



Original/Investigación animal

# Hepatic fatty acid profile of rats fed a triheptanoin-based ketogenic diet

Ingrid Sofia Vieira de Melo<sup>1</sup>, Terezinha da Rocha Ataíde<sup>2</sup>, Suzana Lima de Oliveira<sup>2</sup>, Nassib Bezerra Bueno<sup>2</sup>, Johnnatan Duarte de Freitas<sup>3</sup> and Antônio Euzébio Goulart Sant'Ana<sup>4</sup>

<sup>1</sup>Departamento de Agroindústria, Instituto Federal de Educação Ciência e Tecnologia de Alagoas, Murici, AL. <sup>2</sup>Laboratório de Nutrição Experimental, Faculdade de Nutrição, Universidade Federal de Alagoas, Maceió, AL. <sup>3</sup>Departamento de Tecnologia de Alimentos, Instituto Federal de Educação Ciência e Tecnologia de Alagoas, Maceió - AL. <sup>4</sup>Laboratório de Recursos Naturais, Instituto de Química e Biotecnologia, Universidade Federal de Alagoas, Maceió, AL, Brasil.

## Abstract

**Objective:** the aim of this study was to evaluate the influence of consumption of a ketogenic diet supplemented with triheptanoin, a medium-chain anaplerotic triacylglycerol, on the liver fatty acid profile of Wistar rats.

**Methods:** three groups of male Wistar rats ( $n=10$ ) were submitted to an AIN-93 control diet, a triheptanoin-based ketogenic diet, or a soybean oil-based ketogenic diet for 60 days. Excised livers were subjected to lipid extraction and methylation to obtain fatty acids methyl esters, which were subjected to gas chromatography-mass spectrometry.

**Results and discussion:** compared to the rats fed the control diet, those fed ketogenic diets showed a significant reduction in the concentrations of 9-hexadecenoic and 9-octadecenoic acids, whereas those fed triheptanoin showed increased levels of octadecanoic acid.

**Conclusion:** changes in the liver fatty acid profiles of the rats fed a triheptanoin-based or a soybean oil-based ketogenic diet did not seem to be related to the dietary fat source, but rather to the characteristics of the ketogenic diets themselves.

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Key words: *Fatty acid profile. Ketogenic diet. Medium-chain triacylglycerols. Trienantin.*

## PERFIL DE ÁCIDOS GRASOS DE HÍGADOS DE RATONES ALIMENTADOS CON UNA DIETA CETOGÉNICA BASADA EN TRIHEPTANOÍNA

### Resumen

**Objetivo:** el objetivo de este estudio fue evaluar la influencia del consumo de una dieta cetogénica complementada con triheptanoína, un triacilglicerol de cadena media y anaplerótico, en el perfil de ácidos grasos del hígado de ratones Wistar.

**Métodos:** tres grupos de ratones Wistar machos ( $n=10$ ) fueron sometidos durante 60 días a una dieta AIN-93 de control, una dieta cetogénica basada en triheptanoína o una dieta cetogénica a base de aceite de soja. Los hígados fueron escindidos y sometidos a extracción de lípidos y metilación para obtener los ésteres metílicos de ácidos grasos, que se sometieron a cromatografía de gas-espectrometría de masa.

**Resultados y discusión:** en comparación con los ratones alimentados con la dieta de control, los de ambas dietas cetogénicas mostraron una reducción significativa en las concentraciones de los ácidos grasos 9-hexadecenoico y 9-octadecenoico, mientras que los alimentados con triheptanoína mostraron niveles de ácido octadecenoico aumentados.

**Conclusión:** los cambios en los perfiles de ácidos grasos del hígado de los ratones alimentados con dietas cetogénicas no están relacionados con la fuente de grasa de la dieta (triheptanoína o aceite de soja), sino más bien con la concentración total de lípidos.

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Palabras clave: *Ratones Wistar. Triheptanoína. Ácidos grasos.*

### Abbreviations

FA: Fatty Acids.  
MCT: Medium-Chain Triacylglycerols.  
SCD1: Stearoyl-CoA Desaturase-1.

### Introduction

The ketogenic diet is a feasible therapy for individuals with epilepsy; however, it has poor tolerability,

**Correspondence:** Ingrid Sofia Vieira de Melo.  
Instituto Federal de Educação Ciência e Tecnologia de Alagoas.  
Campus Murici, Conjunto Residencial Astolfo Lopes, s/n.  
Cidade Alta, Murici- AL.  
CEP 57820-000, Brasil.  
E-mail: ingridsofia\_melo@hotmail.com

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and alternative therapies are being continuously investigated<sup>1</sup>. The ketogenic diet rich in medium-chain triacylglycerols (MCTs), is widely used, and is an equally effective variant of the classical ketogenic diet, because it maintains ketosis despite the higher content of carbohydrates<sup>2</sup>.

