Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.
The effect of synthetic and bovine conjugated linoleic acid on energy balance

A thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Nutritional Science at Massey University, Palmerston North, New Zealand

Ann Hayman
1999
ABSTRACT

Conjugated linoleic acid (CLA) is biologically active and has altered body composition in experimental animals. Dietary supplementation with synthetic CLA reduced body fat in mice and rats in a number of studies. The CLA used in previously published research contained mixed isomers, the majority of which were 9c11t-CLA and 10t12c-CLA. The biologically active isomer at the time of starting the trials described in this thesis was assumed to be 9c11t-CLA, due to the prevalence of this isomer in biological tissues.

The two trials in this thesis were designed to investigate the effect of dietary CLA on energy balance. In the first (refer Abstract, section 2.1), synthetic CLA reduced body fat in male BALB/c mice in a dose response manner, over the range 0.25 to 1.0 % w/w CLA in the diet. High levels (1.0 % and 2.0 %) caused a reduction in growth. In the second (refer Abstract, section 3.1) dietary treatments supplemented with synthetic CLA, or bovine CLA in milk fat, at levels similar to the 0.25 % w/w synthetic CLA treatment found to be effective in reducing body fat in mice, had no effect on energy balance in female Sprague-Dawley rats.

The CLA in milk fat contains approximately 86 % of the 9c11t-CLA isomer while synthetic CLA contains approximately 37 %, 9c11t-CLA and 46 % 10t12c-CLA. Results from these two trials support recent evidence from research demonstrating 10t12c-CLA is the biologically active isomer, in relation to energy metabolism and body composition.

9c11t-CLA is the prevalent isomer of CLA found in the human diet. The CLA used in previously published research was chemically synthesised and contained a considerably higher proportion of 10t12c-CLA then found in human food sources.

PREVIOUS PUBLICATION: The study described in Chapter 2 has been previously published as an abstract and displayed as a poster presentation at the Pacific Partners in Nutrition Conference, held at Auckland, New Zealand, September, 1999 (Hayman, et al., 1999).
ACKNOWLEDGEMENTS

I wish to thank my family for support, interest in my work, and for willingly providing me with time to complete this work. Particularly my husband Neil, and Tim and Craig. Thanks also to my parents, for company and meals, during some of the time at Massey University.

Sincere thanks are given to my supervisors Dr Hilary Green (Milk and Health Research Centre) and Dr Rodger Pack (Massey University) for their valued opinions, encouragement, commitment and sharing their many years of experience. Special thanks to Hilary for assistance with experimental protocol, guidance during bench work and comments during draft writing.

The Milk and Health Research Centre (MHRC), which is jointly funded by the New Zealand Dairy Board and New Zealand Dairy Research Institute, funded this work. My employers, the New Zealand Dairy Board, granted study leave. Massey University assisted financially with a Massey Masterate scholarship, as did M&HRC with a scholarship in Milk and Health. I would like to express my appreciation to the M&HRC, NZDB and Massey University for making the opportunity to complete a higher degree possible.

I wish to thank Marie Russel and Florence Chung in the Nutrition Laboratory of the Institute of Food, Nutrition and Human Health (IFN&HH), Massey University, for analytical services in determining fat, protein, ash and moisture.

The mice in the energy balance study described in Chapter 2 were also used in a separate study investigating the effects of CLA on immune function (Zhao, 1999). Thanks are due to Dr Kay Rutherfurd and Hui Zhao for sharing animals and facilities during the mouse CLA feeding trial.

Thanks are due to Bertram Fong and Dr Alastair MacGibbon for CLA analysis and method development carried out at New Zealand Dairy Research Institute.
I would also like to thank the following people;

Dr Linda Schollum, Chris Booth and Anne Broomfield of M&HRC for advise and assistance in working with animals.

The staff at the Small Animal Production Unit for use of facilities.

Margaret Scott and Justine Shoemark at the Food Evaluation Unit of NZ Institute Crop and Food Research Ltd for use of facilities.

Brett Guthrie and John Pedley in the Physiology Laboratory (IFN&HH) for use of facilities and assistance with calorimetry. The musical and humorous working atmosphere in the Laboratory was also much appreciated.

Dr Kay Rutherfurd, for assistance with Animal Ethics approval, and staff in the Immune Laboratory of the M&HRC for assistance in obtaining blood and body composition samples.

Nicky Frearson and Sally Robinson in the Nutrition Laboratory (M&HRC) for assistance with animal and laboratory work.

Dr Phil Pearce (IFN&HH) for the free fatty acid analytical service.

Don Thomas for use of sample preparation facilities at The Poultry Research Centre (IFN&HH).

