Gluconeogenesis and energy expenditure after a high-protein, carbohydrate-free diet[1–3]

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ABSTRACT

Background: High-protein diets have been shown to increase energy expenditure (EE).

Objective: The objective was to study whether a high-protein, carbohydrate-free diet (H diet) increases gluconeogenesis and whether this can explain the increase in EE.

Design: Ten healthy men with a mean (±SEM) body mass index (in kg/m^2) of 23.0 ± 0.8 and age of 23 ± 1 y received an isoenergetic H diet (H condition; 30%, 0%, and 70% of energy from protein, carbohydrate, and fat, respectively) or a normal-protein diet (N condition; 12%, 55%, and 33% of energy from protein, carbohydrate, and fat, respectively) for 1.5 d according to a randomized crossover design, and EE was measured in a respiration chamber. Endogenous glucose production (EGP) and fractional gluconeogenesis were measured via infusion of [6,6-$^2H_2$]glucose and ingestion of ^2H_2O; absolute gluconeogenesis was calculated by multiplying fractional gluconeogenesis by EGP. Body glycogen stores were lowered at the start of the intervention with an exhaustive glycogen-lowering exercise test.

Results: EGP was lower in the H condition than in the N condition (181 ± 9 compared with 226 ± 9 g/d; $P < 0.001$), whereas fractional gluconeogenesis was higher (0.95 ± 0.04 compared with 0.64 ± 0.03; $P < 0.001$) and absolute gluconeogenesis tended to be higher (171 ± 10 compared with 145 ± 10 g/d; $P = 0.06$) in the H condition than in the N condition. EE (resting metabolic rate) was greater in the H condition than in the N condition (8.46 ± 0.23 compared with 8.12 ± 0.31 MJ/d; $P < 0.05$). The increase in EE was a function of the increase in gluconeogenesis ($\Delta EE = 0.007 \times \Delta gluconeogenesis - 0.038$; $r = 0.70$, $R^2 = 0.49$, $P < 0.05$). The contribution of Δgluconeogenesis to ΔEE was 42%; the energy cost of gluconeogenesis was 33% (95% CI: 16%, 50%).

Conclusions: Forty-two percent of the increase in energy expenditure after the H diet was explained by the increase in gluconeogenesis. The cost of gluconeogenesis was 33% of the energy content of the produced glucose. *Am J Clin Nutr* 2009;90:519–26.

INTRODUCTION

Gluconeogenesis, ie, the formation of glucose from noncarbohydrate precursors, remains relatively stable in widely varying metabolic conditions in humans, as was concluded in a recent review by Nuttall et al (1). In the overnight postabsorptive state, circulating glucose is derived from endogenous glucose production, which consists of 2 processes: glycogenolysis (ie, the release of glucose from stored glycogen) and gluconeogenesis. Thus, a change in the glucose production rate in varying metabolic conditions is supposed to be mostly dependent on the rate of glycogenolysis and not gluconeogenesis (1).

However, in rats, gluconeogenesis has been shown to be stimulated when glucose availability was reduced during fasting or with a low-carbohydrate or carbohydrate-free diet; moreover, gluconeogenesis was increased by a high-protein diet (2, 3). Azzout-Marniche et al (4) showed that an increase in the protein content of the diet in rats changed the activity of the enzymes phosphoenolpyruvate carboxykinase and glucose 6-phosphatase, which suggests that liver gluconeogenesis is stimulated by a high-protein diet. In the fed state glucose 6-phosphate was directed toward glycogen synthesis, whereas in the fasted state it was converted to glucose and released from the hepatocyte.

High-protein diets were previously shown to increase energy expenditure (EE) in healthy human volunteers (5–11). Gluconeogenesis has been hypothesized to contribute to this increased EE after a high-protein diet (5, 6, 9, 12). Although gluconeogenesis is thought to be relatively stable in humans, a high-protein diet, especially in the absence of carbohydrates, may stimulate gluconeogenesis (13). Because gluconeogenesis is an energetically costly pathway of protein metabolism with energy costs that are estimated to amount to 20% (6, 12), this process may contribute to an increased EE after a high-protein diet or after a high-protein, carbohydrate-free diet.

The objective was to study whether a high-protein, carbohydrate-free diet (H diet) increases gluconeogenesis and whether this can explain the increase in EE. Therefore, gluconeogenesis and EE were measured when healthy subjects consumed an H diet or a normal-protein (N) diet. To obtain the same baseline condition and to contrast the effects of the 2 diets, body glycogen stores were depleted beforehand by means of an exhaustive glycogen-lowering exercise test. Glucose and insulin concentrations were measured to test whether there was a difference in circulating glucose concentrations and whether the effects on gluconeo-

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genesis could be mediated by insulin—a factor known to influence gluconeogenesis (14).

