Postprandial de novo lipogenesis and metabolic changes induced by a high-carbohydrate, low-fat meal in lean and overweight men[1–3]

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ABSTRACT

Background: Adjustments of carbohydrate intake and oxidation occur in both normal-weight and overweight individuals. Nevertheless, the contribution of carbohydrates to the accumulation of fat through either reduction of fat oxidation or stimulation of fat synthesis in obesity remains poorly investigated.

Objective: The objective of this study was to assess the postprandial metabolic changes and the fractional hepatic de novo lipogenesis (DNL) induced by a high-carbohydrate, low-fat meal in lean and overweight young men.

Design: A high-carbohydrate, low-fat meal was administered to 6 lean and 7 overweight men after a 17.5-h fast. During the fasting and postprandial periods, energy expenditure (EE), macronutrient oxidation, diet-induced thermogenesis, and serum insulin, glucose, triacylglycerol, and fatty acids were measured. To determine DNL, [1-^{13}C]sodium acetate was infused and the mass isotopomer distribution analysis method was applied.

Results: After intake of the high-carbohydrate meal, the overweight men had hyperinsulinemia and higher fatty acid and triacylglycerol concentrations than did the lean men. The overweight group showed a greater EE, whereas there was no significant difference in carbohydrate oxidation between the groups. Nevertheless, the overweight men had a marginally higher protein oxidation and a lower lipid oxidation than did the lean men. DNL was significantly higher before and after meal intake in the overweight men and was positively associated with fasting serum glucose and insulin concentrations. Furthermore, postprandial DNL was positively correlated with body fat mass, EE, and triacylglycerol.

Conclusion: After a high-carbohydrate, low-fat meal, overweight men had a lower fat oxidation and a higher fractional hepatic fat synthesis than did lean men. *Am J Clin Nutr* 2001;73:253–61.

KEY WORDS Obesity, hyperinsulinemia, carbohydrates, fuel metabolism, fractional hepatic de novo lipogenesis, overweight men, lean men, diet-induced thermogenesis, energy expenditure, triacylglycerol, fractional hepatic fat synthesis

INTRODUCTION

The stability of body composition requires that intakes of protein, carbohydrate, and fat are balanced in terms of macronutrient oxidation over time (1, 2). Mechanisms such as postprandial endocrine and metabolic signals arise to give high priority to the adjustment of carbohydrate oxidation to carbohydrate availability (3). In this way, glycogen stores appear to determine the contribution of glucose to the oxidized fuel mix, which influences the rate of protein and fat oxidation (4). Despite this acute and precise regulation, carbohydrate consumption reduces the need to use fat as fuel, so carbohydrate is important in controlling the balance between fat intake and fat oxidation (5). Thus, the level of adiposity may be affected if short-term meal-related factors are sustained over long periods (6).

Obesity is a multifactorial and complex disorder that is characterized by a long-term energy intake above energy expenditure (EE) (7). Many studies examined the regulation of these 2 components to provide an understanding of energy balance in overweight men. In this context, it is well known that fatty acid synthesis is stimulated by high-carbohydrate, low-fat diets in both animals and humans (8–10), but it is generally thought that de novo lipogenesis (DNL) is a quantitatively unimportant metabolic pathway in weight-stable men (11). Nevertheless, DNL may be higher in overweight hyperinsulinemic men than in lean men, depending on the type of carbohydrate in the diet (12).

A widely used technique for assessing in vivo DNL has been indirect calorimetry, which allows estimation of the net conversion of carbohydrate to fat, because DNL is the only metabolic process with a respiratory quotient >1; however, indirect calorimetry measures only net DNL and not the flux through the pathway (11). Glycogen storage was shown to represent the major fate of excess dietary carbohydrate after large carbohydrate loads from a single meal (13, 14) or from several days of surplus intake of energy as carbohydrate (15), showing a minor quantitative role for DNL in the day-to-day storage of surplus energy.

In the past few years, new isotopic methods for studying human hepatic DNL in vivo were developed by measuring the incorpora-

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[2] Supported by the Linea Especial en Nutrición y Obesidad (Special Investigation Project on Nutrition and Obesity), University of Navarra, and Gobierno de Navarra (project 1/98 de OF 32/98).

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Received December 21, 1999.

Accepted for publication July 28, 2000.

TABLE 1
Characteristics of the 13 young male participants[1]

Variable	Lean (n = 6)	Overweight (n = 7)
Age (y)	22.1	20.5
Height (m)	1.74 ± 0.01	1.76 ± 0.01
Weight (kg)	63.5 ± 2.8	96.1 ± 6.21[2]
BMI (kg/m^2)	20.8 ± 0.7	30.8 ± 1.7[2]
Percentage body fat (%)	15.4 ± 1.6	26.7 ± 1.2[2]
Body fat (kg)	9.9 ± 1.3	26.1 ± 3.0[2]
Fat-free mass (kg)	53.6 ± 2.1	69.9 ± 3.3[2]

[1] $\bar{x}$ ± SEM.
[2] Significantly different from lean, $P < 0.01$.

tion of [1-^{13}C]sodium acetate into plasma VLDL fatty acids, which specifically determines the hepatic fraction of de novo synthesized VLDL palmitate (16, 17). Using this method (mass isotopomer distribution analysis; MIDA), several researchers studied the effects of isoenergetic low-fat, high-carbohydrate diets composed of simple and complex carbohydrates (10, 18) or during surplus or deficit carbohydrate diets (15). In these studies, a strong relation between carbohydrate intake and fractional DNL was found, but it remained quantitatively insignificant to the body fat stores. Nevertheless, these trials were performed in healthy nonobese men. Until now, only one study showed that DNL in obese men was correlated with body adiposity (19), but the factors influencing fatty acid synthesis in obese humans remain unclear. An understanding of the processes involved in the development of obesity is necessary so that strategies for its prevention and management can be designed. This can be facilitated by a better ability to identify individuals who are at risk of becoming obese, who may be characterized by a metabolic or genetic susceptibility to weight gain (20). The aim of this study was to determine the fractional hepatic DNL and the relation of this pathway with some metabolic and hormonal factors in lean and overweight men in response to a short fast followed by a high-carbohydrate, low-fat meal.