Roger Kissling (NZDB) and Dr Barbara Kuhn-Sherlock (M&HRC) for statistical advise.
# Table of Contents

Abstract iii  
Previous Publication iii  
Acknowledgements iv  
Table of contents vi  
List of tables xi  
List of figures xiii

## Chapter 1  Review of the Literature

1.1  **Conjugated linoleic acid in the diet**  
1.1.1  Introduction 1  
1.1.2  CLA content of food 2  
1.1.3  Dietary CLA intake 3  
1.1.4  CLA content in the infant diet 4  
1.1.5  CLA content of cow's milk 6  
1.1.6  Effect of processing and storage on dairy product CLA level 9  
1.1.7  Effect of dietary CLA on human blood CLA level 12  
1.1.8  CLA derived from linoleic acid in non-ruminants 12  
1.1.9  CLA in milk from other mammals 14  

1.2  **Biological activity of CLA**  
1.2.1  Biologically active isomer 15  
1.2.2  Cancer 16  
1.2.3  Immune 18  
1.2.4  Atherosclerosis 18  
1.2.5  Bone formation and resorption 19  
1.2.6  Energy partitioning and metabolism 20  
1.2.6.1  Body weight and food intake 20  
1.2.6.2  Body composition 21  
1.2.6.3  Energy metabolism 23
1.3 Measurement of energy balance
1.3.1 Energy balance equation
1.3.1.1 Measurement of energy expenditure
1.3.2 The role of brown adipose tissue in diet induced thermogenesis
1.4 Summary

Chapter 2 Trial 1; Investigation of the Effect of Synthetic Conjugated Linoleic Acid on Energy Balance in Mice

2.1 Abstract
2.2 Experimental materials and procedures
2.2.1 Animals and housing
2.2.2 Mouse diets
2.2.2.1 Relationship of mouse diet CLA level to the New Zealand and Australian dietary CLA level
2.2.3 Experimental procedure
2.3 Physical and chemical methods
2.3.1 Intake of food and energy
2.3.1.1 Food intake
2.3.1.2 Feed efficiency
2.3.1.3 Mouse diet composition
2.3.1.31 Preparation of mouse diets for chemical analysis
2.3.1.23 Compositional analysis
2.3.1.33 Conjugated linoleic acid
2.3.1.34 Fatty acid profile of Tonalin and corn oil
2.3.1.4 Energy intake
2.3.2 Energy Expenditure
2.3.2.1 Calorimetry
2.3.2.2 Activity
2.3.2.3 Sleep/wake pattern
2.3.2.4 Serum free fatty acid content
2.3.3 Body composition
2.3.3.1 Live weight
Chapter 3  Trial 2; Investigation of the Effect of Bovine Conjugated Linoleic Acid on Energy Balance in Rats