SUBJECTS AND METHODS

Subjects

Ten healthy men [body mass index (kg/m^2): 23.0 ± 0.8; age: 23 ± 1 y] were recruited by advertisements placed on notice boards at the university. All subjects underwent a medical screening and all were in good health, nonsmokers, not using medication, and at most moderate alcohol users (≤10 times/wk). Characteristics of the subjects are presented in **Table 1**. Written informed consent was obtained from all participants. The study was approved by the Medical Ethics Committee of the Maastricht University Medical Center. Subject recruitment started in June 2007, and the study was conducted between September 2007 and July 2008.

Maximal power output

After a medical screening, the subjects performed an incremental exhaustive exercise test according to the protocol of Kuipers et al (15) on an electronically braked cycle ergometer (Lode Excalibur, Groningen, Netherlands) to determine maximal power output (W_{max}). Exercise was performed until voluntary exhaustion or until the subject could no longer maintain a pedal rate of >60 rpm. Heart rate was measured continuously with a Polar Sport tester (Polar, Kempele, Finland). Subjects started cycling at 100 W for 5 min. Thereafter, the workload was increased by 50 W every 2.5 min. When heart rate exceeded 160 beats/min, the workload increment was reduced to 25 W/2.5 min. For each subject, W_{max} was calculated as follows:

$$W_{max} = 100 + a \ldots 50 + b \ldots 25 + c \ldots 5 \qquad (1)$$

where a is the number of completed steps at 50 W, b is the number of completed steps at 25 W, and c is the time of the final uncompleted load increment (to the nearest 0.5 min).

Study design

The study had a single-blind, randomized, crossover design. Subjects completed two 36-h sessions in a respiration chamber to measure EE and substrate oxidation when subjects were consuming either an H diet or an N diet. Endogenous glucose production and gluconeogenesis were measured immediately afterward in the postabsorptive state. On both occasions, after a basal blood sample was collected to determine natural abundance, the session started with an exhaustive glycogen-lowering exercise test based on the subject's individual W_{max} in the afternoon (day 1). After a 1.5-d stay in the respiration chamber, endogenous glucose production and gluconeogenesis were measured in the morning on day 3. The 2 sessions were conducted 8 wk apart to preclude influences of enrichment derived from the previous experiment. A flow chart of the experimental session is shown in **Figure 1**.

TABLE 1
Characteristics of the 10 male subjects[1]

	Value
Age (y)	23 ± 1
W_{max} (W)	294 ± 14
Height (m)	1.81 ± 0.02
Weight (kg)	75.5 ± 3.2
BMI (kg/m^2)	23.0 ± 0.8
Body fat (%)	18.0 ± 1.7

[1] All values are means ± SEMs. W_{max}, maximal power output.

The macronutrient compositions of the H and N diets were 30%, 0%, and 70% and 12%, 55%, and 33% of energy from protein, carbohydrate, and fat, respectively. In the H condition, protein intake was 170 ± 5 g/d, carbohydrate intake was 2 ± 0 g/d, and fat intake was 179 ± 5 g/d. Lettuce and mushrooms accounted for a carbohydrate intake of 1.6 ± 0.0 g/d. In the N condition, protein intake was 63 ± 2 g/d, carbohydrate intake was 323 ± 9 g/d, and fat intake was 87 ± 2 g/d. When expressed per kilogram body weight, protein intakes were 2.27 ± 0.06 and 0.84 ± 0.02 g in the H and N conditions, respectively.

A detailed composition of the diet is presented in **Table 2**. To ensure that the perceived appeal of all food items was acceptable and similar between subjects, it was determined beforehand whether the subjects liked all of the food items sufficiently. Subjects were provided with a list of all food items that were to be used in the experiments and had to rate the food items. Food items that were not liked sufficiently (<60 mm on a 100-mm visual analog scale) were replaced by other sufficiently liked food items. The subjects were instructed that, during the experiments, all food items that were offered had to be eaten completely. On the days before the experiments, the subjects consumed their habitual diet. On the last day before an experimental session, the subjects consumed the same diet in both conditions.