SUBJECTS AND METHODS

Subjects

The characteristics of the 13 young men who were studied at the metabolic unit of the Department of Physiology and Nutrition, University of Navarra, are shown in **Table 1**. Men were recruited to fit into one of the following categories: *1*) nonobese [body mass index (BMI; in kg/m^2) < 25] or *2*) overweight or obese (BMI > 27), according to the criteria of the World Health Organization Classification of Overweight and Obesity (21). All the men were healthy and did not have diabetes or other endocrine disease, were nonsmokers, were taking no oral prescription medications, and had had variations in their body weight of < 10% in the previous 3 mo. Skinfold thickness was measured at 3 sites (biceps, triceps, and suprailiac) by the same investigator using a Holtain caliper (CMS Ltd, Crymych, United Kingdom) and the equation of Durnin and Womersley was used to estimate percentage body fat (22). The studies were approved by the Clinical Investigation Ethical Committee of Navarra and informed consent was obtained from all the participants.

Procedures

All the men were studied during a 3-d baseline period followed by 1 experimental day. Each subject was given the following instructions: *1*) to follow an isoenergetic diet during the 3-d baseline period to maintain body weight; *2*) to eat the last meal of the night preceding the experimental day at 2200, allowing a fasting period of 10 h before the experimental day; *3*) to avoid any physical activity during the previous 3 baseline days and to not drink alcohol; and *4*) to come to the metabolic unit by car, bus, or taxi on the morning of the experimental day to minimize physical activity.

On the experimental day, the men entered the metabolic unit between 0730 and 0745 and stayed in until 2000. After the assessment of body weight and body composition and collection of urine samples, the participants lay on a bed for 45 min until resting EE (REE) was determined by indirect calorimetry. Two venous catheters were inserted into an antecubital vein (one in each hand) for blood sampling and for intravenous infusion of [1-^{13}C]sodium acetate. All experiments started at 0800 after an overnight fast of 10 h and finished at 2000. The men received a test formula meal after 17.5 h of fasting (between 1530 and 1545) and the postmeal period was studied during the next 4 h. During the study period, the participants were allowed to move only inside the metabolic unit.

Diets

During the 3-d baseline period, the participants consumed an isoenergetic diet designed to maintain body weight by providing 55% of energy as carbohydrate, 15% as protein, and 30% as fat. The diet was adjusted to the men's energy requirements, which were based on the Harris-Benedict equation (23). The men recorded all food eaten by using diet diaries, which were reviewed carefully by a nutritionist with each subject and analyzed to confirm the amount and composition of energy ingested. Body weights were stable throughout the 3-d baseline period.

On the experimental day, all the men received a high-carbohydrate test meal of precise, known composition. The test meal provided 40% of each subject's daily energy requirements, which were calculated as 1.2 × basal REE as measured by indirect calorimetry on the morning of the experimental day.

The test meal was a liquid formula (Meritene Polvo; Novartis Nutrition, Barcelona, Spain) to which sugar and corn oil were added. The meal provided 80% of energy as carbohydrate (53% as sucrose and 27% as lactose), 17% as protein, and 3% as lipids. The energy and nutrient contents of the test meal are shown in **Table 2**. The test diet formula was well tolerated and totally ingested, and the men showed no evidence of gastrointestinal disturbance.

Respiratory exchange measurements

Indirect calorimetry measurements were assessed with a continuous open-circuit ventilated-hood system (Deltatrac Monitor

TABLE 2
Energy and nutrient contents of the test meal[1]

Variable	Lean (n = 6)	Overweight (n = 7)
Energy (kJ)	3316 ± 86	4140 ± 217
Protein (g)	33.6 ± 0.8	41.9 ± 2.2
Carbohydrate (g)	158.9 ± 4.0	198.0 ± 10.3
Sucrose (g)	104.4 ± 2.7	131.0 ± 6.9
Lactose (g)	53.5 ± 1.3	66.9 ± 3.4
Fat (g)	2.6 ± 0.1	3.3 ± 0.1

[1] $\bar{x}$ ± SEM.

MBM-200; Datex-Engstrom Division, Instrumentarium Corp, Helsinki). The calibration of oxygen, carbon dioxide, and airflow was carried out with reference gas before the beginning of each test. EE, nonprotein respiratory quotient (NPRQ), substrate oxidation, and net lipogenesis were calculated from oxygen consumption, carbon dioxide production (recorded by Deltatrac once per minute and averaged over 20 min), and urinary nitrogen excretion, according to the method and equations described by Ferranini (24). Fasting measurements were taken at 60-min intervals starting at 10 h of fasting and were maintained until 17.5 h of fasting. Postmeal measurements were taken at 30-min intervals over the 240 min after intake of the meal. Cumulative carbohydrate, protein, and lipid oxidation rates; net lipogenesis; and EE expressed as mg/kg fat-free mass (FFM) over the fasted and fed periods were calculated as the areas under the curves compared with basal (10 h fasted) and premeal (17.5 h fasted) absolute values, respectively (25). The areas under the curves were computed with the trapezoidal rule. Diet-induced thermogenesis (DIT) was calculated as the increase in EE above premeal values for 4 h after meal intake and was expressed as a percentage of energy intake (26).

