Glycogen storage capacity and de novo lipogenesis during massive carbohydrate overfeeding in man[1-3]

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ABSTRACT The metabolic balance method was performed on three men to investigate the fate of large excesses of carbohydrate. Glycogen stores, which were first depleted by diet (3 d, 8.35 ± 0.27 MJ [1994 ± 65 kcal] decreasing to 5.70 ± 1.03 MJ [1361 ± 247 kcal], 15% protein, 75% fat, 10% carbohydrate) and exercise, were repleted during 7 d carbohydrate overfeeding (11% protein, 3% fat, and 86% carbohydrate) providing 15.25 1.10 MJ (3642 ± 263 kcal) on the first day, increasing progressively to 20.64 ± 1.30 MJ (4930 ± 311 kcal) on the last day of overfeeding. Glycogen depletion was again accomplished with 2 d of carbohydrate restriction (2.52 MJ/d [602 kcal/d], 85% protein, and 15% fat). Glycogen storage capacity in man is ~15 g/kg body weight and can accommodate a gain of ~500 g before net lipid synthesis contributes to increasing body fat mass. When the glycogen stores are saturated, massive intakes of carbohydrate are disposed of by high carbohydrate-oxidation rates and substantial de novo lipid synthesis (150 g lipid/d using ~475 g CHO/d) without postabsorptive hyperglycemia. *Am J Clin Nutr* 1988;48:240–7.

KEY WORDS Carbohydrate overfeeding, glycogen stores, de novo lipid synthesis, indirect calorimetry, body composition

Introduction

It has long been known from studies involving carcass and organ analysis in animals that the composition of the diet and its availability effect the organism's glycogen reserves. The work of Bergström et al (1) and Hultman and Nilsson (2) using muscle and liver biopsies provided direct measurements of such changes in man. The highest muscle glycogen levels were observed when glycogen stores are first depleted by sustained exercise followed by ingestion of a high-fat diet and then repleted by consumption of a carbohydrate-rich diet, ie, the glycogen loading technique (1).

Skeletal muscles and liver are the principal sites for the storage of glycogen in the body. Liver glycogen concentrations vary with the diet with values in the range of 50–500 mmol glycosyl residues/kg tissue in the postabsorptive state (mean 270 mmol [44 g] glycosyl residues/kg liver) (3). Liver glycogen varies appreciably during the day in relation to the patterns of eating and fasting (2). Glycogen concentrations in biopsy samples from the quadriceps femoris muscle were found to be in the range of 60–120 mmol glycosyl residues/kg with a mean of 85 mmol (14 g) glycosyl residues/kg tissue (4). However, the glycogen concentration in skeletal muscle also depends upon the muscle group being investigated (4). For a 70-kg man with ~40% of his weight as skeletal muscle and a liver weighing 1.8 kg, one can estimate that ~3 mol glycosyl residues or almost 500 g of glycogen are stored in the body. If the highest reported literature values (1, 3) are extrapolated to the whole body, then up to 4.3 mol glycosyl residues or some 700 g of glycogen could be stored in the body.

Although it is known that this value can be increased markedly during glycogen loading, when muscle glycogen levels can reach 2.4 g/100 g wet muscle or more (1), it is often believed that the glycogen stores are normally maintained within a relatively narrow range. However, the capacity for storing large amounts of dietary carbohydrate by conversion to glycogen is in fact considerable (5–7). To assess the upper limit for glycogen storage in man, we performed continuous metabolic balance stud-

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ies for 10 d, which included 7 d during which massive excesses of carbohydrates were ingested.

Subjects and methods

Subjects

Three healthy young men, one of whom was a competition swimmer at university level, (21–22 y, 62–72 kg, 174–180 cm, and 11–14% body fat) with no family history of diabetes or obesity and who were not taking any medication, participated in this study. The subjects were each given a detailed account of the protocol, which had previously been reviewed and accepted by the institutes ethical committee, before they gave their consent to participate.

Protocol

The experiment lasted 14 consecutive days. During the first 3 d the subjects consumed a restricted diet, high in fat and low in carbohydrate, and followed an exercise program. Halfway through this period the subjects were admitted into a respiration chamber in which respiratory exchange measurements were to be continued for 10 d. After 36 h in the chamber the diet was changed to a high-carbohydrate, low-fat diet that was ingested for the following 7 d. During the last 2 d while still in the chamber, the subjects received limited amounts of a high-protein diet (protein-sparing modified-fast [PSMF], ~2.5 MJ or 600 kcal) essentially devoid of carbohydrate. The subjects then left the respiration chamber but continued to consume the high-fat, low-carbohydrate diet in restricted amounts for a further 2 d.

