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## The 24-h carbohydrate oxidation rate in a human respiratory chamber predicts ad libitum food intake<sup>2</sup>

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### Abstract

**Background**—The 24-h respiratory quotient (24-h RQ) and 24-h carbohydrate balance (24-h CHO-Bal) are predictors of weight change. Whether these relations are mediated by the effects of substrate oxidation and balance on food intake is not known.

**Objective**—We tested whether substrate oxidation and balance predict future ad libitum food intake.

**Design**—Substrate oxidation and balance were measured in a respiratory chamber in 112 normoglycemic subjects (83 Pima Indians and 29 whites; 67 men and 45 women) in energy balance for 3 d before tests were performed. The subjects then self-selected their food ad libitum for the following 3 d.

**Results**—The 24-h RQ, 24-h carbohydrate oxidation (24-h CHO-Ox), and 24-h CHO-Bal in the respiratory chamber predicted subsequent ad libitum food intake over 3 d (as a percentage of weight maintenance energy needs; %EN-WM). The 24-h CHO-Ox explained 15% of the variance in %EN-WM. The weight change over the 3-d ad libitum period was associated positively with 24-h CHO-Ox and negatively with 24-h CHO-Bal in the chamber; these associations were no longer significant after adjustment for %EN-WM.

**Conclusion**—Carbohydrate oxidation and balance predict subsequent ad libitum food intake and can influence short-term weight changes, which indicates that carbohydrate balance is a contributing metabolic factor affecting food intake.

### Keywords

Substrate oxidation; nutrient balance; food intake; body weight regulation

## INTRODUCTION

Obesity has reached epidemic proportions in the United States and is threatening to become a global epidemic (1). Metabolic predictors of future weight gain include lower 24-h and resting energy expenditure (2), reduced spontaneous physical activity (3), lower nonexercise activity thermogenesis (4), higher fasting (5) and 24-h (6) respiratory quotient (RQ), lower carbohydrate balance (7), higher insulin action (8,9), lower plasma leptin concentrations (10), reduced levels of sympathetic nervous system activity (11,12), and lower plasma glucose response during an oral-glucose-tolerance test (OGTT; 13).

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RQ is an indicator of the carbohydrate-to-fat oxidation ratio (14). Its positive association with the change in body weight has been interpreted as an indication that subjects who rely less on fat oxidation as a substrate for energy production may have a greater tendency to gain weight, possibly because they are more prone to store excess energy as fat (5,6). Evidence indicates, however, that substrate oxidation rates can also affect food intake.

The negative association between postload blood glucose response and weight change provides support for the role of glucose, with the likely involvement of insulin, as a satiety signal, in that a delayed decline in blood glucose can postpone meal initiation or prompt meal termination, or both, thus leading to a lower overall food intake, as previously suggested (15).

Recently, carbohydrate balance was found to be a much stronger predictor of weight and fat gains in adults than was fat balance (7). This finding may indicate that persons with higher carbohydrate oxidation relative to intake may have a greater tendency to deplete glycogen stores and to experience more hunger, thereby causing the ingestion of more total energy, as previously hypothesized, mainly on the basis of animal studies (16,17). Whether substrate oxidation rates can affect spontaneous food intake in humans, however, has not yet been explored.

The objective of the present study was to investigate the hypothesis that substrate oxidation rates on a weight maintenance diet, as measured in a 24-h respiratory chamber (18), predict ad libitum energy intake during 3 subsequent days, as evaluated by an automated food-selection system (19). The hypothesis that the previously reported relation between postload glucose response and weight change is mediated by the effects of substrate oxidation rates on energy intake was also tested.

## SUBJECTS AND METHODS

### Subjects

One hundred twelve subjects (age:  $33 \pm 8$  y; body fat:  $31 \pm 8\%$ ) were included in this analysis: 83 Pima Indians (47 men and 36 women) were recruited from the Gila River Indian Community (40 miles southeast of Phoenix, AZ), and 29 whites (20 men and 9 women) were recruited from the greater Phoenix area through an advertisement. Before participation, volunteers were fully informed of the nature and purpose of the study, and written informed consent was obtained. The experimental protocol was approved by the Institutional Review Board of the National Institute of Diabetes and Digestive and Kidney Diseases and by the Tribal Council of the Gila River Indian Community. All subjects were found to be free of disease according to physical examinations, medical histories, and laboratory tests. Glucose tolerance was assessed by a 75-g OGTT. Only nondiabetic subjects, according to the American Diabetes Association diagnostic criteria (20), participated in the present study.

