Nutrition 30 (2014) 863–868

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Basic nutritional investigation

Effects of low carbohydrate diets on energy and nitrogen balance and body composition in rats depend on dietary protein-to-energy ratio

Lena Frommelt [a], Maximilian Bielohuby Ph.D. [b], Dominik Menhofer [b], Barbara J.M. Stoehr [b], Martin Bidlingmaier M.D. [b], Ellen Kienzle Prof. [a,*]

[a] Animal Nutrition, Ludwig-Maximilians University, Munich, Germany
[b] Endocrine Research Unit, Medizinische Klinik und Poliklinik IV, Klinikum der Universität, Ludwig-Maximilians University, Munich, Germany

ARTICLE INFO

Article history:
Received 30 August 2013
Accepted 27 November 2013

Keywords:
Low-carbohydrate high-fat diets
Ketosis
Nitrogen balance
Body composition
Rats

ABSTRACT

Objectives: Truly ketogenic rodent diets are low in carbohydrates but also low in protein. The aim of this study was to differentiate effects of ketosis, low carbohydrate (LC) and/or low-protein intake on energy and nitrogen metabolism.
Methods: We studied the nitrogen balance of rats fed LC diets with varying protein contents: LC diets consisted of 75/10, 65/20 and 55/30 percent of fat to protein (dry matter), respectively, and were iso-energetically pair-fed to a control (chow) diet to 12-wk-old male Wistar rats (n = 6 per diet).
Previous studies demonstrated only LC75/10 was truly ketogenic. Food, fecal, and urine samples, as well as carcasses were collected and analyzed for heat of combustion and nitrogen (Kjeldahl method). Blood samples were analyzed for plasma protein, albumin, and triacylglycerol.
Results: All LC groups displayed less body weight gain, and the degree of reduction was inversely related to digestible crude protein intake (daily weight gain compared with chow: LC75/10: −50%; LC55/30: −20%). Nitrogen excretion by urine was related to digestible protein intake (chow: 0.23 ± 0.02 g nitrogen/d; LC75/10: 0.05 ± 0.01 g nitrogen/d). Renal energy excretion was closely associated with intake of digestible crude protein (r = 0.697) and renal nitrogen excretion (r = 0.769). Energy-to-nitrogen ratio in urine was nearly doubled with LC75/10 compared with all other groups. Total body protein was highest with chow and lowest with LC75/10. Rats fed with LC75/10 displayed features of protein deficiency (reduced growth and nitrogen balance, hypoproteinemia, depletion of body protein, and increased body and liver fat), whereas the effects with the non-ketogenic diets LC65/20 and LC55/30 were less pronounced.
Conclusion: These results suggest that truly ketogenic LC diets in growing rats are LC diets that are also deficient in protein for growth.

Introduction

Ketosis induced by low carbohydrate (LC) diets can have beneficial effects on neurologic disorders such as epilepsy in children by modulating adenosine triphosphate-dependent potassium channels in neurons [1]. It has been shown that ketogenic diets can not only be used for epilepsy treatment, but their neuroprotective role also may be beneficial for other neurologic diseases such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis [2]. Ketogenic diets also can enhance mitochondrial function and recently have been considered a clinical treatment option in the field of oncology. Additionally, ketogenic LC diets are used for weight loss in overweight individuals [3,4].

To evaluate the benefits or adverse effects of ketogenic diets in animal experiments it is important to know and to differentiate the effects of ketosis per se and the effects of each of the nutrients contained in the experimental diets, e.g., fat, carbohydrates, or protein. It is important to know how to design a ketogenic LC diet for rats because this diet, in addition to being

* Corresponding author. Tel.: +49 (0) 89 2180 78701; fax: +49 (0) 89 2180 78702.
E-mail address: Kienzle@tiph.vetmed.uni-muenchen.de (E. Kienzle).

