The Human Metabolic Response to Chronic Ketosis Without Caloric Restriction: Physical and Biochemical Adaptation

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To study the metabolic effects of ketosis without weight loss, nine lean men were fed a eucaloric balanced diet (EBD) for one week providing 35–50 kcal/kg/d, 1.75 g of protein per kilogram per day and the remaining 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 with minerals and vitamins. Weight and whole-body potassium estimated by potassium-40 counting (^{40}K) did not vary significantly during the five-week study. Nitrogen balance (N-Bal) was regained after one week of the EKD. The fasting blood glucose remained lower during the EKD than during the control diet (4.4 mmol/L at EBD, 4.1 mmol/L at EKD-4, $P < 0.01$). The fasting whole-body glucose oxidation rate determined by a ^{13}C-glucose primed constant infusion technique fell from 0.71 mg/kg/min during the control diet to 0.50 mg/kg/min ($P < 0.01$) during the fourth week of the EKD. The mean serum cholesterol level rose (from 159 to 208 mg/dL) during the EKD, while triglycerides fell from 107 to 79 mg/dL. No disturbance of hepatic or renal function was noted at EKD-4. These findings indicate that the ketotic state induced by the EKD was well tolerated in lean subjects; nitrogen balance was regained after brief adaptation, serum lipids were not pathologically elevated, and blood glucose oxidation at rest was measurably reduced while the subjects remained euglycemic.

KETONEMIA IN HUMANS results from either a primary reduction in insulin secretion or secondarily from dietary carbohydrate restriction. This latter state, nutritional ketosis, differs from the former by insulin-mediated moderation of blood ketone levels. Whereas protein ingestion during nutritional ketosis reduced ketonemia, neutral fat ingestion has little if any effect on circulating 3-hydroxybutyrate or acetoacetate levels. Chronic ketonemia thus may be maintained independent of total caloric intake, so long as carbohydrate (and to a lesser extent protein) ingestion is restricted.

Nonetheless, most studies of human metabolism during nutritional ketosis have been done during total fasting[1-4] or modified fasting.[5-8] The obligate associated weight loss during these states contributes a complicating metabolic variable to the study of chronic ketosis. When eucaloric diets resulting in ketonemia have been studied,[9-15] dietary periods of 14 days or less have been the rule. This practice appears to be the result of tolerance problems with diets adequate in calories but very low in carbohydrate content. Nausea and weakness have been the most frequent side effects, and thus limited the duration of previous studies. That such brief periods of carbohydrate restriction (14 days or less) do not result in a stable metabolic state is suggested by a recently reported study by Phinney et al[16] showing apparent continuing adaptation to ketosis induced by a protein-supplemented fast for up to six weeks or more.

The only published study of adult humans in a state of chronic ketosis on a controlled diet is that of McClellan and DuBois,[17] and McClellan et al[18] using as subjects two arctic explorers who had previous experience living on a carbohydrate-free diet. Beyond this limited study, only the empiric experience of North American native peoples is available to indicate the ability of humans to maintain health and function during prolonged nutritional ketosis. Both the Plains Indian and Arctic Innuit (Eskimo) peoples lived for all or part of the year on diets extremely limited in carbohydrate, deriving the bulk of their nutrients from animal products consisting of meat and fat.[19]

Beyond the anthropologic interest of such a study, the examination of the human response to prolonged carbohydrate restriction is of significant value because of its use in the treatment of reactive hypoglycemia and childhood seizures.

Thus, in order to better understand the physical and metabolic adaptation to chronic ketosis, we developed a eucaloric ketogenic diet (EKD) that was well tolerated by lean, healthy men, and monitored its effects on resting metabolism and body composition over a four-week period.

MATERIALS AND METHODS

Subjects

Nine lean healthy men between the ages of 20 and 30 years were chosen to take part in this 35-day inpatient project. Details of the

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Received for publication March 15, 1983.

Supported in part by National Institutes of Health grants AM-26349, GM-22691, RR-88, AM-077070, and GM-00455-03.

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0026-0495/83/3208-0005$02.00/0

Table 1. Characteristics of the Subjects

Group and Subject	Age (yrs)	Height (cm)	Initial Weight (kg)	Weight/ Height*	C.H.I.† (%)
Untrained					
RP	25	180	68.1	0.93	107
BD	20	177	75.6	1.06	134
AK	26	196	82.5	0.95	107
DP	22	176	73.6	1.03	150
Trained					
JP	25	180	81.7	1.10	149
WB	20	175	62.8	0.90	111
IK	20	189	75.8	0.98	115
BK	20	192	76.0	0.94	110
MK	26	173	69.1	1.01	129
Mean	23	182	73.9	0.99	124

*Weight for height: actual weight divided by ideal body weight, based upon Metropolitan Life Insurance Co Table, medium frame.[20]

†Creatinine Height Index: mean 24-hour urine creatinine during EBD-1, divided by expected creatinine (23 mg/kg/d), using ideal body weight determined as above.

ages, heights, and initial weights of the subjects are presented in Table 1. The initial four subjects were selected independent of their level of physical training, but all were physically active (one to five hours a week of sports activity). The last five subjects were specifically selected; all were competitive bicyclists at a stable level of training. All subjects kept a daily diary of physical activity, specifying the type, intensity, and any perceived limitations. All subjects passed a complete medical history and physical examination, and demonstrated normal ECG, chest x-ray films and blood screening battery (complete blood cell count, electrolytes, renal function, liver function, blood glucose, and blood lipid profile) before admission to the Clinical Research Center. All details and risks of the study were explained to the subjects verbally and in writing so that they could give signed informed consent before acceptance into the study. The study was approved by the M.I.T. Clinical Research Center Executive Committee and also by the M.I.T. Committee on the Use of Humans as Experimental Subjects.

Diets

For the first seven days of the study, the subjects were given a eucaloric balanced diet (EBD) consisting of either: (1) whole milk supplemented with sucrose and dextrin oligosaccharides, or (2) a lactose-free commercial liquid diet (Ensure, Ross Laboratories, Columbus, OH) supplemented with dextrin oligosaccharides. The EBD daily provided 1.75 g of protein per kilogram of ideal body weight (IBW) based on height and medium frame size from the Metropolitan Life Insurance Company Tables,[20] with the remainder of calories divided as 33% fat and 67% carbohydrate. The caloric intake was determined from a diet history to provide adequate energy to meet daily needs, and ranged from 35 to 50 calories per kilogram per day. The EBD was supplemented with a multiple vitamin containing folate and trace minerals and sodium in the form of bouillon.