Measurement of fatty acid synthesis

Fatty acid synthesis or DNL was measured during the study period by the intravenous infusion of [1-^{13}C]sodium acetate and application of the MIDA method, which was described in detail previously (16, 17). In brief, MIDA is a stable isotope mass spectrometric method for measuring polymerization biosynthesis on the basis of the principles of combinatory probabilities, in which the labeling pattern of a polymer synthesized from a stable isotopically labeled precursor will conform to a predicted binomial or multinomial expansion. Thus, the isotopic enrichment of the precursor pool is calculated from measurements of the product alone. In the case of fatty acid synthesis, the proportions of excess (above natural background abundance) of single-labeled and double-labeled fatty acids are a function of probability (P) that the precursor subunits were labeled isotopically. When P is used for the isotopic enrichment of the acetyl coenzyme A pool, the theoretic ^{13}C enrichment in 16:0 if 100% of the 16:0 were newly formed from this acetate pool is calculated. The actual isotopic enrichment is measured by gas chromatography–mass spectrometry (GC-MS). This value divided by the theoretic maximum values equals the fraction of the fatty acid that is newly formed, and this value represents the dilution of de novo synthesized 16:0 from sources other than DNL. This method requires that newly synthesized (labeled) and preformed (unlabeled) palmitate mix in the liver and communicate with plasma VLDL over the period of the isotope infusion and assumes that the major de novo fatty acid is 16:0 (10).

[1-^{13}C]Sodium acetate (Isomed; CIL, Cambridge, United Kingdom) (>99% pure and >98% enriched) in 1 L 0.5 mol saline solution/L was intravenously infused at a rate of 500 mg/h. Infusion started at 0830 (7 h before the meal test), to ensure a plateau in ^{13}C enrichment of 16:0 before meal intake (10), and continued for a total of 11 h.

At 14 h, after 17.5 h of fasting, and 30, 60, 120, 180, and 240 min after the meal test, 5 mL blood was sampled and put in EDTA on ice. Plasma was obtained by low-speed centrifugation at 4°C for 15 min at 1549 × g and frozen at −40°C until assayed. Fasting VLDL was isolated by density-gradient separation with use of a fixed bucket rotor (Beckman Instruments, Inc, Fullerton, CA). For this, 2.3 mL plasma was overlaid with 5.7 mL saline solution containing EDTA and centrifuged at 20°C and 175 000 × g for 17 h. In the samples obtained after consumption of the meal, chylomicrons were first separated by two 30-min spins at 125 000 × g at 20°C and 1 mL from the top (the chylomicron layer) was removed and replaced with saline solution each time. VLDL was then isolated by a 17-h spin, as described previously. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis was used to confirm the exclusion of chylomicrons.

The lipid was extracted from VLDL with Folch solvent from 2 mL plasma prepared as described above and methylated with 5% methanolic acid, then heated at 50°C for 2 h. The fatty acids methyl esters were extracted with hexane after the addition of water and capped with nitrogen.

GC-MS (GC System HP 6890 coupled with a Mass Selective Detector HP 5973; Hewlett-Packard, Palo Alto, CA) was used to quantitate the molecular ions of methyl 16:0 (mass-to-charge ratios of 270–272) with use of electron-impact ionization. The selected ion-monitoring mode was used and the fractional de novo synthesis was calculated as described in detail previously (17).

Analysis of serum glucose, insulin, triacylglycerol, and fatty acids

Serum glucose was assessed enzymatically (Glucosa PAP; Roche, Madrid, Spain), as were fatty acids (NEFA C, Wako Chemicals GmbH, Neuss, Germany) and triacylglycerol (Unimate 5 Trig, Roche, Spain). Insulin was analyzed by duplicate radioimmunoassay (Coat-A-Count Insulin, Diagnostic Products Corporation, Los Angeles).

Statistical methods

All results are expressed as means ± SEMs. The normal distribution of variables was tested and a Tukey test was used as the post hoc contrast after a two-factor repeated-measures analysis of variance for comparison of time-course measurements. Differences in time-point measurements between groups were analyzed by using Student's t test with Bonferroni correction when homoscedasticity criteria were not achieved. Otherwise, Tukey comparisons were done. Differences between groups for table variables were analyzed with Student's t test. Relations between variables were evaluated with Pearson correlation coefficients and stepwise regression analysis was used for the adjustment of some variables. The postprandial incremental area under the curve was calculated for glucose, insulin, triacylglycerol, and fatty acids with use of the trapezoidal method and with consideration of the fasting premeal values (27). The fasted and fed average postprandial area under the curve for fractional DNL was also calculated. The data were analyzed with use of SPSS (version 7.5; Microsoft, Redmond, WA).

