

A double-blind, placebo-controlled test of 2 d of calorie deprivation: effects on cognition, activity, sleep, and interstitial glucose concentrations¹⁻⁴

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ABSTRACT

Background: Anecdotal information and limited research suggest that short-term caloric deprivation adversely affects cognition. However, this issue has not been studied using double-blind, placebo-controlled procedures, because the formulation of a calorie-deficient feeding regimen identical to one with calories is impossible using ordinary foods. Therefore, test meals varying in caloric content, but indistinguishable in sensory characteristics, were formulated using hydrocolloid-based gels as the principal structural component.

Objective: The purpose of this study was to examine the effects of 2 d of near-total caloric deprivation on cognitive function, satiety, activity, sleep, and glucose concentrations in a controlled environment.

Design: A double-blind, placebo-controlled crossover study of caloric deprivation was conducted in a controlled environment for 48 h. Cognitive function in 27 healthy young subjects was assessed repeatedly with standardized tests of vigilance, reaction time, learning, memory, logical reasoning, mood, and satiety. Wrist-worn monitors were used to assess ambulatory vigilance, activity, and sleep. Interstitial glucose concentrations were assessed continuously with a minimally invasive monitor.

Results: When the subjects received the near calorie-free diets, mean calorie consumption totaled 1311 kJ (313 kcal) over the testing period. During the fully fed treatment sessions, the subjects consumed a mean of 9612 kJ/d (2294 kcal/d), which matched their individual, daily energy requirements. Satiety and interstitial glucose concentrations were lower during the calorie-deprived diet ($P < 0.001$) than during the fully fed diet. There were no detectable effects of calorie deprivation on any aspect of cognitive performance, ambulatory vigilance, activity, or sleep. The mood states assessed, including fatigue, were not affected by calorie deprivation.

Conclusions: Cognitive performance, activity, sleep, and mood are not adversely affected in healthy humans by 2 d of calorie-deprivation when the subjects and investigators are unaware of the calorie content of the treatments. *Am J Clin Nutr* 2008;88:667-76.

INTRODUCTION

Little definitive information is available regarding the effects of short-duration calorie deprivation on cognitive state (for reviews see 1, 2). The effects of acute underfeeding on the cognitive performance of healthy nonobese individuals have not been

examined beyond the effects of a few (up to 3) missed meals. When adults are tested in such studies, the results are equivocal (3-5). The most common finding is impaired mood state, but, in some instances, positive effects of fasting on mood have been reported (6, 7). In several studies in which evidence of the adverse effects of missing several meals on cognitive performance were observed, the subjects had also not consumed any beverages; therefore, simultaneous food and fluid restriction was being assessed (8, 9).

To date, studies of calorie restriction on cognitive function have not been conducted using double-blind, placebo-controlled designs, because the formulation of a calorie-free feeding regimen that is identical to one with calories is impossible with ordinary foods. To address these shortcomings, test diets varying in calorie content were formulated with the use of hydrocolloid-based gels as the primary constituent. These meals were indistinguishable with regard to their sensory properties. In addition, to ensure that any effects observed were not related to the composition of the meals, 2 indistinguishable isocaloric, calorie-adequate diets were tested: a carbohydrate and a carbohydrate-fat regimen.

To quantify the effect of these meals on the physiologic state most closely identified with acute nutritional status, interstitial glucose concentrations, which are highly correlated with plasma glucose concentrations, were assessed continuously using the Continuous Glucose Monitoring System (CGMS), a minimally

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invasive ambulatory technology (10). Previous studies have shown that abnormally low glucose concentrations are associated with the rapid onset of decrements in cognitive performance (11–13). However, plasma glucose concentrations are tightly regulated in healthy individuals and usually only fall to abnormally low concentrations in patients with metabolic abnormalities, such as diabetes or in healthy subjects receiving exogenous insulin (11–14). Among individuals who were in a chronic state of semistarvation due to several months of severe undernutrition in harsh field conditions (mean weight loss of 14%), mean plasma glucose concentrations did not fall below 3.8 mmol/L (68.4 mg/dL) (15, 16). This is slightly above what is generally considered the lowest normal physiologic concentration of plasma glucose, ≈ 3.6 mmol/L (64.8 mg/dL) (17). Since it has been suggested that certain aspects of sleep and hunger may be regulated in part by some of the same hormones and sleep deprivation increases hunger, we also assessed the effects of caloric deprivation on sleep (18). The purpose of this study was to examine the effects of 2 d of near-total caloric deprivation on cognitive function, satiety, activity, sleep, and glucose concentrations in a controlled environment.

SUBJECTS AND METHODS

Subjects

Twenty-seven subjects (25 men and 2 women; mean age 23.6 ± 1.0 ; mean body weight 76.15 ± 2.0 kg) completed the study after providing informed consent. The study was approved by the local institutional review board. Four subjects withdrew because of illness or injury unrelated to the study. All subjects were healthy and had no evidence of diabetes, any other metabolic disorder, or heart disease. Subjects who indicated that they were on a weight-loss diet were excluded from participating. Before the start of each of the 3 test sessions, the subjects were required to abstain from caffeine and sedating antihistamines for ≥ 48 h and alcoholic beverages for 24 h.

Experimental design

A double-blind, placebo-controlled crossover design was used. Each subject participated on 3 occasions separated by ≥ 6 d. All subjects received, in a counterbalanced order, each experimental treatment: 1) a carbohydrate food condition, 2) a carbohydrate-fat combination food condition, and 3) a calorie-deprivation treatment consisting of nonnutritive foods (**Table 1**; See Appendix A under “Supplemental data” in the online issue).