Beginning on the eighth day of the study, the subjects were given a eucaloric ketogenic diet (EKD), providing equal amounts of protein and calories as the EBD (1.75 g and 35–50 calories per kilogram daily, respectively), but containing less than 20 g of carbohydrate daily. Thus 83% to 85% of the total calories provided by the EKD came from fat. The daily diet was composed of three meals and a snack. The subjects were given their choice from five meals prepared from ground beef, breast of chicken, water-packed tuna fish, powdered egg solids, and cheddar cheese. Mayonnaise, heavy cream, sour cream, and cream cheese were used as the primary lipid sources. All protein sources were subjected to acid digestion and Kjeldahl analysis for nitrogen content multiple times to document uniformity. The caloric contents of the fat sources were obtained from standard tables.[21] All food portions were weighed on an analytical balance, and all serving dishes were cleaned with rubber spatulas by the subjects to ensure quantitative accuracy of their intakes. The EKD was supplemented with the same multivitamin as the EBD, but in addition, the subjects received the following daily supplements: 600 mg of calcium; 300 mg of magnesium; 1.0 g potassium as bicarbonate (in addition to the 1–1.5 g provided by the protein sources); 5 g of sodium (minimum) as bouillon or salt in cooking; and a minimum of 2000 mL of noncaloric beverages daily.

Schedule of Events

Figure 1 is a diagram of the study schedule. All subjects were housed for the full 35-day study in the Clinical Research Center. They were allowed to eat one meal away from the Clinical Research Center daily, and to go about their normal activities except as limited by scheduled tests. In the following discussion and tables, the week of the EBD will be referred to as EBD-1, and the subsequent four weeks of the EKD will be referred to as EKD-1, EKD-2, EKD-3, and EKD-4.

Daily Monitoring

Each subject was weighed on a metabolic scale each morning before breakfast. Urine samples for acetone determinations were monitored twice daily by semi-quantitative analysis (Acetest tablets, Ames Division, Miles Laboratories, Elkhart, IN). Blood pressure, pulse, respirations, and temperature were determined twice daily.

Cardiographic Monitoring

In addition to the preadmission ECG, all subjects had weekly resting 12-lead ECGs done, with particular attention paid to possible

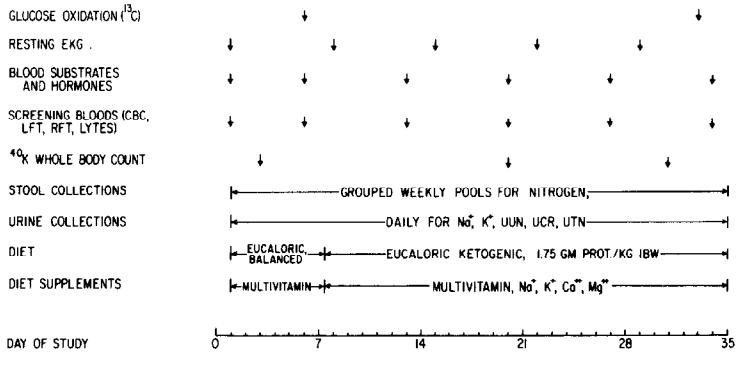

Fig. 1. Study protocol. The vertical arrows indicate the time of individual tests and horizontal arrows indicate continuous processes. Abbreviations: ^{13}C = carbon-13; ^{40}K = potassium-40; LFT = liver function tests; RFT = renal function tests; Lytes = serum electrolytes; UUN = urine urea nitrogen; UCR = urine creatinine; UTN = urine total nitrogen; PROT = protein; IBW = ideal body weight.

rhythm and interval changes. In addition, all subjects were requested to report any perceived irregularities in heart beat (palpitations).

Routine Laboratory Screening

The subjects had blood drawn either weekly (first four subjects) or biweekly (last five subjects) for a battery of laboratory tests. This included whole blood for determinations of hemoglobin, hematocrit, white blood cell and differential counts, and platelet estimate; serum for determinations of levels of sodium, potassium, chloride, bicarbonate, calcium, inorganic phosphorus, uric acid, urea nitrogen, creatinine, total protein, albumin, total and direct bilirubin, serum alanine amino transferase, alkaline phosphatase, total and HDL fraction cholesterol, and triglycerides. All blood was drawn with the subjects supine and with minimal hemostasis. Regular urinalyses wee also obtained on the same schedule. The above determinations were done in the Clinical Research Center core laboratory by routine semiautomated techniques.

Blood Substrates and Hormones

On the same schedule as the above, as illustrated in Figure 1, samples were drawn for determinations of glucose, lactate, 3-hydroxybutyrate, free fatty acids (FFA), amino acids, insulin, and triiodothyronine (T_3). The determination of glucose, lactate, 3-hydroxybutyrate, and amino acids utilized heparinized whole blood deproteinized with iced 30% perchloric acid, separated, and frozen at −20 °C until used for analysis. The amino acid profile was determined on a Dionex D-400 chromatographic analyzer with the individual concentrations determined by automatic integration of peak areas. Results of glutamine and glutamate are not reported because of incomplete separation of these two amino acids by the technique used. The glucose, lactate, and 3-hydroxybutyrate assays were enzymatic determinations taken from the standard published techniques of Bergmeyer.[22]

The determination of FFA was done on heparinized plasma, stored at −20 °C until analysis, utilizing the published procedure of Ho.[23] Samples exceeding 1.5 mmol/L FFA were diluted 50% in 4% fat-free albumin and reanalyzed to confirm the higher concentration readings.

The determinations of insulin and T_3 were made by radioimmunoassay. The T_3 and insulin samples were serum. All samples were kept at −20 °C before analysis. The T_3 assay (done by Dr E. Danforth, Jr, Burlington, VT) utilized a technique published elsewhere.[24] The insulin determinations (done by Dr R. Wannemacher, Fort Detrick, MD) utilized kit assay materials purchased from Cambridge Nuclear Corp (Cambridge, MA).

Total Body Potassium

Estimations of total body potassium were determined at EBD-1, EKD-2, and EKD-4 to check for changes in lean body mass (and glycogen). The technique of ^{40}K counting was used,[25] which employed a sodium iodide crystal/photomultiplier linked to a multichannel analyzer. The subject sat in a tilt chair under the crystal for a 45-minute counting period.

Nitrogen Balance

Nitrogen intake was determined, as noted above, by Kjeldahl analysis of known uniform protein sources, with portion sizes being carefully weighed at the time of meal preparation.

