Effect of Dietary Medium-Chain Triacylglycerol on Serum Albumin and Nitrogen Balance in Malnourished Rats

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Summary  The present study was examined the therapeutic effect of medium-chain triacylglycerol (MCT) in protein-energy malnutrition (PEM). Wistar rats were fed low protein diet containing 70 g/kg of long-chain triacylglycerol (LCT) or MCT for 31 days. The serum albumin concentration in rats fed MCT diet (2.88 ± 0.04 g/dl) were significantly higher compared with those fed LCT diet (2.72 ± 0.04 g/dl) at day 31. Nitrogen balance was higher in rats fed MCT diet (54.1 ± 2.3 mg/day) compared with those fed LCT diet (45.4 ± 2.4 mg/day) during d 10–12. These results suggest that MCT effectively elevates serum albumin concentration and improves nitrogen balance in malnourished rats.

Key Words: rat, medium-chain triacylglycerol, protein-energy malnutrition, albumin

Introduction

Many elderly people, especially those residing at nursing homes or hospitals, tend to suffer from protein-energy malnutrition (PEM) [1]. For example, there is a high prevalence of malnutrition in a group of hospital outpatients (30%) and groups of inpatients (up to 60%) in United Kingdom [2], while up to 85% of older adults in nursing homes are suffering from malnutrition in the United States [3]. PEM is defined as a serum albumin concentration of less than 35 g/l [4]. PEM in elderly people is associated with impaired activity and general physical condition, a high prevalence of infections, increased risk of developing pressure sores, reduced mental capacity and increased mortality rate [5]. So, some measures should be taken against this disorder.

Individual differences of calorie requirement in elderly person are comparatively large and ability of digestion and absorption decline with aging. So, uniform nutritional supports are not necessarily effective. We thought that changing nutritional support from quantitative to qualitative might be effective.

Medium-chain triacylglycerol (MCT), composed of medium-chain fatty acid (MCFA) such as octanoic and decanoic acids, has several unique nutritional and physiological properties. While long-chain fatty acid (LCFA) is absorbed via the intestinal lymphatic ducts and transported as chylomicrons to systemic circulation, MCFA is absorbed via the portal system and rapidly transferred to liver, then is easily oxidized.

MCT was first used in clinical nutrition in the 1950s for dietary treatment of malabsorption syndromes caused by rapid absorption [6]. MCT was also used for rapid calorie supply to postsurgical patients, who run into acute loss of energy [7]. The purpose of this study was to examine whether administration of MCT is also effective to PEM, which is caused by chronic loss of energy in elderly person, using experimental animal model of PEM.

Materials and Methods

Animals

All animals were treated in accordance with the NIH
Guide for the Care and Use of Laboratory Animals [8]. This experiment was performed after getting approval of in-house animal experimental committee. Specific pathogen-free 3-week-old male Wistar rats weighing 37–48 g were purchased from Japan SLC (Shizuoka, Japan) and given a standard commercial diet (Labo MR Stock, Nossan Corporation, Yokohama, Japan) for 3 days. They were housed individually in stainless-steel mesh cages and allowed free access to filtered tap water under controlled conditions (temperature 22 ± 2°C, humidity 50 ± 10%, lights on from 0800 to 2000 h).

Following the acclimation period, the animals were divided into 2 groups of 6–7 animals each and were allowed free access to the semi-purified diets (Table 1) for 31 days. Food intake and body weight was recorded three times a week. At the end of feeding period, the rats were anesthetized with diethyl ether, and blood was collected by decapitation. The serum was separated by centrifugation at 1900 × g for 5 min at 16°C. The lung, heart, liver, spleen, kidney, intestine, testes, epididymal fat, paranephric fat, mesenteric fat, gastrocnemius, plantaris, soleus and diaphragm were removed and weighed. The visceral weight was defined as the sum weight of lung, heart, liver, spleen, kidney, intestine and testis. From each groups, 3–4 rats were randomly selected, and feces and urine were collected during two days from day 10 to day 12, stored at −20°C until analysis.

**Experimental oils and Diets**

Rapeseed oil as long-chain triacylglycerol (LCT) was used as control oil. MCT was purchased from Nisshin OilliO, Ltd (Tokyo, Japan) and used as test oil. Table 2 shows the fatty acids composition of the experimental oils.

Table 1 shows the composition of the experimental diets. Each diet included 70 g/kg of the respective experimental oil. They were prepared by modifying the AIN-93 diet [9]. The proportion of casein lessens from 20% to 5%. To adjust the weight in diets detracted with casein, beta-starch was supplemented.

