5-1993

The Effects of a 30 Day Feeding of Triacetin on Colonic and Jejunal Mucosal Proliferation, Plasma Substrate Concentrations, Carcass Composition and Growth and Development in Rats

Jamie Whoric Lynch

University of Tennessee, Knoxville

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James Bailey, Major Professor

We have read this thesis and recommend its acceptance:

Jay Whelan, Dileep Sachan

Accepted for the Council:

Dixie L. Thompson

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)
To the Graduate Council:

I am submitting herewith a thesis written by Jamie Whoric Lynch entitled "The effects of a 30 day feeding of triacetin on colonic and jejunal mucosal proliferation, plasma substrate concentrations, carcass composition and growth and development in rats." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in nutrition.

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Dr. Jay Whelan

Dr. Dileep Sachan

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and Dean of The Graduate School
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Effects of a 30 day feeding of triacetin on colonic and jejunal mucosal proliferation, plasma substrate concentrations, carcass composition and growth and development in rats

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Jamie Whoric Lynch
May 1993
ACKNOWLEDGEMENTS

I would like to thank my major professor, Dr. James Bailey, for his unending patience with me and my multitude of questions and for the guidance he has given me with this research and in career decisions. I would also like to express my gratitude to my committee members, Dr. Jay Whelan and Dr. Dileep Sachan for their guidance. In addition, I would like to express my thanks to Dr. Effin Graham and Kathleen Malueg of the department of Ornamental Horticulture and Landscape Design for the use of Dr. Graham’s lab and equipment and for teaching me histology. I would like to thank Kim Lyons for her expert assistance with graphs and slides and Paula Campbell and Ann Knightly for their assistance with animal feeding and preparation of samples. I would like to thank my family, Jim and Betty Whoric and Daphne Larkin, for their love and support and for helping me to believe in myself. I especially would like to thank my husband, Andy, for being supportive and helping me through the times I did not think I would make it through. And finally, I would like to thank God for giving me the strength I needed to complete the degree program.
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GENERAL INTRODUCTION

Fat is a necessary nutrient in the diet of all humans. Fat supplies essential fatty acids and fat soluble vitamins. The use of fat versus glucose in the clinical setting has some definite advantages. Less carbon dioxide is produced when fat is oxidized for fuel verses glucose, which is especially important when trying to wean respirator-dependent patients. Because fat is isotonic, it can be infused intravenously to provide a patient with a calorically dense source of energy. In comparison, a glucose solution is hypertonic. Glucose solutions above 5% can cause phlebitis at peripheral infusion sites. Its intravenous use in lieu of glucose has been shown to promote euglycemia in diabetic patients.

Dietary fats are composed primarily of long-chain triglycerides (fatty acid chain length ≥ 14 carbons, LCT). In addition to the aforementioned clinical advantages, the use of LCT have been associated with decreased incidence of fatty liver. However, the clinical use of LCT in intravenous nutrition have been reported to have some undesirable metabolic consequences. They have been shown to decrease bacterial resistance, increase plasma triglycerides and plasma
cholesterol and during long-term use induce hepatic dysfunction. In addition to their undesirable metabolic effects, LCT are insoluble in water and, subsequently, are difficult to incorporate into intravenous solutions.

Due to the undesirable attributes associated with LCT use, medium-chain triglycerides (fatty acid chain length of 6-12 carbons, MCT) have been investigated as a fat source. Because MCT do not require bile acids for absorption, they make an excellent source of lipid when given at low doses for those patients who have problems digesting and absorbing LCT. MCT use has been found to be useful in ketogenic diets for treatment of childhood epilepsy as they induce ketonuria more easily than other dietary fats. MCT are rapidly metabolized and used for energy. However, high doses of MCT have been associated with metabolic abnormalities, such as abnormalities in central nervous system function.

Because of the problems related to the use of LCT and MCT, new lipid alternatives have been investigated. One lipid alternative studied has been the short-chain triglycerides (fatty acid chain length of 2-4 carbons, SCT) and short-chain fatty acids (fatty acid chain length of 2-4 carbons, SCFA). The SCFA, acetate, propionate, and
butyrate, are normally produced in the colon from the fermentation of nondigestible carbohydrate by the colonic bacteria. They are a preferred energy source of the cells in the colon. SCT are easily soluble in water and provide for easy mixing into water-based intravenous solutions. During acute administration (7-10 days), they have proved beneficial. They are cleared from the plasma quickly and metabolized completely for energy. Enteral and intracolonic infusions of SCT and SCFA have been reported to increased nitrogen balance in patients as well as decreased atrophy of the intestinal mucosa. The intravenous use of SCT has been shown to be safe in animals. The safety and effectiveness of SCT in intravenous administration in humans has yet to be determined. SCT do not, however, obviate the use of LCT as they do not provide the essential fatty acids. Recent investigation has indicated that parenteral/enteral use of SCT for up to seven days has desirable effects on ketogenesis, protein metabolism, and the intestinal mucosa. However, their effectiveness in long-term use is not known at this time.

The objectives of this study are to determine the effects of an long-term (30 day) oral diet containing SCT on the intestinal mucosa, plasma substrates, and growth and development in rats. The effects
on the intestine were measured in the jejunum and the colon biochemically and histologically. Biochemical indices included DNA, RNA, and protein. Histological measures included villus height in the jejunum and crypt depth in the colon. The plasma substrate concentrations measured were free fatty acid, total ketone body, pyruvate, lactate, triglyceride, and glucose. Growth and development will be evaluated by comparing body weight (growth), body composition (% water, % protein, % fat, and % ash), and adipocyte cell size and number in the epididymal, inguinal, and perirenal fat depots.
EXPLANATION OF THESIS FORMAT

This thesis contains a general introduction and two separate papers to be submitted for publication. All of the data from the two papers came from one experimental study that I performed at the University of Tennessee, Knoxville under the direction of my major professor, Dr. James Bailey. As needed, some data is duplicated in both studies for clarity.
Part 1: Triacetin feeding for 30 days increased intestinal cellular proliferation without adversely affecting plasma substrate concentrations
ABSTRACT