Energy intake

The diets were prepared by trained dieticians at the Institute of Physiology. The restricted high-fat, low-carbohydrate diet consumed on days 1–3 and 13–14 provided ~6.70 MJ (1600 kcal) composed of 15% protein, 75% fat, and 10% carbohydrate. During the overfeeding period (days 4–10 inclusive) the high-carbohydrate, low-fat diet provided ~15 MJ (3600 kcal, composed of 11% protein, 3% fat, and 86% carbohydrate) on day 4. Energy intake was then increased progressively each day while the composition was kept constant to provide 6.28 MJ (1500 kcal) in excess of the previous day's energy expenditure, which was measured in the respiration chamber (8). By day 10 the energy intake had thus increased to ~21 MJ (5000 kcal). For the next (and last) 2 d in the respiration chamber, the subjects consumed a high-protein, low-calorie diet (PSMF, ~2.5 MJ or 600 kcal). The diet was then changed to the same restricted high-fat, low-carbohydrate diet eaten on days 1–3 for the last 2 d of the experiment spent outside the chamber. Each food item consumed was weighed to the nearest gram with a Mettler P10 balance (Mettler, Greifensee, Switzerland) and its intake was corrected for any residue left on the plate. Energy intake and dietary composition were calculated from food tables (9) with a desk-top computer (HP 9830, Hewlett Packard (Schweiz) AG, Schlieren, Switzerland). The factors 16.74, 37.67, and 16.74 kJ/g (4, 9, and 4 kcal) were used to calculate the metabolizable energy contents of protein, fat, and carbohydrate, respectively. Twenty percent, by weight, of each food item (except for the sugared fruit juices) were set aside and pooled. At the end of each 24-h period, the pooled samples were homogenized. An aliquot was immediately freeze dried (Virtis automatic freeze-drier, Gardiner, NY) and the remainder was frozen and stored at −20 °C.

The frozen sample was dried to constant weight to establish its water content. The residue was ground to a powder that was analyzed for nitrogen (10), extractable fat by the Soxlet method (11), and ash contents. Dietary protein was taken to be 6.25 × total N and the carbohydrate content was calculated by difference (ie, dry weight − [weight protein + fat + ash]).

Much of the excess carbohydrate was provided by sugared fruit juices of known uniform composition and energy content. These were not included in the 20% duplicate sample but were analyzed separately; their energy contents were found to agree closely with those indicated by the manufacturers. The energy content of the fruit juices was added to the gross energy values of the high-carbohydrate diets.

Energy expenditure

During the first 2 d on the high-fat, low-carbohydrate diet, energy expenditure was not measured but physical activity was recorded by a pedometer (Pedoboy #10, Barigo Barometer Fabrik Gmbh, Schweninger, FRG). Heart rate was monitored continuously using a portable heart-rate monitoring instrument (HRM, Difa, Breda, Holland). The subjects performed various types of physical activity, ie, running and swimming, during this period to deplete their muscle glycogen stores. At the end of day 2, each subject was admitted into an open-circuit indirect calorimeter chamber (12) where energy expenditure was measured continuously for the next 10 d. Resting metabolic rate was measured for 1 h each morning with a ventilated-hood system within the chamber (12). Each day the subjects walked for two 30-min periods (beginning at 1130 and 1730) on a treadmill (Quinton Inst Co, Seattle, WA) at 2 mph (3.22 km/h), 5% slope in the morning and 2 mph (3.22 km/h), 10% slope in the afternoon. During the remainder of the day, spontaneous physical activity was allowed within the confines imposed by the chamber but strenuous physical exertion was not permitted.

The subjects were allowed to leave the chamber twice a day for ~30 min after the resting metabolic rate measurements in the morning and again in the afternoon at ~1630 during which time the calibrations of the analyzers were verified and other measurements were performed, eg, body weight, urine collection, etc.

Nutrient balances

Stools were collected from day 1 until several days after the test. At each change in the diet the subject consumed 1 g carmine red as a fecal marker. Stools were weighed, frozen, freeze dried, and analyzed for N, fat, and ash, and carbohydrate was calculated by difference as described above.

Twenty-four-hour nutrient and metabolizable energy intakes were calculated from the food tables (9). The 24-h gross energy intake was also determined on the basis of the direct analysis of the 20% dried duplicate sample and corrected for nutrients losses in the stool collections between the appearance of the fecal markers.

Urine was collected during the day (14 h) and the night (10 h) and was tested for glucose (Gluketur-Test®, Boehringer Mannheim Gmbh, Mannheim, FRG) and total N was analyzed (10). Daily protein oxidation was calculated by summing the urinary N excretions during the day and the night and multiplying this value by 6.25. Twenty-four-hour carbohydrate and fat oxidations were calculated according to classical formulas (13) from the nonprotein respiratory quotient.

