The Human Metabolic Response to Chronic Ketosis Without Caloric Restriction: Preservation of Submaximal Exercise Capability with Reduced Carbohydrate Oxidation

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To study the effect of chronic ketosis on exercise performance in endurance-trained humans, five well-trained cyclists were fed a eucaloric balanced diet (EBD) for one week providing 35–50 kcal/kg/d, 1.75 g protein/kg/d and the remainder of kilocalories as two-thirds carbohydrate (CHO) and one-third fat. This was followed by four weeks of a eucaloric ketogenic diet (EKD), isocaloric and isonitrogenous with the EBD but providing less than 20 g CHO daily. Both diets were appropriately supplemented to meet the recommended daily allowances for vitamins and minerals. Pedal ergometer testing of maximal oxygen uptake ($\dot{V}O_2$max) was unchanged between the control week (EBD-1) and week 3 of the ketogenic diet (EKD-3). The mean ergometer endurance time for continuous exercise to exhaustion (ENDUR) at 62%–64% of $\dot{V}O_2$max was 147 minutes at EBD-1 and 151 minutes at EKD-4. The ENDUR steady-state RQ dropped from 0.83 to 0.72 ($P < 0.01$) from EBD-1 to EKD-4. In agreement with this were a three-fold drop in glucose oxidation (from 15.1 to 5.1 mg/kg/min, $P < 0.05$) and a four-fold reduction in muscle glycogen use (0.61 to 0.13 mmol/kg/min, $P < 0.01$). Neither clinical nor biochemical evidence of hypoglycemia was observed during ENDUR at EKD-4. These results indicate that aerobic endurance exercise by well-trained cyclists was not compromised by four weeks of ketosis. This was accomplished by a dramatic physiologic adaptation that conserved limited carbohydrate stores (both glucose and muscle glycogen) and made fat the predominant muscle substrate at this submaximal power level.

IT IS a commonly held belief that the state of nutritional ketosis induced by carbohydrate restriction impairs the capability for endurance exercise.[1-5] Phinney et al[6] have recently reported a maintenance of the capacity for moderate exercise by untrained obese subjects after a six-week adaptation to nutritional ketosis induced by a protein supplemented fast. A critical difference between previous experiments with exercise during carbohydrate-restricted diets and this recent study was careful attention to the provision of adequate minerals, especially sodium and potassium, during ketosis. Because of the difficulty of isolating the effect of ketosis as a variable on exercise performance during rapid weight loss, however, neither endurance performance nor relative rates of substrate utilization were effectively quantitated by that study.[6] The preceding paper[7] documented the clinical and biochemical tolerance of lean, healthy humans for a eucaloric diet providing 15% of total calories as protein and 85% as fat. In order to better measure the capability for moderate submaximal exercise and the carbohydrate oxidation rate after adaptation to nutritional ketosis, the exercise capabilities of five highly trained endurance bicyclists were studied before and after four weeks of the eucaloric ketogenic diet (EKD) described in our earlier paper.[7]

MATERIALS AND METHODS

Details of the five subjects, their diets, and clinical monitoring are covered in the preceding paper.[7] For the purpose of this study, an elite bicyclist is defined as one capable of attaining a maximal oxygen uptake of 65 mL/kg/min on a pedal ergometer in the laboratory.

Schedule of Events

Figure 1 diagrams the exercise tests and associated metabolic studies during the two diets. In the subsequent text, the term EBD-1 will represent the week of the high-carbohydrate control diet, and EKD-1 through EKD-4 the subsequent four weeks of the ketogenic diet.

All procedures employed in this study, as well as the protocol in its entirety, had prior approval of the Massachusetts Institute of Technology (M.I.T.) Clinical Research Center Executive Committee, the M.I.T. Committee on the Use of Humans as Experimental Subjects, and the Boston University Clinical Research Review Committee. A full explanation of all procedures and their associated risks was provided to each subject verbally and in writing, and signed consent obtained before admission to the study.