Traditionally, MCTs with even-chain fatty acids (FAs) were used in dietary therapies, because they are commonly found in nature; however, MCTs with odd-chain FAs, such as triheptanoin, are more effective for some disorders than the traditional therapy, notably because of its anaplerotic properties<sup>3</sup>. Experimental studies have shown the effectiveness of triheptanoin<sup>4,5</sup>; accordingly, triheptanoin has recently been suggested for inclusion in the dietary therapy of epilepsy<sup>6</sup>. A recent study found that rats treated with pilocarpine in a triheptanoin-based ketogenic diet needed to undergo a higher number of seizures to develop *status epilepticus*, a marker of the establishment of epilepsy, as compared to the control group<sup>7</sup>.

Triheptanoin is a synthetic MCT consisting of 3 heptanoic acid (C7:0) molecules and has excellent anaplerotic properties<sup>8</sup>. Few studies have described the safety and tolerability of chronic consumption of triheptanoin. In humans, there are some clinical trials in children with mitochondrial disorders<sup>9,3</sup>. Also, experimental tests showed that compared to ingestion of soybean oil, in normal or ketogenic proportions, ingestion of triheptanoin has no adverse effect in terms of weight gain, and the results of blood biochemical analysis and histological analysis were normal<sup>10,11,12</sup>.

Nevertheless, dietary FA composition plays a crucial role in human health, and the repercussion of any novel dietary fat source must be investigated<sup>13</sup>. In particular, dietary fat may induce important modifications in the membrane FA composition of several tissues, including the liver, which may cause important metabolic alterations<sup>14</sup>. Hepatic FA composition is a determinant for insulin sensitivity that acts independently of cellular energy balance and stress<sup>15</sup>. In this study, we evaluated the influence of dietary triheptanoin, in ketogenic proportions, on the hepatic FA profile of rats.

## Objective

The aim of this study was to evaluate the influence of consumption of a ketogenic diet supplemented with triheptanoin, a medium-chain anaplerotic triacylglycerol, on the liver fatty acid profile of Wistar rats.

## Methods

### Animals

The use of animals was approved by the Research Ethics Committee of the Federal University of Alagoas; number, 010077/2005/51. We have read the Jour-

nal's position on issues involved in ethical publication and our report is consistent with these guidelines.

Weaned male albino Wistar rats ( $n=30$ ), weighing  $33.46 \pm 5.7$  g (mean  $\pm$  standard deviation) were obtained from the Federal University of Alagoas, Brazil, and were housed in individual cages at a temperature of 20–24°C under a controlled light-dark cycle (12h/12h). The animals were fed with diet and water *ad libitum* for 6 weeks, including the initial adaptation period of 1 week.

### Diet and treatments

The animals were divided into 3 groups as follows: **Control** (AIN-93G; 7g soybean oil/100g diet<sup>16</sup>), **KetoTAG<sub>7</sub>** (triheptanoin-based ketogenic diet; 40g margarine, 4g of soybean oil, and 25.79g triheptanoin/100g diet), and **KetoTAGsoy** (soybean oil-based ketogenic diet; 40g margarine and 29.79g of soybean oil/100g diet; Table I). Margarine was used to achieve consistency and to ensure uniformity of the ketogenic diets.

The ingredients used in preparing the diets were provided by Rhoister (São Paulo, Brazil). Triheptanoin was purchased from Stéarinerie Dubois (Boulogne-Billancourt, France). Margarine, soybean oil, and corn starch were purchased from a local market.

### Euthanasia of animals and liver removal

After 6 weeks, the animals were fasted overnight, were intraperitoneally injected with an anesthetic solution of urethane + chloralose (1000 mg/kg + 40 mg/kg), and were euthanized by cervical dislocation followed by complete opening of the abdominal cavity and liver resection. The left lobe of the liver was sectioned, and a fragment was frozen in liquid nitrogen and stored at -80°C.

### Lipid extraction and methylation

Extraction of lipids from the liver was performed using organic solvents according to the method by Folch et al. (1957)<sup>17</sup>. The lipidic extracts of the livers and lipidic mixture of the diets were subjected to methylation with boron trifluoride (BF<sub>3</sub>) in methanol (20%). The reaction mixture was placed on a heating plate at 40°C and shaken for 30 h.

