Macronutrient disposal during controlled overfeeding with glucose, fructose, sucrose, or fat in lean and obese women[1–3]

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ABSTRACT

Background: Previous short-term studies (≤ 6 h) showed differences in energy expenditure (EE) and macronutrient oxidation in response to overfeeding with different types of dietary carbohydrate. This finding could have implications for obesity.

Objective: We used 96-h continuous whole-body calorimetry in 8 lean and 5 obese women to assess metabolic disposal (energy dissipation and glycogen or fat storage) of a controlled excess of dietary energy supplied as different carbohydrate sources or as fat.

Design: Five dietary treatments were applied in random order: energy balance (control) and overfeeding by 50% of energy requirements with fat (O_{fat}) or predominantly with glucose, fructose, or sucrose (O_{cho}). Macronutrient oxidation rates were assessed from nonprotein gaseous exchanges. Net macronutrient balances were calculated as cumulative differences between intake and oxidation.

Results: Increased EE in response to overfeeding dissipated 7.9% of the energy excess with a variation in EE of < 1.7% across overfeeding treatments (NS). EE during the O_{fat} treatment significantly exceeded that during the control treatment in the lean but not in the obese women. There were no significant differences between lean and obese women in macronutrient oxidation or balances, so data were pooled. O_{cho} induced glycogen storage on day 1 (≈ 100 g) but thereafter progressively stimulated carbohydrate oxidation so that balance was reached on days 3 and 4. Fat oxidation was proportionately suppressed. Of the excess carbohydrate, 74% was oxidized; there were no significant differences between the various O_{cho} treatments. O_{fat} stimulated fat oxidation by 18% and suppressed carbohydrate oxidation. On average, 12% of the excess energy was stored as glycogen and 88% as fat; there was no significant difference between overfeeding treatments.

Conclusion: There was no significant difference in fat balance during controlled overfeeding with fat, fructose, glucose, or sucrose.   *Am J Clin Nutr* 2000;72:369–77.

INTRODUCTION

The rapidly increasing prevalence of obesity in affluent and emerging countries indicates that large numbers of people in the modern world live under conditions of a sustained positive energy imbalance (1). A combination of changes in dietary and activity patterns, especially an increase in high-fat, energy-dense diets and sedentary lifestyles, has been identified as a key component of the problem (2). However, relatively little attention has been paid to the finer details of diet composition.

In recent years, there have been significant changes in the composition of some of the major macronutrients in the diet that might impinge on energy balance and obesity. In particular, there have been major increases in the consumption of highly refined carbohydrates (3). Because of its high oxidative reactivity, carbohydrate (together with alcohol when consumed) plays a dominant role in determining the oxidation and storage of metabolic fuels. We described previously the "oxidative hierarchy" that governs whole-body fuel selection according to the dietary supply of the 4 energy-giving macronutrients (fat, carbohydrate, protein, and alcohol) in many human indirect calorimetry studies in which substrate oxidation rates were measured (4–8). When the ratio of fat to carbohydrate in diets is altered, carbohydrate metabolism is very tightly autoregulated (6–11), in contrast with the regulation of fat metabolism (11–13). During overfeeding with carbohydrate, there is not only an increase in carbohydrate oxidation (6–8, 10, 14, 15) but also a decrease in fat oxidation (6, 15). In contrast, during overfeeding with fat, there is virtually no corresponding increase in fat oxidation (11, 12, 15). Thus, fat can accumulate indirectly in response to overfeeding, even with high-carbohydrate diets (13). The oxidative hierarchy provides an excellent model for predicting the effect of various dietary regimens on fat, glycogen, and protein balance. However, this model has so far considered carbohydrate as a single homogeneous entity.

There is evidence that there are differences in energy expenditure (EE) and macronutrient oxidation when different sources of carbohydrate (eg, glucose, fructose, and sucrose) are compared. Fructose has an especially fast rate of hepatic uptake and its metabolism is independent of insulin (16, 17). Fructose was shown to

TABLE 1
Physical characteristics of the lean and obese women[1]

	Lean (n = 8)	Obese (n = 5)
Age (y)	53.1 ± 0.3	52.4 ± 4.8
Weight (kg)	65.6 ± 6.0[a]	81.0 ± 4.5[b]
BMI (kg/m^2)	25 ± 1.0[a]	31 ± 4.0[b]
Percentage body fat (%)	35.1 ± 5.6[a]	44.8 ± 4.6[b]

[1] $\overline{x}$ ± SD. Values in the same row with different superscript letters are significantly different, $P < 0.05$ (Student's t test).

increase carbohydrate oxidation, reduce fat oxidation, and increase thermogenesis (18–21) more than was an isoenergetic amount of glucose (18–21) or starch (18). Sucrose (the disaccharide of fructose and glucose) was shown to have a similar effect on EE and carbohydrate oxidation to fructose, compared with glucose and starch (18). However, these metabolic responses to different carbohydrates were all recorded in studies of very short duration (6 h), and few data on longer-term effects are available.

In this study we tested whether, under controlled conditions of excess energy balance and physical activity, there is a difference in the potential of different carbohydrates, consumed as simple sugars, to induce fat storage. This is particularly relevant because diets that are low in fat and high in carbohydrate are currently recommended, leading to some speculation that a liberal intake of highly refined carbohydrates may affect health adversely (22). The study used continuous indirect whole-body calorimetry to measure energy balance and macronutrient disposal in lean and obese women during 96 h of overfeeding by 50% of energy requirements in which the excess energy was provided as fat or predominantly as glucose, fructose, or sucrose. The extent and duration of the overfeeding were carefully designed to be within the metabolic limits that would permit the excess energy to be stored totally as fat, largely as glycogen, or as a variable mixture of the 2. We were primarily interested in assessing net fat accumulation with the different dietary regimens.

