Failure to increase lipid oxidation in response to increasing dietary fat content in formerly obese women

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Research Department of Human Nutrition and KVL Centre for Food Research, Royal Veterinary and Agricultural University, DK-1958 Frederiksberg, and Department of Internal Medicine and Endocrinology, Herlev Hospital, University of Copenhagen, DK-220 Copenhagen, Denmark

Astrup, Arne, Benjamin Buemann, Niels Juel Christensen, and Soren Toubro. Failure to increase lipid oxidation in response to increasing dietary fat content in formerly obese women. Am. J. Physiol. 266 (Endocrinol. Metab. 29): E592–E599, 1994—The effect of an increase in dietary fat content on fat and carbohydrate balances and energy expenditure (EE) was studied in nine formerly obese women with genetic predisposition to obesity (postobese) and a closely matched control group. Isocaloric low- (20% fat energy) and high-fat diets (50%) were consumed for 3 days preceding and during a 24-h respiratory chamber stay, whereas a medium-fat diet (30%) was consumed only on the day of measurement. After adjustment for 24-h energy intake to equal 24-h EE, 24-h fat balance was increased when the dietary fat content increased (P < 0.0002). No differences in macronutrient balances were found on the low-fat and medium-fat diets, but on the high-fat diet the postobese women failed to increase ratio of fat to carbohydrate oxidation appropriately (0.59 g/g, 95% confidence interval 0.47–0.67 vs. controls 1.02 g/g, 0.88–1.12; P = 0.002). This caused a positive adjusted fat balance (+11.0 g/day, 2.3–19.6 vs. controls −8.9 g/day, −17.5 to −0.2; P < 0.001) and a negative carbohydrate balance (−41.8 g/day, −69.5 to −14.0 vs. controls +23.2 g/day, −4.6 to +60.5; P < 0.001). Decreasing the dietary fat content increased 24-h EE in the postobese women (P = 0.02), whereas it was unaffected in the control group. Independent of energy balance, an increase in dietary fat content to 50% fat energy results in preferential fat storage, impaired suppression of carbohydrate oxidation, and reduction of 24-h EE in postobese women.

carbohydrate oxidation; energy expenditure; dietary composition; fat intake; fat oxidation; macronutrient balance; obesity; postobese; substrate utilization

OBESEITY TODAY is a prevalent disease in the Western world, and it has become increasingly clear that those who suffer from excessive body fat are at an elevated risk for several health-disabling diseases such as coronary heart disease, diabetes, hypertension, stroke, and cancers (17). Recent research has delivered evidence for obesity being a disorder resulting from an interaction between a genetic predisposition and certain environmental factors (6, 28). Among the environmental factors suspected of triggering the expression of the obesity genes in the susceptible individuals are a diet with a high-fat content, a low level of physical activity, and the use of certain drugs. It has been suggested that dietary fat plays a special role because cross-sectional studies have demonstrated that dietary fat intake is positively related to body fatness independent of energy intake, whereas carbohydrate intake is negatively related to body fatness (32). Given that clear associations between total energy intake and obesity have not been found in prospective studies, recent investigations have focused on differences in dietary composition. A recent study has found that although total energy intake and body weight were similar in children of overweight parents and in children at low familial risk, the predisposed group consumed a larger percentage of energy from fat and a smaller percentage from carbohydrate and subsequently gained more weight (11). Thus consuming a high-fat diet may promote a positive fat balance by mechanisms beyond its energy content. In addition, a prospective study has delivered evidence for a low fat-to-carbohydrate oxidation ratio being a risk factor for subsequent weight gain (35). It is therefore likely that a high dietary fat content promotes a positive energy balance and that this effect may be enhanced in genetically susceptible individuals.

Pathophysiological abnormalities are difficult to assess when the obese state is already established because the changes in body composition increase both energy expenditure (EE) and substrate oxidations. To avoid these difficulties our approach has been to study formerly obese subjects (postobese) with a genetic predisposition.