Maximal Oxygen Uptake Testing

As noted in the schedule of tests (Fig. 1), the trained subjects underwent determination of their maximal oxygen uptake ($\dot{V}O_2$max) twice: once within 10 days before the start of the EKD, and the second time at EKD-3. The $\dot{V}O_2$max was measured on a magnetically braked, pedal-mode ergometer (Warren Collins, Inc, Braintree, MA). Continuously collected expired gas was analyzed with a semiautomated mixing/collecting system,[8] utilizing a Beckman LB_2 CO_2 analyzer and a Beckman E_2 oxygen analyzer, both standardized with reference gas assayed by the Scholander technique.[9] The $\dot{V}O_2$max test procedure required the subject to begin pedaling at a power of 150 W for four minutes, after which the load was increased by 50 W every two minutes until the subject could no

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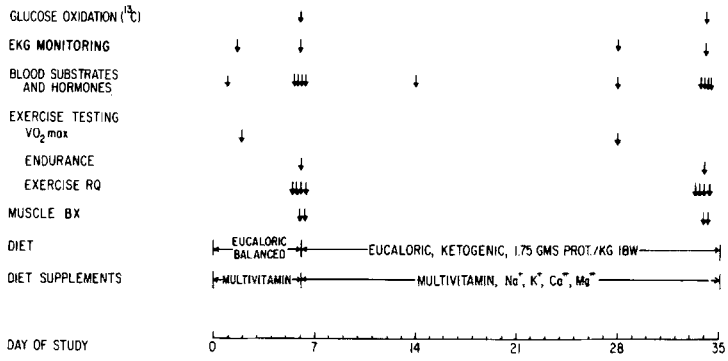

Fig. 1. Study protocol. The vertical arrows indicate the times of individual tests, the horizontal arrows indicate continuous processes. Endurance exercise performed at 62%–64% of $\dot{V}O_2$max. Muscle biopsy specimens (BX) obtained from the vastus lateralis muscle. Abbreviations: ^{13}C, carbon 13; $\dot{V}O_2$max, maximal oxygen uptake; RQ, respiratory quotient; Prot, protein; IBW, ideal body weight.

longer maintain a cadence above 60 rpm. All subjects reached at least 350 W for 60 seconds, and in the initial test all but one exceeded a respiratory quotient (RQ) of 1.0. The $\dot{V}O_2$max was assumed to have been achieved if the oxygen consumption "plateaued" in the final samples. The subject's heart rate was monitored continuously by a combined visual and recording electrocardiograph.

Endurance Testing

At the end of EBD-1 and EKD-4, the five subjects were tested for submaximal endurance at power levels equivalent to 60%–65% of their tested $\dot{V}O_2$max. The endurance tests during both diets were done in the morning after an overnight fast. Following completion of the glucose ^{13}C infusion at rest (procedure described in the previous paper[7]), the subject exercised with the infusion still running and the blood sampling catheter still in place in the opposite arm on the pedal ergometer at a power output estimated to fall in the above range. Respiratory gas analysis was performed as described above for two minutes of each 10 minutes of exercise throughout the endurance test. If the load required adjusting, it was accomplished within the first 30 minutes of exercise during EBD-1. Subjects were given approximately 500 mL of normal saline to maintain patency of the blood-drawing catheter during the study. They were allowed to drink water ad libitum. The glucose ^{13}C infusion was continued for two hours, with the four plateau blood samples and five plateau breath samples obtained between 90 and 120 minutes into the exercise period (or in the final 20 minutes of exercise if exhaustion appeared imminent). Following completion of the two-hour exercise infusion study, however, the subjects were asked to continue at the same power output until they were unable to maintain a pedaling rate above 60 rpm. They were not allowed to time themselves during the endurance rides, thus avoiding artificial time goals being set beyond the two-hour infusion schedule.

Blood samples were drawn during exercise for determination of blood glucose ^{13}C enrichment as noted above. In addition, blood for determination of amino acid, glucose, lactate, FFA, 3-hydroxybutyrate, insulin, and T_3 levels was drawn at rest (Rest), after 30 minutes of exercise (30 min), at exhaustion while still pedaling (Exh), and 15 minutes after cessation of exercise (Post). These samples were analyzed by the techniques described in the preceding paper.[7]