From a knowledge of the composition of energy entering the body, that oxidized, and that leaving the body in urine and fe-

TABLE 1
Body weight changes*

Subject	Age	Height day 1	Start high fat day 4	Start high CHO day 11	Start PSMF day 13	End PSMF day 15	End test
	y	*cm*	*kg*	*kg*	*kg*	*kg*	*kg*
1	22	180	71.9	71.3	76.9	71.5	71.9
2	21	175	70.8	68.6	73.5	69.6	68.8
3	21	173.5	61.8	62.3	65.4	61.7	61.4
$\bar{x}$	21.3	176.2	68.2	67.4	71.9	67.6	67.4
SD	0.6	3.4	5.5	4.6	5.9	5.2	5.4

* High fat, high-fat, low-carbohydrate diet; high CHO, high-carbohydrate, low-fat diet; PSMF, protein-sparing modified fast.

ces (hair and cutaneous losses were assumed to be negligible), it was possible to calculate daily nutrient balances (ie, changes in body composition) during the 10 d of the experiment.

Blood variables

Blood samples were taken in the postabsorptive fasting state at the beginning of and at regular intervals throughout the experiment. They were analyzed for glucose (14), insulin (15), free fatty acids (16) on the Dole extract (17), triglycerides (18), and blood urea N (19). Lipoproteins were separated by electrophoresis (Readysystem AG, Bad-Zurzack, Switzerland) and thyroid hormone concentrations were analyzed by automated radioimmunoassay ARIA II (Becton Dickinson, Orangeburg, NY).

Results

Mean body weight decreased by 0.8 ± 1.4 kg during the 3 d on the restricted, high-fat, low-carbohydrate diet (**Table 1**). After the 7 d of overfeeding the high-carbohydrate, low-fat diet (day 10), body weight had increased by 4.6 ± 1.3 kg (ie, 5.6, 4.9, and 3.2 kg). During the 2 d on the restricted high-protein, low-energy diet (600 kcal/d) 4.4 ± 0.9 kg were lost. Two days later, body weights were the same as at the start of the overfeeding phase of the experiment (ie, 71.3 and 71.9, 68.6 and 68.8, 62.3 and 61.4 kg, respectively).

Energy and nutrient intake

The composition of the diets over the 14-d experiment are presented in **Table 2**. From the ratio of metabolizable energy obtained from direct analysis and food tables, it can be seen that the food tables tended to overestimate energy intake with the high-fat, low-carbohydrate diet and underestimate during the high-carbohydrate, low-fat overfeeding and the high-protein low-energy diets.

When the high-carbohydrate, low-fat diet was initiated, it was necessary to increase the energy intake by 8.8 MJ (2100 kcal, food table data) to obtain a positive energy balance of 6.28 MJ (1500 kcal) during the first 24-h period of overfeeding. On the second day an increase of 1.54 MJ (370 kcal, food tables) was necessary to maintain the same positive energy balance, the increment decreasing gradually to 0.42 MJ/d (100 kcal/d) over the next 3 d (**Fig 1**). During the last 2 d of the high-carbohydrate diet, the increase over the preceding days energy intake rose to 0.95 MJ (227 kcal). This pattern was slightly different when intake was based on the data obtained by direct analysis, where on the fifth day of overfeeding (day 8) the additional energy was 1.94 MJ (464 kcal) and fell to 0 on the last day of overfeeding (day 10). The food tables overestimated protein and carbohydrate intakes during each of the different diets except for the 2 d on the low-energy, high-protein (PSMF) diet (days 11 and 12). The tables overestimated fat intake during the low-energy high-fat, low-carbohydrate diets but considerably underestimated it during the high-carbohydrate, low-fat diet by ~40–80 g/d (670–1339 kJ/d; 160–320 kcal/d).

The substrate balances calculated from substrates entering (direct analysis) and leaving the body are presented in **Table 3**. With the onset of carbohydrate overfeeding, there was a dramatic increase in carbohydrate oxidation (**Fig 2**) from 74 ± 40 g/d (day 3) to 398 ± 87 g/d (day 4). Thereafter carbohydrate utilization (ie, oxidation and that used for de novo lipid synthesis) increased progressively in response to the increase in carbohydrate ingestion, attaining 1010 ± 37 g/d on the last day of overfeeding. Concomitant with the increase in carbohydrate utilization there was a rapid suppression of lipid oxidation.

After an initial decrease in protein oxidation at the beginning of carbohydrate overfeeding (from 104 ± 12 to 82 ± 7 g/d from day 4 to day 5), protein oxidation remained relatively constant until the last 2 d of overfeeding when it increased in proportion with the protein in the diet so that a positive N balance was maintained at ~3.8 g N during the last 5 d of overfeeding.