SUBJECTS AND METHODS

Study design

Thirteen women (8 lean and 5 obese) were recruited from the Medical Research Council Dunn Clinical Nutrition Centre volunteer register and by local advertisement. Characteristics of the women are shown in **Table 1**; percentage body fat was measured by dual-energy X-ray absorptiometry (QDR1000; Hologic, Waltham, MA). All the women were nonsmoking omnivores who, aside from those who were overweight, were otherwise healthy. Some of the women were taking low-dose estrogen as hormone replacement therapy. All the women underwent a medical examination before taking part in the study. Written, informed consent was obtained from each woman, and the study was approved by the Dunn Nutrition Centre Ethical Committee.

Each woman was required to attend the research center on 5 occasions and spend 108 h continuously in a whole-body calorimeter on each occasion. In premenopausal women, each measurement was made in the same phase of the menstrual cycle. The women arrived at the center in the evening, were weighed while they were lightly clad, and entered the calorimeter at 2000. Measurements commenced at 0800 on day 1 after an overnight

equilibration period and finished at 0800 on day 5. While they were in the calorimeter, all the women adhered to an identical fixed light-activity regimen of rest, meals, and exercise. Basal metabolic rate (BMR; in kJ/h) was measured on the mornings of days 1 and 2 from 0800 to 0900 after a 12-h fast. Sleeping metabolic rate (SMR; in kJ/h) was recorded overnight, from 0000 to 0600, on each of 2 consecutive nights (days 2 and 3) for each woman per treatment. The women spent a total of 120 min/d in obligatory standing: 30 min getting up each morning, 5 min before each exercise period, 10 min after each exercise period, and 30 min in preparation for bed. There were 4 periods of 15 min of exercise on a static cycle ergometer at a work rate of 25 W (0.5 kp and 40 rpm). One woman performed the same work but used stepping instead of cycling as her exercise routine. Except for these periods of exercise and standing, the women were required to remain seated and to undertake only sedentary activities. Each calorimeter was comfortably furnished with a desk, an armchair, a bed, a television, a videocassette player, a radio and compact disc player, a washbasin, and a portable toilet.

Dietary treatments

While in the calorimeter, each subject followed 1 of 5 dietary treatments assigned in random order. These included a control treatment in which the energy intake (EI) was calculated as individually measured BMR $\times$ 1.3, to account for controlled EE while the subjects were in the calorimeter. The EIs of the subjects for all the treatments were assigned to the nearest EI of 7.0, 7.5, 8.0, 8.5, or 9.0 MJ/d. During each of the 4 other treatments, EI was calculated as control EI plus 50% overfeeding predominantly with carbohydrate (O_{cho}) or with fat (O_{fat}). Three different sources of dietary carbohydrate were used for the O_{cho} diets: glucose (O_{glu}), sucrose (O_{suc}), and fructose (O_{fru}). Protein intakes were kept constant across all treatments. With the O_{fat} treatment, the extra fat was incorporated directly into the meals. With the O_{cho} treatments, the excess carbohydrate was administered largely as a lemon drink sweetened with the appropriate carbohydrate. Because of concerns about administering too much fructose, the 50% excess energy was provided as 54% fructose and the remainder was provided as fat. Glucose and sucrose were handled similarly. The control diets provided 48% of energy as carbohydrate, 40% as fat, and 12% as protein. The O_{cho} diets provided 50% of energy as carbohydrate, 42% as fat, and 8% as protein. The O_{fat} diet provided 32% of energy as carbohydrate, 60% as fat, and 8% as protein. With each treatment, the subjects were fed 5 meals/d: breakfast, a morning snack, lunch, an evening snack, and dinner. The meals were designed to be as normal as possible and were isoenergetic.

Whole-body calorimetry

The study used the 3 calorimeter chambers at the Dunn Clinical Nutrition Centre to measure oxygen consumption and carbon dioxide production. The calorimeters were ≈3.5 m long, ≈2.8 m wide, and ≈2.1 m high, with a total volume of 23 m^3. The rooms were maintained at a temperature of 24 ± 0.5 °C and ventilated at a rate of 200 L/min, monitored by a type 2100 Rotameter (KDG Mowbray, Slough, United Kingdom) and a vortex-shedding flow meter type VL512 (Delta Controls, West Molesey, United Kingdom). Oxygen concentration was measured by using a paramagnetic analyzer (model 184; Servomex, Crowborough, United Kingdom), carbon dioxide by using a single-beam infrared analyzer (model 1510; Servomex), and water

TABLE 2
Energy intake, expenditure, and balance over 96 h in lean and obese women in response to overfeeding[1]

	Treatment				
	Control	O_{fru}	O_{glu}	O_{suc}	O_{fat}
			kJ		
Lean ($n = 8$)					
Intake	30855 ± 1458	46165 ± 2251	46165 ± 2251	46165 ± 2255	46185 ± 2255
Expenditure	32351 ± 2735[a]	33370 ± 2578[a,b]	33762 ± 2616[b]	33335 ± 2751[a,b]	33637 ± 2461[b]
Balance	−1496 ± 2635[a]	12794 ± 2634[b]	12402 ± 2899[b]	12839 ± 3110[b]	12547 ± 2257[b]
Obese ($n = 5$)					
Intake	33280 ± 1788	49772 ± 2556	49772 ± 2556	49772 ± 2556	49836 ± 2636
Expenditure	34616 ± 1609[a]	36180 ± 1804[b]	36081 ± 1354[a,b]	35670 ± 1478[a,b]	34752 ± 1758[a,b]
Balance	−1343 ± 1453[a]	13591 ± 2746[b]	13691 ± 1271[b]	14101 ± 1887[b]	15083 ± 1944[b]
All subjects ($n = 13$)					
Intake	31787 ± 1953	47552 ± 2910	47552 ± 2910	47558 ± 2911	47589 ± 2949
Expenditure	33222 ± 2557[a]	34451 ± 2643[b]	34654 ± 2446[b]	34233 ± 2557[b]	34066 ± 2209[a,b]
Balance	−1434 ± 2181[a]	13100 ± 2593[b]	12897 ± 2422[b]	13324 ± 2690[b]	13522 ± 2425[b]