Muscle biopsies of the vastus lateralis were done using the technique of Bergstrom[10] before and after endurance exercise at EBD-1 and EKD-4. The muscle glycogen level was determined on two portions of each sample that were dissected free of connective tissue and immediately frozen in liquid nitrogen. The frozen samples were subsequently weighed, dissolved in HCl, neutralized, and the resultant solution analyzed by an enzymatic technique for free glucose.[11] In addition, histologic sections of muscle were obtained from a third portion of each biopsy specimen, mounted in OCT and frozen in isopentane cooled to the temperature of liquid nitrogen. The samples were sectioned and stained for glycogen using PAS reaction and fiber-type differentiation using a stain for myofibrillar adenosine triphosphatase as described by Costill et al.[12]

Statistical Analysis

Data are reported as the mean ± SEM. Tests yielding results suitable for paired comparisons were assessed by *t* test. The remaining data were evaluated by the appropriate two-way and three-way analysis of variance (ANOVA) techniques utilizing the TANVAR program of the PROPHET system.[13] For anticipated comparisons, significance was ascertained by using the least significant differences test,[14] with $P < 0.01$ being the criterion unless otherwise specified.

RESULTS

The EKD was tolerated by all five subjects without difficulty. Multiple monitoring variables, including weight, urinary ketones, and urine creatinine and total nitrogen excretion indicated excellent compliance with the rigid dietary restrictions imposed by the study.

The subjects were asked to continue their normal training throughout the study, and this was monitored by daily diaries of training duration kept by each subject. Four of the five cyclists maintained their previous training intensity for the five-week study. One subject, WB, who had been actively racing for eight months, decreased his weekly mileage from 300 to 100 miles in the month before the study. He attributed this to an increasing sense of chronic fatigue, or "staleness." He did maintain the 100 mile per week schedule throughout the study, however.

Maximal Oxygen Uptake

The results of the control and EKD-3 $\dot{V}O_2$max determinations are presented in Table 1. There was an insignificant decrease in the mean value at EKD-3 compared with the baseline values, representing a change of 2%. This difference is well within the range of error of this test. Two points of interest are to be found in the $\dot{V}O_2$max data, however. First, the greatest decrease in $\dot{V}O_2$max value over the course of the study for any subject was the 7% fall in the case of subject WB, who had slackened his training. Second, there was a marked decrease in the RQ at which the second $\dot{V}O_2$max was achieved. Whereas all subjects in the

Table 1. Maximal Oxygen Uptake ($\dot{V}O_2$max) and Endurance of Trained Subjects after Eucaloric Balanced (EBD) or Ketogenic (EKD) Diets

	$\dot{V}O_2$max				Endurance					
	$\dot{V}O_2$ (L/min)*		RQ†		Duration (min)		RQ		$\dot{V}O_2$ (L/min)	
Subject	EBD-1‡	EKD-3	EBD-1	EKD-3	EBD-1*	EKD-4	EBD-1	EKD-4	EBD-1	EKD-4
JP	5.35	5.31	1.02	0.87	148	232	0.81	0.65	3.30	3.20
WB	4.66	4.34	1.10	0.90	140	89	0.86	0.75	2.61	2.95
IK	5.09	5.13	0.97	0.87	100	130	0.82	0.72	3.10	2.97
BK	5.64	5.45	1.07	0.85	169	121	0.84	0.72	3.79	3.97
MK	4.78	4.78	1.04	0.99	178	181	0.80	0.74	3.09	3.01
Mean ± SEM	5.10 ± 0.18	5.00 ± 0.20	1.04 ± 0.02	0.90 ± 0.02	147 ± 13	151 ± 25	0.83 ± 0.01	0.72 ± 0.02	3.18 ± 0.19	3.21 ± 0.18

*$\dot{V}O_2$ = mean oxygen uptake per minute from 30 minutes to exhaustion.

†RQ = respiratory quotient, mean from 30 minutes to exhaustion.

‡EBD-1 = within three days of admission for $\dot{V}O_2$max, end of first week of eucaloric balanced diet for Endurance; EKD-3,4 = after three and four weeks of eucaloric ketogenic diet, respectively.

control tests exceeded RQ values of 1.0 save one, four of the five reached the listed $\dot{V}O_2$max at EKD-3 with RQ values less than 0.9 ($P < 0.01$).

