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Despite transient ketosis, the classic high-fat ketogenic diet induces marked changes in fatty acid metabolism in rats☆

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Abstract

In contrast to humans, rats on a high-fat ketogenic diet seem incapable of maintaining plasma β-hydroxybutyrate above 1 mmol/L for more than a week. Our goal was to determine whether fatty acid metabolism in rats changes despite the absence of sustained ketosis induced by the ketogenic diet. Fatty acid metabolism was assessed as changes in tissue fatty acid profiles and change in ^{13}C-α-linolenic acid incorporation into plasma, liver, adipose tissue, and brain lipids. Despite loss of ketosis, the ketogenic diet reduced some polyunsaturated fatty acids in adipose tissue (up to 44%) and plasma (up to 90%) but raised polyunsaturates in liver triglycerides by up to 25-fold and raised arachidonic and docosahexaenoic acids in the brain by 15%. Lower tissue incorporation of ^{13}C-α-linolenic acid but higher unlabeled and ^{13}C-labeled docosahexaenoic acid in brain supports the view that the principal changes in fatty acid composition resulted from enhanced mobilization of polyunsaturates from adipose tissue to liver and brain. In the absence of sustained ketosis, changes in fatty acid metabolism resulting in an increase in brain polyunsaturates, particularly docosahexaenoic acid may, nevertheless, contribute to the seizure protection by the ketogenic diet.
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1. Introduction

The high-fat, very low-carbohydrate, "classic" ketogenic diet ameliorates several disorders affecting brain metabolism and function, including intractable epilepsy [1], glucose transporter defects [2], and mitochondriopathies [3]. The ketogenic diet raises plasma ketone bodies (acetoacetate, β-hydroxybutyrate [β-HBA], and acetone) because genes controlling enzymes of ketogenesis, particularly 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) synthase [4,5] and HMG-CoA lyase [6], are activated when plasma insulin is suppressed by low carbohydrate intake. The fatty acid β-oxidation pathway soon becomes saturated, and free fatty acids (FFA) are consequently shunted into ketogenesis, resulting in a sustainable state of mild to moderate ketosis.

Seizure protection while on the ketogenic diet may be due to a direct effect of ketone bodies (eg, acetone) [7], but seizure suppression can occur in animals on the ketogenic diet that no longer exhibit ketosis [8]. Hence, we hypothesized that metabolites other than ketone bodies may be able to contribute to the seizure-suppressing effects of the ketogenic diet. A 2- to 5-fold increase in plasma polyunsaturated fatty acids, particularly arachidonic acid (20:4ω6) and docosahexaenoic acid (22:6ω3), accompanies improved seizure control in children with refractory epilepsy on the ketogenic diet [9]. In these children, seizure protection was not correlated to the degree of ketosis [9]. The possibility that changes in serum polyunsaturates contribute to seizure inhibition while on the ketogenic diet is also supported by the observation that exogenous-infused docosahexaenoic acid has direct seizure-suppressing effects in a rat model [10]. These reports suggest that changes in plasma levels or metabolism of polyunsaturated fatty acids may contribute to the seizure-protective effects of the ketogenic diet, and may occur independently of changes in plasma ketone bodies.

Several groups have shown that, in contrast to children, well-fed rats growing normally generally cannot sustain

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ketosis beyond 7 to 10 days of continuous feeding on the ketogenic diet [8,11-13]. The objective of the present study was therefore to investigate whether changes in tissue fatty acid composition and metabolism remain evident despite the loss of ketosis after 10 days on the ketogenic diet. In addition to evaluating fatty acid profiles in plasma, adipose tissue, liver, and brain, we measured tissue incorporation of a tracer fatty acid, ^{13}C-α-linolenic acid (18:3ω3), and its conversion to docosahexaenoic acid. The tracer study was intended to help establish whether the ketogenic diet affects synthesis of docosahexaenoic acid.