Diets containing either the short-chain triglyceride (SCT) triacetin or long-chain triglyceride (LCT) were fed to rats to determine the effects on intestinal mucosa cells and plasma substrates. Male Sprague Dawley rats were given one of three diets, a control diet containing 5% LCT (by calories) or one of two experimental diets that were 30% lipid (by calories). The lipid component of the LCT group was 100% LCT while that of the SCT group was 95% SCT/5% LCT. Plasma lactate, glucose, and total ketone body concentrations were unaffected by diet. Compared to the LCT and control groups, plasma pyruvate and free fatty acid concentrations were decreased in animals fed SCT. In contrast, plasma triglyceride concentrations were elevated in the SCT group compared to other groups. Intestinal biochemical measures included total DNA, RNA, protein and the protein:DNA ratio. Histological indices measured were villus height in the jejunum and crypt depth in the colon. Mucosal protein concentration was unaffected in the jejunum and colon. Jejunal RNA was significantly decreased in the SCT group compared to other groups. SCT feeding significantly increased the DNA content in the
jejenum and colon thereby lowering the protein:DNA ratio, indicating smaller, more numerous cells. And jejunal villus height and colonic crypt depth were unaffected by diet. Provision of a balanced diet containing 28.5% of the total calories as the SCT triacetin had no adverse effects on metabolic substrates and resulted in smaller and more numerous mucosal cells in the jejunum and colon. These data indicate that the SCT triacetin could provide benefit in the maintenance of gut integrity and in the treatment of certain intestinal disorders.
KEY WORDS: triacetin, short-chain triglycerides, intestine, rats
INTRODUCTION

Short-chain fatty acids (SCFA) are produced in the colon by bacterial fermentation of undigestible carbohydrates, such as cellulose, pectins, and hemicellulose (1). The primary SCFA produced are acetate, propionate, and butyrate. They are found in the human colon in the molar ratios of 60:24:16 and account for about 7% of total body energy maintenance requirements (2). Other reports suggest ranges of molar ratios of acetate, propionate, and butyrate of 75:15:10 to 40:30:20 (3). More than 95% of the SCFA that are produced in the colon are readily absorbed (1). Once absorbed, they are preferred oxidative fuels of the colonocytes (2). They are even preferred over glucose and glutamine (4) and account for 60-70% of the energy requirements of the colonocytes (5). Therefore, it can been seen that the SCFA are an important part of the nutriture of the colonic mucosa.

SCFA in the form of triglycerides, short-chain triglycerides (SCT), are water soluble and can easily be mixed into water-based parenteral solutions. Because of their size, they can bypass the carnitine acyltransferase system that is required for metabolizing the
longer chain fatty acids normally found in food. They are cleared from the plasma quickly. In the intestinal epithelium, the primary metabolic pathway is complete oxidation to CO₂ (6). *In vivo* and *in vitro*, their use increases blood flow to the large bowel (7,8). Furthermore, they increase sodium and water absorption in the large bowel (9,10,11).

Recent research has focused on the trophic effects of SCT and SCFA in the small and large bowel. When given as intracolonic infusions (12,13) and in *in vitro* studies (14), SCFA stimulate epithelial cellular proliferation in the colon. Other evidence suggests that the acute use (7-10 days) of intravenous SCT, in particular triacetin, could be beneficial in stimulating cellular proliferation in the colon and thereby decreasing intestinal atrophy associated with the use of TPN and trauma (15,16).

SCT and SCFA have been shown to be helpful in several disease states. SCFA irrigation in patients with diversion colitis reversed symptoms (17). Intracolonic infusions of SCFA indicated trophic and vasodilatory effects on the colon of patients undergoing Hartmann's procedure (7). In rats with experimentally induced short-bowel syndrome, elemental enteral diets with SCT increased intestinal
adaptation when compared with medium-chain triglycerides (18). Infusions of SCFA promoted the healing of anastomosis in rats (19).

Little is known about the effects of SCT on the intestinal mucosal cells in a non-trauma or non-diseased model (20) or about the safety of the compound when administered longer term. This study was carried out to assess the metabolic effects of the SCT, triacetin, on the intestinal mucosal cells and plasma substrates in rats.
MATERIALS AND METHODS

Animal care and conduct of experiments were approved by the University of Tennessee Animal Care and Use Committee, which follows NIH guidelines on the humane use of laboratory animals. Twenty-four male Sprague Dawley rats weighing 100 ± 10 g were obtained from Harlan (Indianapolis, IN). Rats were housed in individual plastic metabolic cages with ad libitum access to food and water. After an adaptation period of four days, the rats were randomly placed into one of three dietary groups, with eight animals per group. Animals received diets that varied in fat composition. Table 1 shows the composition of the control and experimental diets. The diets contained either long-chain triglyceride (LCT) in the form of corn oil and/or SCT in the form of triacetin (Sigma, St. Louis, MO). All diets contained 22% (% calories) protein. Weight percentages of vitamins, minerals, and fiber were 1.0%, 3.5%, and 5.0% respectively to meet American Institute of Nutrition standards. Animals received one of three diets. The control diet (5% fat by calories) contained lipid that was 100% LCT. The two experimental diets were 30% lipid (by calories). The lipid component of the two
<table>
<thead>
<tr>
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<th>Control</th>
<th></th>
<th></th>
<th>LCT</th>
<th></th>
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<td></td>
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<td>% kcal</td>
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<td>0.4</td>
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</tr>
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<td>10.6</td>
<td>10.0</td>
<td>9.9</td>
<td>10.0</td>
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<td>59.0</td>
<td>42.5</td>
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<td>39.4</td>
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<td>5.0</td>
<td>-</td>
<td></td>
<td></td>
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<td>corn oil(^1)</td>
<td>2.1</td>
<td>5.0</td>
<td>14.4</td>
<td>30.0</td>
<td>0.7</td>
<td>2.0</td>
<td></td>
<td></td>
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<tr>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>19.0</td>
<td>28.0</td>
<td></td>
<td></td>
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<tr>
<td>Mineral Mix(^2)</td>
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<td>-</td>
<td>3.5</td>
<td>-</td>
<td>3.5</td>
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<tr>
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<td>-</td>
<td>1.0</td>
<td>-</td>
<td>1.0</td>
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<td></td>
<td></td>
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<tr>
<td>Choline bitartrate</td>
<td>0.2</td>
<td>-</td>
<td>0.2</td>
<td>-</td>
<td>0.2</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
<td>99.7</td>
<td>100.0</td>
<td>99.8</td>
<td>100.0</td>
<td>98.9</td>
<td></td>
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\(^1\) Fatty acid composition corn oil - palmitate (11.5%), stearate (2.2%), oleate (26.6%), linoleate (58.7%), linolenate (0.8%), other (0.2%)