Endurance

As shown in Table 1, three of the subjects rode the ergometer longer at EKD-4 than at EBD-1, while two stopped sooner after four weeks of the EKD. The mean exercise times for the five subjects of 147 minutes at EBD-1 and 151 minutes at EKD-4 are insignificantly different. The mean $\dot{V}O_2$ during the endurance ride, which was determined from all samples obtained after the initial 30 minutes of exercise (more than 20 samples per subject), showed no difference between the oxygen cost of generating the same power on the two diets. There was, however, a highly significant decrease in the RQ value during the endurance test at EKD-4 compared with the baseline study ($P < 0.01$). Expressing the endurance test $\dot{V}O_2$ as the percent of tested $\dot{V}O_2$max, the EBD-1 value was 62%, the EKD-4 value 64% (difference insignificant). Thus, four weeks of adaptation to the ketogenic diet resulted in no change in endurance performance, while markedly altering the exercise RQ, indicating a dramatic shift in exercising muscle substrate utilization.

Blood Substrates

The blood substrate data obtained during endurance exercise are presented in Table 2. The blood glucose levels rose sharply in the first 30 minutes of exercise at EBD-1 ($P < 0.01$), and fell equally as sharply to exhaustion ($P < 0.01$), followed by a statistically insignificant rise after exercise. The changes with exercise at EKD-4, by comparison, were similar but attenuated. There was a significant rise from resting to 30 minutes ($P < 0.02$), followed by an insignificant fall to exhaustion and no change after exercise. It is noteworthy that, despite the severe carbohydrate restriciton of the EKD, there was a rise in glucose levels in response to exercise and an apparent maintenance of the blood glucose level in the normal range during and after exercise. No instances of exercise-induced hypoglycemia were noted on either diet, as indicated by the lack of symptoms, as well as the lowest tested value being 3.34 mmol/L (MK, at EBD-1 EXH).

The responses of FFA to exercise were roughly similar in degree and direction on both diets, with the exception that the 30-minute values differed significantly ($P < 0.01$), suggesting better lipid substrate availability early in exercise with the EKD. Both exercise bouts resulted in significant rises in FFA levels during exercise (both $P < 0.01$) and again after exercise (both $P < 0.001$).

The major quantitative difference between substrate levels in response to exercise on the two diets occurred in the 3-hydroxybutyrate levels. There was, as expected, a highly significant difference ($P < 0.001$) between the responses to the two diets. With exercise on both diets, there was a rise in 3-hydroxybutyrate, and then further elevation in the period after exercise,

Table 2. Blood Substrates and Hormones During Submaximal Exercise

	EBD-1*				EKD-4*			
Variable	Rest†	30 Min	Exh	Post	Rest	30 Min	Exh	Post
Glucose (mmol/L)	4.40 ± 0.07	5.72 ± 0.25	4.43 ± 0.38	4.73 ± 0.41	4.06 ± 0.18	4.91 ± 0.39	4.63 ± 0.29	4.60 ± 0.26
FFA (mmol/L)	0.68 ± 0.10	0.56 ± 0.10	1.25 ± 0.13	2.84 ± 0.23	0.96 ± 0.12	1.26 ± 0.09	1.60 ± 0.31	3.02 ± 0.31
3-Hydroxybutyrate (mmol/L)	0.04 ± 0.02	0.06 ± 0.02	0.30 ± 0.18	0.46 ± 0.09	1.28 ± 0.35	1.16 ± 0.30	1.45 ± 0.47	2.44 ± 0.46
Lactate (mmol/L)	1.27 ± 0.10	3.08 ± 0.55	2.77 ± 0.61	1.88 ± 0.13	1.25 ± 0.07	2.42 ± 0.35	2.41 ± 0.27	2.22 ± 0.39
Insulin (μU/mL)	9.0 ± 0.3	7.6 ± 0.8	4.3 ± 0.5	9.3 ± 1.3	6.9 ± 0.5	6.2 ± 1.2	6.1 ± 0.9	12.6 ± 2.3
T_3 (ng/mL)	135 ± 6	148 ± 6	139 ± 10	131 ± 5	89 ± 4	111 ± 8	121 ± 12	102 ± 9

Values are given as mean ± SEM. Sample size: n = 5 for all points.