Energy intake

During each experimental session, the subjects were fed in energy balance. The energy content of the first dinner and

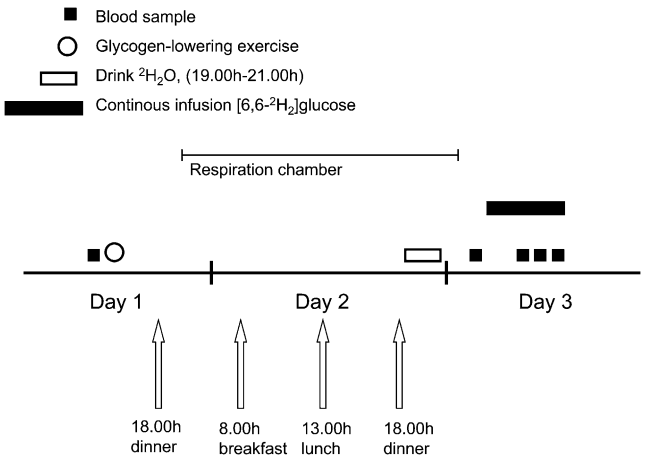

FIGURE 1. Flow chart of an experimental session of the study in which 10 healthy men received a high-protein, carbohydrate-free diet (30%, 0%, and 70% of energy from protein, carbohydrate, and fat, respectively) or a normal-protein diet (12%, 55%, and 33% of energy from protein, carbohydrate, and fat, respectively) for 1.5 d in a randomized crossover design. Energy expenditure was measured continuously in a respiration chamber. Body glycogen stores were lowered beforehand by means of an exhaustive glycogen-lowering exercise test. Postabsorptive endogenous glucose production and fractional gluconeogenesis were measured via combined infusion of [6,6-$^2H_2$]glucose and ingestion of ^2H_2O.

TABLE 2
Composition of the meals in the normal-protein (N) diet and in the high-protein, carbohydrate-free (H) diet

N diet[1]	H diet[2]
Breakfast	Breakfast
Whole-wheat bread	Boiled egg
Low-fat margarine	Bacon
Chocolate spread	Coffee (decaffeinated)/tea
Confiture	Lunch
Coffee (decaffeinated)/tea	Soup
Lunch	Salami
Soup	Tuna
Whole-wheat bread	Garden cress
Low-fat margarine	French cheese
Chocolate spread	Lettuce
Cheese	Mushrooms
Lettuce	Olive oil
Cucumber	Sugar-free syrup
Olive oil	Dinner
Grape juice	Soup
Dinner	Chicken meat
Soup	Tuna
Chinese noodle dish	Garden cress
Cucumber	Cheese
Olive oil	Lettuce
Mixed fruit	Mushrooms
Grape juice	Olive oil
	Sugar-free syrup

[1] Macronutrient composition: 12%, 55%, and 33% of energy from protein, carbohydrate, and fat, respectively.
[2] Macronutrient composition: 30%, 0%, and 70% of energy from protein, carbohydrate, and fat, respectively.

breakfast of the first experimental session was based on the basal metabolic rate (BMR), as calculated with the equation of Harris and Benedict (16), multiplied by an activity index of 1.35. To determine the appropriate level of energy intake to attain energy balance in the respiration chamber, the sleeping metabolic rate (SMR) was calculated during the first night and multiplied by an activity index of 1.35. Energy intake was divided over the meals as 20% for breakfast (0800), 40% for lunch (1300), and 40% for dinner (1800). The subjects did not eat anything after dinner on day 2 until the end of the experiment on day 3.

Glycogen-lowering exercise test

To lower body glycogen stores, the subjects performed a glycogen-lowering exercise test on an electronically braked cycle ergometer. After warming up at 50% of their W_{max} for 5 min, the subjects cycled for 2 min at 90% of their W_{max} followed by 2 min at 50% of their W_{max}; this cycle repeated until the subjects were no longer able to maintain the high-intensity exercise. The maximal intensity was then lowered to 80% of W_{max}. When this intensity also could no longer be maintained, the maximal intensity was decreased to 70% of W_{max}. The test was ended after exhaustion (17). The subjects were allowed to consume water during the exercise test. Heart rate was monitored continuously during the exercise with a Polar Sport tester.

Indirect calorimetry

Oxygen consumption and carbon dioxide production were measured in the respiration chamber (18). The respiration chamber is a 14-m^3 room furnished with a bed, chair, computer, television, DVD player, telephone, intercom, sink, and toilet. The room was ventilated with fresh air at a rate of 70–80 L/min. The ventilation rate was measured by using electronically modified dry gas meters (G6; Schlumberger, Dordrecht, Netherlands). The analysis system consisted of dual pairs of infrared carbon dioxide (ABB/Hartman&Braun Uras, Frankfurt aM, Germany) and paramagnetic oxygen analyzers (model 4100; Servomex, Crowborough, United Kingdom). Data were acquired by using custom-built interfaces (IDEE; Maastricht University, Maastricht, Netherlands), a computer (Apple Macintosh, Cupertino, CA), and graphic programming environment (Labview; National Instruments, Austin, TX).