2. Materials and methods

2.1. Animals and treatments

Procedures for animal housing and treatment were approved by the University of Toronto. Male Wistar rats (Charles River, La Prairie, QC, Canada) aged 40 to 42 days were housed in pairs in plastic cages with wood chip bedding. Lighting was on a light/dark cycle (12:12 hours). The rats had 10 days to adapt to the facility, during which they consumed the control diet. They were then divided into 2 groups (n = 8 per group) and randomly assigned to stay on the control diet or to switch to the ketogenic diet (see Table 1 for fatty acid composition). The feeding trial lasted for 10 days. Fresh diet was supplied in clean dishes every 24 hours. Rats had ad libitum access to water and their respective diets throughout the experiment.

Every 3 or 4 days, 100-μL blood samples were drawn from the tail vein of 4 rats per group using heparinized needles. These samples were immediately centrifuged at 1500 rpm and 7°C, and plasma was separated and stored at −20°C for analysis on the following day. β-Hydroxybutyrate was measured using a spectrophotometric enzyme assay kit (Sigma Chemical Co, St Louis, Mo).

Table 1
Composition of control and ketogenic diets[a]

Ingredient	Control diet (g/kg)	Ketogenic diet (g/kg)
Protein	89.4	154.1
Butter	0	508.3
Soybean oil	69.3	123.6
MCT oil	0	51.5
Canola oil	10.0	5.2
Sucrose	136.1	0
Cornstarch	461.0	0
Dextrin	163.4	32.5
Cellulose	41.0	71.7
Vitamin mix	0.3	0.6
Mineral mix	27.0	48.3
Choline	2.5	4.4

MCT indicates medium-chain triglyceride.

[a] The control diet contained 79.3 g of fat per 1000 g of diet of the following composition: palmitate (9.4%), stearate (4.2%), oleate (27.2%), linoleate (50.6%) and α-linoleneate (8.0%). The ketogenic diet contained 688.8 g fat/1000 g of diet, including 12 carbons (11.3%), myristate (7.3%), palmitate (23.9%), stearate (10.9%), oleate (25.7%), linoleate (16.2%), α-linoleneate (2.4%), and other (2.3%). No other polyunsaturated fatty acids were detectable in the diets except linoleate and α-linoleneate.

On day 9, 6 rats from each group were gavaged with 5 mg of ^{13}C-α-linolenic acid dissolved in 100 μL of olive oil. The remaining 2 rats in each group were gavaged with 100 μL of olive oil and used as references for carbon 13 (^{13}C) background during analysis of isotopic enrichment. On day 10 (24 hours after tracer dosing), all rats were killed by an intraperitoneal injection of 1.3 μL/kg of sodium pentobarbital (MTC Pharmaceuticals, Cambridge, Ontario, Canada). Blood was drawn by cardiac puncture, after which, liver, brain, and perirenal adipose tissue were excised from each rat. Blood was immediately centrifuged and stored at −20°C for later analysis. Livers were stored at −80°C, whereas brain and adipose were stored at −20°C.

2.2. HMG-CoA lyase gene expression

To assess the expression of the enzyme specific to ketone body synthesis (HMG-CoA lyase), a standard Northern blot assay was performed on homogenates of rat liver from the 2 groups. Total RNA was isolated using Trizol reagent (Life Technologies Inc, Gaithersburg, Md) and electrophoresed on 1% agarose gel containing formaldehyde. Total RNA was transferred overnight to a nylon membrane (Ambion, Austin, Tex) and UV cross-linked. Reverse transcription of liver total RNA was catalyzed by 1 μL of Superscript and 1 μL of RNase to form the complementary DNA. A 0.4-kb probe was made using forward (5′-AAGTGGGTGCCGCAGATGGC-3′) and reverse (5′-AGCAGTCAGCATGTCTTTCA-3′) primers based on published sequences [6]. Forward and reverse polymerase chain reaction was carried out in a Perkin-Elmer 480 DNA thermal cycler and Ready-To-Go polymerase chain reaction beads (Pharmacia, Baie d'Urfé, Quebec, Canada). The conditions were set for an initial 5 minutes denaturing step at 94°C followed by 30 cycles of denaturation (94°C for 1 minute), annealing (62°C for 2 minutes), and extension (72°C for 3 minutes + 5 seconds per cycle). Probes were eluted and labeled with ^{32}P-adenosine triphosphate by random priming. After addition of the labeled probe, the blot was washed twice in a solution of sodium chloride/sodium citrate and sodium dodecyl sulfate (SDS) (2× SSC/0.1% SDS) for 5 minutes at 42°C and twice in 0.1× SSC/0.1% SDS at 50°C. The phosphorus 32 signal from the radioactive bands was quantified using a Packard Instant Imager (Canberra Packard Canada, Mississauga, Canada) and normalized to β-actin.