As the diet passed from a hypocaloric high-fat, low-carbohydrate to a hypercaloric high-carbohydrate, low-fat composition, there was not only a large increase in carbohydrate oxidation but also in glycogen storage (339 ± 82 g/d). With each successive day the amount of carbohydrate that was stored decreased even though the amount that was ingested increased (Fig 2). After 4 d of overfeeding, the glycogen stores had become saturated and it was calculated that they had increased by ~770 g. Thereafter carbohydrate balance was maintained near equilibrium. When the diet was devoid of carbohydrate

TABLE 2
Daily composition of the diet calculated by direct analysis and from food tables ($n = 3$, $\bar{x} \pm SD$)

Day	Ingested nutrients from direct analysis			Nutrients excreted in feces from direct analysis			Available nutrients from direct analysis*		
	Protein	Fat	CHO	Protein	Fat	CHO	Protein	Fat	CHO
	g	*g*	*g*	*g*	*g*	*g*	*g*	*g*	*g*
1	86 ± 12	172 ± 8	42 ± 13	13 ± 11	6 ± 3	13 ± 9	72 ± 5	166 ± 7	29 ± 18
2	63 ± 6	134 ± 51	56 ± 28	13 ± 11	6 ± 3	12 ± 9	50 ± 16	128 ± 48	43 ± 20
3	75 ± 9	128 ± 3	52 ± 2	27 ± 16	11 ± 8	24 ± 17	49 ± 24	117 ± 6	28 ± 16
4	103 ± 10	63 ± 20	757 ± 11	21 ± 13	7 ± 2	20 ± 13	82 ± 18	56 ± 19	737 ± 12
5	116 ± 16	104 ± 11	834 ± 92	22 ± 15	7 ± 4	20 ± 17	93 ± 4	97 ± 13	813 ± 78
6	124 ± 12	88 ± 27	861 ± 41	20 ± 3	7 ± 4	18 ± 8	104 ± 9	81 ± 24	843 ± 35
7	125 ± 13	85 ± 16	890 ± 63	23 ± 3	7 ± 2	22 ± 5	102 ± 14	78 ± 14	868 ± 58
8	131 ± 19	98 ± 22	947 ± 78	15 ± 6	4 ± 1	14 ± 6	117 ± 23	94 ± 23	933 ± 84
9	131 ± 2	105 ± 22	939 ± 47	9 ± 5	3 ± 2	7 ± 3	122 ± 6	102 ± 23	932 ± 45
10	133 ± 11	74 ± 20	987 ± 41	9 ± 6	4 ± 3	5 ± 4	124 ± 11	69 ± 23	981 ± 43
11	136 ± 10	16 ± 2	0	11 ± 2	6 ± 1	10 ± 1	126 ± 10	10 ± 2	0
12	136 ± 10	16 ± 2	0	11 ± 2	5 ± 1	10 ± 1	126 ± 10	11 ± 2	0
13	77 ± 22	118 ± 29	43 ± 13	12 ± 2	6 ± 1	10 ± 1	64 ± 20	113 ± 28	33 ± 14
14	79 ± 25	142 ± 22	64 ± 7	14 ± 8	7 ± 3	16 ± 10	65 ± 18	135 ± 21	49 ± 7

Day	Available nutrients from food tables†			Metabolizable energy Direct analysis‡/ food tables
	Protein	Fat	CHO	
	g	*g*	*g*	%
1	79 ± 8	171 ± 17	45 ± 8	96
2	70 ± 8	163 ± 12	59 ± 12	75
3	69 ± 4	142 ± 9	47 ± 2	77
4	114 ± 8	12 ± 1	836 ± 65	94
5	124 ± 5	17 ± 1	900 ± 89	104
6	124 ± 4	13 ± 0	948 ± 61	100
7	127 ± 3	15 ± 5	994 ± 68	98
8	129 ± 14	16 ± 1	989 ± 90	106
9	138 ± 10	20 ± 4	1038 ± 74	104
10	142 ± 13	17 ± 3	1073 ± 44	97
11	112 ± 4	5 ± 1	0	126
12	111 ± 3	5 ± 1	0	116
13	75 ± 19	156 ± 29	43 ± 14	72
14	75 ± 11	155 ± 12	65 ± 17	83

* Nutrients absorbed by the organism based on direct analysis of food and feces.
† Available nutrients in food using Atwaters coefficients which allow for partial losses in feces (20).
‡ From direct analysis, Metabolizable Energy = Gross Energy − (Fecal Energy + Urinary Energy).

(days 11 and 12), carbohydrate was still used as the principal energy substrate to the detriment of the glycogen stores, which decreased by ~700 g during these 2 d.

The initial increase in the glycogen stores by ~500 g was accompanied by an increase in the mean 24-h nonprotein respiratory quotient (**Fig 3**). The mean 24-h nonprotein respiratory quotient exceeded 1.00 (indicative of net de novo lipid synthesis) on day 2 of carbohydrate overfeeding; it continued to increase and reached a value of ~1.15 during the last 3 d of overfeeding when daily fat synthesis from glucose averaged 142 g/d. Even on day 11 when no carbohydrate was present in the diet, the mean nonprotein respiratory quotient was still just > 1.00.