*EBD-1 = end of first week, eucaloric balanced diet; EKD-4 = after four weeks of eucaloric ketogenic diet.

†Rest = resting subject after overnight fast; 30 Min = after 30 minutes of exercise; Exh = at exhaustion, still pedalling; Post = 15 minutes after stopping exercise.

but only the rise after exercise at EKD-4 was significant.

The blood lactate level rose with exercise on both diets ($P < 0.01$). There was a pronounced decline in blood lactate levels after exercise at EBD-1 that was not seen at EKD-4. In this instance, as was the case with glucose, the same exercise intensity at EKD-4 resulted in more attenuated changes in concentration compared with those seen at EBD-1.

Glucose Oxidation

The μ-^{13}C-glucose infusions done at rest at EBD-1 and EKD-4 reported in the preceding paper[7] were continued uninterrupted into the endurance exercise tests of the five trained subjects. The breath $^{13}CO_2$ and plasma ^{13}C-glucose enrichment results are plotted in Figure 2, demonstrating that isotope plateaus were achieved during exercise in both diet periods. The calculated mean glucose oxidation rate between 90 and 120 minutes of exercise (80 and 100 minutes for subject IK) at EBD-1 was 15.1 ± 2.5 mg/kg/min. The corresponding value at EKD-4 (samples taken 70 to 90 minutes for WB) was 5.1 ± 0.9 mg/kg/min. The difference between the two is significant ($P < 0.05$ by paired t test). At a mean $\dot{V}O_2$ of 3.2 L/min, the estimated caloric expenditure during the exercise infusions was 950 kcal/h.[15] The glucose oxidation rate at EBD-1 implies that blood-borne glucose supplied 28% of the energy expenditure during isotope plateau, falling to 9% during the analogous time period at EKD-4.

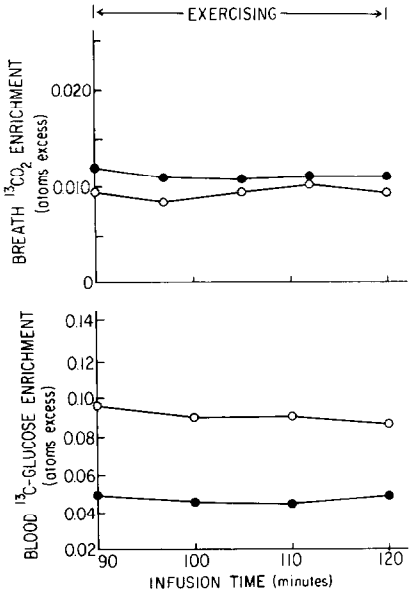

Fig. 2. Carbon 13 enrichment of breath CO_2 and blood (plasma) glucose during ^{13}C-glucose infusion after an overnight fast. Samples were taken after 90–120 minutes of continuous exercise. Solid dots represent studies done after one week of eucaloric balanced diet and open circles after four weeks of eucaloric ketogenic diet.

Muscle Glycogen Before and After Exercise

The results of muscle glycogen analysis are presented in Table 3. The uniformity of the individual values within each time category attest to the homogenous nature of both the subject group and of their exercise efforts in the laboratory setting.

Histologic staining of matched sections of muscle biopsy specimens for fiber type and semiquantitative glycogen content from each sample obtained after exercise demonstrated virtually complete and uniform depletion of the slow-twitch (Type I) fiber glycogen in both diet periods. Thus, the residual 56 and 53 mmol of glucose per kilogram of muscle observed in the samples obtained after exercise represents glycogen contained in fast-twitch fibers, indicating that exhaustion in these endurance tests correlated with slow-twitch fiber glycogen depletion.

By three-way ANOVA, there were significant effects on muscle glycogen levels caused by diet ($P < 0.01$) and exercise ($P < 0.001$), and also an interaction between diet and exercise ($P < 0.01$). The reductions with exercise are both significant (EBD-1, $P < 0.001$; EKD-4, $P < 0.025$), but clearly the reduction at EKD-4 is much less, hence the diet–exercise interaction.