\(^2\) AIN Mineral Mixture 76 (per 1 kg): calcium phosphate dibasic 500 g, sodium chloride 74 g, potassium citrate monohydrate 220 g, potassium sulfate 52 g, magnesium oxide 24 g, manganese carbonate 3.5 g, ferric citrate 6 g, zinc carbonate 1.6 g, cupric carbonate 0.3 g, potassium iodate 0.01 g, sodium selenite 0.01 g, chromium potassium sulfate 0.55 g, sucrose 118 g

\(^3\) AIN Vitamin Mixture 76 (per 1 kg): thiamine hydrochloride 600 mg, riboflavin 600 mg, pyridoxine hydrochloride 700 mg, nicotinic acid 3 g, D-calcium pantothenate 1.6 g, folic acid 200 mg, D-biotin 20 mg, cyanocobalamin 1 mg, retinyl palmitate (250,000 IU/g) 1.6 g, DL-alpha-tocopherol acetate (250 IU/g) 20 g, cholecalciferol (400000 IU/g) 250 mg, menaquinone 5 mg, sucrose 972.9 g
experimental diets was either 100% LCT (LCT group) or 95% SCT/5% LCT (SCT group). The LCT in the third diet was incorporated to prevent essential fatty acid deficiency. All animals received diets for 30 days. Food intake was measured daily until the end of the experiment. Body weights were determined at day 0 and for every 5 days thereafter until day 30. On day 30, animals were euthanized by decapitation.

Blood was collected into Vacutainer (Becton Dickinson, Rutherford, NJ) tubes containing EDTA and centrifuged at 1740 x g for 15 minutes at 4°C. Plasma glucose and plasma triglycerides (TG) were determined using Sigma kit numbers 510-A and 336-10 respectively (Sigma, St. Louis, MO). Total plasma ketone bodies (21), defined as beta-hydroxybutyrate plus acetoacetate, plasma free fatty acids (FFA) (22), and plasma lactate and pyruvate (23) were measured.

The small intestine was dissected from the ligament of Treitz to the cecum. The colon was dissected distal to the cecum to the anus. Each was placed under the tension of a 10 g weight. A jejunum segment was taken from the first 10 cm of the small bowel. Similarly, a colon segment was taken from the first 10 cm of the large
bowel. Segments were flushed with cold saline, stripped of excess fat, and weighed. Intestinal segments were inverted and scraped with a glass slide for the removal of the mucosal cells. The mucosal cells were analyzed for DNA, RNA, and protein content by the modified methods of Burton (24), Fleck and Begg (25), and Lowry (26), respectively. The protein-DNA ratio for each group was calculated as an index of cell size (mg protein/10 cm divided by mg DNA/10 cm).

A 2 cm segment from the jejunum and the colon were also removed and fixed in neutral buffered formalin. The segments were embedded in Paraplast Tissue Embedding Medium (Oxford Labware, St. Louis, MO) using a Tissue - Tek Dispensing Console, Thermal Console, and Crio Console (Miles, Inc., Elkhart, IN). Ten micron cross sections were stained with hematoxylin (27) and eosin using a modified procedure (28). Sections were analyzed using light microscopy to determine villus height in the jejunum and crypt depth in the colon.

All data was analyzed using analysis of variance (ANOVA) with the Statistical Analysis Software (SAS, Cary, NC) system to determine significant differences among groups due to diet. Significance was considered to be $p < 0.05$. When a significant
difference was observed, Duncan’s Multiple Range Test was used to further analyze the differences.
RESULTS

No overt adverse effects of any diet were observed during the 30 days of experimental study. Table 2 shows the effect of dietary lipid composition on body mass and food consumption. Body mass was not significantly different at the beginning of the study period. However, by day 30, the rats in the LCT group weighed significantly more than the animals in the other groups. Caloric consumption per day for each week of study was calculated from the daily intake records (Table 2). Food consumption was not significantly different among groups at week 1. During week 2, both LCT and SCT groups ate significantly more than the control group. During weeks 3 and 4, only the LCT group ate significantly more than the animals in the control group. Food consumption was not significantly different between LCT and SCT groups throughout the study period.