Energy expenditure and substrate oxidation

EE and carbohydrate, fat, and protein oxidation were calculated from the measurements of oxygen consumption, carbon dioxide production, and urinary nitrogen excretion by using the formula of Brouwer (19). Urinary nitrogen excretion was measured during two 12-h periods: from 0700 on day 2 until 1900 on day 2 and from 1900 on day 2 until 0700 on day 3. Samples were collected in containers with 10 mL H_2SO_4 to prevent nitrogen loss through evaporation. Volumes and nitrogen concentrations were measured, the latter with a nitrogen analyzer (Elemental Analyzer; CHN-O-Rapid, Heraeus, Wellesley, MA).

The 24-h EE (total EE; TEE) consists of SMR, diet-induced thermogenesis, and activity-induced EE (AEE). EE and the 24-h respiratory quotient (RQ) were measured from 0700 on day 2 until 0630 on day 3. Activity was monitored by using a radar system based on the Doppler principle. SMR was defined as the lowest mean EE measured over 3 consecutive hours between 0000 and 0600. Resting metabolic rate (RMR) was calculated by plotting EE against radar output. The intercept of the regression line at the lowest radar output represents the EE in the inactive state (RMR) and consists of SMR and diet-induced thermogenesis (11). Diet-induced thermogenesis was calculated by subtracting SMR from RMR. AEE was calculated by subtracting SMR and diet-induced thermogenesis from 24-h EE. Physical activity level (PAL) was calculated by dividing TEE by SMR, and energy balance was calculated by subtracting EE from energy intake.

Body composition

Body composition was determined with a 3-compartment model using the hydrodensitometry and deuterium dilution (^2H_2O) technique (20, 21) and was calculated by using the combined equation of Siri (22).

Endogenous glucose production and fractional gluconeogenesis

Infusion of [6,6-$^2H_2$]glucose and ingestion of ^2H_2O were combined to measure endogenous glucose production and fractional gluconeogenesis. Glucose produced by gluconeogenesis after ingestion of ^2H_2O was labeled with deuterium at the C5 position. Glucose molecules produced by gluconeogenesis and glycogenolysis were labeled with deuterium at the C2 position. The ratio of C5 to C2 enrichment of glucose represents fractional gluconeogenesis. C2 enrichment equals the plasma

^2H_2O enrichment when in the steady state, as was shown by Landau et al (23). Therefore, plasma ^2H_2O enrichment was measured instead of the C2 enrichment of glucose.

To measure fractional gluconeogenesis, the subjects ingested ^2H_2O (99% enriched; Campro Scientific, Berlin, Germany) every 0.5 h between 1900 and 2100 on day 2, up to a total dose of 5 g/kg body water, to achieve a plasma ^2H_2O enrichment of ≈0.5%. Body water was estimated to be 73% of body fat-free mass. Water consumed during the remainder of the study was enriched with 0.5% ^2H_2O to maintain isotopic steady state.

On day 3, a Venflon catheter (Becton Dickinson, Franklin Lanes, NJ) was placed in a superficial dorsal vein of the hand for blood sampling, and another Venflon catheter was placed in a superficial vein of the other arm for intravenous infusion. The hand was placed in a thermostatically controlled hot box at 60°C to obtain arterialized venous blood samples. A blood sample was taken at 0745 to measure the natural abundance of [6,6-$^2H_2$]glucose and glucose and insulin concentrations. Immediately afterward, a primed continuous infusion of [6,6-$^2H_2$]glucose (99% enriched; Cambridge Isotopes, Andover, MA) was started at a rate of 0.11 μmol/kg per minute (prime 11 μmol/kg). At 130, 140 and 150 min after the start of the infusion, blood samples were taken to measure enrichment of [6,6-$^2H_2$]glucose, plasma ^2H_2O enrichment, and deuterium enrichment at the C5 position of glucose.

Gas chromatography and mass spectrometry

Plasma ^2H_2O enrichment was measured by using isotope ratio mass spectroscopy (Optima; Micromass, Manchester, United Kingdom). Enrichments of plasma [6,6-$^2H_2$]glucose and deuterium at the C5 position of glucose were determined as described previously (24). Briefly, the enrichment of plasma [6,6-$^2H_2$]glucose was measured as the aldonitril pentaacetate derivative of glucose in deproteinized plasma. Glucose was monitored at *m/z* values of 187 and 189. The enrichment of [6,6-$^2H_2$]glucose was determined by dividing the peak area of *m/z* 189 by the peak area of *m/z* 187, ie, calculating the *M*+2 tracer-to-tracee ratio and correcting it for natural abundance. To measure deuterium enrichment at the C5 position of glucose, glucose was converted to hexamethylenetetramine as previously described by Landau et al (23). Hexamethylenetetramine was injected into a gas chromatograph–mass spectrometer and was separated on an AT-amine column [30 m × 0.25 mm, film thickness (d_f) 0.25 μm]. Isotopic enrichments were measured on a gas chromatograph–mass spectrometer (model 6890 gas chromatograph coupled to a model 5973 mass selective detector, equipped with electron impact ionization mode; Hewlett-Packard, Palo Alto, CA).