RESULTS

The time course of serum glucose, insulin, triacylglycerol, and fatty acid concentrations during the fasting period (10–17.5 h) and after the single meal intake (0–240 min) are shown in **Figure 1**. As expected, after the high-carbohydrate load, overweight and lean men had significantly higher serum glucose ($P < 0.001$) and insulin ($P < 0.001$) and lower fatty acid concentrations ($P < 0.01$). Compared with the lean men, the overweight men had significantly higher serum glucose concentrations after 14 h (5.33 ± 0.13 and 4.84 ± 0.02 mmol/L, respectively; $P < 0.05$) and

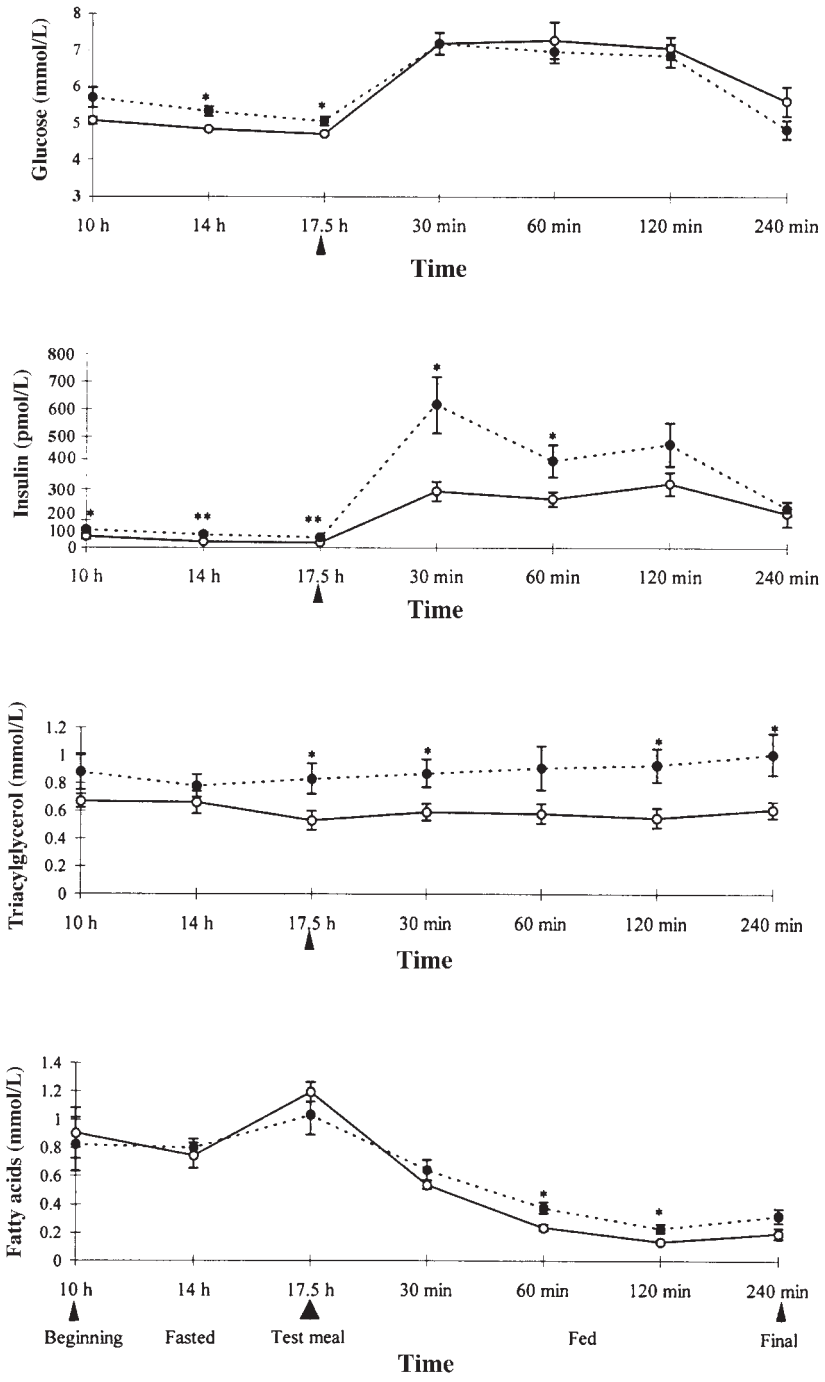

FIGURE 1. Mean (±SEM) time course of serum glucose, insulin, triacylglycerol, and fatty acid concentrations during the fasting period (between 10 and 17.5 h) and after intake of the meal (0–240 min) in lean (○; $n = 6$) and overweight (●; $n = 7$) men.[*, **]Significantly different between the lean and overweight men: [*]$P < 0.05$, [**]$P < 0.01$.

17.5 h (5.06 ± 0.12 and 4.72 ± 0.04 mmol/L) of fasting ($P < 0.05$). However, no significant differences were found after meal intake (4.84 ± 0.25 and 5.61 ± 0.43 mmol/L 240 min postprandially). Insulin concentrations were also significantly higher in overweight men at 14 h (58.4 ± 7.4 compared with 25.5 ± 3.3 pmol/L) and 17.5 h (45.6 ± 5.7 compared with 21.9 ± 1.6 pmol/L) of fasting ($P < 0.01$). In the same way, the overweight men had higher insulin concentrations at 30 min (619.3 ± 122.3 compared with 244.5 ± 42.5 pmol/L) and 60 min (376.7 ± 69.7 compared with 211.3 ± 31.4 pmol/L) after carbohydrate intake ($P < 0.05$) than did the lean men. Just before intake of the meal (17.5 h of fasting), the overweight men had significantly higher ($P < 0.05$) triacylglycerol concentrations (0.83 ± 0.11 compared with 0.53 ± 0.07 mmol/L); this was also the case at 30 min (0.87 ± 0.10 compared with 0.59 ± 0.06 mmol/L), 120 min (0.93 ± 0.12 compared with 0.55 ± 0.07 mmol/L), and 240 min (1.01 ± 0.15