The effects of dietary lipid composition on plasma substrates are shown in Table 3. There were no significant differences in lactate, ketone body, and glucose concentrations among groups. Plasma lactate concentration in the SCT group (33.0 ± 7.8 µM) was significantly lower than the LCT group (74.5 ± 2.8 µM). Plasma FFA
Table 2. Body mass and food consumption of rats fed short- or long-chain triglyceride for 30 days

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Mass (g)</th>
<th>Food Consumption (Kcal/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 30</td>
</tr>
<tr>
<td>Control</td>
<td>125.8 ± 2.3</td>
<td>305.1 ± 8.5</td>
</tr>
<tr>
<td>LCT</td>
<td>115.3 ± 1.0</td>
<td>338.5 ± 4.5&quot;</td>
</tr>
<tr>
<td>SCT</td>
<td>131.7 ± 1.7</td>
<td>304.0 ± 6.4</td>
</tr>
</tbody>
</table>

8 animals per group; all values expressed as means ± SEM
* significantly different from other groups, p < 0.05
** significantly different from control, p < 0.05
Table 3. Plasma substrate concentrations of rats fed short- or long-chain triglyceride for 30 days

<table>
<thead>
<tr>
<th>Group</th>
<th>FFA (mM)</th>
<th>PYR (µM)</th>
<th>LAC (mM)</th>
<th>TKB (µM)</th>
<th>TG (mg/dL)</th>
<th>GLU (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.89 ± 0.07</td>
<td>67.6 ± 5.5</td>
<td>2.63 ± 0.24</td>
<td>47.8 ± 4.9</td>
<td>185 ± 10.9</td>
<td>148 ± 4.7</td>
</tr>
<tr>
<td>LCT</td>
<td>1.01 ± 0.04</td>
<td>74.5 ± 2.8</td>
<td>1.96 ± 0.11</td>
<td>50.6 ± 8.0</td>
<td>187 ± 9.2</td>
<td>157 ± 2.9</td>
</tr>
<tr>
<td>SCT</td>
<td>0.50 ± 0.04*</td>
<td>33.0 ± 7.8**</td>
<td>2.36 ± 0.18</td>
<td>55.0 ± 4.7</td>
<td>225 ± 12.1*</td>
<td>159 ± 6.3</td>
</tr>
</tbody>
</table>

*Significantly different from other groups, p < 0.06
**Significantly different from LCT, p < 0.05

FFA = free fatty acids; PYR = pyruvate; LAC = lactate; TKB = total ketone bodies; TG = triglycerides; GLU = glucose.
8 animals per group; all values expressed as means ± SEM.
were significantly lower in the SCT group (0.50 ± 0.04 mM) compared to the LCT (1.01 ± 0.04 mM) and control (0.89 ± 0.07 mM) groups. Additionally, the plasma triglyceride concentration in the SCT group was significantly higher in the SCT group compared to the LCT and control groups (225 ± 12.1 mg/dL vs 187 ± 9.2 mg/dL and 185 ± 10.9 mg/dL, respectively).

Feeding the SCT diet for 30 days was associated with a significant increase in DNA content of the jejunal and colonic mucosa (Figure 1). In the jejunum, animals consuming the SCT diet had a significantly higher DNA content than did the LCT group (0.55 ± 0.07 mg/10 cm compared to 0.21 ± 0.02 mg/10 cm). The DNA content in the control group (0.43 ± 0.06 mg/10 cm) was also greater than that in the LCT group. There were no significant differences in the DNA content of the jejunum between the SCT and control groups. In the colon, the DNA content was the greatest in the SCT group (0.32 ± 0.04 mg/10 cm compared to LCT 0.21 ± 0.02 mg/10 cm and control 0.19 ± 0.03 mg/10 cm).

The only significant effect of diet on RNA content was observed in the jejunum (Figure 2). Animals in the SCT group had significantly less RNA per 10 cm than the other two groups. Jejunal
Figure 1. Jejunal and colonic DNA concentrations (µg/10 cm) of rats fed short- or long-chain triglyceride for 30 days
* significantly different from other groups, p<0.05
Figure 2. Jejunal and colonic RNA concentrations (µg/10 cm) of rats fed short- or long-chain triglyceride for 30 days
* significantly different from other groups; p<0.05
RNA content was as follows: SCT, 0.53 ± 0.03 mg/10 cm; LCT, 1.06 ± 0.07 mg/10 cm; and control, 0.89 ± 0.08 mg/10 cm. No differences in RNA content of the colonic mucosa were observed. Similarly, no significant differences were noted in protein content of the jejunal or colonic mucosa (Figure 3).

For the jejunal mucosa, the protein:DNA ratio was significantly different among all groups (Figure 4). The smallest ratio was seen in the SCT group (29.5 ± 4.6) with the next two highest values in the control group (47.4 ± 5.2) and LCT group (76.2 ± 5.5). In the colon, there were no significant differences among the protein:DNA ratios (Figure 4). However, the smallest value was once again in the SCT group (47.0 ± 8.7). The control group was second smallest (57.4 ± 4.9), and the LCT group had the highest protein:DNA ratio (60.6 ± 4.2).

Differences in crypt depth in the colonic segments and in villus height in the jejunal segments are shown in Table 4. No significant differences in crypt depth were observed. Mean values for crypt depth for control, LCT, and SCT are 0.24 ± 0.01 µm, 0.21 ± 0.01
Figure 3. Jejunal and colonic protein concentrations (µg/10 cm) of rats fed short- or long-chain triglyceride for 30 days
Figure 4. Jejunal and colonic protein:DNA ratios of rats fed short- or long-chain triglyceride for 30 days
Jejunum

Colon

* significantly different from other groups; p<0.05
Table 4. Colonic crypt depth and jejunal villus height of rats fed short- or long-chain triglyceride for 30 days

<table>
<thead>
<tr>
<th>Group</th>
<th>Crypt Depth (µm)</th>
<th>Villus Height (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.24 ± 0.01</td>
<td>0.56 ± 0.03</td>
</tr>
<tr>
<td>LCT</td>
<td>0.21 ± 0.01</td>
<td>0.54 ± 0.05</td>
</tr>
<tr>
<td>SCT</td>
<td>0.21 ± 0.02</td>
<td>0.49 ± 0.03</td>
</tr>
</tbody>
</table>

8 animals per group; all values expressed as means ± SEM
μm, and 0.21 ± 0.02 μm, respectively. Mean villus height for control
(0.56 ± 0.03 μm), LCT (0.54 ± 0.05 μm), and SCT (0.49 ± 0.03
μm) groups was unaffected by diet.
DISCUSSION