Serial 24-hour urine collections were maintained for the 35 days of the study, with the closed collection bottles stored at 4 °C during the collection period, the volume carefully measured after completion, and aliquots stored at −20 °C before analysis. In addition to Kjeldahl analysis for total nitrogen, each urine sample was assayed for sodium, potassium, urea nitrogen, and creatinine. The consistency of the daily creatinine determinations was used as a criterion of the quality of the collection process. Sodium and potassium outputs were followed as a marker of the adequacy of their dietary supplementation.

Complete stool collections were maintained for the 35-day study, divided into seven-day pooled samples separated by nonabsorbable oral dye markers given at the end of each weekly diet period. After homogenization and dilution to a standard volume, aliquots of the weekly collections were stored at −20 °C until digestion and analysis for total nitrogen by the standard Kjeldahl technique. Estimated daily stool nitrogen losses were made by dividing the weekly total loss by seven.

No direct attempt was made to measure cutaneous nitrogen losses in this study. In view of the rather prodigious sweat losses of the trained (and less frequently, the untrained) subjects, however, a correction factor for sweat nitrogen was added. Consolazio et al[26] determined the whole body sweat nitrogen losses under conditions of high environmental temperature, demonstrating values of roughly 4–5 mg/kg/h for up to seven hours. The assumption of a similar nitrogen content of exercise-induced sweat is supported by the study of Costa et al[27] in men in a temperate climate on a moderate protein intake. A correction factor of 5 mg/kg/h of vigorous daily exercise was added to the basal value of 5 mg/kg/d advocated by Calloway et al.[28]

The resultant equation for the nitrogen balance calculation was:

$$\text{N-Bal} = N_{in} - (\text{urine N} + \text{fecal N} + \text{skin and sweat N})$$

The skin and sweat factor varied from 5–15 mg/kg-day under conditions observed during this study.

Glucose Oxidation Rate Determination

The resting glucose oxidation rate was determined for all subjects after an overnight fast at the end of EBD-1 and EKD-4 using the primed, constant infusion technique of Wolfe et al.[29] Two intravenous catheters were placed in the supine subject, one in each forearm. Both were initially infused with saline, while three samples each of blood and breath were collected for determination of baseline ^{13}C enrichment of glucose and CO_2, respectively. The blood was collected in heparinized tubes and kept on ice until separation of plasma by centrifugation and storage at −20 °C. Breath CO_2 was collected by bubbling 3 L of expired gas through a micropipette tip into 5 mL of 0.1N NaOH in a screw-top glass vial. The vials were kept tightly closed until analysis. The subjects were primed with a 25 mg bolus of μ-^{13}C-glucose (KOR Isotopes Inc, Cambridge, MA; 85% enrichment) and 25 mg (0.30 mmol) of $NaH^{13}CO_3$, followed by an infusion of the same glucose isotope (1.0 mg glucose per milliliter) at 0.382 mL/min controlled by a Harvard syringe pump. Between 60 and 90 minutes from the start of the infusion, three five-minute collections of expired gas were obtained from the supine resting untrained subjects (subjects RP, BD, AK, DP) using a two-way Hans-Rudolph valve and a Douglas bag. Volume was measured in a Chain Gasometer (Warren-Collins, Braintree, MA) and standardized for temperature and barometric pressure. Aliquots of the expired gas were then analyzed for O_2 and CO_2 on a Perkin-Elmer Model 1100 Medical Gas Analyzer. The five-minute collections from the trained subjects (subjects JP, WB, IK, BK, MK) were analyzed by direct uptake into the Chain Gasometer, with the gas directly drawn from the bleed-valve to a Beckman LB2 infrared CO_2 analyzer and a Beckman E_2 paramagnetic oxygen analyzer. Both instruments were standardized with reagent-grade helium (zero) and a reference 4% CO_2/16% O_2 gas mixture, the composition of which was previously accurately determined using a Scholander apparatus.[30]

After 90 minutes of constant infusion of the μ-^{13}C-glucose following the priming bolus, serial samples of blood (every 10 minutes) and 3 L expired gas samples (every seven minutes) were collected for the next 30 minutes and processed as noted above.

The blood glucose enrichment was determined on the plasma samples with precipitation of protein by serial additions of equal volumes of balanced zinc sulfate and barium hydroxide solutions as described by Somogyi,[31] sequential elution through anion and cation exchange columns (Dowex AG1-X8 and AG50W-X8, respectively), and the eluate taken to dryness in a warming oven at 70 °C. The dry samples were combusted at 1000 °C and the resultant CO_2 analyzed for ^{13}C enrichment in a Nuclide Model 3-60 RMS dual inlet, dual collector, isotope-ratio mass spectrometer. Similarly, the breath samples in NaOH were analyzed by acid release of the CO_2, the enrichment of which was then measured in the isotope-ratio mass spectrometer. Both the blood glucose and breath CO_2 enrichment values were corrected by subtraction of the mean baseline (background) enrichment determined from the three preinfusion samples.

To calculate the rate of glucose oxidation, the percent CO_2 from glucose was determined by the equation:

$$\%CO_2 \text{ from glucose} = (\text{Percent } CO_2 \text{ enrichment}/0.8)/\text{Percent enrichment of plasma glucose})$$

The 0.81 factor corrects for the stable rate of bicarbonate retained by humans, determined by primed constant infusion of $NaH^{13}CO_3$.[32] Using the above value, the rate of glucose oxidation is:

$$\text{Glucose oxidation (mg/kg/min)} = \%CO_2 \text{ from glucose} \times \dot{V}CO_2 \times (0.18 \text{ mg}/\mu\text{mol glucose} \div 6\ \mu\text{mol } CO_2/\mu\text{mol glucose}).$$

Statistical Analysis

Data are reported as means + SEM. Tests yielding results suitable for paired comparison were assessed by paired *t* tests. 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.[33] For anticipated comparisons, significance was ascertained by using the least significant differences test,[34] with $P < 0.01$ the criterion unless otherwise specified.

RESULTS

Diet

Of primary and basic importance to this study was the ability of the subjects to tolerate the EKD. Nine subjects were recruited for the study, and all nine completed the five-week dietary protocol. Subjective symptoms associated with the diet were minimal, with most participants noting mild lethargy in the first 7 to 10 days of the EKD, and three of the physically trained subjects noting some early transient limitation of their ability to maintain training schedules. All subjects noted a decrease in stool frequency and volume. Three noted mild constipation. There were no symptoms of nausea, persistent fullness, diarrhea, or flatulence.