Each subject’s daily energy intake during the fully fed treatment conditions was based on his or her calculated daily energy expenditure, as determined on the basis of 1) body weight, 2) the predefined daily activities occurring during testing, 3) metabolic assessment of the standardized treadmill exercise they engaged in during the study, and 4) resting metabolic rate (RMR).

During the test sessions, subjects were restricted to the testing facility and were under continuous investigator supervision to ensure dietary compliance and standardization of all activities and sleep. Environmental conditions were maintained between 20 and 22 °C and 40% and 60% humidity.

Preliminary testing

Before the first experimental test session, the subjects practiced all behavioral tests ≥ 3 times. In addition, each subject wore the CGMS glucose monitor and the vigilance monitor for 24 h to become familiar with these devices. RMR, when subjects were in a fasted state for ≥ 8 h, and energy expenditure, during controlled moderate exercise (walking on a treadmill), were determined during the preliminary phase of the study on the basis of oxygen consumption and carbon dioxide production using a metabolic cart (Parvomedics, Sandy, UT) or a portable metabolic unit (COSMED K4; COSMED, Rome, Italy).

Testing

Each test session lasted for 2.5 d (51 h) (**Table 2**). Subjects reported in the evening (day 0), consumed a balanced dinner meal consisting of normal foods (≈ 4185 kJ, or 1000 kcal; 20% protein, 45% carbohydrate, and 35% fat), and underwent baseline behavioral testing. A vigilance-activity monitor (VigMon II; Precision Control Design, Ft Walton Beach, FL) was placed on the subject’s nonpreferred wrist (19, 20). Ambulatory vigilance was assessed using this monitor at specified intervals when subjects were not engaged in behavioral tests or sleeping. The room lights were extinguished at 2300, and the subjects were awakened by 0630.

After awakening on day 1, the CGMS was placed on the subjects, and a breakfast meal was provided at 0800. The meal consisted of 1 of the 3 treatment conditions, and all subsequent test meals until dinner on day 2 had the same macronutrient composition. Different flavors and colors of each test diet were provided to make it more difficult for the subjects to distinguish between the different types of test meals (21). Water and non-caffeinated diet beverages were available ad libitum, and the subjects were frequently encouraged to consume these beverages

TABLE 1

Composition of a single experimental meal for each diet based on a 11 802-kJ (2820-kcal) mean daily energy requirement

	Diet		
	Carbohydrate	Carbohydrate-fat	Calorie-deprivation
		<i>kJ (kcal)</i>	
Gel	3776.1 (902.3)	3744.3 (894.7)	NA
Placebo gel	NA	27.2 (6.5)	99.2 (23.7)
Sugar-free beverage	31.4 (7.5)	31.4 (7.5)	31.4 (7.5)
Sugar-free gelatin	78.7 (18.8)	78.7 (18.8)	78.7 (18.8)
Sugar-free candy	25.1 (6.0)	25.1 (6.0)	25.1 (6.0)
Exercise beverage	20.9 (5.0)	20.9 (5.0)	20.9 (5.0)
Total	3932.2 (939.8)	3927.6 (938.7)	255.3 (61.0)

¹ NA, not available.

TABLE 2
Daily schedule¹

Time	Day 0	Day 1	Day 2
0600		Subject woke up, body weight measured, CGMS monitor placed	Subject woke up
0700		Blood sample drawn	Body weight measured, blood sample drawn
0800		Experimental meal, vigilance task	Experimental meal, vigilance task
0900		Behavioral testing	Behavioral testing
1000		Vigilance task	Vigilance task
1100		Moderate activity, POMS, vigilance task	Moderate activity, POMS, vigilance task
1200		Experimental meal	Experimental meal
1300		Blood sample drawn	Blood sample drawn
1400		Behavioral testing	Behavioral testing
1500			Moderate activity, POMS
1600		Moderate activity, POMS, vigilance task	Vigilance task
1700	Volunteers report, vigilance-activity monitor placed, body weight measured	Moderate activity, POMS	Behavioral testing
1800	Balanced meal	Experimental meal	Blood sample drawn
1900	Behavioral test practice	Blood sample drawn	Body weight measured, balanced meal
2000	Behavioral test practice	Behavioral testing	CGMS monitor removed, subjects released
2100	Behavioral test practice		
2200	Body weight measured	Body weight measured	
2300	Lights out	Lights out	

¹ POMS, Profile of Mood States; CGMS, Continuous Glucose Monitoring System.

to ensure that they remained fully hydrated. No snacks or any other food or beverages were permitted during the test session. At 0900, a 60-min behavioral test session, which included the Profile of Mood States (POMS) and Satiety Labeled Intensity Magnitude (SLIM) questionnaires, was conducted.

At 1100, 1 h of moderate physical activity was required. The activity consisted of walking on a treadmill at $\approx 40\text{--}45\%$ of the heart rate reserve as previously determined. Lunch was provided at 1200. At 1400, a behavioral test session identical to the first session was conducted. At 1600, another 1 h exercise session was conducted at an intensity level identical to that of the morning session. Dinner was provided at 1800, and a third behavioral test session was conducted at 2000. A similar schedule was followed on the second day of testing until 1700, when a final behavioral test session was conducted (Table 2). At the conclusion of all testing procedures, the subjects were fed a 4185-kJ (1000-kcal) meal and released.