Glucose and insulin concentrations

Blood was distributed into EDTA-containing tubes for measurement of glucose and insulin concentrations. Blood samples were centrifuged at 4°C for 10 min at 3000 rpm. All samples were stored at −80°C until further analysis. Plasma glucose concentrations were measured by using the hexokinase method (Glucose HK 125 kit; ABX Diagnostics, Montpellier, France). Insulin concentrations were measured by using radioimmunoassay (Linco Research Inc, St Charles, MO).

Calculation and statistical analysis

Endogenous glucose production was calculated by dividing the infusion rate of [6,6-$^2H_2$]glucose by the resulting *M*+2 tracer-to-tracee ratio of plasma aldonitril pentaacetate glucose. This was done after correction for natural abundance by subtracting the natural abundance from the measured *M*+2 enrichment and after ascertaining that the *M*+2 tracer/tracee ratios were in steady state. Fractional gluconeogenesis was calculated by dividing deuterium enrichment at the C5 position of glucose by plasma ^2H_2O enrichment. The absolute rate of gluconeogenesis was calculated by multiplying fractional gluconeogenesis by glucose production (24). The mean of the 3 values obtained 130, 140, and 150 min after the start of the infusion was calculated.

Data are expressed as means ± SEMs unless otherwise indicated. A paired *t* test was carried out to test for differences in endogenous glucose production, fractional gluconeogenesis, absolute gluconeogenesis, concentrations of glucose and insulin, EE, and macronutrient balances between the H and N conditions. Furthermore, a paired *t* test was carried out to test whether energy and macronutrient balances were significantly different from zero. To study the possible relation between gluconeogenesis and EE, the difference in gluconeogenesis between the high-protein, carbohydrate-free diet and the normal diet (Δgluconeogenesis), and the difference in EE between the high-protein, carbohydrate-free diet and the normal diet (ΔEE) was calculated. These values were corrected for a potential order of treatment effect by subtracting the mean value of Δgluconeogenesis or ΔEE of individuals with the same order of treatment of each individual value of Δgluconeogenesis or ΔEE, respectively. The values had a normal distribution. Pearson's correlation coefficient was used to test whether there was a linear correlation between Δgluconeogenesis and ΔEE. Subsequently, a linear regression analysis was performed to obtain more information about the exact relation between Δgluconeogenesis and ΔEE. A P value <0.05 was regarded as statistically significant. Statistical procedures were performed by using SPSS 15.0 (SPSS Inc, Chicago, IL).

RESULTS

Endogenous glucose production and gluconeogenesis

Endogenous glucose production, ie, glucose derived from glycogenolysis and from gluconeogenesis, was lower when subjects were in the H condition than when subjects were in the N condition (181 ± 9 g/24 h compared with 226 ± 9 g/24 h; $P < 0.001$), whereas fractional gluconeogenesis was higher (0.95 ± 0.04 compared with 0.64 ± 0.03; $P < 0.001$). As a result, absolute gluconeogenesis tended to be higher when subjects were in the H condition than when subjects were in the N condition (171 ± 10 g/24 h compared with 145 ± 10 g/24 h; $P = 0.06$).

Glucose and insulin concentrations

The fasting blood glucose concentration was lower when subjects were in the H condition than when subjects were in the N condition (4.43 ± 0.13 mmol/L compared with 5.07 ± 0.10 mmol/L; $P < 0.001$). There was no significant difference in fasting insulin concentrations between the H and N conditions

(11.02 ± 3.01 mU/L compared with 13.88 ± 2.12 mU/L, respectively).

Energy expenditure

Energy intake was 10.27 ± 0.28 MJ in the H and the N conditions, and the subjects were in energy balance in both conditions. EE and its components are shown in **Table 3**. RMR was greater in the H condition than in the N condition (8.46 ± 0.23 MJ compared with 8.12 ± 0.31 MJ; $P < 0.05$).

Substrate utilization

The 24-h RQ was lower in the H condition than in the N condition (0.76 ± 0.01 compared with 0.85 ± 0.01; $P < 0.001$). There was a significant difference in protein, carbohydrate, and fat balances between the 2 conditions ($P < 0.001$; **Table 4**). In the H condition, the subjects were in a positive protein balance ($P < 0.001$) and a negative carbohydrate balance ($P < 0.01$), whereas, in the N condition, the subjects were in a negative protein balance ($P < 0.01$), a positive carbohydrate balance ($P < 0.01$), and a negative fat balance ($P < 0.05$).