### Study protocol

On admission to the metabolic ward, the subjects were given a standard weight maintenance diet (20%, 30%, and 50% of daily calories were provided as protein, fat, and carbohydrate, respectively) for 3 d before any tests were performed. The weight maintenance energy needs (EN-WM) on the metabolic ward were calculated for each subject on the basis of weight and sex: for men,  $EN-WM = 9.5 \times \text{weight (in kg)} + 1973$ ; for women,  $EN-WM = 9.5 \times \text{weight (in kg)} + 1745$  (21). To determine the foodstuffs that would be made available for the measurement of ad libitum food intake by an automated food-selection system (*see* below), the subjects were asked to complete a food-preference questionnaire, which consisted of a listing of 80 food items presented in random order. On the basis of a model developed by Geiselman et al (22),

typical breakfast, lunch, dinner, and snack food items were categorized according to a macronutrient self-selection paradigm that varied the fat content of the foods as a percentage of calories with other macronutrients. Foods were categorized as being high in fat ( $\geq 45\%$  kcal) or low in fat ( $< 20\%$  kcal), and within each of these categories, foods were categorized as being high in simple sugar ( $\geq 30\%$  kcal), complex carbohydrate ( $\geq 30\%$  kcal), or protein ( $\geq 13\%$  kcal). In completing this self-administered questionnaire, individuals were asked to assign each food a hedonic rating by using a 9-point Likert scale with the following anchors: never tasted; 1, dislike extremely; 5, neutral; 9, like extremely. Several foods on the list are among the top 10 sources of dietary fat in the United States and include hamburgers, French fries, ham and other luncheon meats, doughnuts, cookies, cakes, candies, bread products, muffins, eggs, and cheeses; the list also reflects items of intake common to Pima Indians (23) and to individuals living in the southwestern United States. During the final 3 d of the study on the metabolic ward and after having spent 24 h in the respiratory chamber, the subjects were asked to self-select all their food with the use of a computer-operated vending machine system.

### Automated food-selection system

The measurement of ad libitum food intake by an automated food-selection system was previously described, validated, and tested for reproducibility (intra-class correlation for energy intake = 0.90,  $P < 0.0001$ ; 19,24–27). Briefly, an automated food-selection system is made up of a refrigerated vending machine (model 3007; U-Select-It, Des Moines, IA) that contains 40 trays. The 40 food items made available to the subjects on each of the 3 d consisted of those foods to which the subject had assigned an intermediate high (between 4 and 8) hedonic rating on the food-preference questionnaire. In addition, a core group of condiments and foods was provided to each subject on each day, which included butter, peanut butter, cream cheese, jams, salad items, salad dressings, crackers, bread, tortillas, Indian fry bread, spices, salsa, orange juice, apple juice, milk, and a 6-pack of soda of the subject's choice. The same selection was offered each day and accommodated the appropriateness of certain foods for breakfast, lunch, dinner, and evening snacks. The subjects had unrestricted access to the vending machine for 23.5 h/d and were asked to follow their typical eating pattern as closely as possible. The refrigerated machines were housed in a separate eating area equipped with a table, a chair, a microwave oven, and a toaster. The subjects were instructed to eat only in the vending room, to eat whatever they wished whenever they desired, and to return the food wrappers and unconsumed food portions to the vending machine. Television viewing during food consumption was prohibited. Daily energy intake (DEI) and protein, fat, and carbohydrate intakes were calculated from the actual weights of the food and condiments consumed by using the CBORD Professional Diet Analyzer Program (version 4.1.11; CBORD Inc, Ithaca, NY). The database was modified to reflect the nutrient content of specific food items as indicated by the manufacturer. The results are presented in Table 1 as the means  $\pm$  SDs of the 3 d. DEI is expressed as mean kcal/d and mean percentage of EN-WM (%EN-WM) on the metabolic ward [(mean daily energy consumed/EN-WM)  $\times$  100].