The present study was undertaken to investigate whether fuel combustion (fat-to-carbohydrate oxidation ratio) adjusts appropriately to equal intake (fat-to-carbohydrate intake ratio) after a few days of consuming diets with realistic differences in dietary fat content. Furthermore, we wanted to study whether postobese women with the genetic predisposition to obesity responded differently from normal women to the dietary changes.

METHODS

Subjects. Nine formerly obese women were recruited to participate in the study. They all had a familial history of obesity (at least one obese 1st degree relative). They underwent a full medical history and physical examination, electrocardiogram with routine hematology and biochemistry, and urine screening tests and were found to be in good health. None had non-insulin-dependent diabetes mellitus or other endocrine diseases. They had normal glucose metabolism as assessed either by a euglycemic hyperinsulinemic glucose clamp or by a standardized meal test. None of the subjects took any medicine apart from contraceptives. They all had been weight stable (±2 kg) for at least 6 mo before the study. A control group of normal subjects without weight problems was selected from healthy volunteers and matched with the group of postobese with regard to sex, age, height, body weight and body composition. Body weight was measured on a decimal scale (Seca model 707, Copenhagen). Body composition was estimated by the bioimpedance method using an Animer (HTS-Engineering, Odense, Denmark). Fat-free mass (FFM)
and fat mass were calculated from the equations of Heitmann (14). The physical characteristics and anthropometry of the two groups are given in Table 1. The study was approved by the Municipal Ethical Committee of Frederiksberg and Copenhagen, and all subjects gave informed consent in accordance with the Helsinki II Declaration.

**Diet program.** All postobese subjects had normalized their body weight by dietary means on a conventional low-fat, high-protein, and high-carbohydrate diet of 4.2 MJ/day. Their initial body weight was 95 ± 18 kg (mean ± SD), and they lost 31 ± 17 kg to reach their normal body weight, which took from 4 to 14 mo. After reaching their ideal body weight (±5%), they attended the department every week for control and for nutritional instruction to prevent relapse. Postobese and control women started with a medium-fat diet providing 30% energy from fat, 55% as carbohydrate, and 15% as protein and were assigned in random order to two different diets separated by a washout period of 1–2 mo: a low-fat diet with 20% of energy from fat, 65% as carbohydrate, and 15% as protein or a high-fat diet with 50% energy from fat, 35% from carbohydrate, and 15% from protein. The sodium content of the diets was similar. The high- and low-fat diets were controlled for 3 days before and during the stay in respiratory chamber, whereas the medium-fat diet was only controlled during the stay. We chose this design because perfect macronutrient balance can be achieved on a medium-fat diet from one day to the next (8), whereas the time required to adjust to the more extreme diets is unknown. All foods were delivered from our metabolic kitchen and either consumed at the department or taken home. Meals were prepared ready to serve. Postobese and control subjects were treated similarly in all aspects of the procedure. Energy requirements of the subjects were computed from equations giving the relation between lean body mass and 24-h EE based on a previous study (4). Because the postobese were expected to have a 3–4% higher 24-h EE when consuming the low-fat diet (4, 19), the energy intake on this diet was adjusted accordingly during the chamber stay.

**Respiratory chamber.** The 24-h EE and substrate oxidation rates were measured in two open-circuit respiratory chambers, which have been described in detail previously (2–4). The two respiratory chambers work independently, each having a floor area of 6.5 m² and a volume of 14.7 m³, equipped with facilities for a pleasant stay. The gas exchange of the subjects was calculated from measurements of oxygen and carbon dioxide concentrations (Hartman and Braun analyzers, Frankfurt, Germany) at the outlet of the chamber and from measured airflow through the chamber. Protein oxidation was calculated from 24-h urinary nitrogen excretion. The room temperature was maintained constant at 24°C in the daytime and at 18°C at night. EE and oxidation of lipid and carbohydrate were calculated using standard equations (7). Heart rate and electrocardiogram were continuously monitored by a telemetry system (Dialoguc 2000, Danica Electronics, Denmark) and stored in a computer for subsequent analysis. Spontaneous physical activity (SPA) in the respiratory chambers was assessed by two microwave radars (Zettler GHz-Doppler Mime 15, Munich, Germany). The measurements were carried out in the follicular phase of the menstrual cycle. A standard protocol with fixed sessions of physical activity, including three bouts of bicycling of 10 min each (75 W), was carried out during the 24-h stay. The subjects were kept under 24-h surveillance by a laboratory technician in the daytime and by trained medical students at night. The 24-h measurement ran from 0900 to 0900 h. Before discharge, body weight and bioimpedance were recorded after voiding, and subsequently the subject was at rest, sitting in an armchair. An indwelling venous catheter was placed in an antecubital vein for 0.5 h before blood samples were taken immediately before and 25 and 50 min after a carbohydrate-rich breakfast (providing 25% of 24-h energy intake). The composition of this meal was kept constant on the three occasions.