2.3. Lipid extraction and fatty acid analysis

Total lipids were extracted using the method of Folch et al [14]. Diheptadecanoyl L-α-phosphatidylcholine, triheptadecanoic acid, cholesteryl heptadecanoic acid, nonesterified heptadecanoic acid, and 5-α-cholestane (Sigma) were added as internal standards to approximately 0.5 g of liver or 1 mL of plasma to quantify phospholipids (PLs), triglycerides (TGs), cholesteryl esters (CEs), FFA, and free cholesterol,

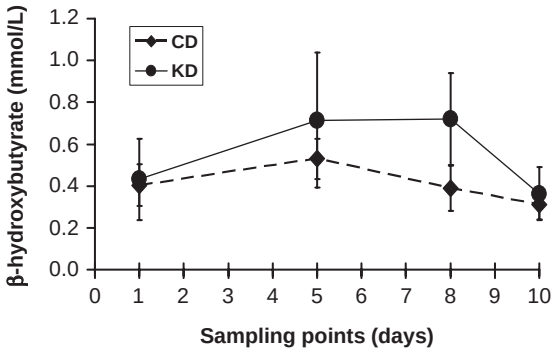

Fig. 1. Effect of the control or ketogenic diet on plasma β-HBA levels. Values are mean ± SD (n = 4 per group). CD indicates control diet; KD, ketogenic diet.

respectively. Only nonesterified heptadecanoic acid and 5-α-cholestane were added to brain samples. No internal standards were added to adipose tissue lipid extracts.

Total lipids of plasma and homogenized samples of liver and brain were extracted into chloroform-methanol (2:1, vol/vol). Lipid classes in the isolated total lipid extracts from plasma and liver (but not brain or adipose tissue) were fractionated by neutral lipid thin-layer chromatography using silica gel plates measuring 20 × 20 cm (Whatman LK6D plates precoated with 250 μm of Silica Gel 60A, Chromatographic Specialties, Brockville, Ontario, Canada). Separate lanes were spotted with PL, FFA, TG, and CE standards. The plates were developed using hexane, diethyl ether, and acetic acid (80:20:1 by volume) in covered tanks for 35 minutes. Lipid bands were identified under ultraviolet light after spraying with 2,7-dichlorofluoroscein and scraped off each plate. Free fatty acid bands were directly methylated in 14% methanolic boron triflouride. PL, TG, and CE bands were saponified in 1N methanolic NaOH for 60 minutes at 90°C. After adding hexane and concentrated HCl, the fatty acid phase was separated and methylated in 14% methanolic boron triflouride at 90°C for 30 minutes. Total lipid extracts from brain and adipose tissue were directly saponified and methylated. Fatty acid methyl esters from all tissues and lipid fractions were analyzed by gas-liquid chromatography using a 30-m × 0.25-mm ID capillary column (J&W Scientific DB-23, Folsom, Calif) in a Hewlett Packard 6890 gas liquid chromatograph (Palo Alto, Calif).

Methyl esters of total fatty acid extracts from adipose tissue, liver, and brain were analyzed for ^{13}C enrichment by gas chromatography–combustion-isotope ratio mass spectrometry (Europa 20-20 with Orchid combustion interface, PDZ Europa, Crewe, Cheshire, UK) [15]. Given the considerable changes in background fatty acid composition in these rats, the ^{13}C enrichment data were expressed as "specific activity" (ng ^{13}C/mg fatty acid).

