>1 slice</td>
<td></td>
</tr>
<tr>
<td>5:30</td>
<td>Spaghetti</td>
<td>1 1/2 cup</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Meat sauce</td>
<td>1/2 cup</td>
<td></td>
</tr>
</tbody>
</table>

(continue on the next page if you need it)

Note, please do not fill in Code column.
<table>
<thead>
<tr>
<th>Time</th>
<th>Food Description</th>
<th>Amount</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>