The results of the present study indicate that a 30 day oral diet with 28.5% of the total calories supplied as the SCT triacetin produce no overt toxic effects in the rat while stimulating cellular proliferation in the jejunum and the colon. The elevated DNA concentration of the jejunal and the colonic mucosal cells observed in the animals fed triacetin demonstrate an increase in the number of cells in these areas as the DNA content of eukaryotic cells is relatively constant. These results agree with previous studies. When used enterally, parenterally, and as intracolonic infusions, SCFA and SCT have been shown to have a trophic effect on the colonocytes and on the jejunal mucosal cells of rats (7,12-16). The protein:DNA ratio is commonly used as an index of cell size. Mucosal protein content was not significantly different among treatment groups. However, DNA concentration was significantly greater in animals fed SCT. Subsequently, the protein:DNA ratio was significantly lowered in the animals fed triacetin. The lower protein:DNA ratio in the SCT group would indicate smaller, more numerous cells in the jejunum and colon.
The mechanism for this increase is cellular proliferation is thought to be the increased availability of the SCFA acetate to the intestinal mucosa, particularly in the colon. Digestion of Triacetin is initiated in the stomach by gastric lipase (29). The SCFA are absorbed in the stomach and primarily in the jejunum into the portal system in the form of FFA (6). Once in the portal system, they are delivered to the liver where they may be used for the synthesis of long-chain fatty acids and LCT, glutamine, and the ketone bodies acetoacetate and beta-hydroxybutyrate (30). As a majority of the SCT are absorbed in the jejunum, they are available to the intestinal mucosa cells for use as oxidative fuel. In fact, the catabolism of SCFA by the intestinal epithelium to CO$_2$ is a predominant pathway (6). This could explain the increased proliferation seen in the jejunum of rats consuming the SCT diet versus those consuming the LCT diet.

Although proliferation increased in the jejunum and colon with SCT feeding, crypt depth and villus height were unaffected by diet. Increased proliferation may not always translate into an increase in the crypt depth or villus height. The cells in the SCT group were smaller and more numerous as previously mentioned. An increased proliferation with smaller cells could result in no change in size of the
total segment (i.e. crypt or villus). Measurements of the size of the crypt and villus most likely correspond to total protein content of the segment. Like crypt depth and villus height, the protein content in the jejunum and colon was also found to be unaffected by diet. The trends in villus height in the jejunum mimic those seen in protein content in the jejunum with both being largest in the control group and smallest in the SCT group. The trends for crypt depth and protein content in the colon are also similar with the SCT and LCT groups being the smallest in crypt depth and the control group having the largest crypt depth.

The changes in RNA concentration seen in the jejunum and colon reflect changes seen in protein content. The amount of RNA present presumably would influence the amount of protein synthesized. In the colon, the amount of RNA increases from the control group to the SCT group. This trend is also present in protein concentration of the colon with the control group having the least protein and the SCT group containing the most protein. This trend can also be observed in the jejunum. For the most part, decreasing amounts of RNA in the jejunum correspond to decreasing amount of protein.
It is important to note that the provision of the SCT diet did not have a significant anorexic effect when compared to the control and LCT groups. Intake for the four weeks of study was not significantly different between the SCT and LCT groups. The LCT animals actually consumed more Kcal/d for all four weeks when compared to the control animals. During weeks 1 and 2, the caloric consumption of the LCT and SCT groups were almost identical. However, during weeks 3 and 4, the caloric consumption of animals consuming the SCT diet tended to be lower than those consuming 30% LCT. This difference may be due to the unpalatability of the SCT diet. The differences in food consumption is reflected in ending body mass with the LCT group weighing more on the average than the other two groups.

Thirty day feeding of SCT did not have any overt adverse effects. Plasma lactate concentrations were unaffected while the pyruvate concentrations were decreased in the SCT group compared to the LCT group. This decrease in pyruvate concentrations could be explained in two possible ways. The increased availability of acetate with SCT feeding could increase acetyl-CoA levels, thereby increasing citrate synthesis via the citric acid cycle. Acetyl-CoA is a known
inhibitor of pyruvate kinase, and citrate is a known inhibitor of phosphofructokinase (31). Inhibition of both enzymes could contribute to a decreased pyruvate concentration. Plasma glucose and total ketone body concentrations were unaffected. Increased availability of the SCFA acetate have been previously shown to increase ketone body production by the liver (32). In the study, triacetin was infused as the only source of calories for three hours to dogs at 70% above the resting energy expenditure. The reason for the lack of increase in ketone body concentration in the present study is due to a much lower amount of SCT being administered to the animals (28.5% of total calories). When incorporated into a balanced diet, the lack of increase in ketone body concentration is observed. Another factor contributing to the lack of increase in ketone bodies may be due to a large uptake of acetate by the intestine and a possible increase in LCT synthesis from acetate. Other observed effects on plasma substrates included a decrease in the plasma FFA concentration. As the SCFA acetate is absorbed in the FFA form (6), the plasma FFA concentration would be expected to increase. Plasma triglyceride concentration was significantly increased in the group consuming the SCT diet. This increase could be due to increased
synthesis of LCT from acetate. Previous studies of Zucker rats consuming diets containing MCT reported elevated triglyceride concentrations (33) compared to animals receiving equivalent diets of LCT. The fatty acid synthase activity in these animals was also elevated, suggesting increased lipogenesis. In addition, the adipose tissue lipoprotein lipase activity was depressed in animals receiving MCT diets vs those receiving LCT diets. All of these factors could contribute to increased concentrations of TG in the plasma.

The importance of SCFA in the nutriture of the colonic mucosa has already been established. Furthermore, SCFA and SCT have shown clinical significance. As previously mentioned, their use has been associated with a reversal of symptoms in diversion colitis in humans (17), increased intestinal adaptation in rats with short-bowel syndrome (18), and increased healing of anastamosis in rats (19). Future research needs to focus on the effects on longer-term oral diets with SCT in these as well as other intestinal defects.