Plasma, liver, and brain cholesterol was recovered after saponification of the total lipid extracts, derivatized using trimethylsilyl chloride and analyzed using capillary gas chromatography, as previously described [16].

2.4. Statistical analysis

All statistical analysis was performed on Statistical Analysis Software (version 8.02, SAS Institute, Cary, NC). Treatment differences between the control and ketogenic diet groups were detected using an unpaired t test. Statistical significance was accepted at $P < .05$.

3. Results

3.1. General

The rats in both groups had similar and normal growth throughout the experiment. Final body weight was 321 ± 15 g in the controls and 318 ± 15 g in the ketogenic diet group (not significantly different). In rats on the ketogenic diet, plasma β-HBA increased by 1.7-fold on days 5 and 8, but this increase was not significant relative to the controls and was no longer detectable on day 10 (Fig. 1). Liver HMG-CoA lyase expression after 10 days on the ketogenic diet did

Table 2
Fatty acid percentage composition of peri-renal adipose tissue of rats consuming a control or ketogenic diet

Fatty acid	Control diet	Ketogenic diet
14:0	1.4 ± 0.1	4.1 ± 0.5*
16:0	24.3 ± 1.8	25.8 ± 0.8
18:0	3.5 ± 0.3	5.2 ± 0.4*
16:1ω7	5.6 ± 1.2	4.3 ± 0.5*
18:1ω9	31.3 ± 1.3	33.2 ± 0.8*
18:2ω6	26.4 ± 3.2	21.3 ± 0.8*
20:4ω6	0.3 ± 0.1	0.2 ± 0.03*
18:3ω3	2.9 ± 0.4	2.0 ± 0.1*
22:6ω3	0.09 ± 0.06	0.05 ± 0.01*

Values are mean ± SD (n = 7 or 8 per group). Totals are calculated by adding sums of saturated fatty acids, monounsaturated fatty acids, and ω3 and ω6 polyunsaturated fatty acids. 14:0 indicates myristic acid; 16:0, palmitic acid; 18:0, stearic acid; 16:1ω7, palmitoleic acid; 18:1ω9, oleic acid: 18:2ω6, linoleic acid; 20:4ω6, arachidonic acid; 18:3ω3, α-linolenic acid; 22:6ω3, docosahexaenoic acid.

* $P < .05$, means are significantly different.

Table 3
Brain fatty acid profile of rats consuming a control or ketogenic diet

Fatty acid	Control diet	Ketogenic diet
Brain total fatty acids (mg/g)		
14:0	0.06 ± 0.01	0.1 ± 0.05*
16:0	7.4 ± 0.2	7.9 ± 0.5
18:0	4.6 ± 0.2	4.4 ± 0.2
18:1ω9	7.0 ± 0.7	7.5 ± 0.5
18:2ω6	0.4 ± 0.1	0.5 ± 0.1
20:4ω6	4.1 ± 0.4	4.8 ± 0.5*
18:2ω6	ND	ND
22:6ω3	5.6 ± 0.6	6.4 ± 0.7*
Total fatty acids	33.1 ± 2.1	35.9 ± 2.3*

Values are mean + SD (n = 7 or 8 per group). Totals are calculated by adding sums of saturated fatty acids, monounsaturated fatty acids, and ω3 and ω6 polyunsaturated fatty acids. 14:0 indicates myristic acid; 16:0, palmitic acid; 18:0, stearic acid; 18:1ω9, oleic acid; 18:2ω6, linoleic acid; 20:4ω6, arachidonic acid; 18:3ω3, α-linolenic acid; 22:6ω3, docosahexaenoic acid; ND, not detected.

* $P < .05$, means are significantly different.