During the 6 d during which lipid synthesis exceeded fat oxidation, net de novo lipogenesis amounted to a total of ~580 g. Because in addition to de novo lipogenesis some fat was provided in the diet (~85 g/d), the overall fat gain was ~1.1 kg.

The principal blood variables are presented in **Table 4**. The hypocaloric high-fat, low-carbohydrate diet caused both plasma glucose and insulin concentrations to decrease and free fatty acids to increase. During carbohydrate overfeeding plasma glucose rose initially but was maintained at the control value obtained at the beginning of the experiment by the rising plasma insulin concentrations. Plasma triglycerides increased 10-fold during carbohydrate overfeeding. This increase was also re-

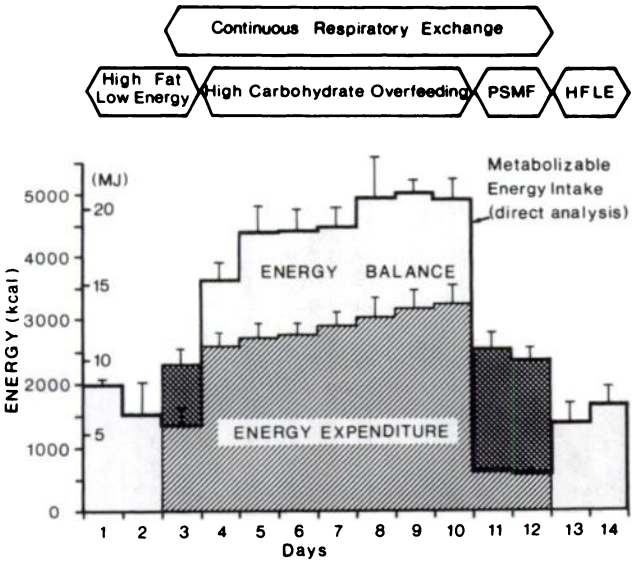

FIG 1. Experimental protocol (PSMF = protein-sparing modified fast; HFLE = high-fat, low-energy diet [~7 MJ]) and the changes in daily metabolizable energy intake —, energy expenditure ▨, positive energy balance □, and negative energy balance ■ (n = 3, $\bar{x} \pm$ SD).

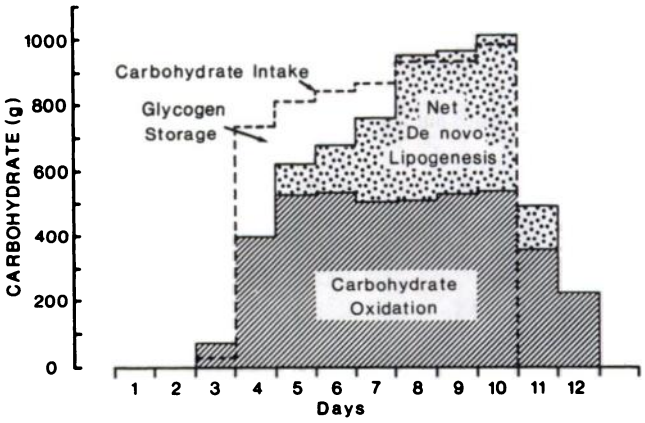

FIG 2. Daily carbohydrate intake (– – –) and its disposal (oxidation, glycogen storage, and conversion to lipid) during 7 d of progressive carbohydrate overfeeding (n = 3).

flected by changes in the lipoprotein fractions where it can be seen that the very-low-density lipoproteins (VLDLs) increased from 20 to 70%. Blood urea N was decreased on the high-carbohydrate, low-fat diet, increased markedly on the hypocaloric high-protein diet, and returned to control concentrations at the end of the experiment. Only very slight changes were observed in the thyroid hormone concentrations and these can be explained by the short duration of the experiment.

Discussion

After 3 d of hypocaloric high-fat, low-carbohydrate diet combined with a rigorous exercise program, the body's glycogen stores can be assumed to be very low. During carbohydrate overfeeding the rate of glycogen storage was initially large, decreasing as the stores became saturated. The maximum increases in stored glycogen observed were 1146, 629, and 654 g (in subjects 1, 2, and 3, respectively) with a mean of 810 g. Saturation of the glycogen stores occurred on day 4 of carbohydrate overfeeding for subjects 2 and 3 and on day 5 for subject 1. When the glycogen stores had increased by ~500 g (end of day 2 of overfeeding), carbohydrate oxidation and storage became insufficient to dispose of all of the ingested carbohydrate. The excess was disposed of by conversion to fat, ie, de novo lipogenesis. During the last 3 d of overfeeding, total carbohydrate utilization (ie, oxidation and glucose conversion to fat) was very similar to that which was ingested. Within the limitations of the methods used, these results demonstrate that carbohydrate balance was again achieved.