In summary, providing a diet with 28.5% of its total energy as the SCT triacetin increases cell number in the jejunum and colon of rats without any adverse effects on plasma substrate concentrations.
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Part 2: Triacetin feeding for 30 days decreased adipocyte diameter without adversely affecting growth and development
ABSTRACT

This study determined the effects of a 30 day feeding of either the short-chain triglyceride triacetin (SCT) or long-chain triglyceride (LCT) on growth and development, carcass composition, and adipose tissue cellularity in rats. Male Sprague Dawley rats were given one of three diets, a control diet containing 5% fat (by calories) or one of two experimental diets that were 30% fat (by calories). The source of the fat calories in the experimental groups was 100% LCT (LCT group) or 95% SCT/5% LCT (SCT group). Caloric consumption was higher in the LCT and SCT groups compared to the control group during weeks 1 and 2. During weeks 3 and 4, the animals in the LCT group consumed more calories than those in the control group. There were no significant differences in caloric intake between the LCT and SCT groups throughout the study. There were no significant differences in body weight at the onset of the study; however, the LCT group weighed significantly more than the other two groups at the end of the study. In all three fat pads, the SCT group had a significantly lower pad mass compared to the LCT group. Mean fat cell size was smaller in all fat depots of animals fed SCT. Perirenal fat
cell number was significantly lower in the LCT and SCT groups compared to the control group. Cell number in the other fat pads (epididymal and inguinal) was not significantly affected by diet. The % water, % lipid, and % protein in the eviscerated carcass was not significantly different among groups. Average daily caloric consumption was not significantly different between the LCT and SCT groups. Provision of 28.5% of the total calories as SCT during a 30 day feeding resulted in no overt adverse affects while decreasing adipocyte size in three fat depots and adipocyte number in the perirenal pad compared to the control group. These changes in adipocyte cellularity were present without significant changes in total carcass lipid content.
KEY WORDS: triacetin, short-chain triglycerides, adipocyte, growth, rats
INTRODUCTION

As shown in Part 1, the SCT, triacetin, has trophic effects on the jejunum and colon of rats. Because of this trophic effect, recent research has focused on the use of SCFA and SCT in the treatment of certain intestinal disorders (1,2,3,4). Thus, SCT have a potential role as an oral, enteral, and parenteral nutrient. However, little evidence exists regarding the effects of feeding SCFA or SCT on whole animal nutriture and carcass composition.

Adipocyte cell size and cell number as well as total fat depot mass must be considered to determine the effects of diet on total body adiposity. Cell number has been found to increase during the life span up to a certain age in rats (5). After adulthood, increases in adipose tissue cellularity have been found to increase with overfeeding (6) and high-fat feeding in rats (14,7) especially in the perirenal depot, which has been found to be particularly plastic with regards to cell number (8,9). The exact mechanisms for increases in cellularity of adipose tissue are now known. It has been suggested that adipocyte hypertrophy results in the adipocyte reaching a "critical
fullness" which then stimulates new adipoblasts for filling with lipid (14). It has been suggested that this process may take place in humans (10). Adipocyte cell size is influenced by species, age, diet, and physical activity (11). Bjorntorp suggests that changes in total fat depot mass and cell size are influenced by genetic factors and environmental factors (15). Genetic factors include species and gender. Two primary environmental factors include adipocyte lipoprotein lipase (LPL) activity and corticosteroids. LPL activity influences the amount of triglyceride that is taken up by the fat depot which in turn influences the size of the adipocytes.

A limited number of studies have been carried out using the SCT triacetin as a starch substitute. Animals fed diets containing up to 77% SCT (by calories) for up to 26 weeks grew poorly compared to animals fed control diets (12,13,14). Other evidence exists that providing a diet of 49% SCT (by calories) for 20 days results in decreased but not significant differences in growth compared to control animals (15). At high levels of SCT feeding, growth was depressed; whereas, at lower levels, weight gain was lower but not to a significant effect. These studies reported using very high levels of lipid in the diet. A better use of SCT in studies such as these would
be incorporating SCT into diets at nutritionally appropriate levels.

Because SCT may be an important nutrient in certain digestive diseases, it is necessary to determine the effects of its long-term use of growth and development and on body composition. Therefore, the present study was carried out to determine the effects of a 30 day feeding of a SCT diet on growth and development, carcass composition, and adipocyte cell size and number in rats.
MATERIALS AND METHODS

Animal care and feeding of diets was conducted as previously stated in Part 1. On day 30, animals were euthanized by decapitation.

Carcasses were eviscerated and frozen. Carcasses were ground and mixed with a blender by adding equivalent weights of water. Aliquots were removed and lyophilized for determination of percent moisture. A moisture free sample was removed and percent lipid was determined using a modified soxhlet method (16).

Percent protein was determined using the Kjeldahl method (17). Another sample of the dried carcass was burned in a muffle furnace at 550° C for percent ash determination (18).

Three adipose tissue depots, epididymal, inguinal, and perirenal, were dissected as previously described (13) for analysis of cell size and cell number. A 30-40 mg segment from each pad was incubated in 2 mL of 0.05 mol/L collidine-HCl buffer, pH 7.4, plus 3 mL of 20-30 g/L osmium tetroxide (Electron Microscopy Sciences, Fort Washington, PA) in collidine buffer (19). Tissue samples were incubated at 37° C for 24 hours followed by another 48 hours at
room temperature. Adipocytes were separated by filtering through a 275 µm screen followed by a 25 µm screen (Tetko, Lancaster, NH). The first screen filtered out large debris while the second screen collected the adipocytes. Therefore, all cells between 25 and 275 µm were used for further analysis. The cells were washed off the screen with 0.154 mol/L NaCl into a preweighed beaker to a total weight of 220 g. Triton X-100 (octyl-phenoxy polyethoxyethanol, Sigma, St. Louis, MO) was added to the saline at 0.1 g/L to prevent cellular clumping. The adipocytes were analyzed for cell size and cell number using a Coulter Counter and Coulter Channelyzer 256 (Hialeah, FL).