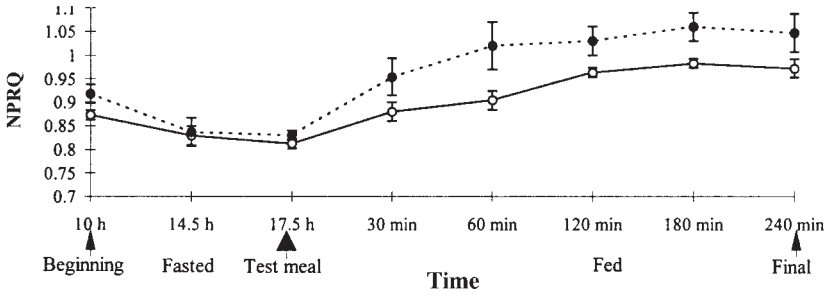

FIGURE 2. Mean (±SEM) time course of nonprotein respiratory quotient (NPRQ) during the fasting period (between 10 and 17.5 h) and after intake of the meal (0–240 min) in the lean (○; $n = 6$) and overweight (●; $n = 7$) men.

compared with 0.61 mmol/L) after carbohydrate intake. There were no significant differences in fasting serum fatty acids between the 2 groups during the fasting period. Nevertheless, after intake of the meal, the overweight men had significantly higher values ($P < 0.05$) at 60 min (0.37 ± 0.04 compared with 0.23 ± 0.02 mmol/L) and 120 min (0.23 ± 0.03 compared with 0.13 ± 0.01 mmol/L) than did the lean men. The postprandial changes in these serum measurements, which were calculated as the incremental area under the curve compared with premeal values (measured before meal intake), were not significantly different between the lean and the overweight men for glucose, insulin, fatty acid, and triacylglycerol concentrations (data not shown).

The fasting and postprandial NPRQs during the study are shown in **Figure 2**. There were no significant differences in basal (10-h fasted) NPRQ (0.91 ± 0.02 compared with 0.87 ± 0.01). After carbohydrate intake, NPRQs increased in the 2 groups ($P < 0.01$); the overweight men started to reach values >1 at 60 min postprandially until the end of study. Also, NPRQs in the overweight men tended to be higher 60 min after the meal (1.02 ± 0.05 compared with 0.90 ± 0.02; $P = 0.067$) and at 180 min (1.06 ± 0.03 compared with 0.98 ± 0.01; $P = 0.092$) than did the lean men.

The cumulative fasting (7.5 h) REE and postprandial (4 h) EE, nutrient oxidation, and total postprandial net lipogenesis and DIT are shown in **Table 3.** During the fasting period, there were no significant differences between the lean and the overweight men in EE and nutrient oxidation. After load intake, EE was significantly higher in the overweight men than in the lean men ($P < 0.01$). There were no significant differences in carbohydrate oxidation between the groups. However, in the overweight men lipid oxidation tended to be lower ($P = 0.055$), protein oxidation tended to be higher ($P = 0.066$), and net lipogenesis values were significantly higher ($P < 0.05$), although there was not a positive net fat gain. DIT tended to be higher in the overweight men ($P = 0.071$) and was positively correlated with postprandial EE ($r = 0.654$, $P = 0.015$) but not with carbohydrate or protein oxidation as expressed in mg/kg FFM.

The individual data used to calculate de novo fractional palmitate synthesis (f) just before meal intake (17.5 h fasted) and at 4 h after meal intake are shown in **Table 4.** The time course of fasted and fed fractional hepatic DNL is plotted in **Figure 3**. Fasting DNL was significantly higher in the overweight men at 14 h (2.63 ± 0.19% compared with 1.01 ± 0.27%; $P < 0.01$) and at 17.5 h (2.93 ± 0.23% compared with 1.67 ± 0.34%; $P < 0.05$). After carbohydrate intake, both groups had significantly higher DNL ($P < 0.01$) with a significant time × body type interaction ($P = 0.005$). The lean men reached a maximum DNL of 5.20 ± 0.93% 60 min after load intake, whereas the overweight men had a higher DNL over the 4 h postprandially. The overweight group reached a maximum DNL of 8.52 ± 1.04% at 180 min compared with 3.18 ± 0.43% in the lean group. At 240 min postprandially, the overweight group had a DNL of 8.28 ± 1.23% compared with 3.16 ± 0.75% in the

TABLE 3
Cumulative fasted and postprandial energy expenditure (EE), nutrient oxidation, diet-induced thermogenesis (DIT), and net lipogenesis[1]

Variable	Lean ($n = 6$)	Overweight ($n = 7$)	P^2
Fasting			
REE (kJ·kg FFM^{-1}·7.5 h^{-1})	34.5 ± 0.5	32.6 ± 1.2	0.229
Carbohydrate oxidation (mg·kg FFM^{-1}·7.5 h^{-1})	720.4 ± 110.1	837.9 ± 141.0	0.535
Lipid oxidation (mg·kg FFM^{-1}·7.5 h^{-1})	425.9 ± 39.5	312.0 ± 63.0	0.170
Protein oxidation (mg·kg FFM^{-1}·7.5 h^{-1})	278.2 ± 44.1	311.1 ± 64.7	0.694
Postprandial			
EE (kJ·kg FFM^{-1}·4 h^{-1})	24.9 ± 0.3	29.8 ± 1.2	0.004
DIT (%)	3.56 ± 0.8	6.65 ± 1.2	0.071
Carbohydrate oxidation (mg·kg FFM^{-1}·4 h^{-1})	850.8 ± 55.9	826.9 ± 76.6	0.812
Lipid oxidation (mg·kg FFM^{-1}·4 h^{-1})	169.8 ± 56.6	30.8 ± 25.2	0.055
Protein oxidation (mg·kg FFM^{-1}·4 h^{-1})	337.5 ± 42.6	472.3 ± 54.2	0.066
Postprandial net lipogenesis (g/4 h)	−11.05 ± 1.93	−2.87 ± 2.7	0.038

[1] $\bar{x}$ ± SEM. Cumulative fasting and fed values were calculated as the area under the curve over the last 7.5 h of fasting and 4 h after meal intake. REE, resting energy expenditure; FFM, fat-free mass.