Table 4
Plasma fatty acid profiles of rats consuming a control or ketogenic diet

	Free fatty acids (μg/mL)		Triglycerides (μg/mL)	
	Control diet	Ketogenic diet	Control diet	Ketogenic diet
14:0	1.1 ± 0.7	0.5 ± 0.4	4 ± 1	4 ± 1
16:0	26.7 ± 11.1	13.1 ± 4.2*	130 ± 50	70 ± 34*
18:0	8.6 ± 2.6	9.7 ± 3.6	15 ± 4	33 ± 40
18:1ω9	10.2 ± 7.8	2.0 ± 1.6*	114 ± 51	50 ± 34*
18:2ω6	10.1 ± 7.6	2.0 ± 1.8*	127 ± 58	41 ± 10*
20:4ω6	2.1 ± 1.4	0.9 ± 0.8*	18 ± 6	18 ± 15
18:3ω3	1.0 ± 1.0	0.1 ± 0.1*	9 ± 5	2 ± 1*
22:6ω3	0.4 ± 0.3	0.2 ± 0.1	10 ± 4	13 ± 16
Total fatty acids	66.2 ± 35.3	30.9 ± 10.7*	475 ± 196	259 ± 160*

Values are mean ± SD (n = 7 or 8 per group). Totals are calculated by adding sums of saturated fatty acids, monounsaturated fatty acids, and ω3 and ω6 polyunsaturated fatty acids. 14:0 indicates myristic acid; 16:0, palmitic acid; 18:0, stearic acid; 18:1ω9, oleic acid; 18:2ω6, linoleic acid; 20:4ω6, arachidonic acid; 18:3ω3, α-linolenic acid; 22:6ω3, docosahexaenoic acid.

* $P < .05$, means are significantly different.

not differ from control values (data not shown). Brain, liver, and plasma cholesterol concentrations did not differ significantly (control vs ketogenic diet): 16.6 ± 0.5 vs 16.2 ± 0.6 mg/g for brain; 10.7 ± 0.3 vs 11.0 ± 0.1 mg/g for liver; and 0.54 ± 0.03 vs 0.55 ± 0.03 mg/mL for plasma.

3.2. Fatty acid profiles

Compared with the controls, adipose tissue fatty acids of rats in the ketogenic diet group contained a significantly higher proportion of myristic, stearic, and oleic acids, but 20% to 44% less polyunsaturates (particularly linoleic, arachidonic, α-linolenic, and docosahexaenoic acids; $P < .05$; Table 2). Brain arachidonic acid and docosahexaenoic acid were both significantly higher in rats on the ketogenic diet, thus raising the proportion of total ω3 and total ω6 polyunsaturates in the brain by about 15% each ($P < .05$; Table 3).

Plasma total FFAs were 53% lower in rats on the ketogenic diet with reductions of up to 90% for some individual fatty acids such as α-linolenic acid ($P < .05$; Table 4). Plasma TGs were also about 45% lower on the ketogenic diet, an effect which significantly reduced the concentration of all fatty acids in plasma TG except arachidonic acid and docosahexaenoic acid (Table 4). Plasma PL and CE concentrations and their respective fatty acid profiles did not differ between the 2 groups (data not shown).

Liver TGs were 3.4-fold higher in rats on the ketogenic diet group with increases in the ω3 and ω6 polyunsaturates being the most marked, including a 24-fold increase in arachidonic acid and a 26-fold increase docosahexaenoic acid ($P < .01$; Table 5). Total liver CE concentration was nonsignificantly higher in the ketogenic diet group but several individual fatty acids within the CE fraction, including myristic, palmitic, stearic, γ-linolenic, arachidonic, and docosahexaenoic acids, were increased by 1.6- to 5-fold ($P < .05$; Table 5). Apart from 4-fold higher myristic acid and 50% lower palmitoleic acid, no significant differences were seen in liver FFA in rats on the ketogenic diet ($P < .05$; data not shown). There were also no statistical differences in the fatty acid profile or concentration of liver PL across the 2 groups (data not shown).