[1] $\overline{x}$ ± SD. Values in the same row with different superscript letters are significantly different, $P < 0.05$ (ANOVA with Scheffe's post hoc test). O_{fru}, 50% overfeeding predominantly with fructose; O_{glu}, 50% overfeeding predominantly with glucose; O_{suc}, 50% overfeeding predominantly with sucrose; O_{fat}, 50% overfeeding with fat.

vapor by using an optical condensing dew point meter (type 1100ap; General Eastern, Watertown, MA). Data were collected by using a systems voltmeter with an 18-channel scanner (type 7062; Solartron, Farnborough, United Kingdom) into a personal computer through a measurement coprocessor (Hewlett-Packard, Palo Alto, CA). The carbon dioxide and oxygen analyzers for each calorimeter were manually calibrated against bottled nitrogen (zero readings), 1% carbon dioxide in air (carbon dioxide analyzer span), and fresh air (oxygen analyzer span) before subject entry. Every 3 h thereafter, an automatic sequence of calibration was conducted. Between these calibrations, calorimeter air was sampled every 300 s and ventilation air was sampled every 30 min. The results were computed by using the fast-response equations of Brown et al (23).

Substrate oxidation calculations

Pooled urine samples were collected at predetermined times throughout each day and duplicate aliquots were frozen (−20°C) to determine nitrogen excretion, from which net protein oxidation was calculated (LECO FP-248 Nitrogen/Protein Determinator; LECO Instruments UK Ltd, Cheshire, United Kingdom). The net oxidation of fat and carbohydrate was calculated from oxygen consumption, carbon dioxide production, and nitrogen excretion; it was assumed that the ratios of carbon dioxide production to oxygen consumption were 1:0, 0:71, and 0:835 for carbohydrate, fat, and protein, respectively. The volumes of oxygen consumed per gram of substrate were assumed to be 0.746, 2.01, and 0.952 g/L for carbohydrate, fat, and protein, respectively (24, 25).

Statistics

Data from the first 12 h of each 108-h treatment period in the calorimeter were eliminated from the analysis of energy and macronutrient balances. Energy balance, EE, macronutrient balance, and macronutrient oxidation rates for the residual 96-h periods were analyzed by using analysis of variance (ANOVA) with DATA DESK 4.1 (Data Desk Inc, Ithaca, NY); the main variables were dietary treatment, subject group (lean versus obese), time, and the interactions among these 3. The effect of individual sub-

ject on the analyses was taken into account by nesting individual subject within subject group in the ANOVA model. When ANOVA resulted in a significant effect of the main variables, the data were further tested by using Scheffe's post hoc analysis.

The precision of the estimates, and hence the power of the study to detect subtle differences in macronutrient storage, can be calculated from first principles, on the basis of the known precision of the gas exchange measurements, or from the intrasubject CV across repeat runs. The former yields precision estimates (±1 SD) for 96-h measurements of ≈0.3% for EE, ≈170 kJ (10 g) for carbohydrate, and ≈200 kJ (5 g) for fat. The repeated-measures analysis could be performed across the 3 carbohydrate runs because the post hoc analysis indicated no difference in response. This yielded intraindividual variabilities (between-run SD) of only 144, 328, and 248 kJ for energy, carbohydrate, and fat, respectively; because this latter estimate includes any real differences between responses to the different carbohydrates, and any possible differences in the subjects' metabolic body size occurring between runs that were sometimes months apart, it must represent the outside limit of the true precision of the estimates. The precision is further enhanced by a factor of >2 (ie, root mean square of 5 and 8) for comparisons of lean and obese women, and by 3.6 (ie, √13) for comparisons across treatments in the groups combined. Thus, the ANOVA did not lack discriminatory power and the experiment would have been able to detect very small differences had they existed.

RESULTS

Energy balance

The measured EEs of all women were constant across all 4 d in the calorimeter (data not shown) and was significantly affected both by subject group (lean versus obese) and by diet; there was a potentially important group × diet interaction associated with the O_{fat} treatment (**Table 2**). EE was consistently 7–8% higher in the obese than in the lean subjects ($P < 0.001$) with all dietary treatments except O_{fat}, for which the difference was only 3% (NS). This was because the O_{fat} treatment had no

TABLE 3
Basal and sleeping metabolic rate in the lean and obese women in response to overfeeding[1]

	Treatment				
	Control	O_{fru}	O_{glu}	O_{suc}	O_{fat}
	kJ/d				
Basal metabolic rate					
Lean	5773 ± 504	5923 ± 562	6001 ± 489	5784 ± 489	5960 ± 370
Obese	6395 ± 527[2]	6439 ± 325[2]	6153 ± 242[2]	6378 ± 316[2]	6206 ± 284[2]
Sleeping metabolic rate					
Lean	5500 ± 432	5656 ± 471	5727 ± 473	5678 ± 451	6033 ± 669
Obese	6170 ± 290[2]	6369 ± 252[2]	6255 ± 194[2]	6239 ± 193[2]	6161 ± 256[2]

[1] $\overline{x}$ ± SD. O_{fru}, 50% overfeeding predominantly with fructose; O_{glu}, 50% overfeeding predominantly with glucose; O_{suc}, 50% overfeeding predominantly with sucrose; O_{fat}, 50% overfeeding with fat.
[2] Significantly greater than in lean women, $P < 0.001$ (ANOVA).

significant effect on EE in the obese group compared with the control treatment. This apparent failure of obese women to respond to fat overfeeding was reported previously (26). However, although there was a significant effect of the O_{fat} treatment in the lean women but not in the obese women, the difference between groups was not significant.