Diets

The 3 diets—carbohydrate, carbohydrate-fat, and calorie-deprivation—were composed of specially prepared foods and liquid supplements as well as commercial products (Table 1). The principal components of all diets were gels varying in macronutrient composition but formulated so that their rheological properties (viscosity) and taste were comparable. By using hydrocolloids as the principal structural component of all the gel matrices, diets could be formulated that were sufficiently similar so that the double-blind condition of the study could be maintained. Before the study was conducted, various formulations of the gels were tested for palatability and acceptability, and the optimal versions were selected. In addition, blind taste tests were conducted to verify that the 3 gels were indistinguishable. The principal gel for the carbohydrate treatment consisted of starch and maltodextrin. The carbohydrate-fat gel was similar in composition to the carbohydrate treatment but included polyunsaturated lipids. A

hydrocolloid-based gel with artificial sweeteners and flavors was the principal component of the calorie-deprivation treatment.

Cognitive, sleep, mood, and satiety assessments

A battery of cognitive tests and self-report questionnaires were repeatedly administered (Table 2). The cognitive tests were selected to assess a spectrum of cognitive functions shown to be sensitive to nutritional and environmental manipulations (22–29). All tests were administered on notebook computers (CFV21P; Panasonic, Secaucus, NJ) unless otherwise noted.

Scanning Visual Vigilance Task

The Scanning Visual Vigilance Task assesses vigilance and the ability to sustain attention during long, boring, continuous tasks that generate minimal cognitive load (23, 25). The subjects continuously scanned a computer screen to detect an infrequent, difficult-to-detect stimulus that appears at random intervals and locations for 2 s. On average, a stimulus was presented once per minute. On detection of the stimulus, the subjects pressed the space bar as rapidly as possible. Whether a stimulus was detected and time required for detection was recorded. Responses before or after stimulus occurrence were false alarms. The test lasted 20 min.

Four-Choice Reaction Time Test

The Four-Choice Reaction Time Test assesses the ability to respond rapidly and accurately to simple visual stimuli. A series of visual stimuli at 1 of 4 different spatial locations were presented (22). The subjects indicated the correct spatial location of each stimulus by pressing 1 of 4 adjacent keys. Correct and incorrect responses, reaction time, premature errors (responding before stimulus presentation), and time-out errors (response latency > 1 s) were recorded. A total of 400 stimuli were presented and took 5 min to complete.

Repeated Acquisition Test

This test assesses learning and short-term memory (26). The subjects learned a sequence of 12 keystrokes using the 4 arrow keys of the computers. The outline of a rectangle was presented at the beginning of a trial. Each correct response filled in a portion (1/12th) of the rectangle from left to right with a solid square. A key press was considered to be correct when the subject made a response, which corresponded to the predetermined correct answer for that location (sequence) in the bar. Each incorrect key-stroke blanked the screen for 0.5 s. When the screen reappeared, the subject was at the same place in the rectangle. Subjects learned the correct sequence by trial and error. When a sequence was correctly completed, the rectangle was filled, and an empty rectangle reappeared for the next trial. A session ended when the subject completed 15 correct sequences. Incorrect keystrokes and time-to-complete each trial were recorded. The time to complete this task was ≈ 10 min.

Grammatical Reasoning Test

The Grammatical Reasoning Test was adapted from the Baddeley Grammatical Reasoning Test and assesses language-based logical reasoning. It has been used to assess the effects of various treatments on cognitive function (27, 30, 31). On each trial, the letters AB or BA follow a statement. The subjects decided whether or not each statement correctly described the order of the 2 letters. The "T" key on the keyboard was pressed for correct (statement is true), and the "F" key was pressed for incorrect (statement is false). Statements can be positive or negative or active or passive, and a given letter may precede or follow the other letter. A session consisted of 32 trials and took 5 min to complete.

Matching-to-Sample Test

This test assesses short-term spatial memory (working memory) and pattern recognition skills (24, 26). An 8×8 matrix of a red and green checkerboard pattern was presented for 10 s, then removed, and then followed by a variable delay of 8 or 16 s. Two matrices were then presented: the original matrix and a matrix with the color of 2 squares reversed. The subjects attempted to select the original matrix. The task consisted of 30 trials, ≈ 15 for each delay. A response (left or right arrow key) was required within 10 s, or a time-out error was recorded. Correct matches were recorded, as was reaction time. This test took 5 min to complete.

Psychomotor Vigilance Test

The Psychomotor Vigilance Test is a test of simple visual reaction time (32). A series of stimuli were presented at random intervals on a screen, and the subject responded as rapidly as possible when a stimulus appeared. Response time, false alarms, and the number of lapses (long duration responses) were recorded. Performance lapses refer to the instances when a subject failed to respond in <500 ms. This test was administered on a Personal Digital Assistant (PDA; Sony Clié NR70V; Sony Corp, New York, NY).

Procedure for monitoring vigilance, activity, and sleep

The monitors used were slightly larger than a wristwatch and were worn on the nondominant wrist (VigMon II; Precision Control Design Inc) (19, 20). Each contains a microprocessor,

nonvolatile memory, an accelerometer, and other sensors. They assess vigilance, assess patterns of rest and activity, and provide an indirect measure of sleep. The monitors were programmed to provide a vibratory stimulus at random intervals, averaging ≈ 10 times/h. A sequence of up to 3 stimuli, similar to the vibration of a pager or cell phone, was emitted. The subjects were required to push a small button on the monitor in response. The subjects were initially presented a difficult-to-detect stimulus (30% vibration for 200 ms) and given 6 s to respond. Then, a second slightly longer and more intense stimulus (60% vibration for 400 ms) was presented, and 5 s were given to respond. A third more intense stimulus (100% vibration for 1200 ms) was then presented, and 9 s was given to respond. As soon as the subject responded to any of the stimuli in a sequence, it was recorded as a "hit," and no additional stimuli were presented until the next stimulus sequence began. If the subject did not respond at all, it was recorded as a "miss." Correct responses, false alarms, and latency to respond were recorded. Monitors were worn throughout each test session, but the vigilance task was only activated at specific times.