3.3. ^{13}C enrichment in ω3 polyunsaturates

With the exception of 35% higher ^{13}C enrichment in docosahexaenoic acid in brain, ^{13}C enrichment 24 hours after dosing with ^{13}C-α-linolenic acid was 34% to 66%

Table 5
Liver fatty acid profile of rats consuming a control or ketogenic diet

	Triglycerides (μg/g)		Cholesterol esters (μg/g)	
	Control diet	Ketogenic diet	Control diet	Ketogenic diet
14:0	56 ± 25	930 ± 436*	3 ± 2	15 ± 12*
16:0	3885 ± 2210	9699 ± 2831*	155 ± 50	252 ± 72*
18:0	227 ± 74	1751 ± 646*	100 ± 72	167 ± 36*
18:1ω9	2878 ± 1815	8499 ± 3363*	186 ± 157	185 ± 76
18:2ω6	1907 ± 1461	8490 ± 4103*	107 ± 85	179 ± 69
18:3ω6	22 ± 21	275 ± 178*	2 ± 1	5 ± 2*
20:4ω6	24 ± 17	567 ± 365*	45 ± 31	132 ± 57*
18:3ω3	104 ± 80	598 ± 325*	9 ± 8	9 ± 5
22:6ω3	17 ± 26	442 ± 300*	5 ± 3	11 ± 6*
Total fatty acids	9722 ± 5993	33260 ± 13342*	679 ± 353	1029 ± 318

Values are mean ± SD (n = 7 or 8 per group). Totals are calculated by adding sums of saturated fatty acids, monounsaturated fatty acids, and ω3 and ω6 polyunsaturated fatty acids. 14:0 indicates myristic acid; 16:0, palmitic acid; 18:0, stearic acid; 16:1ω7, palmitoleic acid; 18:1ω9, oleic acid; 18:2ω6, linoleic acid; 18:3ω6, γ-linolenic acid; 20:4ω6, arachidonic acid; 18:3ω3, α-linolenic acid; 22:6ω3, docosahexaenoic acid.

* $P < .05$, means are significantly different.

Table 6
^{13}C-enrichment in ω3 fatty acids of adipose tissue, liver, and brain or rats on a control or ketogenic diet 24 hours after dosing with 5 mg of ^{13}C-α-linolenic acid

		Control	Ketogenic diet	% Change
Adipose tissue	18:3ω3	9.1 ± 2.4	3.1 ± 2.8*	−66
Liver	18:3ω3	4295 ± 980	2543 ± 527*	−41
	20:5ω3	2736 ± 589	1712 ± 220*	−37
	22:5ω3	2911 ± 674	854 ± 158*	−71
	22:6ω3	997 ± 457	335 ± 141*	−66
	22:6ω3/ 18:3ω3	0.23	0.13	−43
Brain	22:5ω3	451 ± 477	297 ± 57*	−34
	22:6ω3	62 ± 21	84 ± 4*	+35

Values are the mean ± SD of n = 6 per group (ng ^{13}C/mg fatty acid). 18:3ω3 indicates α-linolenic acid; 20:5ω3, eicosapentaenoic acid; 22:5ω3, docosapentaenoic acid; 22:6ω3, docosahexaenoic acid.

* $P < .05$, means are significantly different.

lower in all ω3 polyunsaturates of adipose tissue, liver, and brain in rats on the ketogenic diet ($P < .01$; Table 6). Some ω3 polyunsaturates were either not detectable, that is, α-linolenic acid in brain or ^{13}C enrichment could not be detected, so these fatty acids are not shown in Table 6. The ratio of ^{13}C enrichment in docosahexaenoic acid compared with α-linolenic acid was decreased by 43% in the liver of rats on the ketogenic diet (Table 6), but could not be measured in brain owing to undetectable α-linolenic acid in brain.

4. Discussion

The novel aspect of this study is that despite minimal and transient ketosis, 10 days on the ketogenic diet left a major imprint on plasma and organ fatty acid profiles. Some of the fatty acid changes, such as raised myristic acid in various tissues (but not plasma) of the ketogenic diet group, probably reflected the higher intake of myristic acid in large amount of butter in the ketogenic diet. However, several other changes in tissue fatty acid profile involve polyunsaturates, which were either not present in the diet (docosahexaenoic acid and arachidonic acid) or were much higher in the ketogenic diet but were lower in some tissues of rats in that group (Tables 2-5). The first such trend is exemplified by all the polyunsaturates disproportionately decreasing in adipose tissue and plasma FFA. The second and opposite trend involved arachidonic acid and docosahexaenoic acid rising modestly in brain but dramatically in liver TG. Hence, these represent significant changes in fatty acid distribution that were independent of differences in the fatty acid composition of the diet. In liver and brain, these are changes in fatty acid concentration, not percentage of composition, so they incorporate a change in the amount of the lipid pool (TG, FFA, etc) itself.