TABLE 3
Daily substrate balance (g) of the three subjects ($\bar{x} \pm$ SD)*

	Substrate entering the system			Substrate oxidation and disappearance from the system			Balance		
Day	Protein	Fat	CHO	Protein	Fat	CHO	Protein	Fat	CHO
1	72 ± 5	166 ± 7	29 ± 18						
2	50 ± 16	128 ± 48	43 ± 20						
3	49 ± 24	117 ± 6	28 ± 16	101 ± 16	164 ± 46	74 ± 40	−52 ± 31	−47 ± 41	−46 ± 48
4	82 ± 18	56 ± 19	737 ± 12	104 ± 12	49 ± 62	398 ± 87	−23 ± 29	7 ± 43	339 ± 82
5	93 ± 4	97 ± 13	813 ± 78	82 ± 7	−30 ± 38	622 ± 96	11 ± 27	127 ± 25	192 ± 53
6	104 ± 9	81 ± 24	843 ± 35	82 ± 17	−45 ± 37	677 ± 98	23 ± 8	126 ± 28	166 ± 74
7	102 ± 14	78 ± 14	868 ± 58	81 ± 23	−81 ± 17	792 ± 83	22 ± 16	160 ± 12	76 ± 31
8	117 ± 23	94 ± 23	933 ± 84	88 ± 23	−140 ± 52	950 ± 158	29 ± 10	235 ± 66	−18 ± 123
9	122 ± 6	102 ± 23	932 ± 45	101 ± 7	−137 ± 26	962 ± 102	22 ± 3	239 ± 17	−30 ± 64
10	124 ± 11	69 ± 23	981 ± 43	98 ± 7	−149 ± 14	1010 ± 37	26 ± 5	218 ± 20	−29 ± 6
11	126 ± 10	10 ± 2	0	115 ± 11	−43 ± 84	491 ± 61	11 ± 1	54 ± 86	−491 ± 61
12	126 ± 10	11 ± 2	0	145 ± 4	84 ± 20	223 ± 59	−20 ± 7	−73 ± 22	−223 ± 59
13	64 ± 20	113 ± 28	33 ± 14	137 ± 21					
14	65 ± 18	135 ± 21	49 ± 7	121 ± 6					

* Negative values for fat oxidation represent de novo fat synthesis.

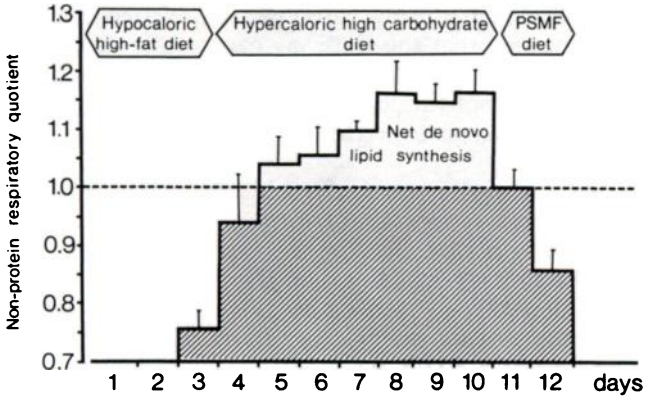

FIG 3. Average 24-h nonprotein respiratory quotients on day 3 of a high-fat, low-carbohydrate diet, during carbohydrate overfeeding, and for 2 d while on a PSMF devoid of carbohydrate. (n = 3, $\bar{x} \pm$ SD).

By adding the negative carbohydrate balances at the end of the experiment, it was possible to obtain a value for the amount of glycogen utilized. During this period 897, 891, and 752 g were utilized by subjects 1, 2, and 3, respectively. These results suggest that subjects 2 and 3 had not depleted their glycogen stores completely before carbohydrate overfeeding began and that they still contained ~100–200 g at that time.

From these data it would seem that the glycogen stores can maximally accommodate 800–900 g of carbohydrate and perhaps as much as 1–1.1 kg in trained athletes. These results are among the highest glycogen-storage values reported in the literature. Hedman (21) used respiratory-exchange measurements to calculate carbohydrate oxidation and hence glycogen depletion in four well-fed, trained cross-country skiers who skied to exhaustion and he calculated values ranging from 315 to 445 g carbohydrate. However, by using known values for the glycogen content of muscle and liver, he calculated that his subjects (mean body weight 72 kg) should have had maximal values of 700 g glycogen. Björntorp and Sjöström (22) came to the same conclusion using similar calculations but suggested that a further 100 g could be stored with 2 wk of carbohydrate overfeeding or by using the glycogen-loading technique. Bergström et al (1) reported values in the range 500–800 g in some of their subjects who followed the glycogen-loading technique. If this glycogen was derived from muscle, as suggested by Olsson and Saltin (23), the addition of liver glycogen would increase their values to those observed in the present study.