Dietary compliance with the study was excellent, as indicated by stable daily weights and consistent urinary nitrogen excretion. The best evidence that the subjects did not eat extra (unsanctioned) dietary carbohydrate during the EKD were the urine and blood ketone levels. All subjects maintained continuous urinary ketone levels while on the EKD. In addition, resting serum 3-hydroxybutyrate levels after an overnight fast, initially at a mean concentration of 0.06 mmol/L during the EBD, rose to weekly consecutive means of 2.34, 2.12, 2.82, and 1.58 mmol/L for the four weeks of the EKD. The lowest recorded resting serum value for any subject was 0.66 mmol/L.

Body Composition

After an initial weight loss during the first few days of the controlled diet (EBD) by four of the subjects, there were no dramatic changes in weight by any subject. Two subjects lost more than 2 kg after day 2 of the study (subject DP, 2.5 kg; subject JP, 2.0 kg) and one subject gained 1.7 kg (subject WB). There was no brisk loss at the time of the EKD initiation (see Table 2). This lack of change suggests an absence of a profound diuresis associated with the onset of ketosis in this group of subjects, although they were given extra sodium chloride (up to a total of 7 g sodium daily) on beginning the EKD to avoid hypovolemic symptoms. Thus, the EKD resulted in a mean loss of body weight of 0.7 kg in the first seven days, a further drop of 0.4 kg in the second week, with a slow regain of 0.6 kg mean weight in the subsequent two weeks of the EKD. None of the weekly mean changes was significant by two-way ANOVA.

Whole body ^{40}K counting was done three times during the study (see Fig. 1) in all subjects except subject RP, in whom it was done only twice (at the end of EBD-1 and again at EKD-4). The mean value for all nine subjects are listed in Table 2. Although there appeared to be a fall from 180 g to 175 g of whole body potassium after two weeks of the EKD, neither this fall nor the subsequent rise to 177 g achieved significance by 2-way ANOVA.

The results of the nitrogen balance calculations, expressed as weekly means for all nine subjects, are presented in Table 2. In addition, the mean daily nitrogen balance is depicted in Fig. 2. The 1.3 g/d positive balance during EBD-1 was followed by a significant change to -1.0 g/d ($P < 0.01$ by two-way ANOVA) in EKD-1. The subsequent rise to $+0.9$, $+0.7$, and $+1.9$ g/d positive balance in EKD-2, EKD-3, and EKD-4 achieved significance only in the last week (EKD-1 $\neq$ EKD-4, $P < 0.01$). The diagrammatic plot of daily nitrogen balance (Fig. 2) indicates the rapid response to the withdrawal of carbohydrate from a eucaloric diet while holding nitrogen and calorie intakes constant. This is consistent with the findings of Silwer[15] in studies of humans on low-carbohydrate, moderate protein diets. Although the mean nitrogen balance fell to -3.1 g/d on the second

Table 2. Body Composition

Subject	Diet and Week of Study*				
	EBD-1	EKD-1	EKD-2	EKD-3	EKD-4
Weight (kg)					
RP	68.3	67.9	67.1	67.4	68.1
BD	75.4	74.3	73.9	74.2	74.1
AK	81.6	81.2	81.3	81.4	81.1
DP	73.2	72.1	71.7	71.2	71.3
JP	81.4	79.6	78.2	78.6	78.5
WB	63.4	63.5	63.3	64.2	64.8
IK	75.6	74.9	74.7	74.7	75.1
BK	76.3	75.0	75.7	76.1	76.6
MK	69.4	69.2	68.7	69.5	70.1
Mean ± SEM	73.8 ± 2.0	73.1 ± 1.9	72.7 ± 1.9	73.0 ± 1.8	73.3 ± 1.7
Nitrogen Balance (g/d)					
RP	2.9	−0.3	2.0	3.2	3.8
BD	1.4	−0.7	0.1	1.8	0.3
AK	1.9	1.3	4.5	2.4	3.6
DP	−2.2	−4.0	−1.0	−1.9	−0.8
JP	−0.5	−2.3	−2.0	0.6	2.7
WB	0.1	−2.5	−1.3	−1.3	0.4
IK	1.8	−0.1	1.1	0.6	2.9
BK	5.5	0.5	4.1	2.0	2.4
MK	1.1	−1.1	0.3	0.4	1.7
Mean ± SEM	1.3 ± 0.4	−1.0 ± 0.3	0.9 ± 0.3	0.7 ± 0.3	1.9 ± 0.2
^{40}Potassium Count (g)†					
RP	140		—		157
BD	183		176		171
AK	178		183		177
DP	202		185		183
JP	209		189		183
WB	163		161		169
IK	166		172		174
BK	194		189		204
MK	182		174		175
Mean ± SEM	180 ± 7.1		175 ± 3.4		177 ± 4.3

*EBD-1 = first week of study, eucaloric balanced diet; EKD-1,2,3,4 = subsequent four weeks of eucaloric ketogenic diet.

†^{40}Potassium Count = whole body potassium content estimation by natural isotope counting.

Weight and nitrogen balance values are each means of the seven daily values from each week.

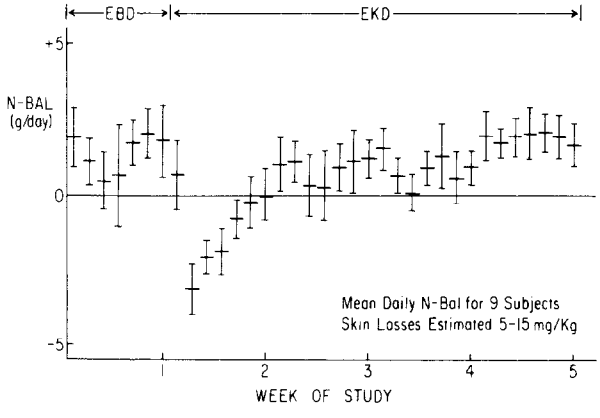

Fig. 2. Measured nitrogen balance. Intake determined by weighed portion intake, with food aliquots assessed for nitrogen by Kjeldahl analysis. Excretion measured by serial complete urine and stool collections, with cutaneous losses estimated at 5 mg/kg/d basal, plus 5 mg/kg for each hour of daily exercise. EBD = eucaloric balanced diet; EKD = eucaloric ketogenic diet. Values shown are mean ± SEM, n = 9.

day of the EKD, there was a rapid adaptation to the carbohydrate-free diet, resulting in a return to mean positive balance on the seventh day of the EKD, after which it remained positive for the remaining three weeks of the study. The mean daily nitrogen balance for all nine subjects for the 35-day balance period was 0.5 g/d. This implies an average net gain in lean body mass over the duration of the study of approximately 400 g.