3.1 Abstract 76

3.2 Experimental materials and procedures 78

3.2.1 Animals and housing 78

3.2.2 Rat diets 78

3.2.3 Experimental procedure 81
3.3 Physical and chemical methods

3.3.1 Intake of food and energy

3.3.1.1 Food intake

3.3.1.2 Feed efficiency

3.3.1.3 Rat diet composition

3.3.1.31 Preparation of rat diet samples for chemical analysis

3.3.1.32 Compositional analysis

3.3.1.33 Fatty acid profile, including conjugated linoleic acid

3.3.1.4 Energy intake

3.3.1.5 Faecal gross energy

3.3.1.6 Digestible energy

3.3.2 Energy expenditure

3.3.2.1 Calorimetry

3.3.2.11 Preliminary studies

3.3.2.12 Calorimetry measurements on trial rats

3.3.2.13 Activity

3.3.3 Body composition

3.3.3.1 Live Weight

3.3.3.2 Measurement of body composition

3.3.3.21 Body composition by dissection

3.3.3.22 Body composition by chemical analysis

3.3.3.22.1 Preparation of samples for chemical analysis

3.3.3.22.2 Fat determination

3.3.3.22.3 Nitrogen determination

3.3.3.22.4 Dry matter determination

3.3.3.22.5 Ash determination

3.3.3.22.6 Formula used to calculate rat body composition

3.4 Results

3.4.1 Data analysis

3.4.1.1 Data excluded from analysis

3.4.2 Food intake

3.4.3 Energy expenditure
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.4.3.1</td>
<td>Diet induced thermogenesis</td>
<td>97</td>
</tr>
<tr>
<td>3.4.4</td>
<td>Body composition</td>
<td>98</td>
</tr>
<tr>
<td><strong>3.5</strong></td>
<td><strong>Discussion</strong></td>
<td>103</td>
</tr>
<tr>
<td>3.5.1</td>
<td>Food intake</td>
<td>103</td>
</tr>
<tr>
<td>3.5.2</td>
<td>Energy expenditure</td>
<td>105</td>
</tr>
<tr>
<td>3.5.3</td>
<td>Body composition</td>
<td>106</td>
</tr>
<tr>
<td>3.5.4</td>
<td>CLA isomers</td>
<td>106</td>
</tr>
<tr>
<td>3.5.5</td>
<td>Milk samples</td>
<td>107</td>
</tr>
<tr>
<td><strong>Chapter 4</strong></td>
<td><strong>General discussion</strong></td>
<td>109</td>
</tr>
<tr>
<td>References</td>
<td></td>
<td>114</td>
</tr>
<tr>
<td>Appendices</td>
<td></td>
<td>122</td>
</tr>
</tbody>
</table>
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Ingredient composition of mouse diets</td>
<td>35</td>
</tr>
<tr>
<td>2.2</td>
<td>Protocol for trial 1</td>
<td>36</td>
</tr>
<tr>
<td>2.3</td>
<td>Mouse activity score</td>
<td>43</td>
</tr>
<tr>
<td>2.4</td>
<td>Fatty acid composition of corn oil and Tonalin</td>
<td>52</td>
</tr>
<tr>
<td>2.5</td>
<td>Composition of mouse diets</td>
<td>52</td>
</tr>
<tr>
<td>2.6</td>
<td>Feed intake, weight gain and feed efficiency</td>
<td>53</td>
</tr>
<tr>
<td>2.7</td>
<td>Oxygen consumption, oxygen consumption/bodyweight, carbon dioxide production,</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>respiratory quotient, energy equivalence of oxygen, metabolic rate and activity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>score for mice after two weeks of CLA feeding trial</td>
<td></td>
</tr>
<tr>
<td>2.8</td>
<td>Oxygen consumption, oxygen consumption/body weight, carbon dioxide production,</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>respiratory quotient, energy equivalence of oxygen, metabolic rate and activity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>score and serum free fatty acid for mice at the end of CLA feeding trial</td>
<td></td>
</tr>
<tr>
<td>2.9</td>
<td>Live weight, weight gain, carcass weight and comparison of group means</td>
<td>62</td>
</tr>
<tr>
<td>2.10</td>
<td>Body fat, relative and absolute, by dissection and comparison of group means</td>
<td>63</td>
</tr>
<tr>
<td>2.11</td>
<td>Body fat, relative and absolute, by chemical analysis, and gross energy</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>content and comparison of group means</td>
<td></td>
</tr>
<tr>
<td>2.12</td>
<td>Lean body mass; moisture, protein and ash and comparison of group means</td>
<td>69</td>
</tr>
<tr>
<td>2.13</td>
<td>Lean body mass and comparison of group means</td>
<td>70</td>
</tr>
<tr>
<td>3.1</td>
<td>Ingredient composition of rat diets</td>
<td>79</td>
</tr>
<tr>
<td>3.2</td>
<td>Protocol for trial 2</td>
<td>81</td>
</tr>
<tr>
<td>3.3</td>
<td>Rat activity score</td>
<td>88</td>
</tr>
<tr>
<td>3.4</td>
<td>Trial diet composition</td>
<td>93</td>
</tr>
<tr>
<td>3.5</td>
<td>Diet fatty acid profile</td>
<td>94</td>
</tr>
</tbody>
</table>
3.6 Feed intake, weight gain and feed efficiency for whole trial 95
3.7 Energy intake, faecal energy and digestible energy in week four of trial 95
3.8 Rate of oxygen consumption, rate of carbon dioxide production, respiratory quotient, energy equivalence of oxygen and metabolic rate for fasted rats 96
3.9 Rate of oxygen consumption, rate of carbon dioxide production, respiratory quotient, energy equivalence of oxygen and metabolic rate for fed rats 96
3.10 Difference in fed and fasted respiratory quotient, energy equivalence of oxygen and metabolic rate between fed and fasted rats 97
3.11 Mean oxygen consumption, carbon dioxide, respiratory quotient and metabolic rate for all rats grouped together when fed or fasted 98
3.12 Live weight and body fat, relative and absolute, by dissection and chemical analysis 101
3.13 Components of lean body mass; moisture, protein, ash, lean body mass relative and absolute, by chemical analysis 102

Appendix Table 1.1 CLA content of food 122
Appendix Table 1.2 CLA content of dairy products 124
Appendix Table 1.3 CLA content of breast milk 126
Appendix Table 1.4 CLA content of infant formula 126
Appendix Table 1.5 Feeding trials investigating the CLA content of cow’s milk 127
Appendix Table 1.6 Effect of CLA supplemented diet on body weight, food intake and feed efficiency in animal feeding trials 129
Appendix Table 2.1 Ingredient composition of pre-acclimatisation and acclimatisation diets 133
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fig 1.1</td>
<td>Chemical structure of linoleic acid and 9c11t-CLA</td>
<td>1</td>
</tr>
<tr>
<td>Graph 2.1</td>
<td>Metabolic rate versus activity</td>
<td>58</td>
</tr>
<tr>
<td>Graph 2.2</td>
<td>Body fat (dissection) % versus diet CLA %</td>
<td>65</td>
</tr>
<tr>
<td>Graph 2.3</td>
<td>Body fat (chemical) % versus diet CLA %</td>
<td>66</td>
</tr>
<tr>
<td>Graph 2.4</td>
<td>Gross energy versus diet CLA %</td>
<td>67</td>
</tr>
<tr>
<td>Graph 2.5</td>
<td>Body fat (dissection) % versus body fat (chemical) %</td>
<td>68</td>
</tr>
</tbody>
</table>