**Analysis of plasma**

Serum concentration of total protein was measured by Biuret method and albumin was measured by BCG method (A/G B-Test Wako; Wako Pure Chemicals, Osaka, Japan). Transferrin was estimated by TIA method (SRL, Tokyo, Japan). Ketone bodies and free fatty acids were estimated enzymatically (SRL, Tokyo, Japan). Urea nitrogen was estimated using urease-UV method (SRL, Tokyo, Japan). Amino acids were estimated by HPLC (SRL, Tokyo, Japan).

**Analysis of N-balance**

Collected feces were freeze-dried and powdered using a commercially available small electric coffee mill. Nitrogen concentration was determined using the Kjeldahl method [10] in feeds, carcass, viscera, feces and urine. N balance was calculated from dietary N intake (NI), fecal N excretion (FN) and urinary N output (UN) using following equations:

\[ \text{N balance} = \text{NI} - \text{FN} - \text{UN} \]

**Statistical analysis**

All results were expressed as the mean value ± standard error (SE). Statistical significance of the difference among values was analyzed by Student’s t-test.

**Results**

**Growth, body composition and body fat content**

There were no differences in initial body weight, final body weight, food intake and energy intake between the LCT and MCT groups (Table 3).

| Table 1. Composition of the experimental diets |
|-----------------------------------------------|
| Ingredients | LCT (g/kg) | MCT (g/kg) |
| Beta-Corn Starch | 547.5 | 547.5 |
| Lactic Casein | 50.0 | 50.0 |
| Alfa-Corn Starch | 132.0 | 132.0 |
| Sucrose | 100.0 | 100.0 |
| Rapeseed oil | 70.0 | 10.0 |
| MCT | 0.0 | 60.0 |
| Celluose fiber | 50.0 | 50.0 |
| Mineral mix (AIN-93G) | 35.0 | 35.0 |
| Vitamine mix (AIN-93) | 10.0 | 10.0 |
| L-Cystine | 3.0 | 3.0 |
| Choline bitetrtrate | 2.5 | 2.5 |

1 Contains 0.02% (w/w) tert-Butylhydroquinone.

| Table 2. Fatty acid composition of the experimental oils |
|--------------------------------------------------------|
| Fatty acid | LCT (g/100 g total fatty acids) | MCT (g/100 g total fatty acids) |
| C 8:0 | ND1 | 74.4 |
| C10:0 | ND | 25.6 |
| C16:0 | 4.6 | ND |
| C18:0 | 2.1 | ND |
| C18:1 | 58.7 | ND |
| C18:2 | 20.7 | ND |
| C18:3 | 9.9 | ND |
| C20:1 | 1.6 | ND |
| Others | 2.4 | ND |

1 Number of carbon atoms: number of double bonds.  
2 ND = not detected.
There were no differences in visceral weight, visceral fat weight (epididymal, paranephric and mesenteric fat), lower extremity muscle weight (gastrocnemius, plantaris and soleus) and diaphragm weight between the LCT and MCT groups (data not shown).

**Blood analysis**

There were no differences in plasma total protein concentration between the LCT and MCT groups (Table 4). However, plasma concentration of albumin and transferrin were significantly higher in the MCT group than in the LCT group.

Plasma concentration of total amino acids, essential amino acids, nonessential amino acids and glycogenic amino acids were significantly lower in the MCT group than in the LCT group (Table 5).

No difference was observed in the plasma free fatty acids concentration between two groups. Serum total ketone bodies and beta-hydroxybutylic acid were 1.4 times higher and acetoacetic acid was 1.7 times higher in the MCT group though there was no significant difference (Table 6).

**Nitrogen balance**

Nitrogen balance of d 10–12 was significantly higher in the MCT group than in the LCT group (Table 7). Visceral nitrogen content of d 10–12 was significantly higher in the MCT group than in the LCT group. No significant difference was observed in other parameter.

**Discussion**

In this study, we examined whether MCT intake is effective in PEM, which is frequent chronic energy and protein...
loss in elderly person.

Fed a low protein diet, serum albumin concentration in the MCT group was significantly higher than in the LCT group, suggesting that MCT intake has a preventive effect on PEM. In addition, N-balance was higher in the MCT group than in the LCT group and this may be a primarily cause of above-mentioned preventive effect of MCT.

As nitrogen equilibrium of adult healthy subject is nearly equal, their internal protein pool is constant. In some elderly person however, nitrogen equilibrium is negative, because of anorexia, deflection of palatability, lowering ability of digestion and absorption accompanied by aging. In these subjects, internal protein pool gradually decreases and results in loss of body weight, decreasing mass of muscle or visceral protein, lowering concentration of serum albumin. Some of these are diagnosed with PEM.