The rise in muscle glycogen levels from EBD-1 after exercise to EKD-4 before exercise achieved significance ($P < 0.02$), implying that the subjects were able to maintain and regenerate muscle glycogen while on the EKD despite continued training. The extent of glycogen depletion with exercise was 90 and 20 millimoles of glucose per kilogram of muscle at EBD-1 and EKD-4, respectively. As the mean intensity and duration of exercise with the different diets were almost identical, this implies a mean rate of glycogen mobilization (as glucose) of 0.61 mmol/kg/min at EBD-1

Table 3. Effects of Diet and Exercise on Muscle Glycogen Levels

	Glycogen Level (mmol glucose/kg wet wt muscle)			
	EBD-1*		EKD-4*	
Subject	Pre-Ex†	Post-Ex	Pre-Ex	Post-Ex
JP	144.8	59.2	81.7	41.5
WB	124.9	57.8	82.6	60.1
IK	179.7	62.9	67.5	63.5
BK	120.8	38.1	80.7	58.4
MK	142.8	46.6	65.1	57.6
Mean ± SEM	143 ± 10	53 ± 5	76 ± 4	56 ± 4

*EBD-1 = end of first week, eucaloric balanced diet; EKD-4 = after four weeks eucaloric ketogenic diet.

†Pre-Ex = resting subject after overnight fast, before exercise; Post-Ex = 15–30 minutes after exhaustion from endurance exercise.

and 0.13 mmol/kg/min at EKD-4. Thus, the rate of glycogen utilization was reduced to 21% of the control value after four weeks of adaptation to the EKD.

Hormonal Response to Exercise

The results of the hormone response to exercise with the two different diets are presented in Table 2. The insulin value at EBD-1 (Exh) was lower than both the EBD-1 (Rest) and EBD-1 (Post) levels ($P < 0.01$). The EKD-4 (Rest) insulin level did not differ significantly from that at EBD-1 (Rest). There was not a significant fall in insulin during exercise at EKD-4. The rise in insulin after exercise at EKD-4, however, was very marked, differing from the EKD-4 resting and exercise values ($P < 0.001$ in all instances), and also exceeding the EBD-1 (Post) value ($P < 0.05$). Thus, in comparison to EBD-1, the insulin levels remained stable during exercise at EKD-4, but responded to the cessation of exercise with an accentuated rise. It is interesting to note, however, that during the time of this sharp insulin rise after exercise at EKD-4, the plasma glucose concentration remained unchanged. Across this same time interval at EKD-4, the blood 3-hydroxybutyrate level rose sharply, raising the possibility that this contributed to the insulin rise after exercise.

There was no significant change in T_3 levels with exercise at EBD-1. Starting from a much lower resting value at EKD-4 ($P < 0.001$) compared with EBD-1 (Rest), the serum T_3 level rose at EKD-4 (30 min) ($P < 0.02$) and EKD-4 (Exh) ($P < 0.001$). The level after exercise appeared to fall, but although it was no longer significantly different from EKD-4 (Rest), it was also not a significant decline from EKD-4 (Exh). It should be noted that T_3 binds to serum proteins, and this may have a role in the minor (insignificant) rise in T_3 seen with exercise at EBD-1. The magnitude of the rise seen with exercise at EKD-4, in the range of 50% above baseline at EKD-4 (Exh), was far beyond that explainable by hemoconcentration, however, indicating that it was indeed a true physiologic response.

DISCUSSION

The most striking result of this study was the ability of highly trained endurance athletes to maintain their level of training and perform the extent of endurance exercise observed at EKD-4. This is in contradiction to the results of four previous studies allowing 14 days or less of dietary adaptation, which demonstrated markedly impaired performance with carbohydrate-restricted diets.[2-5] The results of these studies are summarized in Table 4 along with the data of this project. In all four cases, dietary and methodologic differences between those studies and this one make direct comparison difficult. Specifically, the high fat diet of Christensen and Hansen[2] was severely protein deficient, and thus unsuitable for prolonged adaptation. In the other extreme, the high-fat diet used by Bergstrom et al[3] contained an estimated 46 g of nitrogen per day, a potentially toxic protein intake. The low-carbohydrate diet of Pruett[4] was not carbohydrate restricted to the extent necessary to result in nutritional ketosis, and that of Galbo et al[5] marginally so.