Profile of Mood States Questionnaire

Mood states were assessed with the POMS, a standardized, 65-item, paper-and-pencil questionnaire consisting of 6 factor-analytically derived subscales: vigor-activity, fatigue-inertia, depression-dejection, confusion-bewilderment, tension-anxiety, and anger-hostility (33). The subjects were asked to rate each adjective with regard to how they were feeling "right now" on a scale of 0 (not at all) to 4 (extremely). It is sensitive to a wide variety of nutritional manipulations, environmental factors, sleep loss, and subclinical doses of various drugs (26, 34–36). It took <5 min to complete.

Satiety Labeled Intensity Magnitude Questionnaire

Satiety and hunger were assessed by using the Satiety Labeled Intensity Magnitude (SLIM) questionnaire once during each cognitive test session (37). The subjects rated their current level of satiety by placing a slash mark along a vertical line containing 11 descriptive phrases of satiety ranging from "greatest imaginable fullness" to "greatest imaginable hunger." The test was administered using paper and pencil.

Continuous interstitial glucose concentrations

The MiniMed CGMS (model MMT-7310X, Medtronic, Northridge, CA) was used to assess interstitial glucose concentrations (10). It is a waist-worn, pager-sized device that can be

TABLE 3
Energy and macronutrient intakes over 2 d of testing ($n = 27$)¹

	Diet		
	Carbohydrate	Carbohydrate-fat	Calorie-deprivation
Energy (kJ)	19132 \pm 466.3	19320 \pm 480.2	1311 \pm 37.3
(kcal)	4566 \pm 111.3	4611 \pm 114.6	313 \pm 8.9
Protein (g)	7.4 \pm 0.6	8.5 \pm 0.4	8.6 \pm 0.4
Carbohydrate (g)	1120.8 \pm 27.8	835.3 \pm 20.8	59.5 \pm 1.6
Fat (g)	0 \pm 0	134.9 \pm 3.4	0 \pm 0

¹ All values are $\bar{x} \pm$ SEM.



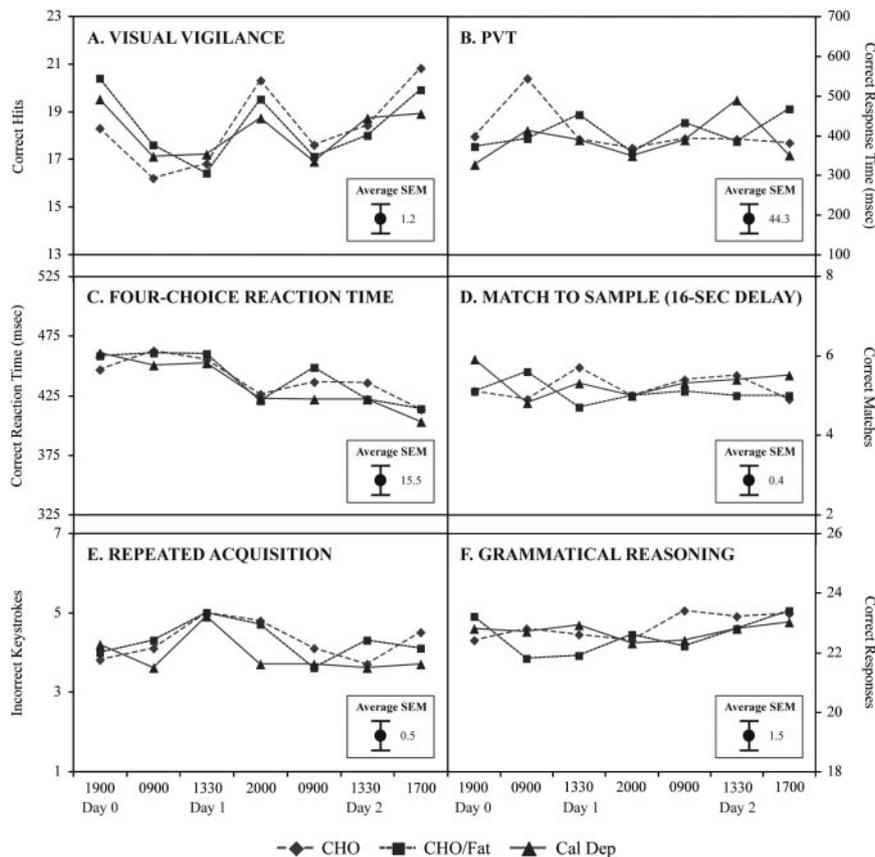


FIGURE 1. Mean cognitive performance in response to each treatment condition [carbohydrate (CHO), CHO-fat combination (CHO/Fat), and calorie-deprivation treatment consisting of nonnutritive foods (Cal Dep)] at all test sessions for the 6 cognitive tests administered on notebook computers or Personal Digital Assistants: Scanning Visual Vigilance Task, Psychomotor Vigilance Test (PVT), Four-Choice Reaction Time Test, Matching-to-Sample Test, Repeated Acquisition Test, and Grammatical Reasoning Test.

worn for up to 72 h to provide continuous information on glucose concentrations (38, 39). The device uses a glucose oxidase-based sensor to measure extracellular fluid glucose in subcutaneous tissue and is calibrated against corresponding blood glucose concentrations. The sensor is inserted through a sterile 22-gauge needle into the subcutaneous tissue of the anterior abdominal wall with the use of a spring-loaded device. The needle is withdrawn after insertion, and the sensor is taped in place. Sensor output is carried by cable to a pager-size monitor worn on a belt that analyzes sensor data every 10 s and records average values every 5 min. The data from the devices were downloaded to a personal computer. The devices were calibrated based on 3 blood samples collected daily (as shown in Table 2), which were analyzed by using the glucose oxidase method on a Beckman Coulter DXC600 (Beckman, Fullerton, CA). Insulin concentrations were also assessed in the blood sample by immunoassay with chemiluminescent detection with the Diamond Diagnostics DPC Immulite 2000 (Holliston, MA).