Serum Minerals and Bicarbonate

The mean concentrations of serum sodium, potassium, bicarbonate, calcium, and phosphorus are reported in Table 3. Note that the values listed for weeks EKD-1 and EKD-3 represent only the four untrained subjects. As expected, the serum sodium levels remained very stable and in the normal range. Four subjects sustained potassium levels below 3.7 mEq/L during the EKD while receiving 25 mEq/d

Table 3. Serum Electrolytes and Blood Formed Elements

Test	Point in Study*					
	Preadmit	EBD-1	EKD-1	EKD-2	EKD-3	EKD-4
Sodium (mEq/L)	143 ± 0.8	140 ± 0.9	144 ± 1.3	143 ± 1.2	142 ± 0.8	141 ± 0.6
Potassium (mEq/L)	4.1 ± 0.1	4.6 ± 0.2	4.0 ± 0.1	3.9 ± 0.1	4.0 ± 0.1	4.9 ± 0.2
Calcium (mg/dL)	9.9 ± 0.1	9.5 ± 0.1	9.7 ± 0.3	9.7 ± 0.1	9.9 ± 0.3	9.5 ± 0.1
Phosphorus (mg/dL)	4.0 ± 01	3.6 ± 0.1	3.6 ± 0.2	3.9 ± 0.2	4.2 ± 0.2	3.9 ± 0.1
Bicarbonate (mEq/L)	26 ± 0.6	26 ± 0.7	22 ± 1.4	22 ± 0.5	24 ± 1.5	24 ± 0.8
Hematocrit (%)	44 ± 0.8	41 ± 0.6	44 ± 0.4	41 ± 0.8	44 ± 1.6	41 ± 0.5
WBC count ($\times 10^3/\mu L$)	5.3 ± 0.4	5.1 ± 0.6	6.0 ± 1.3	4.9 ± 0.5	5.2 ± 0.5	4.5 ± 0.5
Granulocytes (%)	51 ± 2	54 ± 2	60 ± 4	52 ± 2	52 ± 5	52 ± 5
Lymphocytes (%)	37 ± 2	36 ± 2	34 ± 7	33 ± 2	41 ± 5	37 ± 4

Values are given as mean ± SEM. Sample size: EKD-1, EKD-3, n = 4; all others n = 9.

*Preadmit = preadmission; EBD-1 = first week of study, eucaloric balanced diet; EKD-1,2,3,4 = subsequent four weeks of eucaloric ketogenic diet.

supplementation as potassium bicarbonate, with the lowest value noted being 3.4 mEq/L. When potassium concentrations below 3.7 mEq/L were observed, the supplementation for these subjects was increased to 50 mEq/d, which uniformly sufficed to return the level in the subsequent week above 3.7 mEq/L.

Both serum calcium and phosphorus levels were uniformly stable in all subjects during the study. No values of either mineral level were noted outside the normal range. In conjunction with this, no subject reported any disturbance of muscle function during the study, either fasciculation or cramping.

The serum bicarbonate level dropped sharply with initiation of the EKD, from the EBD-1 level of 26 mEq/L to 22 mEq/L at EKD-2 ($P < 0.01$ compared with EBD-1). By the fourth week of the EKD, the serum bicarbonate level had risen back to 24 mEq/L ($P < .05$ compared with EKD-2). Thus, although there was a significant fall followed by recovery in the first four weeks of the EKD, serum bicarbonate levels remained in the normal clinical range, and hence the initiation of ketosis was not accompanied by acidosis.

Formed Elements of Blood

Antecedent to and during this five-week study, both groups of subjects had between 450 and 500 mL of blood drawn for testing. Over this time span, there was no clinically significant change in hematocrit. The higher values at EKD-1 and EKD-3 were drawn only from the untrained subjects, suggesting that highly trained cyclists have lower hematocrit values than untrained controls, which is analogous to the similar situation previously reported for elite runners.[35]

The WBC count appeared to fall in all subjects except one (subject BK) over the course of the EKD, but the change was not significant. Although the mean value fell below the lower limit of normal at EKD-4, there was no evidence of increased susceptibility to infection. Neutropenia has been previously noted in fasting subjects with and without folate supplementation[36] and also in the context of modified fasting.[37] Whereas the studies cited above note reductions in neutrophils of as much as 60%, no such reduction was seen in this study.

Liver Function

The serum alkaline phosphatase and albumin levels remained unchanged and in the normal range throughout the study (see Table 4). Three of the nine subjects had borderline to slightly elevated serum alanine amino transaminase (SGPT) levels on initial screening or after EBD-1. In the absence of other disturbances of

Table 4. Liver and Renal Function Tests

Test	Point in Study*					
	Preadmit	EBD-1	EKD-1	EKD-2	EKD-3	EKD-4
Albumin (g/dL)	4.8 ± 0.06	4.6 ± 0.03	5.0 ± 0.12	4.7 ± 0.07	4.9 ± 0.28	4.5 ± 0.05
Total Bilirubin (mg/dL)	1.3 ± 0.3	1.1 ± 0.2	1.1 ± 0.3	1.0 ± 0.1	0.9 ± 0.2	1.0 ± 0.1
SGPT (U/L)†	16 ± 2.6	16 ± 2.7	26 ± 2.4	22 ± 3.5	32 ± 5.2	17 ± 2.6
Alkaline Phosphatase (U/L)†	39 ± 4	41 ± 4	42 ± 8	35 ± 4	45 ± 6	37 ± 4
BUN (mg/dL)	17 ± 1.0	16 ± 0.7	12 ± 1.2	14 ± 0.6	14 ± 1.7	14 ± 0.6
Creatinine (mg/dL)	1.1 ± 0.06	1.0 ± 0.05	1.2 ± 0.06	1.4 ± 0.06	1.1 ± 0.17	1.2 ± 0.07
Uric Acid (mg/dL)	7.2 ± 0.4	5.9 ± 0.4	12.6 ± 1.2	11.0 ± 0.5	11.7 ± 0.6	8.9 ± 0.6

Values are given as mean ± SEM. Sample Size: EKD-1 & 3 n = 4, all others n = 9.

*Preadmit = preadmission; EBD-1 = first week of study, eucaloric balanced diet; EKD-1,2,3,4 = subsequent four weeks of eucaloric ketogenic diet.

†SI units.

liver function (except subject RP with known Gilbert's syndrome), these subjects continued through the study without significant change in this variable.