[2] Difference between lean and overweight men.

TABLE 4
Fractional de novo lipogenesis (VLDL triacylglycerol 16:0) calculated by mass isotopomer distribution analysis

Subjects	EM1[1]	EM2[2]	EM2:EM1	*P*[3]	A1[4]	*f*[5]
	$\times 10^{-1}$	$\times 10^{-1}$		*MPE*[6]	*MPE*	%
Lean (n = 6)						
1						
Fasted[7]	0.0119	0.0041	0.3443	0.0312	0.1331	0.94
Fed[8]	0.0320	0.0158	0.4944	0.0601	0.2066	1.62
2						
Fasted	0.0541	0.0239	0.4418	0.0503	0.1861	2.99
Fed	0.0757	0.0386	0.5097	0.0629	0.2117	3.70
3						
Fasted	0.0142	0.0092	0.6438	0.0861	0.2438	0.64
Fed	0.0096	0.0025	0.2612	0.0139	0.0673	1.50
4						
Fasted	0.0175	0.0049	0.2796	0.0178	0.0838	2.15
Fed	0.0367	0.0175	0.4763	0.0568	0.2000	1.91
5						
Fasted	0.0308	0.0138	0.4488	0.0516	0.1891	1.69
Fed	0.0713	0.0401	0.5629	0.0724	0.2268	3.28
6						
Fasted	0.0255	0.0101	0.3948	0.0413	0.1633	1.62
Fed	0.1551	0.1113	0.7174	0.0980	0.2547	6.52
Overweight (n = 7)						
1						
Fasted	0.0517	0.0367	0.7104	0.0969	0.2538	2.20
Fed	0.1715	0.0769	0.4485	0.0516	0.1890	9.28
2						
Fasted	0.0541	0.0239	0.4418	0.0503	0.1861	2.99
Fed	0.0874	0.0448	0.5123	0.0634	0.2125	4.26
3						
Fasted	0.0425	0.0138	0.3254	0.0273	0.1201	3.61
Fed	0.0332	0.0084	0.2550	0.0125	0.0614	5.49
4						
Fasted	0.0175	0.0049	0.2796	0.0178	0.0838	2.15
Fed	0.0046	0.0112	0.2643	0.0145	0.0702	6.67
5						
Fasted	0.0598	0.0271	0.4535	0.0525	0.1911	3.22
Fed	0.1140	0.0439	0.3851	0.0394	0.1580	7.34
6						
Fasted	0.0756	0.0835	0.5097	0.0629	0.2117	3.70
Fed	0.2534	0.1181	0.4662	0.0549	0.1962	13.20
7						
Fasted	0.0125	0.0030	0.2421	0.0098	0.0489	2.67
Fed	0.2767	0.1868	0.6752	0.0913	0.2489	11.73

[1,2] Proportions of excess of single-labeled and double-labeled 16:0.
[3] Isotopic enrichment of acetate precursor.
[4] Asymptotic enrichment of M1 isotopomer if 100% of 16:0 were synthesized from acetyl-coenzyme A units at enrichment *P*.
[5] Fractional de novo synthesis.
[6] Mole percent excess.
[7] Value after 17.5 h of fasting just before meal intake.
[8] Value 4 h after meal intake.

lean group ($P < 0.01$). Also, the average area under the curve for DNL (Figure 3) was significantly higher ($P < 0.01$) in the overweight men than in the lean men in the fasting state (2.33 ± 0.53% compared with 4.87 ± 0.37%) and after the meal intake (3.12 ± 0.47% compared with 5.90 ± 0.61%).

After 14 h of fasting, a significant positive association was found between DNL and serum glucose (r = 0.699, P = 0.008) and insulin values (r = 0.932, P = 0.001). Nevertheless, after intake of the meal there were no relations between insulin or glucose concentrations with DNL in the 2 groups. During the postprandial period **(Figure 4)**, there was a significant positive correlation between the average DNL with percentage body fat (r = 0.578, P = 0.038) and triacylglycerol increase (r = 0.668, P = 0.013). DNL and EE were also correlated when both variables were adjusted for differences in FFM (r = 0.557, P = 0.048).

DISCUSSION

The present study was designed to investigate the short-term influence of a high-carbohydrate, low-fat load on fractional hepatic DNL and its relation to macronutrient utilization in lean and overweight men. Glycogen stores may affect the relative contributions of glucose and fatty acids to the metabolic fuel mix used as an energy source (9, 28). Therefore, in this study, participants received an isoenergetic diet during the 3 d preceding the test meal to maintain constant glycogen stores before assessing the effect of a large carbohydrate load. Because the influence of the last meal essentially ends after 10 h of fasting, the postabsortive NPRQ may give an indication of the state of the body glycogen stores (14), which was not significantly different between the lean and the overweight men.