Energy costs of gluconeogenesis

There was a linear correlation between Δgluconeogenesis and ΔEE: Pearson's correlation coefficient was 0.70 ($P < 0.05$). The equation of the relation between Δgluconeogenesis and ΔEE was as follows:

$$\Delta EE = 0.007 \times \Delta\text{gluconeogenesis} - 0.038 \qquad (2)$$

where ΔEE is the difference in EE (in MJ) between the H condition and the N condition, and Δgluconeogenesis is the difference in absolute gluconeogenesis (in g) between the H condition and the N condition ($r = 0.70$, $R^2 = 0.49$, $P < 0.05$; **Figure 2**).

On average, in the H condition, 26 g extra glucose was produced through gluconeogenesis that resulted in an increase in EE of 0.144 MJ. The increase in EE after the H diet compared with the N diet was 0.340 ± 0.132 MJ. Thus, the contribution of increased gluconeogenesis to increased EE was 42%. Because the energy content of 26 g glucose is 0.442 MJ, the energy cost to produce glucose through gluconeogenesis was 33% of the energy content of glucose (95% CI: 16%, 50%).

TABLE 3
Energy expenditure in 10 healthy men during consumption of a normal-protein (N) diet or a high-protein, carbohydrate-free (H) diet for 36 h after an exhaustive glycogen-lowering exercise test[1]

	N diet[2]	H diet[3]
	MJ/d	*MJ/d*
Total energy expenditure	10.06 ± 0.34	10.09 ± 0.31
Sleeping metabolic rate	7.38 ± 0.23	7.50 ± 0.25
Resting metabolic rate	8.12 ± 0.31	8.46 ± 0.23[4]
Diet-induced thermogenesis	0.74 ± 0.10	0.96 ± 0.12
Activity-induced thermogenesis	1.94 ± 0.17	1.63 ± 0.15
Energy balance[5]	0.21 ± 0.17	0.17 ± 0.12

[1] All values are means ± SEMs.
[2] Macronutrient composition: 12%, 55%, and 33% of energy from protein, carbohydrate, and fat, respectively.
[3] Macronutrient composition: 30%, 0%, and 70% of energy from protein, carbohydrate, and fat, respectively.
[4] Significantly different from the N diet group, $P < 0.05$ (paired t test).
[5] Energy balance = 24-h energy intake − 24-h energy expenditure (total energy expenditure).

DISCUSSION

Both gluconeogenesis and EE increased when healthy subjects with low body glycogen stores were consuming the H diet for 1.5 d. The increase in EE was a function of the increase in gluconeogenesis. A major part, 42%, of the increased EE during the H diet was explained by increased gluconeogenesis. The plasma insulin concentration was not affected differently by the 2 diets, nor was there a relation between the change in insulin concentration and the change in gluconeogenesis after the 2 diets. Although insulin is known to be able to influence gluconeogenesis (14), insulin was not responsible for a change in glucose production or gluconeogenesis in the present study.

The infusion of [6,6-$^2H_2$]glucose combined with ingestion of ^2H_2O is a valid method for assessing postprandial endogenous glucose production and fractional gluconeogenesis (23, 25, 26). An equilibration time of 15 h has been shown to be sufficient for ^2H_2O to be equally distributed throughout the body water and to measure gluconeogenesis in a steady state (27). Although gluconeogenesis was previously shown to be relatively stable under various metabolic conditions and was influenced minimally by a low-carbohydrate diet for 11 d or a high-protein diet for 6 mo (1, 28, 29), the present study showed that the relative contribution of gluconeogenesis to endogenous glucose production increased dramatically under conditions of a high-protein, carbohydrate-free diet and low body glycogen stores. Because body glycogen stores probably were not completely restored within this relatively short period by a high-protein, carbohydrate-free diet (30), the rate of glycogenolysis decreased dramatically. Therefore, the relative contribution of gluconeogenesis increased to levels comparable with previous observed values after prolonged fasting (23, 31, 32). Moreover, absolute gluconeogenesis also tended to be higher.

With the H diet, the contribution of increased gluconeogenesis to increased EE was 42%. Although other energy-requiring pathways in protein metabolism, such as protein synthesis, may contribute to the increase in EE after a high-protein diet (6, 33, 34), the results of the present study showed that gluconeogenesis contributes to a major part (42%) of the increased EE. The remaining variance may be explained by other energy-requiring pathways in protein metabolism, eg, protein synthesis, protein oxidation, and ureagenesis. The energy costs of protein synthesis and protein breakdown have been estimated from theoretical values to be 3.6 and 0.7 kJ/g, respectively (6, 12, 33, 34). Nevertheless, they have not actually been measured, and the contribution of these pathways to increased EE with a high-protein diet requires further study. Previously, it has been shown from a theoretical perspective that an increased demand on protein and amino acid turnover for gluconeogenesis by a low-carbohydrate diet increases EE (13). The energy cost to produce glucose through gluconeogenesis was 33% (95% CI: 16%, 50%) of the energy content of glucose. Hall (12) previously estimated, based on published data, the energetic efficiency of gluconeogenesis to be 0.8, which suggests that the energy cost of