**Laboratory analyses.** Blood was sampled without stasis in ice-cooled syringes and centrifuged at 4°C. For determination of catecholamines blood was collected in tubes containing reduced glutathione and ethylene glycol-bis( P-aminoethyl ether)-N,N,N',N'-tetraacetic acid. The tubes were centrifuged immediately and the plasma was stored at −80°C until determination of catecholamines by a radioenzymatic method (9). All plasma samples were coded and analyzed in a random order to avoid any systematic error attributable to the order of analysis.

**Statistical analyses.** Results are given as means and 95% confidence intervals (CI). Data on energy expenditure were adjusted for differences in FFM using the equation \( E_{Eadj} = E_{act} + [(FFM_{mean} - FFM_{act}) \cdot a] \), where \( E_{act} \) is the actual unadjusted EE, \( FFM_{mean} \) the total group mean, \( FFM_{act} \) is the actual FFM, and \( a \) is the slope and \( b \) the intercept derived from linear regression analysis between FFM and EE in the total group of subjects (EE = \( a \cdot FFM + \beta \)). Lipid-to-carbohydrate oxidation ratio (LCOR) and nutrient balances were adjusted for differences in 24-h energy balance by the same method, which has been described by Ravussin and Bogardus (23). All

| Table 1. Anthropometry of study groups |
|-------------------------------|-------------------|-------------------|
|                               | Postobese         | Controls          |
| Age, yr                       | 39 (33–45)        | 36 (31–42)        |
| Sex (F/M)                     | 9/0               | 9/0               |
| Ht, cm                        | 167 (161–170)     | 167 (164–171)     |
| Diet Type, % fat              |                   |                   |
|                               | 30                | 50                |
|                               | 20                | 20                |
|                               | 30                | 50                |
| Body wt, kg                   | 65.6 (62.3–68.9)  | 63.7 (60.4–67.0)  |
| Fat-free mass, kg             | 46.8 (44.9–48.8)  | 46.4 (44.4–48.3)  |
| Fat mass, kg                  | 18.0 (16.6–21.0)  | 17.4 (15.2–19.6)  |

Values are means with 95% confidence intervals (CI) in parentheses. Body weight and composition were measured in the morning on completion of the 24-h stay in a respiratory chamber, after maintaining the specified diet for 4 days. There were no significant differences between study groups or diet effects within groups assessed by multifactor analyses of variance (MANOVA).
group comparisons were made by a multifactor analysis of covariance on unadjusted data using Tukey’s test for subsequent pairwise comparisons. These tests and simple linear and multiple regression analyses were performed with Statgraphics software (Graphic Software Systems, Rockville, MD).

RESULTS

Body weight. The postobese and control subjects were well matched with respect to body weight and body composition (Table 1). Although the study was carried out over a period of a year the subjects succeeded in maintaining a constant body weight so that no differences were present during the different diets.

Energy expenditure and spontaneous physical activity. Twenty-four-hour EE was influenced by diet composition in postobese women but not in control women (Table 2), but the group × diet effect was not significant. With the high-fat diet 24-h EE was similar in the two groups, but it increased in the postobese by 1.4% with the medium fat diet and by 4% with the low-fat diet. The diet-dependent increase in 24-h EE in the postobese group persisted after adjustment for differences in FFM, fat mass, and SPA. More than 80% of the increase in 24-h EE on the medium- and low-fat diets occurred in the daytime, whereas sleeping EE was uninfluenced (Table 2). The diet effect on daytime EE in the postobese remained after adjustment for SPA (P = 0.03).