Body weight

Body weight was assessed periodically with a calibrated scale while the subjects were seminude (shorts and T-shirts) just after voiding.

Data analysis

Descriptive statistics were computed across treatment conditions and assessment times for each dependent variable with the

notebook computer and PDA-administered cognitive tests, the 6 factors of the POMS, and the SLIM questionnaire. For the wrist-worn vigilance monitor reaction time and correct response variables, means were calculated by summing the data collected during 3 discreet time intervals when the vigilance task was active: 0800–0845, 1045–1200, and 1600–1700. For the data collected by CGMS monitors, glucose concentrations were derived every 5 min based on a computer program (MiniMed CGMS, version 1.7A; Com-Station software, Northridge, CA), which fit raw CGMS output to glucose concentrations determined by the blood glucose assays. These data were then averaged into 3- or 4-h time blocks. Activity data were collected by the vigilance-activity monitor in 1-min blocks of time and downloaded for analysis by using the ACT Millennium and Action-W programs (Ambulatory Monitoring Inc, Ardsley, NY). Total activity counts during the waking day (0600–2300) and the sleep period (2300–0600 h) were derived, and descriptive statistics were computed. A validated algorithm determined, in 1-min intervals, whether the subject was awake or asleep (40, 41).

All data were analyzed with repeated-measures ANOVA, with within-subjects factors for treatment condition (carbohydrate, carbohydrate-fat, calorie-deprivation) and time. If a dependent variable was assessed on the evening of day 0, the data were included in the analysis. Post hoc analyses across treatment conditions were performed by using the Tukey test. When appropriate, selected individual contrasts were conducted by using *F* tests across the time factor at each treatment level. A measure of effect

TABLE 4

Statistical significance and effect size: cognitive, ambulatory, vigilance, and mood variables

	<i>P</i>		<i>P</i>		<i>P</i>	
	Treatment	Partial η^2	Time	Partial η^2	Treatment \times time	Partial η^2
Scanning Visual Vigilance Task						
Correct hits	0.906	0.004	0.000	0.179	0.274	0.045
False-positive results	0.100	0.085	0.934	0.012	0.277	0.044
Reaction time	0.985	0.001	0.566	0.030	0.235	0.047
Four-Choice Reaction Time Test						
Correct hits	0.662	0.016	0.000	0.144	0.078	0.060
Premature errors	0.294	0.046	0.000	0.148	0.515	0.035
Time-out errors	0.787	0.009	0.068	0.072	0.168	0.051
Correct reaction time (s)	0.829	0.007	0.000	0.319	0.453	0.037
Repeated Acquisition Test						
Incorrect keystrokes	0.572	0.022	0.001	0.143	0.683	0.030
Time-to-complete (s)	0.948	0.002	0.000	0.159	0.850	0.023
Grammatical Reasoning Test						
Correct responses	0.886	0.005	0.336	0.046	0.581	0.035
Incorrect responses	0.892	0.005	0.476	0.037	0.686	0.031
Time-out errors	0.813	0.009	0.008	0.113	0.746	0.029
Correct reaction time (s)	0.977	0.001	0.000	0.165	0.557	0.036
Matching-to-Sample Test (8-s delay)						
Correct matches	0.597	0.020	0.580	0.031	0.669	0.030
Time-out errors	0.493	0.028	0.188	0.056	0.181	0.052
Correct reaction time (s)	0.812	0.008	0.080	0.072	0.053	0.066
Matching-to-Sample Test (16-s delay)						
Correct matches	0.629	0.023	0.933	0.015	0.632	0.039
Time-out errors	0.800	0.011	0.436	0.047	0.167	0.065
Correct reaction time (s)	0.662	0.020	0.007	0.135	0.938	0.022
Palm Psychomotor Vigilance Test						
Correct responses	0.193	0.064	0.110	0.066	0.673	0.030
False starts	0.775	0.010	0.001	0.142	0.653	0.031
Correct response time (ms)	0.284	0.049	0.088	0.070	0.572	0.034
Vigilance Monitor						
Correct responses	<0.001	0.887	0.73	0.048	0.51	0.078
Reaction time (s)	0.52	0.064	0.36	0.101	0.99	0.024
Profile of Mood States						
Tension	0.341	0.040	0.000	0.150	0.248	0.046
Depression	0.707	0.013	0.031	0.084	0.478	0.036
Anger	0.878	0.005	0.001	0.137	0.495	0.035
Vigor	0.244	0.053	0.000	0.275	0.326	0.042
Fatigue	0.520	0.025	0.002	0.126	0.118	0.055
Confusion	0.468	0.029	0.114	0.063	0.211	0.048
Satiety Labeled Intensity Magnitude						
Score	<0.001	0.427	<0.001	0.671	<0.001	0.196

size, partial η^2 , was also computed. Statistical analyses were performed by using SPSS for WINDOWS, version 15.0 (SPSS Inc, Chicago, IL).