0899-9007/$ - see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.nut.2013.11.009

high in fat content, is low in both carbohydrate and protein content, both of which help to induce a state of ketosis in the rats. Therefore, LC diets with varying protein and fat content have been tested for their ability to induce ketosis in growing rats [5]. It has been demonstrated that only LC diets with an extremely high fat content, but rather low protein were truly ketogenic in rats, presumably because LC diets with moderate to high protein content provided sufficient glucoplastic amino acids for gluconeogenesis to prevent ketosis. It is difficult to differentiate between effects of the low protein intake or even a mild protein deficiency and effects of ketosis when using LC diets with very low protein content. This problem appears especially in experiments with growing rats because the requirement for protein is higher during times of growth rather than during maintenance. In this study, we tested LC diets with different protein contents in growing rats and a special LC diet with matched protein content to the control diet (LC 55/30, to ensure that rats on this LC diet had the same protein intake as in the control diet). The aim of this study was to determine the effects that result from protein malnutrition and effects that result from ketosis. This findings help to receive more detailed information how to design and how to evaluate effects of ketogenic LC diets in rats.

The effect of low protein intake in LC diets may be enhanced by the need for amino acids for gluconeogenesis. It has been demonstrated that increasing the protein content in LC diets alleviates negative effects on growth and reproduction in various species [6,7]. In the present study, we analyzed parameters such as nitrogen balance, renal energy excretion, and body composition to differentiate between the effects of low protein intake, LC intake, and ketosis in rats consuming the same LC diets with varying protein and fat content as previously studied to investigate their effect on induction of ketosis [5].

Materials and methods

Diets and animal husbandry

Four experimental diets were investigated. Composition of the diets is provided in Table 1. Semipurified diets were purchased from Kliba Nafag (Kliba Nafag, a business unit of PROVIMI KLIBA SA, Kaiseraugst, Switzerland). The control diet (chow) contained moderate amounts of carbohydrates and fat. Protein in the chow diet was according to requirements for growing rats [8]. Overall, the dietary composition of the chow diet was typical for standard rodent diets and followed the nutrient composition of the reference diet of the American Institute of Nutrition [9].

Three LC diets with increasing percentages of fat (55%, 65%, and 75% in dry matter [DM]) and decreasing percentages of protein (30%, 20%, and 10 % in DM, respectively), hereafter referred to as LC55/30, LC65/20, and LC75/10 were investigated. LC55/30 was matched to the chow diet with respect to the protein-to-metabolizable energy (ME) ratio (ME predicted by Atwater factors). The only protein source was sodium casein in all diets. The only fat source was beef tallow for all LC diets. Fat in the chow diet originated to 50% from beef tallow and 50% from soybean oil. In the chow diet, the only carbohydrate source was starch.

For minerals, trace elements and vitamins, the ratio (based on a nutrient-to-energy ratio) was matched in the LC diets to the same ratio as in the chow diet, using ME estimated by Atwater factors as recommended for semipurified diets [10]. To ensure that lysine was not growth-limiting in the LC75/10 diet, a small amount was added (in g/kg DM: chow: 16; LC75/10: 8; LC65/20: 17; LC55/30: 25). The content of nutrients in DM is provided in Table 1.

Twenty-four male intact Wistar Unilever rats (Harlan Laboratories, six animals per trial, 12 wk old at start of feeding experimental diets) were used. All animals had free access to tap water and standard natural laboratory diet (Ssniff, Soest, Germany) for the first 10 d following delivery, to allow acclimatization to the new environment. Rats were housed in an open system in individual Makrolon type III cages at 21 ± 1.5°C (relative humidity 55 ± 15%, 100% fresh air) and maintained on a 12-h artificial light and 12-h dark cycle throughout the study. Before digestion trials, rats were adapted for at least 7 d to their respective diet. Food allowance was free choice for the chow diet. All other groups were pair-fed on an iso-energetic basis to the chow diet, with the estimated ME as described previously as a basis. The resulting DM intake is shown in Table 2. Food intake was measured daily. During the digestion trials, litter was removed from the cages and rats were kept on stable metal grids (Tecniplast Deutschland GmbH, Hohenpeissenberg, Germany). Feces were collected and weighed in the morning and evening for 5 to 7 d consecutively. Feces were stored immediately at −23°C until analysis. After 4 wk on the respective diets, rats were given access to food for 1 h after lights out and then fasted for 6 h (to standardize gastrointestinal filling in the three groups) before being sacrificed under an ultra-short isoflurane anesthesia. Rats were dissected and livers were excised, carefully freed from adherent tissues, and immediately frozen on dry ice until further analysis. The gut was emptied and then processed with the remaining body parts. Carcasses were dissected in small pieces and dried by lyophilization. The dried material was ground and frozen until analysis. All procedures were approved by the Upper Bavarian Government's ethical committee for animal experiments and followed the national and European Union laws for animal protection.