When norepinephrine (NE) was taken into account it was a significant covariate, and (multiple analysis of variance, P = 0.006) the difference in 24-h EE between postobese and control subjects was no longer significant (P = 0.34). NE accounted for most of the effect of dietary composition on 24-h EE (NE, P < 0.0005; diet effect, P = 0.12). The effect of dietary composition on daytime EE was also entirely explained by differences in NE.

Analysis of the covariables influencing 24-h EE by stepwise multiple regression analysis showed that 76% of the variance in 24-h EE could be explained by FFM (34%, P < 0.0001), plasma NE (32%, P < 0.0001), SPA (6%, P = 0.002), and dietary lipid to carbohydrate intake ratio (LCIR; 4%, P = 0.048). The effect of plasma NE and LCIR could be only attributed to the postobese group.

Sixty-three percent of the variation in daytime EE could be accounted for by five covariates: FFM (35%, P = 0.0005), SPA (17%, P < 0.0001), fat mass (4%, P = 0.02), group membership (postobese vs. controls, 6%, P = 0.01), and a subject constant (1%, P = 0.01). After taking NE into account 73% of the variation in daytime EE was explained by four covariates only: NE (33%, P < 0.0001), FFM (29%, P < 0.0001), SPA (9%, P = 0.001), and LCIR (3%, P = 0.05); no difference between postobese and control subjects remained (group membership, P = 0.73).

Nutrient oxidations and balances. On the low-fat diet both postobese and control subjects oxidized lipid and carbohydrate in a ratio (g/g) corresponding to that of the diet (Fig. 1). The controls also succeeded in increasing LCOR appropriately when the fat content of the diet was increased, so that LCOR was almost identical to LCIR. By contrast, the postobese subjects failed to increase LCOR sufficiently to match LCIR, and the difference was most pronounced on the high-fat diet (group × diet effect: P = 0.002). This difference was also present without adjustment of LCOR for differences in 24 h energy balance (0.65 g/g, 95% CI 0.53–0.78 vs. controls.

Table 2. Influence of changes in diet composition on energy expenditure and spontaneous physical activity in postobese and controls

<table>
<thead>
<tr>
<th>Diet Type, % fat</th>
<th>20</th>
<th>30</th>
<th>50</th>
<th>Diet effect</th>
<th>Group difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-h EE, kJ/day</td>
<td>Postobese</td>
<td>Controls</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>8,401* (7,946–8,837)</td>
<td>8,086 (7,603–8,569)</td>
<td>8,090* (7,635–8,545)</td>
<td>0.021</td>
<td>0.028</td>
</tr>
<tr>
<td>Adjusted 24-h EE, kJ/day</td>
<td>Postobese</td>
<td>Controls</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>8,206* (7,750–8,661)</td>
<td>8,094 (7,579–8,489)</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>8,094 (7,579–8,489)</td>
<td>8,028</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daytime EE, kJ/12.5 h</td>
<td>Postobese</td>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7,963 (7,467–8,459)</td>
<td>8,077 (7,581–8,573)</td>
<td>8,028</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>7,560* (7,467–8,459)</td>
<td>8,028</td>
<td></td>
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<tr>
<td>Sleeping EE, kJ/5 h</td>
<td>Postobese</td>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7,467 (7,746–8,681)</td>
<td>8,077 (7,560–8,496)</td>
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<tr>
<td></td>
<td>7,579 (7,746–8,681)</td>
<td>8,028</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPA, %</td>
<td>Postobese</td>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.4 (6.1–6.8)</td>
<td>7.3 (7.0–7.6)</td>
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<td></td>
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<tr>
<td></td>
<td>6.1 (5.8–6.5)</td>
<td>6.4 (6.1–6.7)</td>
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</tbody>
</table>