Dietary treatment had a highly significant overall effect on EE ($F = 6.6$, $P < 0.001$). With the overfeeding treatments, EE increased by only 2.6–5.0% in the lean women and by only 1.0–4.0% in the obese women in response to the 50% increase in EI. The average total thermic effect of the 50% overfeeding in all subjects was 7.9 ± 4.9% (computed as Æexpenditure/Æintake × 100). Variations in response to overfeeding between subjects were small. The average incremental EE across the O_{cho} runs (ie, O_{cho} EE − control EE) showed a between-subject SD of just 779 kJ/d, which is equivalent to 2.25% of the total EE.

Both the lean and the obese women were in slightly negative energy balance after 96 h of the control diet with averages of 1496 kJ (−374 kJ/d) and 1343kJ (−336 kJ/d), respectively (Table 2). As intended, energy balance was significantly more positive with the overfeeding treatments than with the control treatment, representing between 37% and 40% of EE ($F = 812$, $P < 0.001$) (Table 2). There was a significant interaction between subject group and treatment, which was caused by the higher energy balance with the O_{fat} treatment in the obese women ($F = 3.52$, $P < 0.05$).

Basal and sleeping metabolic rate

For each subject, BMR and SMR were constant across both days of measurement within each dietary treatment. There was a significant effect of subject group (lean versus obese) on BMR ($F = 30$, $P < 0.001$), although there was no significant effect of dietary treatment and no significant interaction between subject group and dietary treatment on BMR. This meant that BMR was consistently higher in the obese than in the lean women, by between 2.5% and 9.7%, across all dietary treatments (**Table 3**). SMR was also significantly affected by subject group ($F = 44$, $P < 0.001$). Although there was no significant effect of dietary treatment on SMR and there was no significant interaction between subject group and dietary treatment, SMR with the control diet and the O_{cho} treatment were between 8.5% and 11% greater in the obese than in the lean women. In contrast, SMR was only 2.1% greater in the obese than in the lean women with the O_{fat} treatment (Table 3).

Protein balance

Because protein intake was held constant across all treatments, the only effects on protein balance were those mediated by the other substrates, and the results are therefore not tabulated in detail. In summary, with the control diet, protein balance remained within 5 g/d on days 1–4 and overall balance was not significantly different from zero. The responses to the O_{cho} treatments were very similar, with a negative balance (4–9 g) on day 1 followed by positive balances of 6–10 g/d on days 2–4. The O_{fat} treatment was similar to the other overfeeding treatments except on day 1, when protein balance was neutral. Previously, we observed positive protein balances in response to energy overfeeding at a constant protein intake (6).

Carbohydrate balance

There were no significant differences in carbohydrate oxidation or balance between the lean and obese women (**Table 4**). Therefore, the aggregate results are discussed. Carbohydrate oxidation was greatest during the O_{cho} treatments and lowest during the O_{fat} treatment ($F = 125.8$, $P < 0.001$). The significant suppression of carbohydrate oxidation with the O_{fat} treatment led to a positive carbohydrate balance, even though the overfeeding was entirely in the form of fat.

Despite the increase in oxidation rate, all the women were in positive carbohydrate balance during all of the overfeeding treatments. The positive balance induced by each O_{cho} treatment was similar at 1801–2132 kJ over 96 h (equivalent to ≈100–125 g glycogen). Glycogen storage with the O_{fat} diet was lower at 1157 kJ (≈70 g glycogen), but not significantly so.

Daily and cumulative balance values over the 4-d experiments for the O_{suc} and control treatments are shown in **Figure 1**. (In this and subsequent figures, the O_{suc} treatment is used simply as an example of the O_{cho} diets because all these diets induced similar changes.) The daily carbohydrate imbalance with O_{suc} asymptotically approached zero as carbohydrate oxidation gradually increased until it exactly matched intake. This caused glycogen storage to plateau at a new level ≈110 g above the initial value. In **Figure 2**, the same O_{suc} values are replotted against the O_{fat} results. The O_{fat} treatment also caused an initial storage of carbohydrate (through a slight fat-induced suppression of carbohydrate oxidation on day 1), but the cumulative glycogen storage after 96 h was only half that with the O_{suc} treatment. The cumulative carbohydrate balance plots for all 5 treatments are shown in **Figure 3**.