In line with several previous reports [8,11-13], in the present study, the ketogenic diet failed to induce significant ketosis in well-nourished young rats. Even the nonsignificant ketosis that did occur was transient and disappeared within 10 days. Other studies clearly show that rats can sustain ketosis while on a high-fat ketogenic diet [8,17,18] so the conditions preventing or permitting sustained ketosis in rats on a ketogenic diet deserve a brief comment. Two conditions seem common to those studies showing that the ketogenic diet can induce sustained ketosis in rats: either a high intake of medium chain TG (fatty acids of 8-10 carbons) is necessary or long-term underfeeding (or anorexia) resulting in negative energy balance and low weight gain. Sensitivity to anorexia is higher in weanling rats (21-22 days old) than later on, so to avert the confounding effects of impaired weight gain, we waited to start the ketogenic diet until the rats were 50 to 52 days old. These parameters affecting the magnitude and duration of ketosis have not been studied in detail so their thresholds to maintain (or prevent) prolonged ketosis are presently still unknown.

The only other published report describing tissue fatty acid profiles found similar effects on the fatty acid profiles of adipose tissue and liver TG in rats that also underwent transient ketosis while on a ketogenic diet [19]. Despite several consistencies with the present results, one notable difference was a fall in plasma FFA in the present study but a rise in the previous report [19]. This difference remains difficult to explain, but the other study used quite different dietary fats, a factor which may or may not be relevant.

Dietary or infused polyunsaturates such as α-linolenic, arachidonic, eicosapentaenoic, or docosahexaenoic acid have beneficial effects in reducing seizure tendency not only in animal models [10,20,21] and cell culture [22] but also in humans [23]. Certainly, there are ways in which the ketogenic diet could reduce seizures through a mechanism that involves ketone bodies themselves acting on neuronal excitability [7]. However, some children have low to negligible ketosis while on the ketogenic diet yet are protected against seizures, whereas others may be in moderate to even high ketosis but do not achieve seizure control [24]. In experimental models, these effects occur in the absence of intentionally manipulating ketosis, so whether the ketogenic diet can reduce seizures through a mechanism that includes a change in the plasma and tissue content of polyunsaturates, particularly a rise in brain docosahexaenoic acid, requires further investigation [25].

The magnitude of the marked change in polyunsaturates and the opposite trend in these changes occurring in plasma FFA and adipose tissue on the one hand, compared with brain and liver TG on the other, imply net fatty acid flux from adipose tissue via blood to liver TG and other tissues, including brain. The difference in fatty acid content and profile between the control and ketogenic diets was not responsible for the marked change in tissue distribution of arachidonic acid and docosahexaenoic acid because these fatty acids were not present in either diet. The brain was capable of responding to this tissue redistribution of

polyunsaturates by selective incorporation of modest but significant amounts of arachidonic and docosahexaenoic acids. The lower specific activity of docosahexaenoic acid in the liver but higher specific activity in the brain (Table 6) supports tissue redistribution rather than higher synthesis of docosahexaenoic acid under these experimental conditions. The previously reported preferential mobilization of polyunsaturates from adipose tissue [26] seems likely to have played a role in the marked shift in homeostasis of tissue polyunsaturates we have observed here.

One potentially interesting outcome of our study that is currently under investigation by our group is that a ketogenic diet incorporating a higher amount of $\omega 3$ polyunsaturates would be predicted to be more efficient for seizure control, not only because α-linolenic acid is more ketogenic than most other common fatty acids [25] but also because the present data suggest that docosahexaenoic acid, which has direct seizure-inhibiting properties [10], seems to be modestly redistributed toward the brain while on the ketogenic diet.

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