The mean total serum bilirubin levels did not change significantly over the five weeks of the study (see Table 4). Six of the nine subjects began with normal total and direct (conjugated) bilirubin levels, and these remained stable across both diets. Three of the subjects (RP, IK, and BK) had Gilbert's syndrome, the latter two cases previously undiagnosed, and their elevated preadmission and EBD-1 bilirubin values normalized progressively over the four weeks of the EKD.

Renal Function

Renal function was assessed by serial testing of the serum urea nitrogen (BUN), creatinine, and uric acid levels, which are shown in Table 4. The changes observed in the BUN did not achieve significance. The creatinine level rose significantly ($P < 0.01$) by EKD-2, remaining elevated ($P < 0.01$) compared with EBD-1 at EKD-4. No mean creatinine value for any week of the EKD exceeded the upper limits of normal (1.5 mg/dL) and the highest individual recorded value was 1.7 mg/dL.

The serum uric acid level rose sharply with initiation of the EKD, ($P < 0.001$ at EKD-2), and then began to decline by EKD-4 ($P < 0.001$ compared with EKD-2); but still remained above the level of EBD-1 at EKD-4 ($P < 0.001$). All subjects exceeded the normal range (mean value: 11.0 mg/dL; upper limit: 8.0 mg/dL) by EKD-2, which is an expected response to any ketogenic regimen as a result of the interference of ketones with uric acid excretion via the renal organic acid secretory pathway.[38] As the subjects were screened for a family history of gout before acceptance into the study, this malady was not a problem during the rapid changes in uric acid level induced by the EKD. Neither was there any evidence for renal stone formation in any subject during or for six months after the study.

Serum Lipids

The serum lipid data are presented in Table 5. There was a significant rise in cholesterol levels by EKD-4 ($P < 0.001$) compared with EBD-1), although the peak mean value of 208 mg/dL stayed within the normal range. There was no significant change in the absolute level of the cholesterol HDL fraction. The apparent decline in the serum triglyceride levels during the four weeks of the EKD was not significant.

Blood Substrates

The concentrations of blood glucose, free fatty acids, 3-hydroxybutyrate, and lactate drawn after an overnight fast from all nine subjects at rest are listed in Table 5. It is clear that marked changes in energy-yielding substrate concentrations occurred upon initiation of the EKD. The drop in blood glucose from EBD-1 to EKD-2 was significant ($P < 0.001$) and remained so ($P < 0.01$) at EKD-4, although no resting value for any subject was found below the normal range.

The serum free fatty acid level rose in response to the EBD as well as the EKD. Although the rise from the preadmission level to EKD-2 was highly significant ($P < 0.005$), the rise from EBD-1 to EKD-2 was less profound ($P < 0.05$). The elevation in the 3-hydroxybutyrate level appeared much more responsive to the diet change, with both the EKD-2 and EKD-4 levels differing from the preadmission and EBD-1 values by $P < 0.001$. The difference between EKD-2 and EKD-4, on the other hand, was not significant. No significant changes in resting serum lactate levels were measured during the study, with all values remaining within the normal range.

Blood Amino Acids

The data for those amino acid levels showing a significant change with diet are presented in Figure 3. The major changes in blood amino acid concentrations

Table 5. Blood Substrates and Lipids

Test	Point in Study*					
	Preadmit	EBD-1	EKD-1	EKD-2	EKD-3	EKD-4
Glucose (mmol/L)	4.77 ± 0.12	4.57 ± 0.08	3.72 ± 0.10	4.11 ± 0.10	3.77 ± 0.22	4.06 ± 0.12
Free Fatty Acids (mmol/L)	0.51 ± 0.09	0.69 ± 0.08	0.84 ± 0.29	0.95 ± 0.13	0.71 ± 0.08	0.93 ± 0.07
3-Hydroxybutyrate (mmol/L)	0.07 ± 0.02	0.06 ± 0.02	2.34 ± 0.44	2.12 ± 0.38	2.82 ± 0.84	1.58 ± 0.25
Lactate (mmol/L)	0.78 ± 0.06	1.16 ± 0.08	1.01 ± 0.25	1.02 ± 0.27	0.57 ± 0.03	1.18 ± 0.16
Total Cholesterol (mg/dL)	169 ± 9	159 ± 9	171 ± 7	182 ± 9	205 ± 17	208 ± 11
High Density Lipoprotein (mg/dL)		40 ± 4.4		36 ± 3.4		40 ± 7.7
Triglycerides (mg/dL)	91 ± 10	107 ± 17	84 ± 16	86 ± 10	113 ± 13	79 ± 12

Values are given as mean ± SEM. Sample Size: EKD-1 & 3 n = 4, all others n = 9. FFA, cholesterol, and triglycerides were determined from serum; substrates were determined from blood.

*Preadmit = preadmission; EBD-1 = first week of study, eucaloric balanced diet; EKD-1,2,3,4 = subsequent four weeks of eucaloric ketogenic diet.

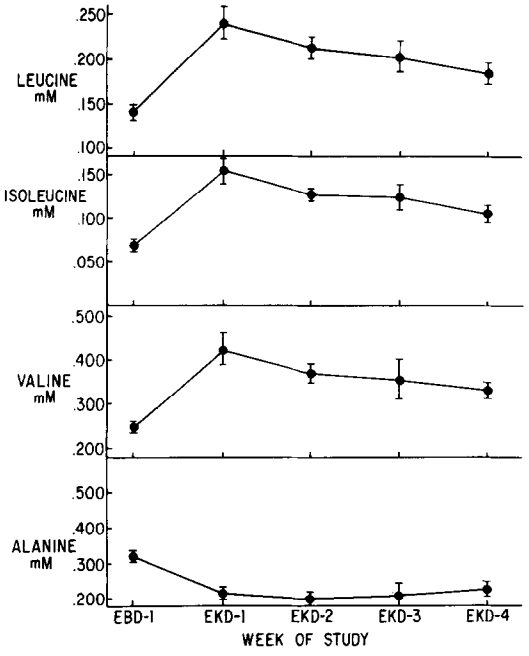

Fig. 3. Blood amino acids from resting subjects after an overnight fast. The EBD-1 value was obtained after one week of a controlled balanced diet, the EKD-1 through EKD-4 values determined after one to four weeks of the eucaloric ketogenic diet. Only those amino acids yielding significant changes with diet are shown.