TABLE 4
Carbohydrate intake, oxidation, and balance over 96 h in the lean and obese women in response to overfeeding[1]

	Treatment				
	Control	O_{fru}	O_{glu}	O_{suc}	O_{fat}
	kJ				
Lean (*n* = 8)					
Intake	14 765 ± 792	23 062 ± 1116	23 062 ± 1116	23 062 ± 1116	14 782 ± 728
Oxidation	15 504 ± 1675[a]	21 450 ± 1530[b]	20 548 ± 1344[b]	21 241 ± 1118[b]	13 903 ± 1163[a]
Balance	−740 ± 1763[a]	1612 ± 1393[b]	2514 ± 1407[b]	1821 ± 1119[b]	879 ± 995[a,b]
Obese (*n* = 5)					
Intake	16 004 ± 818	24 900 ± 1341	24 900 ± 1341	24 900 ± 1341	15 964 ± 846
Oxidation	15 772 ± 1879[a]	22 729 ± 2030[b]	23 379 ± 1604[b]	23 132 ± 1580[b]	14 360 ± 1054[a]
Balance	231 ± 1816[a]	2171 ± 2425[b]	1521 ± 2140[b]	1768 ± 2175[b]	1603 ± 1495[b]
All subjects (*n* = 13)					
Intake	15 241 ± 991	23 769 ± 1480	23 769 ± 1480	23 769 ± 1480	15 236 ± 951
Oxidation	15 607 ± 1683[a]	21 942 ± 1777[b]	21 637 ± 1991[b]	21 968 ± 1574[b]	14 079 ± 1101[c]
Balance	−366 ± 1776[a]	1827 ± 1781[b]	2132 ± 1713[b]	1801 ± 1519[b]	1157 ± 1207[b]

[1] $\overline{x}$ ± SD. Values in the same row with different superscript letters are significantly different, $P < 0.05$ (ANOVA with Scheffe's post hoc test). O_{fru}, 50% overfeeding predominantly with fructose; O_{glu}, 50% overfeeding predominantly with glucose; O_{suc}, 50% overfeeding predominantly with sucrose; O_{fat}, 50% overfeeding with fat.

Fat balance

There were no significant differences in fat oxidation or balance between the lean and obese subjects for any of the treatments (**Table 5**). This lack of difference, despite the slightly elevated EE in the obese group (Table 2), is explained by the fact that the increased EE was made up of small increases in fat and carbohydrate oxidation.

Fat oxidation was greater during the O_{fat} treatments than during the O_{cho} and control treatments in both lean and obese women (Table 5). In the lean subjects, fat oxidation was significantly higher with the O_{fat} diet than with all other treatments (Scheffe's post hoc test; $P < 0.001$). In the obese women, fat oxidation was significantly higher with the O_{fat} treatment than with any of the O_{cho} treatments (Scheffe's post hoc test; $P < 0.001$) but was not significantly different from the control treatment

(Table 5). This again strengthens the observations of Astrup et al (26), who previously noted an impaired response of obese subjects to high-fat diets. As anticipated, all overfeeding treatments induced a positive fat balance compared with the control treatment ($P < 0.001$) but, importantly, there was no significant difference in fat balance between any of the macronutrients.

Daily and cumulative fat balances for the O_{suc} treatment are compared against the control treatment in **Figure 4**. The small acceleration in fat accumulation after day 1 resulted from the slight delay in the induction of carbohydrate oxidation over the first 2 d (Figure 1) and the greater suppression of fat oxidation when carbohydrate oxidation reaches its maximal value. The changes in fat balance with the O_{suc} and O_{fat} treatments are compared in **Figure 5**. Although fat storage was marginally greater with the O_{fat} treatment because of the lower glycogen storage
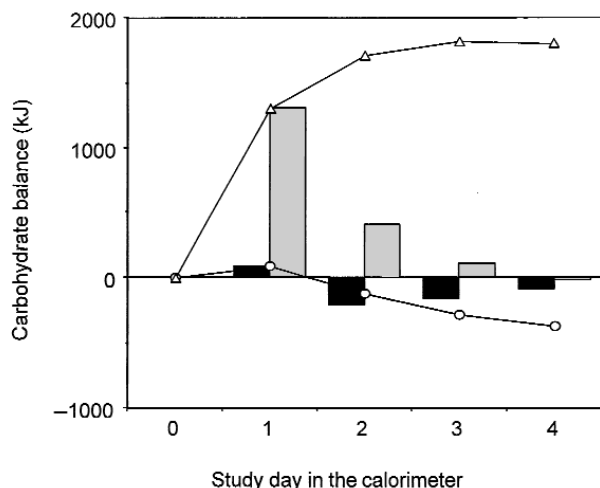

FIGURE 1. Daily and cumulative changes in carbohydrate balance during the control treatment (solid bars and circles) and during overfeeding by 50% of energy requirements predominantly with sucrose (shaded bars and triangles). The bars represent daily balance; the lines represent cumulative balance. Data were pooled for the lean and the obese women, *n* = 13.
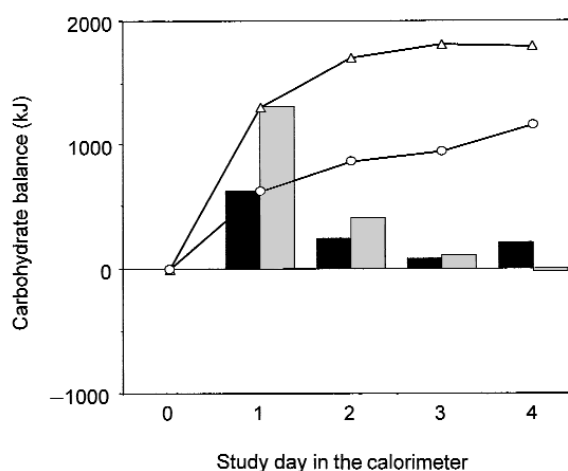