### Gas chromatography-mass spectrometry analysis

The FA methyl esters were analyzed by gas chromatography-mass spectrometry (GC-MS) using a Shimadzu chromatograph (GC-17A), a SPB-5 column (30m  $\times$  0.25mm  $\times$  0.25 $\mu$ m), and at temperatures of 250°C

**Table I**  
Composition of control, ketoTAGC<sub>7</sub>, and ketoTAGsoy diets

Constituents (g/kg)	Diets		
	Control <sup>a</sup>	ketoTAGC <sub>7</sub>	ketoTAGsoy
Cornstarch	495,9	0	0
Dextrinized cornstarch	132,0	0	0
Casein	200	200	200
Fiber	50	50	50
Mineral mix AIN-93G	35	35	35
Vitamin mix AIN-93	10	10	10
L-Cystine	3	3	3
L-Methionine	1,6	1,6	1,6
Choline bitartrate	2,5	2,5	2,5
tert-Butylhydroquinone (mg/kg)	14	139,58	139,58
Soybean oil	70	40	297,9
Triheptanoin	0	257,9	0
Margarine	0	400	400
Energy (kcal/100 g)	3.941,6	7.003,7	7.081,1

<sup>a</sup>Reeves, 1997.

and 310°C of the injector and interface, respectively, with helium as the carrier gas (1 mL/min, 50 kPa). Samples (1 µL) were injected using the split control mode, with a ratio of 30:1. MS was performed using the Shimadzu equipment (GCMS-QP5050A) at 70 eV. GCMS LabSolutions v1.01 software was used. The percentage of the chromatographic peak area was used for FA quantification.

### Statistical analysis

The parametric assumptions of normality (Lilliefors' test) and homoscedasticity (Levene's test) were tested. When these assumptions were met, ANOVA was performed and Tukey's-HSD *post hoc* test was performed; when this was not the case, the Kruskal-Wallis test was performed with Dunn's *post hoc* test. Significance was accepted at the  $p < 0.05$  level.

### Results

The FA profile of the hepatic tissue of rats is shown in table II, and the FA profile of the diets fed to the animals is shown in table III.

Our results showed that the concentrations of hexadecanoic, 9-hexadecenoic, and 9-octadecenoic in the liver were significantly higher in the animals in the Control group than in the KetoTAGsoy group, and the octadecanoic acid concentrations were higher in the

KetoTAGC<sub>7</sub> group than in the Control group (Table II). In addition, the 9,12-octadecadienoic acid concentration was higher in the KetoTAGsoy group than in other experimental groups ( $p < 0.001$ ). Also, there were differences between the concentrations of 9-hexadecenoic, 9-octadecenoic, and octadecanoic acids in Control and KetoTAGC<sub>7</sub> groups ( $p < 0.001$ ). This study conducted in Maceió-Alagoas, in 2010.

### Discussion

The changes in the hepatic FA profile of rats administered ketogenic diets rich in triheptanoin or soybean oil did not seem to be related to the dietary oil, but with the ketogenic diet itself.

The main finding of our study is that the levels of 9-hexadecenoic and 9-octadecenoic FA in the KetoTAGsoy and KetoTAGC<sub>7</sub> groups were significantly lower than those in the Control group (Table II). The synthesis of these FAs is catalyzed by the enzyme stearyl-CoA desaturase 1 (SCD1), the activity of which is essential for the synthesis of hepatic triglycerides because incorporation of a monounsaturated FA in the carbon backbone of glycerol is essential<sup>18</sup>. This finding suggests that compared to the control diet, the ketogenic diet may induce a decrease in the activity of the SCD1 enzyme.

SCD1 deficiency in mice induces loss of body mass by increased hepatic FA oxidation and reduces triacylglycerol synthesis and storage through decreased

**Table II**  
Mean scores of different fatty acid percentages of liver tissues of rats in Control, KetoTAGC<sub>7</sub> or KetoTAGsoy groups. Values are expressed as Mean [SEM]