Although the values reported in this study may seem surprising the metabolic balance data does agree with the observed body weight changes. At the end of the overfeeding period after the glycogen stores had been reduced slightly by the high rate of lipogenesis and carbohydrate oxidation, body weight had increased by 4.6 kg and 700 g glycogen remained. Assuming that glycogen is stored with two to four times its weight of water (23, 24), ~2.1–3.5 kg of the change in body weight can be accounted for. Cumulative gains of body fat by de novo lipogenesis and from that which was provided in the diet amounted to 1.1 kg fat. Thus with the 665 g increase in lean body mass indicated by a gain of 133 g protein, we can account for the 4.6 kg increase in body weight. In a similar manner it is possible to account for 70% of the weight lost during the 2-d hypocaloric diet at the end of the experiment.

Initially large glycogen storage (340 g/d) on the day 1 of carbohydrate overfeeding was observed during carbo-

TABLE 4
Blood variables measured during the experiment ($\bar{x} \pm$ SD)*

	Day 1	Day 4 end high fat	Day 6	Day 9	Day 11 end high CHO	Day 13 end PSMF	Day 15 end test
Glucose (mmol/L)	4.9 ± 0.3	4.0 ± 0.4	5.1 ± 0.2	4.9 ± 0.2	4.8 ± 0.3	4.9 ± 0.2	4.8 ± 0.3
Insulin (pmol/L)	86 ± 43	50 ± 7	115 ± 36	136 ± 29	144 ± 50	57 ± 14	57 ± 14
Free fatty acids (μmol/L)	587 ± 399	984 ± 537	321 ± 335	399 ± 434	488 ± 504	700 ± 500	687 ± 283
Triglycerides (mmol/L)	1.3 ± 1.2	0.8 ± 0.4	1.4 ± 0.4	5.3 ± 0.8	8.6 ± 1.0	3.5 ± 1.2	0.9 ± 0.3
Cholesterol (mmol/L)	4.8 ± 0.5	5.3 ± 1.2	4.2 ± 0.8	4.7 ± 0.8	5.8 ± 0.9	6.4 ± 0.9	6.6 ± 1.2
Lipoproteins							
VLDL pre β (%)	18 ± 5	18 ± 8	—	56 ± 16	68 ± 24	—	16 ± 3
LDL β (%)	53 ± 8	59 ± 10	—	28 ± 11	20 ± 16	—	64 ± 10
HDL α (%)	29 ± 6	23 ± 4	—	16 ± 4	12 ± 9	—	20 ± 8
BUN (mmol/L)	6.3 ± 2.3	6.9 ± 1.4	4.4 ± 1.0	4.2 ± 0.8	4.2 ± 0.9	10.0 ± 1.4	7.0 ± 1.5
T_3 total (nmol/L)	2.22 ± 0.27	1.86 ± 0.38	2.26 ± 0.21	2.53 ± 0.28	2.39 ± 0.58	2.10 ± 0.31	2.29 ± 0.46
T_4 total (nmol/L)	96 ± 20	117 ± 20	104 ± 21	87 ± 11	100 ± 36	87 ± 18	101 ± 13
TSH (mU/L)	2.6 ± 0.9	2.0 ± 1.3	3.4 ± 1.5	3.5 ± 1.6	2.7 ± 1.4	2.2 ± 0.8	2.2 ± 1.0

* High fat, high-fat, low-carbohydrate diet; high CHO, high-carbohydrate, low fat diet; PSMF, protein-sparing modified fast; VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; BUN, blood urea nitrogen; T_3, triodothyronine; T_4, thyroxine; TSH, thyroid stimulating hormone.

hydrate refeeding after starvation (2) and after exhaustive exercise (25). The decreasing ability of the body to store glycogen may be mediated by inhibition of glycogen synthetase activity with increasing glycogen concentrations (25, 26).

We (6, 7) and others (5) demonstrated that humans can ingest relatively large amounts of carbohydrate without initiating de novo lipid synthesis at rates exceeding concomitant fat oxidation. These results are consistent with in vitro data demonstrating very low fatty acid synthase activity in human liver and adipose tissue (27) even after the ingestion of a carbohydrate-rich diet for 3 d. However, these authors did observe elevated fatty acid synthase activities in certain situations where long-term fat-free diets were being received, eg, parenteral nutrition. It is precisely under such conditions that high rates of de novo lipid synthesis were observed with indirect calorimetry (28).