FIGURE 2. Daily and cumulative changes in carbohydrate balance during overfeeding by 50% of energy requirements with fat (solid bars and circles) and overfeeding by 50% of energy requirements predominantly with sucrose (shaded bars and triangles). The bars represent daily balance; the lines represent cumulative balance. Data were pooled for the lean and the obese women, *n* = 13.
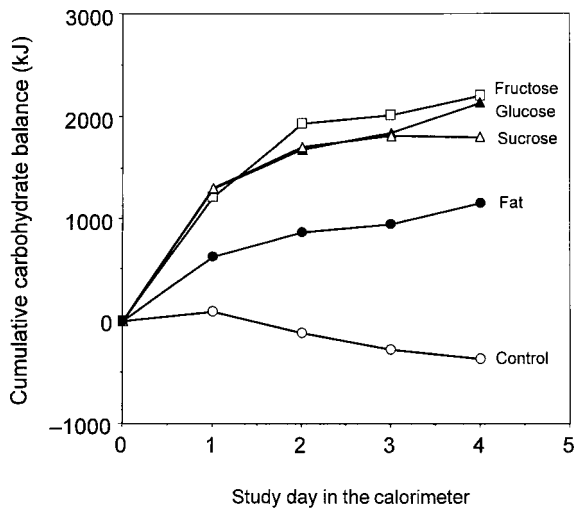
FIGURE 3. Cumulative changes in carbohydrate balance across all treatments. Data were pooled for the lean and the obese women, $n = 13$.

(Figures 2 and 3), the difference was not significant. The similarity in the cumulative fat balance curves for all 4 overfeeding diets is illustrated in **Figure 6**.

DISCUSSION

The key conclusion of this study was that there were no significant differences in 96-h energy or macronutrient balance between overfeeding regimens that used the monosaccharides glucose and fructose, the disaccharide sucrose, or fat. This strengthens predictions arising from our previous work on the oxidative hierarchy model of fuel selection (4–6) and now extends this model to include simple sugars. The result is at variance with observations on the short-term thermogenic response to glucose, fructose, and fat (18–21) and with observations that excess fat consumption promotes more efficient fat storage than does excess carbohydrate feeding (5, 27, 28).

Our experimental design had several advantages over previous methods. First, the intensive protocol, involving five 4-d periods of continuous whole-body calorimetry, provided high-precision estimates of energy and macronutrient balance. Second, the subjects' nutrient intakes and physical activity patterns were tightly controlled to ensure that all measured effects represented true metabolic responses to the different compositions of the overfeeding diets. Third, the incremental energy was supplied as single macronutrients added to a normal mixed diet instead of using the artificial situation of meals consisting of pure glucose, fructose, or fat, which is often used in thermogenic studies (11, 21). Fourth, the 96-h time frame ensured that any short-term perturbations in glycogen stores had time to exert their downstream autoregulatory effects on fuel selection.

Although the obese subjects had a slightly greater absolute EE than did the lean subjects, the difference was <10% and there were no significant differences in response to overfeeding. There was slight evidence that the obese women did not increase EE in response to the fat overfeeding, as claimed previously by Astrup et al (26), but the difference was not significant when compared directly with the results for the lean women. Additionally, although BMR and SMR were significantly higher in the obese than in the lean women, there was no significant effect of overfeeding on either of these metabolic measurements. The slight difference in overall EE was the only evidence of constitutive differences between the lean and obese women and, because all other differences were nonsignificant, the data were pooled for further investigations of the treatment effects.

Note that the response of EE to overfeeding was very constant. For the lean and obese women combined, the control 96-h EE of 33.22 MJ increased to 34.45, 34.65, 34.23, and 34.07 MJ for the O_{fru}, O_{glu}, O_{suc}, and O_{fat} treatments. Thus, the range between group mean responses to the different overfeeding treatments was only 0.58 MJ (equivalent to just 145 kJ/d or <2% of daily expenditure). The between-subject variability in response to overfeeding was also only 2.25% when expressed relative to total EE. This is considerably lower than that observed in some other overfeeding studies (29) and almost certainly reflects the very high precision of whole-body calorimetry and the use of a rigorous protocol that constrained physical activity.

TABLE 5
Fat intake, oxidation, and balance over 96 h in the lean and obese women in response to overfeeding[1]

	Treatment				
	Control	O_{fru}	O_{glu}	O_{suc}	O_{fat}
			kJ		
Lean ($n = 8$)					
Intake	12 325 ± 469	19 407 ± 948	19 407 ± 948	19 407 ± 948	27 675 ± 1320
Oxidation	13 197 ± 2941[a]	8571 ± 2372[b]	9715 ± 3313[b]	8657 ± 2116[b]	16 328 ± 2495[c]
Balance	−873 ± 2923[a]	10 836 ± 2562[b]	9692 ± 3469[b]	10 750 ± 2274[b]	11 346 ± 2590[b]
Obese ($n = 5$)					
Intake	13 224 ± 802	20 860 ± 983	20 860 ± 983	20 860 ± 983	29 860 ± 1598
Oxidation	14 567 ± 3312[a]	10 432 ± 2850[b]	9045 ± 2487[b]	8838 ± 2236[b]	16 924 ± 3132[a,c]
Balance	−1343 ± 3141[a]	10 427 ± 2635[b]	11 814 ± 2213[b]	12 022 ± 1967[b]	12 936 ± 2703[b]
All subjects ($n = 13$)					
Intake	12 670 ± 741	19 966 ± 1178	19 966 ± 1178	19 966 ± 1178	28 515 ± 1758
Oxidation	13 724 ± 3030[a]	9512 ± 2805[b]	9457 ± 2929[b]	8726 ± 2070[b]	16 557 ± 2644[c]
Balance	−1054 ± 2886[a]	10 454 ± 2448[b]	10 508 ± 3131[b]	11 239 ± 2173[b]	11 957 ± 2645[b]