Values are means with 95% CI in parentheses. EE, energy expenditure (adjusted 24-h was adjusted for differences in fat-free mass); SPA, spontaneous physical activity expressed as % of time with activity detectable by radar recorded. Data were analyzed by MANOVA. In case of a significant group effect, a diet effect was analyzed separately in each groups with Tukey’s post hoc test. *+ Values in a row with unlike symbols were significantly different, P < 0.05. NS, not significant.
Fig. 1. Mean and 95% confidence interval (CI) adjusted ratio of lipid to carbohydrate oxidation (CHO) in relation to 3 different lipid to carbohydrate intakes in postobese women (n = 9) and controls (n = 9). Lipid-to-carbohydrate oxidation ratio (LCOR) was adjusted for differences in 24-h energy balance. Lipid energy content of low-, medium- and high-fat diets is indicated. LCOR increased in both groups [multifactor analysis of variance (MANOVA), P < 0.0001], but the increase was impaired in postobese group when dietary lipid content was increased (diet x group interaction, P = 0.002).

1.00 g/g, 0.87–1.12; P = 0.002) and when plasma NE concentration was included as a covariate (P = 0.003).

The corollaries for unadjusted macronutrient balances are shown in Table 3, and fat and carbohydrate balances adjusted for differences in 24-h energy balance are presented in Fig. 2. There was an overall diet effect on 24-h fat balance (P = 0.03), which was further enhanced after adjustment for differences in 24-h energy balance (P = 0.0002). The controls were in slightly negative adjusted lipid balance with the low- and medium-fat diets and close to zero balance with the high-fat diet (Fig. 2). By contrast, the adjusted 24-h lipid balance was negative with the low-fat diet in the postobese (-14.6 g/day, -23.9 to -5.9), in balance with the medium-fat diet (-7.1 g/day, -15.7 to +1.6), and in positive lipid balance with the high-fat diet (+11.0 g/day, 0.03 to +23.3). Carbohydrate balance was different from that of control group on high-fat diet (P < 0.001). Carbohydrate balance was different from that of control group on high-fat diet (P < 0.001).

Values are means and 95% CI.

Table 3. Macronutrient balances, heart rate, and free triiodothyronine index in postobese and controls during changes in diet composition

<table>
<thead>
<tr>
<th>Diet Type, % fat</th>
<th>20</th>
<th>30</th>
<th>50</th>
<th>P</th>
<th>Diet effect</th>
<th>Group difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-h Fat balance, g/day</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Postobese</td>
<td>-11.0</td>
<td>-12.4</td>
<td>+5.5</td>
<td></td>
<td>0.03</td>
<td>NS</td>
</tr>
<tr>
<td>Controls</td>
<td>-13.8†</td>
<td>-20.1</td>
<td>+5.8†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-h Carbohydrate balance, g/day</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postobese</td>
<td>-26.8</td>
<td>-39.0</td>
<td>-26.4§</td>
<td></td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Controls</td>
<td>-20.1</td>
<td>+8.2</td>
<td>+14.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean 24-h heart rate, beats/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Postobese</td>
<td>74.3</td>
<td>73.6</td>
<td>71.9</td>
<td></td>
<td>NS</td>
<td>0.0002</td>
</tr>
<tr>
<td>Controls</td>
<td>69.8</td>
<td>69.5</td>
<td>69.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free T₃ index, AU</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postobese</td>
<td>1.49*</td>
<td>1.31†</td>
<td>1.27†</td>
<td></td>
<td>0.001</td>
<td>NS</td>
</tr>
<tr>
<td>Controls</td>
<td>1.48*</td>
<td>1.57†</td>
<td>1.32†</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Values are means with 95% CI in parentheses. T₃, triiodothyronine in arbitrary units. Fat and carbohydrate balances were calculated separately for each subject. Hence the sum of the mean values expressed in energy units may differ despite a constant protein balance. Data were analyzed by MANOVA. In case of a group effect a post hoc analysis was carried out separately in each group with Tukey's test. *†‡ Values in a row with unlike symbols were significantly different, P < 0.05. §P < 0.001, difference between postobese and controls. NS, not significant.
g/day, +2.3 to +19.6), which was significantly different from the control group (P < 0.001).