TABLE 4
Energy intake and energy expenditure, macronutrient intake and oxidation, and energy and macronutrient balances in 10 healthy men during consumption of a normal-protein (N) diet or a high-protein, carbohydrate-free (H) diet for 36 h after an exhaustive glycogen-lowering exercise test[1]

	N diet[2]			H diet[3]		
	Intake	Expenditure/oxidation	Balance	Intake	Expenditure/oxidation	Balance
		MJ/d			*MJ/d*	
Energy	10.27 ± 0.28	10.06 ± 0.34	0.21 ± 0.17	10.27 ± 0.28	10.09 ± 0.31	0.17 ± 0.12
Protein	1.15 ± 0.03	1.41 ± 0.08	−0.26 ± 0.06[4]	3.13 ± 0.08	2.34 ± 0.12	0.78 ± 0.07[5,6]
Carbohydrate	5.67 ± 0.16	4.42 ± 0.37	1.26 ± 0.32[4]	0.03 ± 0.00	1.03 ± 0.24	−1.00 ± 0.23[4,6]
Fat	3.44 ± 0.10	4.23 ± 0.28	−0.79 ± 0.28[7]	7.11 ± 0.20	6.71 ± 0.25	0.40 ± 0.27[6]

[1] All values are means ± SEMs.
[2] Macronutrient composition: 12%, 55%, and 33% of energy from protein, carbohydrate, and fat, respectively.
[3] Macronutrient composition: 30%, 0%, and 70% of energy from protein, carbohydrate, and fat, respectively.
[4,5,7] Significantly different from zero (paired *t* test): [4]$P < 0.01$, [5]$P < 0.001$, [7]$P < 0.05$.
[6] Significantly different from the N diet, $P < 0.001$ (paired *t* test).

gluconeogenesis is 20%. This value of 20% is lower than the value of 33% that we observed but was within the 95% CI. Taken together, the observed increase in gluconeogenesis contributed 42% to the increase in EE after the H diet, and the energy cost of gluconeogenesis was 33%.

The contribution of the oxidation of the separate macronutrients to total EE was 23%, 10%, and 67% and 14%, 44%, and 42% of energy from protein, carbohydrate, and fat, respectively, whereas the macronutrient intake was 30%, 0%, and 70% and 12%, 55%, and 33% of energy from protein, carbohydrate, and fat in the H condition and the N condition, respectively. In the N condition, there was a positive carbohydrate balance, and, considering that the subjects performed exhaustive glycogen-lowering exercise, it is likely that surplus carbohydrates were stored as glycogen (35, 36). Macronutrient oxidation was relatively well adjusted to intake, also after the extremely high fat intake with the H diet. Although fat stores are less controlled and adaptation of fat oxidation to fat intake normally is not abrupt, the body is able to rapidly increase fat oxidation to the level of fat intake in a glycogen-depleted state (17, 36, 37). The glycogen-lowering exercise may also have affected the protein balance in the N condition. A protein intake of 12% of energy was not enough to obtain protein balance, whereas, in a previous study with a comparable diet, subjects were in protein balance during a diet with 10% of energy from protein (5). Glycogen depletion has been shown to increase rates of muscle proteolysis and branched-chain amino acid oxidation (38) and probably was the reason for the relatively increased protein oxidation, hence a negative protein balance, with the N diet. Thus, in both the H and N conditions, macronutrient oxidation was relatively well adjusted to macronutrient intake, except that there was a positive carbohydrate balance in the N condition. On the days before the experiments, the subjects consumed their habitual diets, which were adequate in protein (39). Glycogen-lowering exercise has been shown to increase the adaptation rate of substrate oxidation to macronutrient intake; therefore, a longer period to adapt to the diet was not required (17).