RESULTS

Mean energy and macronutrient intakes on each diet over the 2 test days are provided in **Table 3**. There were no changes in any aspect of the Scanning Visual Vigilance Task, Four-Choice Reaction Time Test, Repeated Acquisition Test results, Grammatical Reasoning Test, Matching-to-Sample Test, or Psychomotor Vigilance Test results associated with diet as demonstrated by the absence of significant main effects of diet and diet-by-time interactions (**Figure 1**; **Table 4**; *See Appendix B under "Supplemental data" in the online issue*). Ambulatory vigilance performance and all derived activity and sleep variables did not vary with diet, with the exception of vigilance correct responses, but

the absence of a significant diet-by-time interaction factor demonstrated that the effect was attributable to a pretreatment difference (**Table 4**; *See Appendixes A and B under "Supplemental data" in the online issue*). There were no significant changes in any aspect of the mood states assessed: vigor, fatigue, depression, confusion, tension, and anger associated with diet (**Figure 2**). Many aspects of cognitive performance tended to decline somewhat over time, probably reflecting boredom associated with confinement to the laboratory (**Figure 1**; *See Appendix B under "Supplemental data" in the online issue*). Changes in mood state over time corroborated these observations, with the mood states of tension, depression, anger, vigor, and fatigue significantly worsening over the course of each session (**Figure 2**; *See Appendix B under "Supplemental data" in the online issue*). However, it should be noted that a few variables on several tests, such as the Four-Choice Reaction Time Test, appeared to



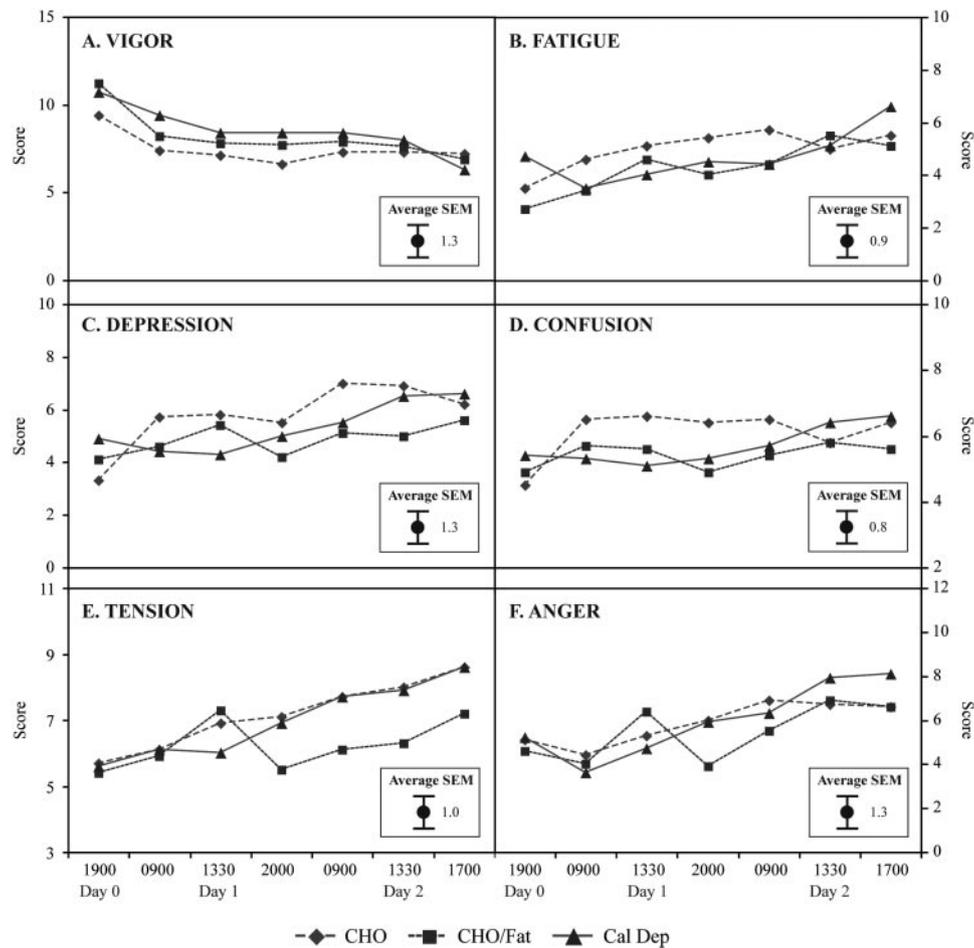


FIGURE 2. Mean mood state in response to each treatment condition [carbohydrate (CHO), CHO-fat combination (CHO/Fat), and calorie-deprivation treatment consisting of nonnutritive foods (Cal Dep)] at all test sessions as assessed by the Profile of Mood States: vigor, fatigue, depression, confusion, tension, and anger.

improve slightly over time, probably because of a practice effect. Table 4 also provides partial η^2 for each parameter, a measure of effect size.

Self-reported satiety, assessed with the SLIM questionnaire, varied with diet and time as demonstrated by a significant main effect of diet ($P < 0.001$), diet-by-time interaction ($P < 0.001$), and post hoc testing (Figure 3; Table 4). Levels of satiety were greater when subjects received the carbohydrate and carbohydrate-fat diets compared with the calorie-deprivation diet, but there were no differences in satiety when the carbohydrate diet was compared with the carbohydrate-fat diet (Figure 3).

Interstitial glucose concentrations varied as a function of diet (Figure 4). Consumption of the calorie-deprivation diet was associated with lower glucose concentrations ($P < 0.001$), and there were significant differences between the carbohydrate and carbohydrate-fat diets at various time intervals ($P < 0.001$; Figure 4). Plasma insulin also varied with diet; higher concentrations were observed when the subjects consumed the carbohydrate and carbohydrate-fat diets than when they consumed the calorie-deprivation diet ($P < 0.001$), but there were no significant differences between the calorically adequate diets. Exit interviews conducted with subjects at the conclusion of testing showed that the subjects were unable to reliably distinguish between treatment conditions.