There was no significant diet effect on adjusted carbohydrate balance (Fig. 2), but it was generally lower in the postobese women (group effect, P = 0.017), the difference being most marked on the high-fat diet (P < 0.001). Protein balance did not differ between subjects groups and it was uninfluenced by diet (data not shown).

Plasma catecholamine and thyroid hormone concentrations. Plasma NE concentration increased postprandially (P < 0.0001) and was also influenced by the preceding diet (P = 0.003), being highest with the low-fat diet, intermediate with the medium-fat diet, and lowest with the high-fat diet (Fig. 3). Fasting NE levels were 15% lower on the medium-fat diet, and 36% lower on the high-fat diet, than on the low-fat diet. NE concentrations were generally elevated in the postobese women (P = 0.0003), but they responded normally to changes in dietary fat content (group x diet interaction, P = 0.88) and to the meal test (group x meal interaction, P = 0.41).

In stepwise multiple regression analysis NE was positively influenced by the breakfast meal (P < 0.0005), inversely related to LCIR (P = 0.0009), and also related to group membership, i.e., postobese vs. controls (P = 0.015).

Plasma epinephrine concentration was lower in the postobese than in the controls (P < 0.0001). Epinephrine increased slightly postprandially (P = 0.04) and was also influenced by the antecedent diet (P = 0.001). With the medium-fat diet, epinephrine was lower than with the other two diets (Fig. 3). The postobese and control groups responded similarly to the test meal and to the antecedent diet.

Free triiodothyronine index did not differ between subject groups, but it was significantly higher in both groups after the low- than the high-fat diet (Table 3).

Heart rate. The 24-h heart rate measured continuously by telemetry was higher in the postobese group than in the control group (P = 0.0002), but it did not change significantly with dietary fat content (Table 3). In an analysis of the factors with influence on 24-h heart rate in a stepwise linear regression analysis, SPA, plasma NE, and group membership together explained 52% of the variance in 24-h heart rate. Notably, the group difference in heart rate between postobese and control subjects was fully explained by the effect of dietary fat content on 24-h EE in the postobese women, because 24-h EE could account for 46% of the variation in 24-h heart rate (P < 0.0001). This indicates that the higher 24-h heart rate in the postobese subjects consuming the medium- and low-fat diets could be attributed to the higher EE.

DISCUSSION

It was previously thought that the body was “energy blind” and that calories from fat, carbohydrate, and protein contributed with the same value to energy balance. There is, however, accumulating evidence to suggest that during ad libitum conditions energy balance is achieved by a separate regulation of carbohydrate, fat, and protein balances (12, 13). Regulation of carbohydrate balance has the highest priority in this
hierarchy, which is appropriate because the limited size of the glycogen stores are only sufficient to cover carbohydrate oxidation for a few days. The control of carbohydrate balance is supposed to be tightly controlled, partly through appetite signals generated from the glycogen stores. This concept has gained strong support from a large body of experimental work (24), and the background for our study was to look for a possible macronutrient imbalance in obesity-prone women. Indeed, we found that isocaloric changes in dietary fat content had markedly different effects in postobese and matched control subjects with regard to 24-h fat and carbohydrate balances, and the findings were independent of adjustments made for the minor differences in 24-h energy balance. When the adjusted balances were measured after 3-day consumption of the low-fat diet, postobese and control subjects were indistinguishable, since both groups achieved a deficit on fat balance, whereas they maintained equilibrium on carbohydrate balance. On the medium-fat diet the postobese women resulted in a surplus on fat balance among the postobese individuals, whereas the controls remained in a slightly negative fat balance. In the postobese group the preferential storage of fat on the high-fat diet was caused by a failure to increase fat oxidation sufficiently to balance with the consumed fat, which resulted in a deposition of 11 g/day in the fat stores. The accompanying negative carbohydrate balance must have brought about a reduction in glycogen stores, which is supposed to be a potent signal for decreased satiety and increased hunger (24).

In the present design we controlled energy intake to equal expenditure during the chamber stay, but under free living conditions the deficit on carbohydrate balance imposed in the postobese women by the high-fat diet may be anticipated to be covered by an increase in total energy intake. To cover the actual deficit on carbohydrate balance by additional intake of the high-fat diet would result in further deposition of 19 g fat.