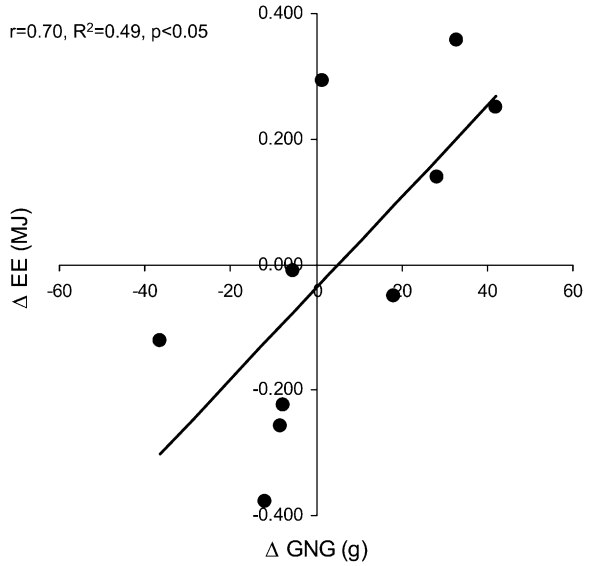

FIGURE 2. Relation between the difference in energy expenditure (ΔEE) and the difference in postabsorptive gluconeogenesis (ΔGNG) in healthy men (n = 10) during consumption of a high-protein, carbohydrate-free (H) diet (30%, 0%, and 70% of energy from protein, carbohydrate, and fat, respectively) or a normal-protein (N) diet (12%, 55%, and 33% of energy from protein, carbohydrate, and fat, respectively) for 36 h after an exhaustive glycogen-lowering exercise test. ΔGNG = absolute GNG in the H condition (g) – absolute GNG in the N condition (g); ΔEE = EE in the H condition (MJ) – EE in the N condition (MJ). Differences were corrected for a potential order of treatment effect by subtracting the mean value of ΔGNG or ΔEE of individuals with the same order of treatment of each individual value of ΔGNG or ΔEE, respectively. Equation that results from linear regression: $\Delta EE = 0.007 \times \Delta GNG - 0.038$ ($r = 0.70$, $R^2 = 0.49$, $P < 0.05$).

In healthy volunteers, the process of hepatic autoregulation normally regulates endogenous glucose production tightly; a change in the rate of gluconeogenesis is compensated for by a reciprocal change in the rate of glycogenolysis so that total endogenous glucose production essentially does not change (31, 40, 41). However, the H diet reduced endogenous glucose production dramatically, which resulted in a lower blood glucose concentration. Apparently, the up-regulation of gluconeogenesis was not sufficient to maintain glucose concentrations. Over time, hepatic autoregulation may be restored again. Hultman and Bergstrom (30) showed that, although extremely slowly, a high-protein diet with a very low carbohydrate content restored glycogen stores. In the present study, carbohydrate balance was −55 g, whereas endogenous glucose production was 181 g. Hence, ≈125 g of the glucose endogenously produced was not immediately used for oxidation and was probably stored as glycogen to restore body reserves. It may be that, after some time, the

contribution of glycogenolysis to endogenous glucose production increases again. Further research is needed to determine whether hepatic autoregulation will be restored.

The strength of this study was that it was the first to measure simultaneously the effects of an H diet on endogenous glucose production and gluconeogenesis as well as on EE. The observed relation between the difference in gluconeogenesis and the difference in EE between the 2 diets allowed conclusions about the contribution of gluconeogenesis to EE. Moreover, for the first time the actual energy costs of gluconeogenesis were calculated. One of the limitations of the study was that the methods used did not allow for gluconeogenesis to be measured in the fed state because of the presence of futile cycles and the isotopic dilution of the precursor by unlabeled pools of metabolites (42). However, in the fed state, the gluconeogenic rate was shown to be only modestly changed depending on the composition of the diet. Hence, the power to observe differences may be reduced (1). Another limitation was that the 2 diets differed not only in protein content, but also in carbohydrate and fat contents. This is inevitable, because when the contribution of one macronutrient is changed, the contributions of the other macronutrients always change to maintain the same total energy intake. Because the main question to be answered was whether gluconeogenesis can be increased, the carbohydrate content of the diet, hence carbohydrate availability, should be low to be able to sensitively study the acute effects of a high protein intake on gluconeogenesis (1). A protein intake of 30% of energy was chosen as being representative of high-protein diets studied in energy balance (43, 44). The remaining energy intake in the H condition had to be from fat. Because fat is a thermoneutral ingredient, ie, it does not increase diet-induced EE, it is not likely that the higher fat intake affected EE (45). Previously, a high-fat, low-carbohydrate diet resulted in decreased basal endogenous glucose production. Unfortunately gluconeogenesis was not measured in this study (46). Because a high fat intake does not increase EE, it is not expected that an increased fat intake increases EE via increased gluconeogenesis.

In conclusion, increased gluconeogenesis contributes to increased EE after consumption of an H diet for 1.5 d following a decrease in body glycogen stores. Forty-two percent of the increase in EE after the H diet was explained by an increase in gluconeogenesis. The energy cost of gluconeogenesis was 33% of the energy content of glucose.

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The authors' responsibilities were as follows—MABV, MSW-P, and KRW: designed the study; MABV collected and analyzed the data and wrote the manuscript; and MSW-P and KRW: contributed to interpretation of the data and reviewed the manuscript. None of the authors had a personal or financial conflict of interest.

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