Cell diameter distributions were calculated for 10 µm intervals from 20 µm to 110 µm. The percent adipocytes in each interval was calculated and plotted to give distributions of cell size for each fat pad. All other data was analyzed using analysis of variance (ANOVA) with SAS (Statistical Analysis System) to determine significant differences among groups due to diet. Significance was considered to be p < 0.05. If the effect was determined to be statistically different, Duncan’s Multiple Range Test was used to further evaluate the differences (20).
RESULTS

As shown in Part 1, no overt adverse effects of any diet were observed during the 30 days of experimental study. Table 2 (Part 1) shows the effect of dietary lipid composition on body mass and food consumption. Body mass was not significantly different at the beginning of the study period. However, by day 30, the rats in the LCT group weighed significantly more than the animals in the control or SCT groups. And even though there was no significant difference in food consumption for animals consuming 30% LCT and SCT throughout the study period, the SCT group did consume less food than the LCT group during weeks 3 and 4.

The effects of dietary lipid composition on carcass composition are shown in Table 1. There were no significant differences in % water, % lipid, and % protein among groups. The only significant difference in carcass composition was seen in % ash. Carcasses in the control group were significantly higher in % ash than those in the LCT and SCT groups.

Total fat depot mass was affected by diet as shown in Table 2. In the epididymial fat pad, the animals consuming the LCT diet had the
Table 1. Carcass composition of rats fed short- or long-chain triglyceride for 30 days

<table>
<thead>
<tr>
<th>Group</th>
<th>% water</th>
<th>% lipid</th>
<th>% protein</th>
<th>% ash</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>54.8 ± 0.94</td>
<td>32.1 ± 1.86</td>
<td>8.93 ± 0.37</td>
<td>9.91 ± 1.44*</td>
</tr>
<tr>
<td>LCT</td>
<td>54.6 ± 1.03</td>
<td>33.6 ± 4.41</td>
<td>8.62 ± 0.67</td>
<td>8.55 ± 0.55</td>
</tr>
<tr>
<td>SCT</td>
<td>53.1 ± 2.25</td>
<td>30.3 ± 4.44</td>
<td>8.29 ± 0.58</td>
<td>8.67 ± 1.01</td>
</tr>
</tbody>
</table>

* animals per group; values expressed as means ± SEM

significantly different from other groups, p < 0.05
Table 2. Epididymal, inguinal, and perirenal fat depot mass of rats fed short- or long-chain triglyceride for 30 days

<table>
<thead>
<tr>
<th>Group</th>
<th>Epididymal (g)</th>
<th>Inguinal (g)</th>
<th>Perirenal (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.04 ± 0.18</td>
<td>5.37 ± 0.29</td>
<td>3.04 ± 0.25</td>
</tr>
<tr>
<td>LCT</td>
<td>4.00 ± 0.22*</td>
<td>6.08 ± 0.40**</td>
<td>3.73 ± 0.37**</td>
</tr>
<tr>
<td>SCT</td>
<td>2.97 ± 0.13</td>
<td>4.61 ± 0.14</td>
<td>2.59 ± 0.20</td>
</tr>
</tbody>
</table>

§ animals per group; all values expressed as means ± SEM
* significantly different from other groups, p < 0.05
** significantly different from SCT
pad with the greatest mass. It’s mass was 24% greater than the mass of the pad in the control group and 26% greater than the mass of the pad in the SCT group. In the inguinal fat pad, the SCT group had a pad mass that was 24% lower than that of the LCT group. The only difference in pad mass in the perirenal pad was between the SCT and LCT groups. The pad mass of the animals consuming the SCT diet was significantly lower (31%) than the pad mass of those consuming the LCT diet.

Adipocyte size distributions for epididymal, inguinal, and perirenal fat pads are shown in Figures 1, 2, and 3 respectively. In the epididymal fat depot (Figure 1), it is evident that SCT feeding decreased adipocyte size. All distributions appear monophasic with the control group peaking between 65 - 75 µm, the LCT group peaking around 75 µm, and the SCT group peaking between 35-45 µm. The mean cell size was also significantly affected by diet. The SCT group had a significantly lower (p < 0.05) mean cell size (51.4 ± 0.6 µm) compared to the control group (70.3 ± 1.0 µm) and LCT group (74.1 ± 1.8 µm). In the inguinal fat depot (Figure 2), SCT feeding also resulted in smaller adipocytes. The mean cell size in the SCT group (53.6 ± 1.8 µm) was significantly lower (p < 0.05) than the mean
Figure 1. Adipocyte size distribution in the epididymal fat depot of rats fed short- or long-chain triglyceride for 30 days
CONTROL
Mean Cell Size:
70.3 ± 1.0

LCT
74.1 ± 1.8

SCT
51.4 ± 0.6
Figure 2. Adipocyte size distribution in the inguinal fat depot of rats fed short- or long-chain triglyceride for 30 days
CONTROL
Mean Cell Size:
61.7 ± 2.0

LCT
68.1 ± 2.0

SCT
53.6 ± 1.8

% OF TOTAL FAT CELLS
Adipocyte Diameter (um)
Figure 3. Adipocyte size distribution in the perirenal fat depot of rats fed short- or long-chain triglyceride for 30 days
PERIRENAL CELL SIZE DISTRIBUTION

CONTROL
Mean Cell Size:
70.4 ± 1.2

LCT
72.0 ± 1.3

SCT
55.5 ± 0.9
cell size in the control group (61.7 ± 2.0 \( \mu m \)) and the LCT group (53.6 ± 2.0 \( \mu m \)). The adipocyte distribution in the SCT group also shows smaller cells with it peaking between 35-45 \( \mu m \). LCT feeding resulted in a biphasic distribution with two peaks around 25 \( \mu m \) and 65-75 \( \mu m \). The adipocyte distribution peaking between 65-75 \( \mu m \).