Table 1
Feed composition in dry matter

Diet		Chow	LC75/10	LC65/20	LC55/30
Energy (MJ/kg)	GE	20.1	33.6	32.1	30.9
	DE*	18.4	28.8	27.4	26.2
	ME†	17.5	28.4	26.5	25.0
Weende analyses (%)	Fat	5.2	76.1	66.0	56.9
	Protein	20.9	9.6	19.7	28.0
	Fiber	4.8	6	6.3	6.7
	Ash	3	5.6	5.2	4.9
Carbohydrates (%)	Starch	23.8			
	Sugar	37.3			
Minerals (g/kg)	Ca	5.4	9.6	9.1	8.5
	P	2.7	5.8	5.3	5.2
	Na	2.1	4.3	3.8	3.6
	K	4.1	7.4	6.5	6.2
Trace elements (mg/kg)	Fe	107.7	162.8	163.9	187.4
	Zn	60.7	94.6	97	91
	Cu	7.8	22.5	13	10.8
	I‡	0.8	1.4	1.4	1.3
	Mn‡	16.7	30.8	30.2	28.8
	Se‡	0.3	0.6	0.5	0.5
Vitamins‡ (IE/kg)	Vit. A	4300	8000	7600	6800
	Vit. D	1075	2000	1900	1700
	Vit. E	113	200	190	170
(mg/kg)	Vit. K	4.6	8.6	8.2	7.3
	Vit. B_1	6.5	12	11.4	10.2
	Vit. B_2	6.9	12.8	12.2	10.9
	Vit. B_6	7.5	14	13.3	11.9
	Vit. B_{12}	0.05	0.1	0.1	0.09
	Niacin	35.5	66.1	62.8	56.2
	Pantothenic acid	17.3	32.2	30.6	27.4
	Folic acid	2.6	4.8	4.5	4
	Biotin	0.2	0.4	0.4	0.3
	Choline	1215	1975	2000	2020

DE, digestible energy; GE, gross energy; LC, low carbohydrate; ME, metabolizable energy

* Experimentally determined.

† DE-5.2 kJ per g digestible protein (digestible protein was experimentally determined: [protein content of feed (g/kg) × apparent digestibility of protein (%)/100] × 5.2/1000).

‡ As labeled by manufacturer.

Analyses

Combustion heat of food (gross energy, GE), feces, urine, and carcasses was determined using an adiabatic bomb calorimeter (IKA-Calorimeter C4000; IKA-Analysentechnik Janke & Kunkel GmbH & Co., Staufen, Germany). GE was determined three times in each sample of food, feces, and carcass and six times in urine. Coefficient of variation within analysis was calculated and if it exceeded

Table 2
Body weight and feed intake during balance trial and collection period*

Diet	n	BW (g)	DM intake (g/kg BW/d)
Chow	6	393.3 ± 15.4[a]	41.4 ± 1.6[a]
LC75/10	6	363.4 ± 12.8[b]	26.3 ± 1.2[b]
LC65/20	6	379.2 ± 15.5 [ab]	26.9 ± 1.0[b]
LC55/30	6	395.6 ± 11.1 [ac]	32.2 ± 1.6[c]

BW, body weight; DM, dry matter; LC, low carbohydrate

* Means not sharing superscript letter are significantly different ($P < 0.05$).