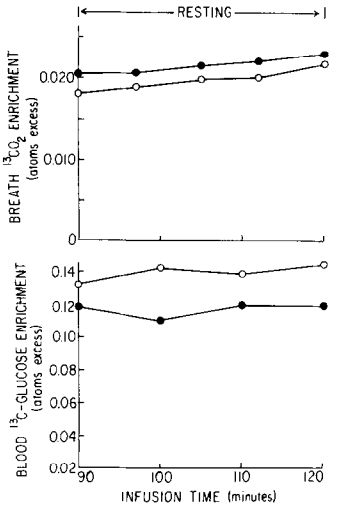

Fig. 4. Carbon 13 enrichment of breath CO_2 and blood (plasma) glucose during ^{13}C-glucose infusion after an overnight fast. Solid dots represent studies done after one week of eucaloric balanced diet and open circles after four weeks of eucaloric ketogenic diet.

occurring with the diet were seen in the branched-chain amino acids (BCAA) and alanine.

The fasting blood leucine level rose sharply with the ketogenic diet, differing from both the preadmission and EBD-1 values ($P < 0.001$) at EKD-2. By EKD-4, the level had fallen significantly ($P < 0.05$) compared with EKD-2, but remained above EBD-1 ($P < 0.01$). Similar patterns were also observed for blood isoleucine and valine levels, which both exceeded the preadmission and EBD-1 values by $P < 0.001$ at EKD-2 and EKD-4. As with leucine, however, both the isoleucine and valine levels fell significantly ($P < 0.01$ and $P < 0.05$, respectively) at EKD-4 compared with EKD-2.

The resting blood alanine level behaved in the opposite manner, remaining stable between admission and EBD-1, then falling sharply at EKD-2 and EKD-4 ($P < 0.001$ at both times compared with pre-admission and EBD-1). The rise from EKD-2 to EKD-4 did not achieve significance.

Glucose Oxidation

The rate of blood glucose oxidation in the fasting subjects at rest was calculated from the data presented graphically in Figure 4. There was a slight rise in breath $^{13}CO_2$ enrichment between 90 and 120 minutes during the studies done on both diets. This does not appear to be due to a similar rise in the plasma ^{13}C-glucose, which maintained a stable plateau during both infusions. Using the mean value for the breath $^{13}CO_2$ enrichment, the calculated mean rate of glucose oxidation at EBD-1 was 0.71 mg/kg/min, falling significantly to 0.50 mg/kg/min ($P < 0.01$) at EKD-4. This implies a basal glucose oxidation rate of 54 g/d after four weeks of the EKD, compared with 76 g/d after a similar overnight fast during the EBD. While these values represent low estimates of absolute glucose oxidation because of the lack of a completely stable plateau in the breath $^{13}CO_2$ enrichment, they are a valid comparison of the rate change induced by the diet change.

Hormonal Response

The changes induced in the fasting insulin and 3,5,3′-triiodothyronine (T_3) levels with the transition to the EKD are shown in Table 6.

There was an overall change in serum insulin levels within the points EBD-1, EKD-2, and EKD-4 by two-way ANOVA ($P < 0.025$). Although by individual testing the only significant change was from EKD-2 to EKD-4, it is clear from the summated data on all subjects, including the data on the untrained subjects at EKD-1 and EKD-3, that there was a rise in the fasting insulin levels with initiation of the EKD that lasted for three weeks. Only at EKD-4 did it fall towards the lower values that would have been expected for subjects on a carbohydrate-free diet, based on previous experience with a protein-supplemented fast.[16]

The resting, fasting T_3 level fell dramatically with initiation of the EKD, having already achieved a significant decline in the untrained subjects by EKD-1,

Table 6. Blood Hormones

Test	Point in Study*					
	Preadmit	EBD-1	EKD-1	EKD-2	EKD-3	EKD-4
Insulin (μU/mL)		10.7 ± 0.8	13.7 ± 0.9	12.6 ± 0.6	12.6 ± 0.9	9.0 ± 1.0
T_3	162 ± 6	144 ± 5	111 ± 6	90 ± 4	98 ± 9	86 ± 4

Values are given as mean ± SEM. Sample size: EKD-1, 3 n = 4; all others n = 9.

*Preadmit = preadmission; EBD-1 = first week of study, eucaloric balanced diet; EKD-1,2,3,4 = subsequent four weeks of eucaloric ketogenic diet.

but continuing to fall in EKD-2 before achieving a plateau. Thus, there was a highly significant decline in the active thyroid hormone level ($P < 0.001$ at EKD-2 and EKD-4) with the institution of the EKD, reaching a stable level of approximately 60% of the EBD-1 value.

Cardiac Function

All nine subjects had weekly electrocardiograms done to screen for changes induced by the metabolic effects of the EKD or mineral deficiency. There were no changes in rhythm, conduction, or repolarization (QT interval) noted in any subject.

Refeeding After Diet

All subjects were fed a diet containing 100–150 g of carbohydrate in the first day after termination of the EKD. After this they were advised to limit large carbohydrate loads for the next two days. The only notable phenomenon after the diet was a transient 2–5 kg weight gain in the first three days, most likely caused by the combined antinatriuretic effect of reversing ketosis and glycogen supercompensation.[39]

DISCUSSION

This study clearly establishes that a carbohydrate-free eucaloric diet can be made tolerable for lean healthy humans. Although the reasons for this success are conjectural, there are two points that separate this study from previous ones utilizing high-fat, low-carbohydrate diets.

The first (and probably the most important) point is control of the salt intake of the subjects. Whereas other investigators had withheld,[40] not controlled,[9-11,13,15,41] or not specified[14] adequate sodium intake, in this study subjects were encouraged to take salt and bouillon on a quantity sufficient to maintain a daily urinary sodium output of 200 mEq while on the EKD. This quantity, representing between 4 and 5 g daily, is not excessive relative to the average American intake. It is interesting to note, however, that the subjects had to be encouraged to take salt in quantities beyond their perceived need (taste) to maximally limit deficiency symptoms of lethargy or fatigue during exercise. This level of sodium supplementation was well tolerated by the subjects and appears to have been a critical factor in avoiding the nausea, fatigue, and orthostatic symptoms that appear to have interfered with previous studies using low-carbohydrate eucaloric diets.

The second point separating this study from some of the previous work with low-carbohydrate diets is the moderate protein intake provided the subjects. Previous studies have used diets providing as much as 375 g/d of protein,[11] raising the possibility of metabolically significant nitrogen overload.

The protein level of the EKD, 1.75 g/kg/d, appears on the average to be adequate to allow nitrogen balance to be regained within a week after removing carbohydrate from the diet. This conclusion is based on the calculated nitrogen balance, weights, and ^{40}K counting. The differences between the calculated slight overall positive nitrogen balance, as opposed to the slight decrements in weight and total body potassium can be explained by mild reductions in whole body water and glycogen. Thus the data are compatible with the subject group as a whole, being in slight positive nitrogen balance over the course of the four weeks of EKD.