In this dietary intervention, the overweight men had fasted hyperglycemia and hyperinsulinemia and higher concentrations of serum triacylglycerol, as found previously in obese individuals (29, 30), as well as a higher fasting DNL. After intake of the high-carbohydrate load, the overweight men had a higher DNL accompanied by increased triacylglycerol concentrations and higher fatty acid concentrations despite the elevated serum insulin concentrations. EE was higher in the overweight men, as reported previously by other researchers (1, 30), and both the lean and the overweight men increased their carbohydrate oxidation, presenting no significant differences in postprandial cumulative carbohydrate oxidation. However, in the overweight men, lipid oxidation tended to be lower and protein oxidation tended to be higher than in the lean men, as found previously (20, 31).

It is known that dietary carbohydrate promotes its own oxidation (9, 13, 32) and that under normal conditions DNL is not a major way to increase body fat stores, as found by other investigators using indirect calorimetry (20, 31). Most short-term studies (13–15, 30) in which carbohydrate was added to mixed diets, even in large quantities, did not show net DNL, based on indirect calorimetry, because storage as glycogen was concluded to represent the fate of excess dietary carbohydrate. In the present study, NPRQ was high throughout the postprandial period, reaching values slightly >1 in the overweight group after the first 60 min, although there was no positive postprandial net lipogenesis, and remained <1 in the lean men, as found in other studies (13, 14). This information indicates that there was no net gain in body fat after the administration of a large carbohydrate load.

Using tracer techniques, several authors assessed the effect of carbohydrate on DNL both in isoenergetic diets (10, 15, 18) and during surplus-carbohydrate diets (15, 33) and reported that carbohydrate consumption produced a dose-dependent increase in fractional DNL. Nevertheless, it remained unimportant to body fat stores because it represented only a few grams per day, when the absolute rate of lipogenesis was measured. However, those studies included only lean men and an area of uncertainty was whether the carbohydrate intake could produce

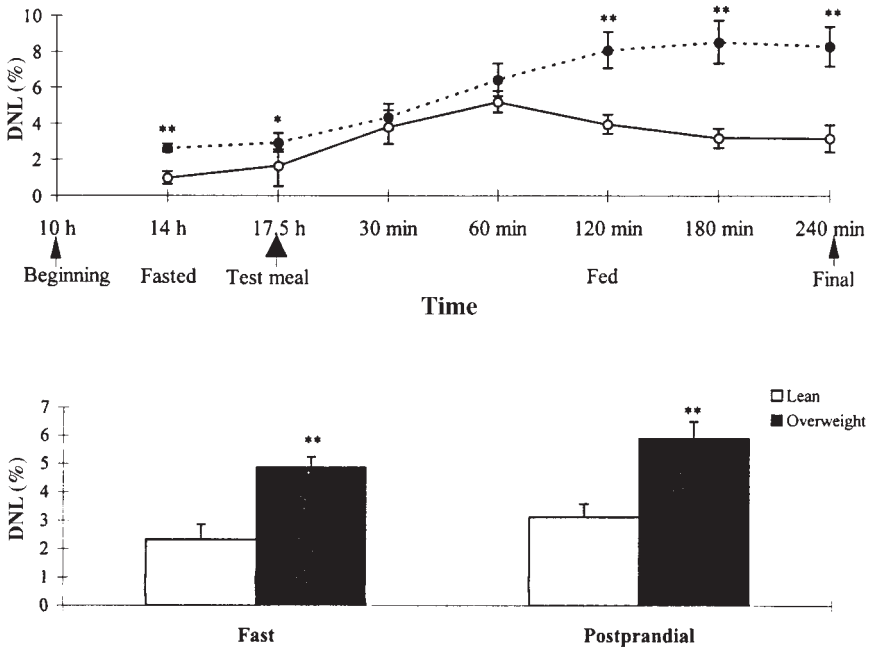

FIGURE 3. Mean (±SEM) time course of fractional hepatic de novo lipogenesis (DNL) during the fasting period (between 10 h and 17.5 h fasted) and after intake of the meal (0–240 min) in the lean (○; $n = 6$) and overweight (●; $n = 7$) men. Average DNL was calculated as the average fasted and fed area under the DNL curve, taking into account basal and premeal values and dividing by the number of measurements. *,**Significantly different between the lean and overweight men: $^{*}P < 0.05$, $^{**}P < 0.01$.

a higher stimulation of DNL in overweight humans. Furthermore, it was seen that insulin-resistant men showed a modestly increased fractional DNL, but the absolute rate of DNL accounted for only a few extra grams of fat per day (19, 34). In the present study, when a stable isotope technique was used it was evident that DNL occurred at NPRQs < 1, even with only a single carbohydrate meal. Indeed, this metabolic pathway was qualitatively higher in overweight individuals, although with no positive net lipogenesis. In this context, it should be pointed out that because of the short-term nature of this experiment, these results represent the fractional hepatic DNL concerning to palmitic acid synthesis, which was measured for a postprandial period of 4 h. The absolute rate of DNL (15) could not be measured because of the lack of a prolonged steady state response, which did not allow measurement of the quantitative role of the higher hepatic DNL found in the overweight men. Nevertheless, these results show that overweight hyperinsulinemic men had a stimulated hepatic DNL, both during fasting and after carbohydrate intake, which is in agreement with data reported previously (34).

On the other hand, hyperinsulinemia and the increase in plasma glucose or fatty acids may contribute to the higher triacylglycerol production (35). In this way, the postprandial increased fractional DNL was accompanied by an increase in triacylglycerol serum concentrations. Recent evidence indicates that increasing dietary carbohydrates can increase the DNL contribution to VLDL triacylglycerol under certain conditions, and it has been reported that obese hyperinsulinemic persons exhibit a DNL contribution that is ≈3-fold higher than in healthy subjects, although it still represents <10% of VLDL triacylglycerol production (16, 17, 19).