From the present design we cannot entirely exclude the possibility that the postobese habitually consumed a diet with a lower fat content than the controls, and they therefore required more time to adapt their fat oxidation to the high-fat diet. Although we found no indications of noncompliance among the postobese, neither we can rule out that, on the days before the respiratory chamber stay, they had a lower adherence to the high-fat diet due to fat avoidance. To rule these alternatives out another study design is required.

Thomas et al. (30) examined the effect of 7 days of unrestricted consumption of a low-fat diet (26% fat energy) and a high-fat diet (52% fat energy) in lean and obese subjects. Lean subjects had a better ability than the obese subjects to increase fat oxidation in response to the high-fat diet, and on day 7 of the high-fat diet a strong positive correlation was found between fat intake and fat oxidation in lean subjects (r = 0.78, P < 0.01) but not in the obese subjects (r = 0.02, not significant). Lipid oxidation is known to be increased by the obese state (3, 26), so that possibly the failure of the obese subjects to increase fat oxidation further could be due to a ceiling effect. Our results, however, show that the inability is not present only in the obese state but persists after normalization of body composition.

The present study also demonstrates that, even in normal women, under conditions where energy intake is closely concordant with energy expenditure, the energy-yielding nutrients consumed for 4 days are not necessarily oxidized in similar proportions. The failure to make rapid adjustments in the oxidative pattern causes macronutrient imbalances to emerge. Thus the normal women maintained a daily fat balance only on the high-fat diet, whereas fat oxidation exceeded fat intake when they consumed the medium- and low-fat diets, which resulted in a deficit on fat balance of 20 g/day, and carbohydrate balance was maintained or slightly positive (Fig. 3). Hence our results are concordant with the findings of cross-sectional (32), longitudinal (18), and intervention studies (16, 21, 30, 31) pointing out that differences and changes in dietary fat content are associated with small corresponding changes in body weight. Furthermore, it offers a sensible explanation as to how dietary fat by an action independent of its energy density promotes hyperphagia and fat gain. That the obesity-promoting effect of a high-fat diet is especially pronounced in susceptible individuals, where weight gain eventually may progress to obesity, may be explained by the finding that the postobese preferentially channeled the ingested fat to the stores.

Obese subjects generally have an increased preference for high-fat foods (10). If this preference results in selection of high-fat foods in subjects who also lack the ability to increase fat oxidation in response to a high-fat diet, these two mechanisms may act in concert to produce severe obesity. In addition, hyperphagia induced by high palatability of high-fat foods may also contribute to promote a positive fat balance in nonobese subjects (25). Inevitably, the positive fat balance caused by increased dietary fat content results in expansion of body fat stores and brings about a proportional increase in plasma concentrations of free fatty acids and triglycerides (4). The increased circulating level of lipid substrate causes insulin resistance, which in turn raises lipid oxidation (5). Thus body fat stores grow until the greater substrate availability of lipids has increased the ratio of lipid to carbohydrate oxidation to a level commensurate with the lipid-to-carbohydrate ratio of the habitual diet. According to this concept, the expansion of body fat stores is an adaptation to a low-fat oxidation or/and a high dietary fat content by which the body may reach a new steady state (12, 13). The magnitude of the increase in body fat for a given increase in dietary fat content may thus depend on the flexibility of the oxidative pattern to adjust to equal the composition of the fuel mixture, and larger weight gains are required in the postobese women than in the controls to increase fat oxidation sufficiently. It is likely that the genetic susceptibility to obesity is linked to differences in this flexibility and to their preference for a high-fat diet. Hill et al. (15) found that obese subjects developed a negative fat
balance when consuming a low- (20% energy as fat) and high-fat diet (45%) for 7 days. They achieved only a positive fat balance on an extremely high-fat diet providing 60% of energy as fat, suggesting that their habitual diet contained at least 45% fat energy. In addition, because a given increase in dietary fat content brings about a larger fat gain in susceptible individuals, the reverse change, i.e., a reduction in dietary fat content, would consequently a priori be expected to bring about larger weight loss in obese than in normal weight subjects. Actually, intervention studies have shown that obese subjects lost more than twice the amount of body fat than controls during a 20-wk low-fat diet consumed ab libitum (15).