[1] $\bar{x}$ ± SD. Values in the same row with different superscript letters are significantly different, $P < 0.05$ (ANOVA with Scheffe's post hoc test). O_{fru}, 50% overfeeding predominantly with fructose; O_{glu}, 50% overfeeding predominantly with glucose; O_{suc}, 50% overfeeding predominantly with sucrose; O_{fat}, 50% overfeeding with fat.
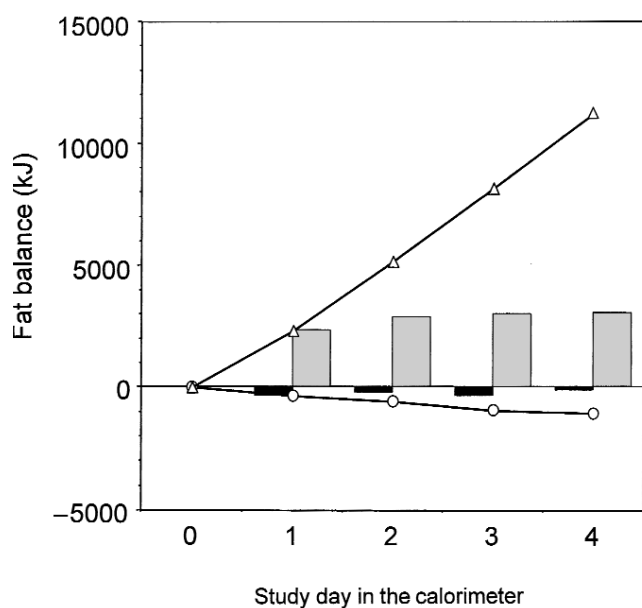
FIGURE 4. Daily and cumulative changes in fat balance during the control treatment (solid bars and circles) and during overfeeding by 50% of energy requirements predominantly with sucrose (shaded bars and triangles). The bars represent daily balance; the lines represent cumulative balance. Data were pooled for the lean and the obese women, $n = 13$.

The mean difference between the overfeeding treatments and the control was 1129 kJ/96 h, which represented 7.9% of the excess energy (computed as the mean intake on the overfeeding runs minus the mean expenditure on the control runs to adjust for the slight inadvertent underestimation of the control requirements; Table 2). The absence of a different thermogenic response to the various fuels runs contrary to observations based on experiments using single nutrient loads and short-term follow-up (18–21). However, this result is in line with our own and others' observations based on mixed-meal protocols with nutrient supplements and measurements made over ≥24 h (7, 15, 30). We concluded previously that, in a real-life setting, the thermogenic response to fat and carbohydrate is virtually identical (5, 31), and we now extend this conclusion to cover simple sugars.

The results for macronutrient oxidation are entirely in line with predictions arising from our oxidative hierarchy model of fuel selection (4, 6, 32, 33). This predicts alterations in whole-body fuel selection according to the metabolic reactivity of the major energy-giving fuels. In brief, alcohol is highly reactive and dominates oxidative pathways, forcing a suppression of the oxidation of other fuels when it is present (4). Carbohydrate and protein come next in the hierarchy and have similar reactivities. There is some dispute as to which is dominant (34), but we have shown clear evidence in favor of carbohydrate, at least under certain circumstances (6). The reactivity of these macronutrients allows them to exert autoregulatory control over their own level of oxidation, whereby a raised intake stimulates an automatic increase in oxidation. Fat, on the other hand, comes at the base of the hierarchy and exerts much weaker autoregulatory control on its own utilization rate.

These principles can be seen clearly in the current data for fat and carbohydrate (given that protein was held constant and alcohol was absent). Carbohydrate oxidation increased greatly in response to carbohydrate overfeeding (from 15.61 to 21.94, 21.64, and 21.97 MJ for fructose, glucose, and sucrose, respectively; Table 4). Fat oxidation was suppressed (from 13.72 to 9.51, 9.46, and 8.72 MJ, respectively; Table 5) even though total EI was in excess. This is a clear illustration of the now universal finding that carbohydrate always takes precedence over fat in modulating fuel selection. The autoregulatory adjustments in carbohydrate oxidation are necessary to maintain glycogen stores within tight limits (35). This was achieved largely because an 8-MJ excess carbohydrate intake was reduced to ≈2 MJ glycogen storage (equivalent to ≈125 g glycogen; Table 4). Almost all of this glycogen storage occurred on day 1, with minimal imbalance on subsequent days (Figure 1). Again, this is a universal observation (6, 36) that may reflect a time lag in the induction of carbohydrate oxidative pathways or the need for glycogen stores to first be perturbed to generate feedback control. An important consequence of this new study is that we have now confirmed what we assumed previously, namely, that different carbohydrates behave in an essentially identical manner.

The response to fat overfeeding was slightly unexpected insofar as fat oxidation increased in response to increased fat intake. There was an increase from 13.72 to 16.56 MJ in response to an increase in intake from 12.67 to 28.52 MJ (Table 5). Carbohydrate oxidation was also significantly suppressed, from 15.61 to 14.08 MJ (Table 4). However, these effects are small compared with the autoregulatory power of carbohydrate because the increased fat oxidation disposed of only 18% of the excess intake, whereas for carbohydrate the value was 74%. This weak, but nonetheless present, autoregulatory influence of fat strengthens the results of earlier studies by others (37).