Fatty Acids (%)	Groups		
	Control (n=7)	KetoTAGC <sub>7</sub> (n=9)	KetoTAGsoy (n=7)
Dodecanoic	0.0329 [0.0145]	0.1556 [0.0671]	0.0871 [0.0331]
Tetradecanoic	0.9357 [0.1389]	0.5233 [0.1470]	0.2500 [0.0578] <sup>a</sup>
Pentadecanoic	0.0970 [0.0339]	0.1640 [0.0341] <sup>c</sup>	0.0330 [0.0164] <sup>b</sup>
Hexadecanoic	36.3686 [4.1831]	26.8300 [2.4167]	20.5914 [1.0524] <sup>a</sup>
Heptadecanoic	0.1043 [0.0464]	0.2056 [0.0565]	0.0414 [0.0201]
Octadecanoic	13.2286 [1.7042]	29.1144 [4.3476] <sup>a</sup>	20.6071 [2.8860]
9-hexadecenoic	3.9886 [0.4760]	0.2878 [0.2030] <sup>a</sup>	0.0743 [0.0356] <sup>a</sup>
9-octadecenoic	23.2110 [2.2952]	12.239 [1.8884] <sup>a</sup>	12.8490 [0.8957] <sup>a</sup>
9,12-octadecadienoic	14.2529 [0.4473]	14.6489 [2.4285] <sup>c</sup>	34.8814 [2.4861] <sup>a,b</sup>
9,12,15-octadecatrienoic	0.1443 [0.0691]	0.0822 [0.0532]	0.2357 [0.0691]
5,8,11,14-eicosatetraenoic	7.2643 [2.9659]	15.2378 [4.5650]	10.1000 [1.3947]
ΣSaturated	8.4611 [2.2009]	11.6179 [1.9624]	6.9350 [1.5835]
ΣMonounsaturated	13.6000 [2.8938]	6.2633 [1.7173] <sup>a</sup>	6.4614 [1.8231] <sup>a</sup>
ΣPolyunsaturated	7.2204 [1.7714]	9.9896 [2.1523]	15.0723 [3.3814]

<sup>a</sup>Significant differences compared to Control group (p<0.05). <sup>b</sup>Significant differences compared to KetoTAGC<sub>7</sub> group (p<0,05). <sup>c</sup>Significant differences compared to KetoTAGsoy group (p<0,05).

**Table III**  
Percentages values of most abundant fatty acids in the diets offered to Control, KetoTAGC<sub>7</sub> and KetoTAGsoy groups

Fatty Acids (%)	Diets		
	Control	KetoTAGC <sub>7</sub>	KetoTAGsoy
Heptanoic	ND <sup>a</sup>	96,21	ND
Dodecanoic	ND	0,81	1,64
Hexadecanoic	24,18	0,62	14,29
9,12-octadecadienoic	39,21	0,69	54,79
9-octadecenoic	32,33	0,71	21,89
Octadecanoic	4,41	0,95	7,40

<sup>a</sup>Not detected.

expression of lipogenic genes, which makes these animals more sensitive to insulin and resistant to diet-induced obesity<sup>19</sup>. Interestingly, compared to animals in the Control group, those in the ketogenic diet groups gained less weight during the experiment; however, in the last week of the study, no significant differences were observed between the mean weights of the groups<sup>12</sup>. Studies on a rat model of the metabolic syndrome showed that these animals also have increased levels of monounsaturated FAs and lower SCD1 activity<sup>20</sup>. Nonetheless, further studies are required to

investigate the possible effect of ketogenic diets on the activity of such enzyme.

The significantly larger amounts of octadecanoic acid in the KetoTAGC<sub>7</sub> group than in the Control group (Table II) might also be explained by SCD1 activity. Under normal conditions, this FA is rapidly metabolized via SCD1 and becomes 9-octadecenoic acid<sup>21</sup>. A lower SCD1 activity induced by the ketogenic diet could explain the increased levels of octadecanoic acid in the KetoTAGC<sub>7</sub> group. Nevertheless, the levels of this FA in the KetoTAGsoy group did not differ significantly from those in the Control group.

The higher concentrations of 9,12-octadecadienoic acid in the KetoTAGsoy group than in the other groups may be explained by the dietary fat source. Since this FA is not endogenously synthesized, its concentration in the liver tissue is a reflection of dietary content, shown here by the significant predominance of this FA in the diet offered to the KetoTAGsoy group (Table III).

Further, the lower concentration of hexadecanoic acid in the KetoTAGsoy group than in the Control group, and the unexpected absence of significant differences between the Control and KetoTAGC<sub>7</sub> groups as well as the KetoTAGC<sub>7</sub> and KetoTAG-Soy groups, despite the distinct concentrations of hexadecanoic acid in the diets (Tables II and III), might be explained by the metabolic features of this FA. Hexadecanoic acid is synthesized endogenously and is the main product of the FA synthase complex; thus, examination of its metabolism and fate is difficult.

In summary, our study shows that ingestion of triheptanoin does not alter the hepatic FA profile of rats. The data of the present study contributes to the spectrum of scientific evidence, which attests the reliability and safety of the therapeutic use of triheptanoin, even in ketogenic proportions.

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