The other major difference in procedure is that in three of the above four studies, intermittent exercise (45 to 55 minutes per hour) at 70%–75% of $\dot{V}O_2$max was utilized. Because of the rest periods (which time is included in the reported duration) and the ability of cyclists to recruit fast-twitch fibers at these slightly higher power levels, the reported times to exhaustion in these studies tend to be longer. Continuous exercise was employed in our study to allow the glucose oxidation determination, which required a steady-state that could not be achieved with intermittent exercise.

Despite these differences in study design, it appears

Table 4. Comparison of Diet and Endurance Exercise Studies

Investigator	Year	% Calories CHO	% Calories Fat	Adaptation Time (days)	Power Load (W)	$\dot{V}O_2$ (%/max)	Endurance Test Duration (min)
Christensen and Hansen[2]	1939	83	3	3–7	176	—	210 C*
		4	94	3–7	176	—	88 C
Bergstrom et al.[3]	1967	82	?	3	—	75	167 I†
		5	54	3	—	75	57 I
Pruett[4]	1970	83	9	14	—	70	193 I
		56	30	14	—	70	187 I
		24	60	14	—	70	164 I
Galbo et al[5]	1979	77	10	4	—	70	106 I
		10	76	4	—	70	64 I
Phinney‡	1981	57	29	>7	186	62	147 C
		<2	85	28	186	64	151 C

*C = continuous endurance exercise.

†I = Interrupted endurance exercise.

‡Results of study reported herein.

from previous studies that exercise performance is limited early in the period of adaptation to eucaloric, carbohydrate-restricted diets. The study by Christensen and Hansen[2] especially invites comparison in this regard. Thus, the results of the current project would indicate that, given a eucaloric ketogenic diet (containing a reasonable protein content and adequate supplementation including the major minerals), adaptation occurs, reversing the apparent exercise limitation over a period of four weeks. Whether further adaptation in exercise tolerance occurs beyond four weeks cannot be inferred from this study.

One point on diet adaptation and maintenance of function worthy of note was the stability of cardiac rhythm uniformly noted in all phases of this project, both at rest and during exercise. In more than 20 hours of intermittent cardiac monitoring during rest and exercise while the subjects were in ketosis, no instance of arrhythmia was observed. This would thus appear to separate the EKD from the low-carbohydrate protein-isolate weight reduction diets reported to induce arrhythmias.[16] As this referenced study dealt with subjects made severely mineral deficient, however, the arrhythmias that they noted in all probability were unrelated to the ketogenic state.[17]

The mechanism through which the subjects in this study adapted to be able to perform prolonged endurance exercise appears to be a simultaneous maximization of glucose storage during periods of rest and limitation of its mobilization during exercise. The slight elevation in fasting insulin seen during the first three weeks of the EKD, which is different from the response seen in fasting and protein-supplemented fasting,[6,18] may be an interim response in highly trained subjects that facilitates the storage of available glucose as liver and muscle glycogen, explaining in part the relatively high muscle glycogen levels noted before exercise at EKD-4.[19]

In addition, other subtle hormonal mechanisms may be responsible for a limited rate of glycogen release. It is known that circulating catecholamines play a role in muscle glycogen mobilization.[20] Although they were not measured in this study, DeHaven et al[21] have noted reduced catecholamine levels in ketotic subjects on a carbohydrate-free protein-supplemented fast, and this may function as a regulatory mechanism that reduces the impetus for glycogen mobilization. Similarly, it has been demonstrated that pharmacologic hyperthyroidism increases muscle glycogen mobilization in exercising dogs.[22] If thyroid hormone, particularly T_3, plays a role in the regulation of muscle glycogen in euthyroid humans, then the observed fall in T_3 levels from EBD-1 to EKD-2 may also contribute to the adaptation to conserve muscle glycogen.

Whatever the mechanisms that balance the restriction and promotion of muscle glycogen utilization, however, the degree of net carbohydrate sparing with exercise after four weeks of the EKD is striking, with muscle glycogen mobilization reduced more than four-fold and blood glucose oxidation falling three-fold. It is of interest to note that a blood glucose oxidation rate of 5.1 mg/kg/min implies an average 50–60 g glucose expenditure during the 151-minute EKD-4 exercise period. At the estimated caloric expenditure of 950 kcal/h, with 85% of the energy from triglycerides, this implies a glycerol release rate of 22 g over the whole exercise period, providing substrate for up to 40% of the glucose consumed during this exercise. This source, coupled with gluconeogenic carbon salvaged from lactate, pyruvate, and glucogenic amino acids provide an ongoing capability for glucose production adequate to meet the needs of the system in the context of extensive limitations on its use, especially during exercise.