The physiologic purpose of these alterations in fuel selection is to channel energy to and from the appropriate storage
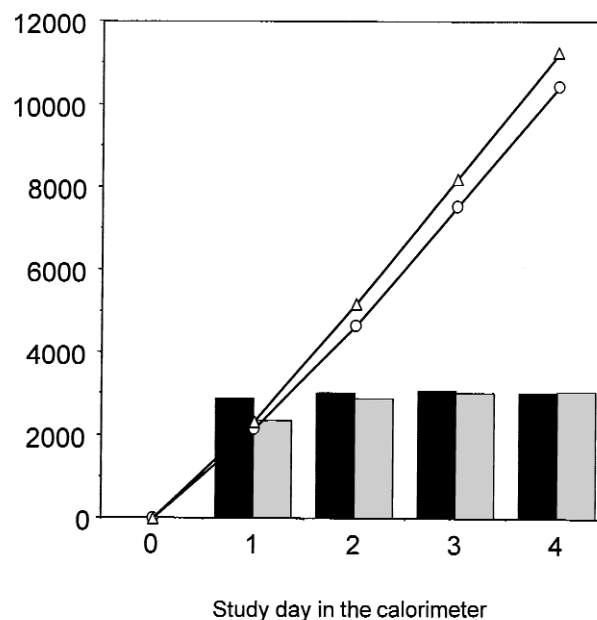

FIGURE 5. Daily and cumulative changes in fat balance during overfeeding by 50% of energy requirements with fat (solid bars and circles) and overfeeding by 50% of energy requirements predominantly with sucrose (shaded bars and triangles). The bars represent daily balance; the lines represent cumulative balance. Data were pooled for the lean and the obese women, $n = 13$.
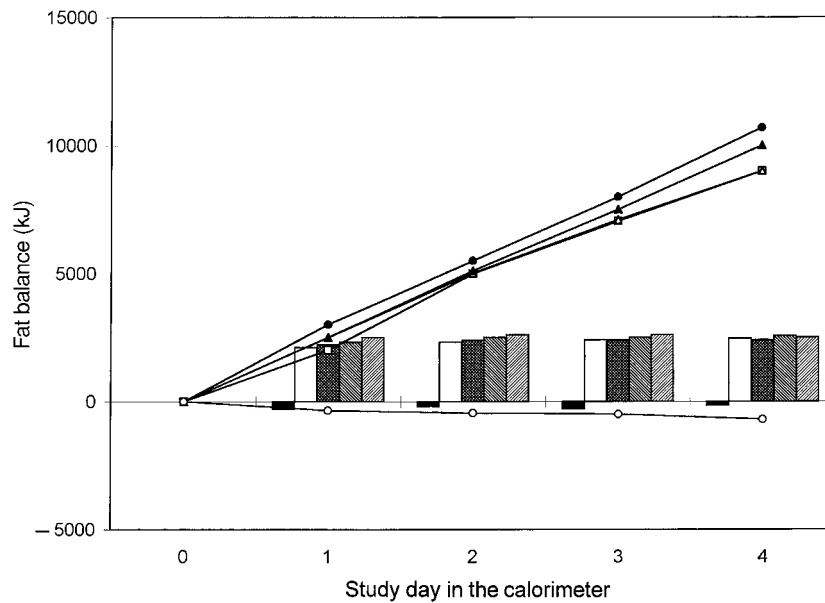
FIGURE 6. Daily and cumulative changes in fat balance across all treatments: control (solid bars and open circles), overfeeding by 50% of energy requirements predominantly with fructose (open bars and open triangles), overfeeding by 50% of energy requirements predominantly with glucose (striped bars and open squares), overfeeding by 50% of energy requirements predominantly with sucrose (heavy hatched bars and solid triangles), and overfeeding by 50% of energy requirements with fat (light hatched bars and solid circles). The bars represent daily balance; the lines represent cumulative balance. Data were pooled for the lean and the obese subjects, $n = 13$.

compartments under all circumstances that may confront the organism. As discussed above, there is an obligate need to regulate glycogen within a relatively narrow window, and adipose tissue has evolved as the main energy reservoir. Thus, any imposed energy imbalance will ultimately be buffered by fat stores once any short-term changes in glycogen have resolved. The outcome of this logic, combined with the insignificant differences in thermogenic response seen in the current study, is that alterations in fat stores are almost identical, irrespective of whether the energy excess is supplied as fructose, glucose, sucrose, or fat (Figures 5 and 6). The degree and duration of overfeeding in the current study were intentionally designed to allow all of the excess to be stored as fat or a proportion to be stored as glycogen. Storing the entire energy excess (14.3 MJ over 96 h) as glycogen would have required an increase in glycogen stores of ≈850 g, which would not be feasible. About half of this value could probably have been achieved, but in practice only 13% (1.9 MJ) was stored as glycogen.

The results of this study apply when energy and macronutrient intakes are controlled and confirm our view that any differential effects of diet type on fat balance are mediated through effects on appetite and food intake and not through differences in their metabolic actions with respect to disposal or total EE (31, 38). These results do not compromise the view that high-fat diets are generally more fattening than are high-carbohydrate diets because of their increased energy density, which promotes passive overconsumption, even when palatability is constant (39). Similarly, the results do not compromise the view that appetite control is less effective in response to high-fat diets, particularly in certain vulnerable subgroups of people (40). In fact, these new results strengthen the view that fat is the macronutrient most likely to lead to fat deposition by disproving the suspicion that a high-fructose diet may disproportionately stimulate fat storage.

We conclude that there is no evidence for differential effects of excess glucose, fructose, or sucrose in relation to fat balance when these carbohydrates were fed under controlled conditions and that their net effect is similar to an excess of dietary fat. The current findings are important because they focus attention on the issue of energy density, which we have elsewhere argued to be critical (41–43), by removing concerns about putative metabolic effects relating to energy balance. It has been argued elsewhere that the simultaneous setting of targets for reducing intakes of fat and simple sugars is mutually antagonistic and hence self-defeating (44). The results of the current study, together with our extensive previous research on the differential effects of dietary fat and carbohydrate, continue to strengthen the view that fat is the more fattening substrate under ad libitum free-living conditions (45).

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