Increases in DNL induced by carbohydrate feeding may participate in triacylglycerol production through several mechanisms (35, 36), such as directly contributing to the VLDL triacylglycerol fatty acid pool to be secreted or indirectly increasing the efficiency of reesterification of serum fatty acids. However, this latter mechanism does not appear to have been explored yet.

In this study, the contribution of DNL to the VLDL triacylglycerol production was not measured, but the positive association between hepatic DNL and increase in triacylglycerol suggests that the higher serum triacylglycerol concentrations in the overweight men could have originated from the higher DNL accompanied by higher fatty acid reesterification. Indeed, the overweight men showed a higher availability of fatty acids to tissues because they showed higher postprandial fatty acid concentrations, but fat oxidation was lower than in the lean men. Because fat oxidation was diminished, the higher circulating fatty acids would also have led to higher hepatic reesterification and triacylglycerol production (35).

Taken together, these results—the qualitatively higher hepatic DNL with lower fat oxidation, the correlation between DNL and increased serum triacylglycerol, and the greater availability of fatty acids to tissues despite lower fat oxidation by tissues—all point to an impairment of tissue fuel selection (utilization of fatty acids) in the overweight group, in whom a carbohydrate load produced a fat-sparing effect more pronounced than in lean men and so a less negative fat balance than in lean men.

Interestingly, DNL was positively correlated with postprandial EE, which could have been because the overweight men had the highest DNL and EE values after carbohydrate intake; this was accompanied by a significant correlation of both EE and DNL with body fat. Because the absolute rate of DNL (in g/4 h) was not measured, it cannot be stated that the higher EE showed by overweight men is attributed to the energy cost of DNL.

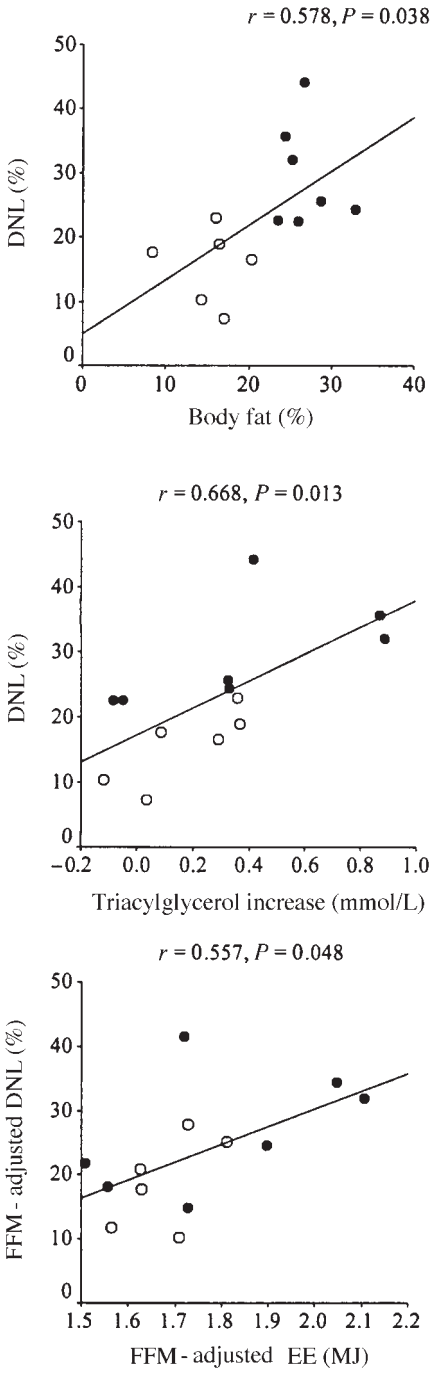

FIGURE 4. Relation between the total average postprandial area under the curve of de novo lipogenesis (DNL), percentage body fat, triacylglycerol increase, and cumulative energy expenditure [EE; adjusted for differences in fat-free mass (FFM)] during the postprandial period in the lean (○; $n = 6$) and overweight (●; $n = 7$) men.

Although there are clear differences between carbohydrate and fat intakes, the fact remains that obesity can develop from overeating simple carbohydrates, especially in persons who show a higher hepatic fat synthesis and lower fat oxidation during carbohydrate overfeeding. Because the heavier participants in this study were already overweight, it is uncertain whether these responses to carbohydrate intake contribute to the development of obesity, but it clearly could contribute to the maintenance of their overweight state. Nevertheless, this study is the first to address the differences between lean and overweight men in fat synthesis and fat oxidation in response to carbohydrate intake. The results of this study also confirm that short-term meal-related factors might be important to the establishment of a positive fat balance during periods of simple carbohydrate overfeeding.

The growing importance of understanding the role of carbohydrate intake on body weight control, type 2 diabetes, and hyperlipidemia, especially the effects produced by simple sugars, should lead to future experiments that focus on direct measures of lipolysis, absolute rates of hepatic DNL, and hepatic fatty acid reesterification during carbohydrate feeding. Future investigations should be conducted that take account of genetic background, which may explain some discrepancies and differences among studies (6, 21, 37) and the large variations between and within both groups for some variables.

We thank to Richard Neese from the Department of Nutritional Sciences, University of California, Berkeley, for his technical support and helpful discussions in the mass isotopomer distribution analysis method.

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