In this light, the moderated responses of insulin, glucose, and lactate to exercise at EKD-4 compared with EBD-1 are not totally unexpected. Thus, less modulation of the gluco-regulatory processes appear necessary to maintain fuel homeostasis across exercise at EKD-4. Whether a cause or an effect of this, the pattern of use of metabolic fuel after ketoadaptation is much the same at rest and during endurance exercise, as evidenced by RQ values near 0.7 in both states. That the control processes through which this was accomplished are complex, however, is demonstrated by the observation that the FFA levels during exercise at EBD-1 and EKD-4 were quite similar (1.25 *v* 1.60 mmol/L at Exh). Yet, there was a dramatic difference in exercise RQ, suggesting a substrate control mechanism more complex than the glucose–fatty acid cycle as proposed by Randle et al.[23]

There are indications in the results of this study that the price paid for such extreme conservation of carbohydrate during exercise appears to be a limitation on the intensity of exercise that can be performed. Although resting muscle glycogen and blood glucose levels were normal at EKD-4, at EKD-3 there was a marked attenuation of the RQ value at $\dot{V}O_2max$, suggesting a severe restriction on the ability of subjects to do anaerobic work. This does not appear to be a function of differential accretion of glycogen by different fiber types, as the muscle biopsy specimens obtained before exercise showed the fast-twitch fibers to be qualitatively replete with glycogen at EKD-4. Thus, the controlling factor does not appear to be the presence or absence of substrate within the fiber. Rather, it is more likely a restriction on substrate mobilization or fiber recruitment. The result, in any case, is a throttling of function near $\dot{V}O_2max$, appar-

ently by limitation of carbohydrate utilization. This appears to occur in exchange for a more ready use of fatty acids at moderate submaximal power levels, ie, at or below 65% of $\dot{V}O_2$max.

The extent of reduction in blood glucose oxidation by exercising muscle observed in this study is in contradiction to conclusions drawn by Jansson.[24] Although Jansson used moderately well-trained subjects exercised on an ergometer at 65% of $\dot{V}O_2$max, the carbohydrate-restricted diet was given for five days rather than the four-week study reported here, and the technique used to determine the glucose consumption rate required measurement of differences in arteriovenous glucose concentration. That no change in the glucose oxidation rate was observed between high- and low-carbohydrate diets was most likely due to the shorter duration of the exercise period (30 minutes *v* 151 minutes) and the relatively brief time allowed for dietary adaptation.

A further indication of metabolic adaptation to chronic ketosis is seen in the oxygen consumption data presented in Table 1. There is less than a 1% rise in $\dot{V}O_2$ at the same power output at EKD-4 compared with EBD-1, while the rate of lipid substrate utilization is roughly doubled at EKD-4. Since the oxygen cost of a calorie liberated from fat is 8% greater than one liberated from carbohydrate, the data suggest a slightly greater efficiency of caloric utilization during fat-powered exercise at EKD-4.

In conclusion, this study demonstrates that for elite bicyclists, both maximal oxygen uptake and aerobic endurance capacity for continuous exercise at 64% of that $\dot{V}O_2$max are not comprised after four weeks of the EKD. This maintenance of function is accomplished by a major conservation of carbohydrate substrate during exercise, three-fold and four-fold for glucose and glycogen, respectively, with increased lipid oxidation providing the difference.

These results do not contradict the current understanding that exhaustion correlates with muscle glycogen depletion (in this case, specific to Type-I fibers). It does demonstrate, however, that metabolic adaptation to limit carbohydrate oxidation can facilitate moderate submaximal exercise during ketosis to the point that it becomes comparable to that observed after a high-carbohydrate diet. Because muscle glycogen stores require many days for repletion, whereas even very lean individuals maintain appreciable caloric stores as fat, there is potential benefit in this keto-adapted state for athletes participating in prolonged endurance exercise over two or more days.

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