Body weight at the conclusion of each test session varied as predicted on the basis of the caloric condition and absorptive rate (Table 5). A mean difference in weight loss of 0.8 kg between the calorie-deprivation condition and the fed conditions is consistent with the energy deficit induced by that treatment (5326 kJ, or 1272 kcal). The small decrease in weight (0.6 kg) in the 2 fully fed conditions over the 2 d of testing could be attributed to more rapid gastrointestinal emptying, which results when humans are fed liquid rather than solid diets (42, 43).

DISCUSSION

This study showed that 2 d of near-total calorie deprivation does not adversely affect the cognitive performance, activity, sleep, and mood of healthy young adults. The results are consistent with several previous studies that found that up to 3 missed meals had little effect on most aspects of the cognitive performance of healthy adults (4, 5). This study confirms and extends those findings by demonstrating that a variety of cognitive functions of healthy young adults, including vigilance, choice reaction time, learning, memory, and reasoning, as assessed by the tests used in this study, are resistant to calorie restriction for 2 full days. Unlike previous studies, neither the subjects nor the investigators were aware of the treatment conditions, and subjects

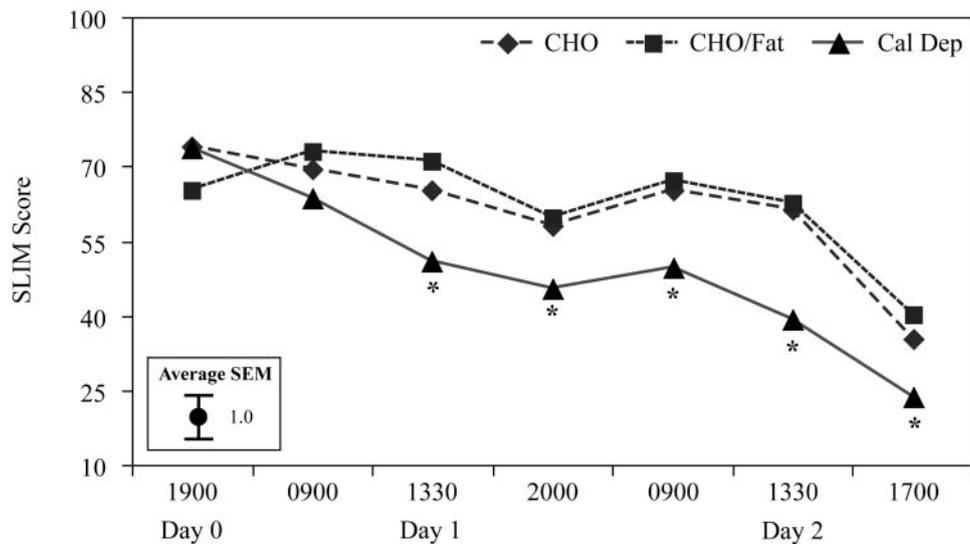


FIGURE 3. Change in Satiety Labeled Intensity Magnitude (SLIM) scores in response to all 3 treatment condition [carbohydrate (CHO), CHO-fat combination (CHO/Fat), and calorie-deprivation treatment consisting of nonnutritive foods (Cal Dep)] over the course of the study. Cal Dep was significantly different ($P < 0.05$) from the other 2 diet conditions at the times indicated by an asterisk. The time-by-treatment interaction was also significant ($P < 0.001$). CHO, carbohydrate; Cal Dep, calorie deprivation.

were continuously monitored to ensure that they fully complied with the experimental procedures. The tests used in this study were previously shown to be sensitive to a wide variety of nutritional and environmental factors, including caffeine, sleep loss, cold stress, and low doses of antihistamines (22–29, 33, 44).

The absence of deterioration in cognitive performance and mood was consistent with the relatively stable concentrations of interstitial glucose present during the 48 h of calorie restriction. In no instance did mean concentrations of glucose fall below clinically acceptable values (17). In studies of healthy subjects, in whom low concentrations of glucose have been associated with impaired cognitive function, glucose was reduced to clinically hypoglycemic concentrations by administration of exogenous insulin (11–14). Typically, glucose concentrations must fall

to 3.6 mmol^{-1} before degradation in cognitive performance is observed in either normal subjects or diabetic patients (14, 45, 46).

This study does not contradict studies conducted with total food and fluid restriction that find decrements in cognitive function during a single day of fasting, as the hydration state of subjects in the current study was maintained at normal levels as demonstrated by the lack of any significant differences in body weight across the diet groups beyond that predicted from the energy deficit. Food deprivation can induce mild dehydration unless investigators ensure maintenance of adequate hydration state because a substantial proportion of daily water intake is typically taken in food (47). Dehydration without food deprivation rapidly produces degradation in cognitive function in a few hours (48–50). Therefore, when impaired cognitive performance