In the context of this return to nitrogen balance, the findings of Silwer[15] are not readily explained. Utilizing diets with protein contents up to 2.3 g/kg/d and total caloric intakes and distributions comparable to those used in this project, he was unable to demonstrate a return of the 24-hour urine total nitrogen level back to baseline levels in most instances. The dichotomy in the results between Silwer's study and this project might be explained by a difference as minor as a lack of adequate salt (Silwer noted that his subjects complained of fatigue and weakness), or the vitamin and mineral supplements used in the EKD may have allowed optimum use of the dietary nitrogen. Further, while Silwer was unable to demonstrate a decrease in nitrogen excretion with administration of a base load, a recent report by Fery and Balasse[42] of reduced blood alanine levels after intravenous bicarbonate administration suggests the additional possibility that the base load given with the EKD supplements (calcium carbonate, magnesium oxide, and potassium bicarbonate) contributed a minor protein sparing effect.

In view of the tests done to screen for ill effects of the

EKD, the remarkably benign nature of a diet providing 85% of calories as fat is notable. After four weeks there was no measurable impairment of hepatic, renal, cardiac, or hematopoietic function. The serum uric acid level, elevated by competition from ketone bodies for excretion, was almost back to normal by that time. The rise in the serum cholesterol level, however, appeared sustained, with no suggestion of a compensatory elevation of the HDL fraction (see Table 5). The lack of elevation in triglyceride levels during the EKD was not unexpected, as the vast majority of the neutral fat intake enters the circulation in the form of chylomicrons, which are readily cleared in muscle and adipose tissue by lipoprotein lipase. There appeared to be little requirement for liver triglyceride synthesis on the EKD, as the VLDL cholesterol level stayed low and, as noted, total triglycerides did not rise. The diet, to be sure, was not designed to be particularly low in cholesterol, with a dietary composition allowing for accurate nitrogen determination and palatability being the two major concerns. Depending upon the specific meal choices selected, the daily cholesterol intake may have approached 2 g for some subjects. In addition, as the diet was essentially free of fiber, it is possible that the stool losses of cholesterol were decreased secondary to reduced intraluminal binding.

The directly measured 30% fall in the rate of glucose oxidation in resting, over-night fasted subjects after adaptation to the EKD is of interest in two respects. First, it indicates the level of metabolic fine-tuning possible in the sparing of carbohydrate substrate. In a state in which glucose oxidation is occurring at approximately 3 g/h at EBD-1, this is reduced to 2 g/h at EKD-4. This occurs despite an apparent maintenance of the resting metabolic rate across the diet change, as indicated by a stable rate of resting oxygen consumption at EKD-4 compared with EBD-1 (unpublished data). Although it was not measured directly, it is presumed that there was a major shift from glucose to ketone body oxidation by the brain, as occurs in total fasting.[3] A moderate decrease in blood glucose, coupled with a small rise in FFA concentration would also act to spare glucose oxidation as a result of the glucose-fatty acid cycle.[43]

Another point of metabolic interest in this study is the sharp decline in the T_3 level without an associated reduction in the resting oxygen uptake (as noted above) or symptoms of functional hypothyroidism (cold intolerance, dry skin, increased need for sleep). This suggests a dissociation between the T_3 level as measured and the metabolic rate. This phenomenon has recently been reported in a similar context by Otten et al,[44] and in a different context by Acheson and Berger.[45] This latter group blocked T_4 conversion to T_3 with iopanoic acid and demonstrated an unchanged metabolic rate by direct and indirect calorimetry.

A major motivation for the undertaking of this study was the indication from the experience of arctic explorers,[46,47] as well as previous work by this author and coworkers,[16] that human adaptation to carbohydrate restiction is a prolonged process. This is supported by the fact that in this study, while blood glucose, 3-hydroxybutyrate, and plasma free fatty acid levels as well as the nitrogen balance had stabilized by the end of the first week of the EKD, some measure of hormonal and metabolic adaptation continued to occur. This is indicated by the continued two-week fall in the serum T_3 levels, the slight decline in the serum bicarbonate levels for two weeks, the slight elevation in the serum insulin levels for three weeks of the EKD, and the lack of a plateau in the downward sloping plots of the serum uric acid and blood branched-chain amino acid levels.

In conclusion, this study demonstrates that the EKD, providing daily intakes of 1.75 g protein per kilogram and adequate mineral supplements, was well tolerated by lean, healthy male subjects. Nitrogen balance was regained after seven days, resulting in no significant change in weight or lean body mass after four weeks. Over this period, there were no changes in hepatic, renal, or cardiac function of clinical significance. Further, although glucose oxidation in subjects at rest after an overnight fast was measurably reduced compared with baseline values, metabolic adaptation to the EKD was incomplete after four weeks, as evidenced by continuing normalization of the fasting branched-chain amino acid and uric acid levels. Thus, it is not clear at what point in time the adaptation in substrate utilization and hormonal response is complete. Further investigation of the EKD for durations beyond the arbitrarily chosen four-week point of this study will yield a better understanding of the time required and the extent to which human metabolism adapts to chronic ketosis.

ADDENDUM

A recent report by Fery et al[48] examined the short-term (three-day) adaptation to eucaloric carbohydrate restriction using a diet similar to that reported here. Their results are in excellent agreement with the early adaptation data (EKD-1) from our study, except that these authors observed a fall in serum insulin with carbohydrate restriction analogous to that seen with fasting. The potential explanations for this difference are that the insulin response to this diet may differ from 3 to day 7; theirs was a mixed female-male subject population; and/or the probable difference in average level of aerobic training between the two

groups resulted in opposite insulin responses to similar diets.

ACKNOWLEDGMENT

We wish to thank Dr C. Maletskos of the M.I.T. Radioactivity Center for performing the ^{40}K counting, Dr Robert Wannemacher of the US Army Medical Research Unit at Ft. Detrick, MD for the insulin assay, and Dr Elliot Danforth Jr of the Metabolic Unit, University of Vermont College of Medicine, Burlington, VT for the T_3 assay. The authors are also indebted to Maureen Quirk for assistance in dietary planning; the staff of the M.I.T. Clinical Research Center for nursing support; Michael Willcutts and Dr William Rand for assistance with statistical evaluation; Mary Trerice, John Palombo, Mindy Sherman, Elvira Zalitis, Maureen O'Connell, Ann Marie Bielitz, and Dennis Rohrbach for technical assistance; Paula Zaborski for typing the manuscript; and the nine stalwart subjects who made the study possible.

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