By extrapolating from in vitro data, Björntorp and Sjöström (22) also concluded that de novo fatty acid synthesis from carbohydrate is a quantitatively insignificant pathway in the whole human organism. They presumed that it remained so even during carbohydrate overfeeding where excess carbohydrate would cause hyperglycemia and hyperinsulinemia and eventually glucose intolerance. In the present study, postprandial plasma glucose and insulin concentrations were not measured during the carbohydrate overfeeding days but no glucosuria was ever observed. However, fasting concentrations were measured every other morning. Fasting glycemia was normal (up to 5.1 mmol/L) but insulin concentrations rose from 50 ± 7 to 144 ± 50 pmol/L.

In addition to providing an assessment of the body's maximal glycogen storage capacity, this study also demonstrates that de novo lipogenesis can become a major metabolic pathway for the disposal of excess glucose carbons. This is not only evident from the respiratory exchange data but also from the increases observed in plasma triglyceride concentrations and the proportion of plasma VLDL. Because very little triglyceride was provided in the diet at this time, it must have originated as newly formed triglyceride in the liver, which is the principal site of de novo lipid synthesis in man (29). Our laboratory and others (5, 27, 30) showed that de novo lipogenesis does not contribute to increasing the body fat stores even when very large amounts of carbohydrate (500 g) are occasionally consumed. Glycogen storage followed by high subsequent rates of glucose oxidation can easily accommodate the daily ingestion of relatively large amounts of carbohydrate without there being a need to convert carbohydrate to fat.

Our data suggest that glycogen stores must increase by ~500 g before appreciable de novo lipogenesis begins. Provided that massive amounts of carbohydrate continue to be ingested, the glycogen stores become saturated so that the only way of disposing of additional excess carbohydrate is by fat synthesis in addition to maximal use of glucose for energy generation. Although it has been suggested that the capacity for de novo lipogenesis is limited even during carbohydrate overfeeding, we demonstrated that this pathway can readily dispose of nearly 500 g of glucose per day. Furthermore, the large excess of carbohydrate entering the organism did not even cause excessive increases in circulating glucose concentration.

As shown in Figure 1, the subjects' energy expenditures increased markedly during the carbohydrate overfeeding period. Because the experimental protocol aimed at initially depleting their glycogen stores, the subjects' energy expenditure on day 3 is somewhat less than their maintenance energy expenditure. The latter can be estimated at 10 MJ/d (2400 kcal/d), ie, the observed energy expenditure on day 3 (9.66 MJ/d) plus 10% of the energy deficit on that day (ie, 3.96 MJ × 10% = 0.4 MJ), to account for the thermic effect of that amount of food. By day 7 on the high-carbohydrate, low-fat diet, their 24-h energy expenditures had increased by 3.5 MJ/d (840 kcal/d). This 35% increase is one of the most substantial diet-induced increases in energy expenditure demonstrated in man. It is of interest to assess how much of this food-induced thermogenesis is due to the obligatory costs incurred for nutrient storage. Considering that fasting blood glucose levels remained in the normal range and that lipogenesis occurred during the entire day, we assumed that 80% of the carbohydrate consumed was converted to glycogen before being used for energy production or lipogenesis. Carbohydrate stored as glycogen requires the expenditure of 2 mol ATP per glucose moiety converted into glycogen plus 0.5 mol for the cost of active transport in the gut and other phenomena, such as digestive enzyme synthesis and gut motility (31). Because glucose oxidation yields 36 mol ATP, the cost of glycogen synthesis consumes 2.5/36 or 7% of the glucose stored as glycogen. The transformation of glycogen into fatty acids, the subsequent esterification before export from the liver, and then triglyceride storage in adipose tissue consume additional ATP, estimated at 18%. Thus ~25% of the energy of the glucose channelled into de novo lipogenesis can be expected to be needed for this process.

Of the energy consumed in excess of maintenance energy, 75% was retained and 25% dissipated. Such a high rate of dissipation of energy consumed in excess can only be brought about by conditions leading to the induction of high rates of carbohydrate conversion into fat. Indeed, the increase in daily energy expenditure was only 8% during the day 1 before lipogenesis became necessary for the storage of some of the excess carbohydrate calories consumed. Subsequently, when the nonprotein respiratory quotient became markedly > 1.0, energy expenditure rose further, causing the dissipation of nearly 30% of the calories consumed in excess.

Finally, our findings indicate that the body's glycogen stores are far from completely filled under normal ad libitum conditions. If the glycogen stores are not limited by physical saturation of the glycogen storage capacity, one can more readily envision that individual differences and responsiveness to food palatability and accessibility may

influence to a considerable extent the range within which glycogen stores are spontaneously maintained. In turn this will affect the relative contributions that glucose and free fatty acids tend to make to the metabolic fuel mix used for energy generation and the conditions for which the steady state of body weight maintenance tends to be achieved (32).

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