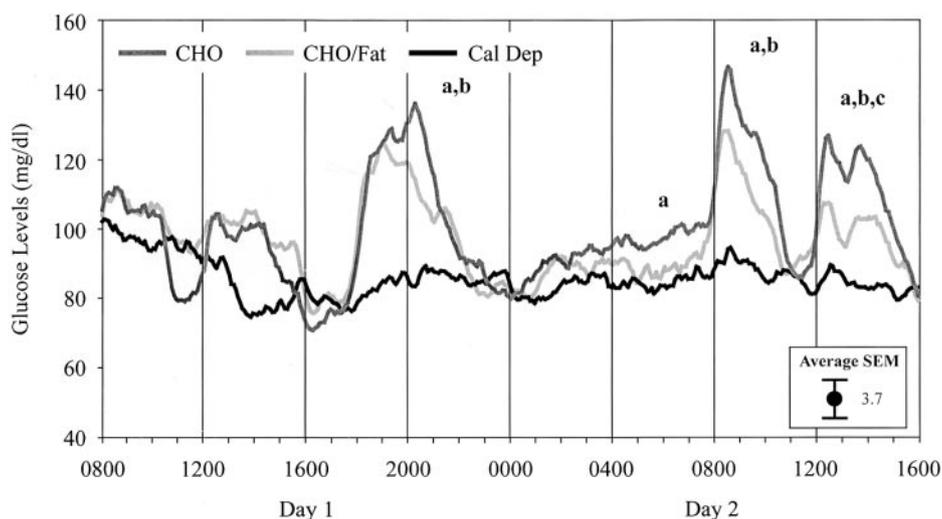


FIGURE 4. Interstitial glucose concentrations at 5-min intervals for all treatment conditions [carbohydrate (CHO), CHO-fat combination (CHO/Fat), and calorie-deprivation treatment consisting of nonnutritive foods (Cal Dep)]. Lowercase letters represent significant differences ($P < 0.05$) between specified treatment diets at 4-h time blocks: a, CHO/Fat compared with Cal Dep; b, CHO compared with Cal Dep; c, CHO/Fat compared with CHO. There were significant effects of treatment and time ($P < 0.001$) and a significant time-by-treatment interaction ($P < 0.001$). The vertical lines at 4-h intervals represent the blocks of time used for statistical analysis.



TABLE 5
Mean body weight of the subjects¹

Day and time	Diet		
	Carbohydrate	Carbohydrate-fat	Calorie-deprivation
Day 1, 0630 (kg)	76.7 ± 2.0 ¹	76.7 ± 2.0	77.1 ± 2.0
Day 2, 1830 (kg)	76.1 ± 1.9	76.1 ± 1.9	75.7 ± 1.9
Loss (kg)	0.6	0.6	1.4
Loss (%)	0.8	0.8	1.8

¹ $\bar{x} \pm \text{SEM}$ (all such values).

is observed in studies of individuals who are neither eating nor drinking, it is likely that the impairment is attributable to dehydration alone or to the combination of dehydration and food deprivation (9). The results of this study should not be interpreted to contradict those of studies in children and adolescents reporting that missed breakfast meals may impair cognitive function, because these populations have higher metabolic rates and less energy reserves (51, 52).

One finding of this study that appears to be inconsistent with previous work, and with anecdotal observations of some individuals, is the absence of an effect of food deprivation on mood state. In several previous studies, 24 h of food deprivation increased how jittery, nervous, or sad individuals reported feeling (4, 7). In this study, 48 h of food deprivation did not affect the POMS, a mood questionnaire known to be sensitive to the effects of a wide variety of nutritional and other factors, including low doses of caffeine, over-the-counter allergy medications (diphenhydramine), sleep deprivation, and environmental stress (21, 23, 26–28, 34–36). The lack of effects on mood state in this study are consistent with the lack of effects of calorie restriction on cognitive performance observed in this and other studies, because degradation in cognitive performance is typically associated with degraded mood state (21, 53, 54). It therefore appears that the double-blind, placebo-controlled procedures used in this study prevented subjects, based on their expectations, from feeling that they “felt worse” when in a calorie-restricted state (21). As noted above, exit interviews indicated that subjects were unable to distinguish the treatment conditions. The absence of differences in mood across conditions confirms that individuals were blind to the treatment condition. Subjects as a group reported differences in satiety across treatment conditions; but to distinguish treatment conditions based on the perception of satiety would require subjects to remember their satiety levels and compare them accurately across several weeks of testing.

The results of the cognitive performance, activity, sleep, and mood measures used in this study are internally consistent, because the comparison of the calorie-deprivation diet with 2 different control diets, which varied in macronutrient composition but were of equal calorie content, resulted in identical negative results. Furthermore, administration of the energy-balanced diets, carbohydrate alone or carbohydrate and fat, were themselves indistinguishable with regard to effects on cognitive performance, mood, or appetite.

The differences in interstitial glucose concentrations between the calorie-adequate and calorie-deprivation diets were as expected. In addition, the higher peak concentrations of glucose produced after some of the carbohydrate-fat meals than after the

matching carbohydrate meals are consistent with previous reports (55, 56). One limitation of this study was the limited number of cognitive performance tests. Although the test battery used assessed a wide range of cognitive functions, from simple to complex, it was not possible to assess all functions potentially affected by calorie deprivation. Future studies of calorie deprivation should measure variables not assessed in the current study. It should also be noted that sleep was assessed indirectly by using wrist-based activity monitors, which are not as definitive a measure of sleep compared with waking state as is polysomnography. Also, the restrictive environment of the laboratory may have limited this study's ability to detect changes in activity associated with calorie deprivation.

It should be noted that preservation of cognitive function during periods of restricted availability of food is a highly adaptive mechanism. If adult human brain function rapidly deteriorated as a consequence of underfeeding, the ability to obtain food would be significantly degraded, which from a survival or evolutionary perspective would not be desirable.

We thank Paul Maguire and Holly McClung for the development and preparation of the hydrocolloid-based gels. The subjects participated in these studies after giving their free and informed voluntary consent. The investigators adhered to the policies for protection of human subjects as prescribed in Army Regulation 70-25, and the research was conducted in accordance with the provisions of 32 CFR Part 219.

The authors' responsibilities were as follows—HRL: designed and directed all aspects of the study and wrote the manuscript; CMC: responsible for the overall conduct of the study and contributed to the manuscript; PJN: contributed to the data management and analysis and manuscript preparation; GEA: responsible for selected behavioral test procedures; MDK and BCN: responsible for the design and conduct of the biochemical aspects of the study; and FMK: provided guidance regarding the experimental design and the statistical analyses. None of the authors had a personal or financial conflict of interest.

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