The reason for the failure to increase fat oxidation in response to a high-fat diet in predisposed individuals is unknown. Prospective studies have supported the notion that a deficient fat oxidative capacity may contribute to the mechanism of weight gain (27, 35). A high insulin sensitivity was found to be associated by an increased risk of subsequent weight gain in non-diabetic Pima Indians (29). Weight gain was strongest correlated to initial glucose oxidation, and the following weight gain brought about insulin resistance, which would decrease carbohydrate oxidation and increase fat oxidation and consequently limit further weight gain. A reduced proportion of fat-combusting slow type I muscle fibers (33) could be a part of the underlying mechanism. Results suggesting an altered balance between uptake and release of free fatty acids in adipose tissue in a direction favoring fat storage have been found in obesity-prone subjects (20, 34, 35), further tending to reduce availability of fat substrate and hence increase carbohydrate oxidation. It is likely that the reduced level of the lipolytic hormone epinephrine found in the postobese in this study and in obesity (8) may contribute to a lower lipid mobilizing ability in the normal weight state.

In agreement with previous studies (1, 15, 19), 24-h energy expenditure was uninfluenced by changes in dietary fat content in the control group. By contrast, in the postobese women 24-h energy expenditure was increased by the low-fat diet and decreased by the high-fat diet as compared with the medium-fat diet. This is similar to the observation by Lean and James (19), who found that a low-fat diet increased 24-h energy expenditure of postobese above the levels of the control group and a high-fat diet suppressed energy expenditure below normal levels. We recently studied the mechanisms responsible for the stimulatory effect of a medium-fat diet on energy expenditure and found that postobese subjects had 50% higher levels of plasma NE concentrations than the controls and that this difference entirely could account for the 8% higher 24-h energy expenditure and 12% higher 24-h heart rate in the postobese (2). In the present study the magnitude of the diet effect on energy expenditure in the postobese was smaller, amounting to only 1.4% on the medium-fat diet and 4% on the low-fat diet compared with the high-fat diet. When plasma NE concentrations were taken into account, the higher levels in the postobese group and the increase in plasma NE concentration with decreasing fat-to-carbohydrate ratio fully explained the altered responsiveness to dietary changes in the postobese group. The statistical association, however, does not prove that NE was responsible for the effect on energy expenditure. The postobese also had a higher 24-h heart rate, and although the increase observed with reduction in dietary fat-to-carbohydrate ratio did not reach statistical significance (Table 3), 24-h energy expenditure was a highly significant covariate that entirely explained the group difference and any possibly diet effect. It is well established that heart rate changes synchronously with energy expenditure and are used as a rough index of energy expenditure in field studies. Thus the observed group difference in heart rate may be due to the higher energy expenditure of the postobese.

The finding that both free triiodothyronine index and plasma NE concentrations were increased by decreasing the lipid to carbohydrate ratio of the antecedent diet in both study groups is most likely attributable to the carbohydrate content of the diet. It is well established that carbohydrate and carbohydrate-rich meals activate the sympathetic nervous system acutely, but the observed effect of the antecedent diet has not previously been reported. Our results thus confirm that the conflicting levels of plasma NE reported in obesity partly may be attributed to differences in antecedent dietary fat-to-carbohydrate ratio.

Apart from the etiological importance the results may also have therapeutic implications. By a decrease in the dietary fat content from 50 to 30% energy in the postobese group, the surplus on fat balance was eliminated, and the low-fat diet caused a deficit on fat balance. Furthermore, 24-h energy expenditure was increased by 4% by the low-fat diet in the postobese group. Thus decreasing the dietary fat-to-carbohydrate ratio normalizes the otherwise unbalanced macronutrient balances, suggesting that obesity may be regarded as a carbohydrate deficiency syndrome and that an increase in dietary carbohydrate content at the expense of fat is the appropriate dietary part of a therapeutic strategy.

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