The malnourished state has long been recognized as a potential precipitating factor in the development of hypoalbuminemia [11]. When serum albumin decreased by 1 g/l, the odds of being classified as malnourished increased 1.1 times [3]. So, serum albumin is a marker of nutritional status [12]. In this study, serum albumin concentration was higher in the MCT group than in the LCT group, suggesting that nutritional status of the MCT group was improved.

Serum albumin has been the most common index of nutritional status [13]. But, half-life of albumin is very long, and its total body pool is by far the largest among the plasma proteins [14]. In addition, its concentration is influenced by many factors independent of nutritional factors such as infections [15] and trauma [16] (by an increase in the transcapillary escape rate of albumin), hydration status (by haemodilution), liver function (by a decrease in synthesis) and kidney disease (by albumin losses). Thus, the relevance of albumin as a nutritional parameter has repeatedly been questioned [17]. On the other hand, half-lives of transferrin is relatively short. PEM is characterized by serum transferrin concentration [13]. In the present study, serum concentration of transferrin was higher in the MCT group than in the LCT group, also suggesting the improvement of nutritional status in the MCT group.

In case of fasting or glucoprivation, there is a flux of alanine from muscle to liver to support hepatic gluconeogenesis [18], and amino acids derived from muscle protein are also an important substrate for gluconeogenesis [19]. So, in such case, plasma pools of amino acids are increased [20]. In PEM, the concentrations of essential amino acids (EAA) decrease, while those of nonessential amino acids (NEAA) increase, and the EAA/NEAA ratio (E/N ratio) falls [1]. In our study, serum concentration of NEAA and glycogenic amino acids was lower in the MCT group. It also supports the above-mentioned speculation that the nutritional status in the MCT group was improved.

Medium-chain fatty acids, generated by MCT hydrolysis, are metabolized rapidly in mitochondria and yield a large amount of acetyl-CoA, which are converted to ketone bodies and are released to blood [21]. So, continuous intake of MCT, compared with LCT, results in increased concentrations of blood ketone bodies [22]. Ketone bodies are produced in the liver, mainly from the oxidation of fatty acids, and are exported to peripheral tissues for use as an energy source [23] when glucose is not readily available. Ketone bodies are always present in the blood and there levels increase during fasting [24]. During fasting, ketone bodies tend to suppress gluconeogenesis and protect the protein stores [25]. In this study, serum concentrations of total ketone bodies were higher in the MCT group than in the LCT group by about 40%. Maybe these increased ketone bodies were act as alternative fuel and suppress protein breakdown.

Nitrogen excretion was suppressed in premature infants fed MCT-containing formula [26]. In burned rat receiving the chemically structured triacylglycerol with MCT and LCT, protein energy expenditure was decreased while whole energy expenditure remained stable [27]. It suggests that probably non-protein energy expenditure was increased due to rapid oxidation of MCT, and protein utilization was spared. As a result, nitrogen balance was higher in the rat receiving structured lipid. Moreover, serum albumin concentration was higher in the structured lipid receiving rat [28]. In our study, nitrogen balance in the MCT group was higher during the day 10–12, and serum albumin concentration of this group was also higher at d 31. Judging from these observations, increasing concentration of serum albumin resulting from administration of MCT might be due to increased nitrogen balance of these animals.

In this study, we test the possibility of exploitation of MCT in prevention or curing of PEM, and showed that MCT administration might has the improvement effect of hypoalbuminemia and low nitrogen balance, which are typical and diagnostic symptom of PEM.

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Table 7. Nitrogen balance in rats fed experimental diets

| Parameter          | LCT       | MCT       |
|--------------------|-----------|-----------|
| Nitrogen intake    | 71.8 ± 4.2| 83.5 ± 2.6|
| Fecal nitrogen     | 11.3 ± 1.9| 13.9 ± 0.7|
| Urinary nitrogen   | 14.9 ± 2.3| 15.8 ± 1.6|
| Nitrogen balance   | 45.4 ± 2.4| 54.1 ± 2.3*|
| Carcass nitrogen   | 2847 ± 124| 2925 ± 112|
| Visceral nitrogen  | 336 ± 13  | 369 ± 5*  |

* Values are means ± SEM. Significantly different from LCT group: *p<0.05.
Abbreviations

EAA, essential fatty acid; FN, fecal nitrogen; LCFA, long-chain fatty acid; LCT, long-chain triacylglycerol; MCF, medium-chain fatty acid; MCT, medium-chain triacylglycerol; NEAA, non-essential fatty acid; NI, nitrogen intake; PEM, protein-energy malnutrition; UN, urea nitrogen.

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