The distributions for adipocyte diameter in the perirenal fat depot are similar to those seen in the inguinal depot. SCT feeding once again resulted in smaller adipocytes. The mean cell size in the SCT group (55.5 ± 0.9 \( \mu m \)) was significantly smaller compared to the control group (70.4 ± 1.2 \( \mu m \)) and the LCT group (72.0 ± 1.3 \( \mu m \)). The adipocyte diameter distribution in the LCT group is biphasic with peaks around 25 \( \mu m \) and 85-95 \( \mu m \). The adipocyte diameters in the control group peak between 75-85 \( \mu m \). In the SCT group, the distribution is monophasic with a peak in cell diameter between 35-45 \( \mu m \).

Total adipocyte number for the epididymal, inguinal, and perirenal fat pads are shown in Table 3. Cell number in the epididymal and inguinal fat pads were unaffected by diet. However, in the perirenal pad, the LCT and SCT groups had a significantly reduced cell number compared to the control group.
Table 3. Epididymal, inguinal, and perirenal fat cell number of rats fed short- or long-chain triglyceride for 30 days

<table>
<thead>
<tr>
<th>Group</th>
<th>Cell Number*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Epididymal</td>
</tr>
<tr>
<td>Control</td>
<td>30.98 ± 2.84</td>
</tr>
<tr>
<td>LCT</td>
<td>27.20 ± 1.85</td>
</tr>
<tr>
<td>SCT</td>
<td>36.03 ± 3.06</td>
</tr>
</tbody>
</table>

8 animals per group; all values expressed as means ± SEM
* means x 10^6
** significantly different from other groups, p< 0.05
DISCUSSION

Supplying SCT to the diet of rats at 28.5% of total calories did inhibit growth and development somewhat; however, the difference was not statistically significant extent, as discussed in Part 1. These results are in agreement with McAtee et al. (20) but do not support other results of high fat SCT feedings (12-15).

Overall, carcass composition was unaffected by dietary lipid composition. Percent water, lipid, and protein were not significantly different among groups. As this is the first known documented study of the effect of SCT feeding on carcass composition, these results can only be compared to carcass composition from the feeding of other lipid constituents. Results of a study by Turkenkopf et al. indicate that medium-chain triglyceride (MCT) and LCT feeding at 65% of calories did not alter % nonlipid in eviscerated carcasses of lean and obese Zucker rats while MCT feeding in lean Zucker rats lowered % lipid compared to control animals (21).

In all three fat depots, the animals consuming 30% LCT had significantly greater fat pad mass compared to animals consuming the SCT diet. These results reflect the differences in mean adipocyte
diameter as the SCT group had significantly smaller adipocytes in all three fat depots. Even though the LCT group consumed more Kcal/d during weeks 3 and 4, the difference was not significant. And during weeks 1 and 2, the caloric intakes of the LCT and SCT groups was almost identical. This leads to the question if the SCT diet was less energy efficient than the LCT diet. As alterations in adipose tissue LPL activity can lead to changes in cell size, cell number, and depot mass (11), decreased adipocyte LPL activity in the SCT group could be contributing to the decrease seen in depot mass. With high-fat MCT feedings, LPL activity was lower in the adipocytes of obese Zucker rats compared to controls (1).

Adipocyte cell size distributions were determined from 20 µm to 110 µm. Cells below 20 µm may not be adipocytes. Adipose tissue contains adipocytes, blood cells, endothelial cells, pericytes, fibroblasts, and small fat cells, which some investigators have called preadipocytes. Of all these cells, 33-67% are adipocytes (15). Adipocytes and preadipocytes have been differentiated by size with adipocytes being > 20 µm and preadipocytes being < 20 µm (10). Therefore, in order to ensure the measurement of only adipocytes, a lower limit of 20 µm was used.
Epididymal adipocyte distributions were monophasic for all dietary treatments. But in the inguinal and perirenal fat depots, the distributions were biphasic in the LCT group, meaning that in these depots there were small and large adipocytes. Biphasic distributions in the adipose tissue of rabbits (22) and rats (23, 16) have been documented in epididymal, inguinal, and perirenal depots. However, changes in distributions of adipocyte diameter were noted with aging and dietary restriction (16).

The total cell number in the epididymal and inguinal fat depots was unaffected by diet. This was not the case in the perirenal fat pad. In this depot, 30% LCT and SCT feeding resulted in a significant reduction in the number of adipocytes present. Of the fat depots studied in this study, the perirenal depot has been found to be the latest maturing and most dynamic depot (13). Perirenal cell number has been found to increase with aging (14). In the present study, the increases in cell number in the perirenal depot that occur with aging were decreased with increased dietary lipid. These results conflict with the results of other studies which report increases in cell number with high-fat feeding (12).

In summary, SCT have some potential clinical advantages. SCT
are water soluble and can be easily mixed into water-based parenteral formulations, have a trophic effect on the intestinal epithelium and may be useful in the treatment of certain intestinal diseases. Because of the potential use of SCT as a clinical nutrient, it is necessary to determine the effects of SCT feeding on growth and development and body composition. The results of the present study indicate that SCT feeding for 30 days resulted in decreased mean adipocyte size in the epididymal, inguinal, and perirenal depots with concomitant decreases in total depot mass and body mass. These affects were achieved without significantly affecting carcass composition or caloric consumption.
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VITA

Jamie Whoric Lynch was born in Tullahoma, Tennessee on August 23, 1967. She graduated from Tullahoma High School in the spring of 1985. After attending the University of Tennessee at Knoxville from fall of 1985 until spring of 1991, she graduated with a Bachelor of Science in Nutrition and Food Science with a minor in music performance. In the summer of that year, she attended an eleven month internship in clinical dietetics at the University Medical Center in Tucson, Arizona. She then returned to the University of Tennessee at Knoxville, and in May 1993, she received a Master of Science degree in Nutrition Science. While at the University of Tennessee, she passed the registration exam and is now a Registered Dietitian.

She is presently working at Baptist Hospital of East Tennessee in the Nutrition Services department as a clinical dietitian.