### Respiratory chamber

The method for measuring energy expenditure and substrate oxidation in the respiratory chamber was previously described (18). In brief, volunteers entered the chamber at 0745 after having fasted overnight and remained therein for 23 h. Meals were provided at 0800, 1130, 1700, and 2000 (evening snack). Because of the confinement within the chamber, only 80% of EN-WM on the metabolic ward were provided in the respiratory chamber, as previously described (18). The rate of energy expenditure was measured continuously, calculated for each 15-min interval, and then extrapolated for the 24-h interval (24-h EE). Spontaneous physical activity was detected by radar sensors and expressed as the percentage of time over the 24-h period in which activity was detected. Sleeping metabolic rate was defined as the average energy expenditure of all 15-min periods between 2330 and 0500 during which spontaneous

physical activity was <1.5%. Carbon dioxide production ( $VCO_2$ ) and oxygen consumption ( $VO_2$ ) were calculated for every 15-min interval and were extrapolated for the 24-h interval. The 24-h RQ was calculated as the ratio of 24-h  $VCO_2$  and 24-h  $VO_2$ . The substrate balances were calculated from the 24-h energy intake, 24-h EE, and 24-h RQ. Carbohydrate and fat oxidation rates were calculated from the 24-h RQ, accounting for protein oxidation (calculated from the measurement of 24-h urinary nitrogen excretion; 28). To calculate DEI, energy expenditure and substrate oxidation were measured after  $\geq 3$  d on the weight maintenance diet and  $\geq 1$  d before the 3-d unrestricted access to the vending machines. Body weights before and after the chamber stay were recorded ( $\bar{x} \pm SD$  change in body weight:  $-0.82 \pm 0.86$  kg).

### Dual-energy X-ray absorptiometry

Body composition was measured by dual-energy X-ray absorptiometry (DPX-L; Lunar Corp, Madison, WI). Percentage body fat (%BF), fat mass (FM), and fat-free mass (FFM) were calculated as previously described (29).

### Analytic measurements

Plasma glucose concentrations were measured with the glucose oxidase method (Beckman Instruments, Fullerton, CA). Plasma insulin concentrations were measured by automated radioimmunoassay (Access; Beckman Instruments).

### Statistical analysis

Statistical analyses were performed by using the procedures of the SAS statistical package (version 8.2; SAS Institute Inc, Cary, NC). Unless otherwise specified, all data are expressed as means  $\pm$  SDs. The areas under the curve (AUC) for the plasma glucose and insulin concentrations during the OGTT ( $AUC_{OGTT}$ ) were calculated by the trapezoidal method. Race and sex differences in the general, anthropometric, metabolic, and energy intake characteristics were evaluated by Student's *t* test and chi-square analyses for continuous and categorical variables, respectively. During the ad libitum period, a repeated-measures analysis of variance was used to detect any effect of time on energy intake. The relations between variables were assessed by Spearman's correlation, linear regression analyses, or both. The amount of variance in the dependent variable (ie, DEI or %EN-WM) accounted for by the independent variables (ie, general, anthropometric, and metabolic characteristics) was quantified by stepwise multiple regression analyses. On the basis of the results of previous studies, the 24-h EE and sleeping metabolic rate were adjusted for age, sex, FFM, FM, and race (18,30), whereas the 24-h RQ was adjusted for age, sex, energy balance, %BF, and race (6) by using general linear regression models before correlation analyses. Similarly, the 24-h carbohydrate and fat oxidation rates (24-h CHO-Ox and 24-h Fat-Ox, respectively) were adjusted for race, sex, age, FM, FFM, and energy balance by using general linear regression models before correlation analyses.

## RESULTS

General, anthropometric, metabolic, and energy intake and expenditure characteristics of the study population are shown in Table 1. There was no difference in the sex distribution between the races (chi-square analysis:  $P = 0.2$ ). There were no racial differences in the anthropometric and metabolic variables, except for fasting plasma insulin concentrations and insulin  $AUC_{OGTT}$ , which were higher in Pima Indians ( $\bar{x} \pm SD$ :  $39.6 \pm 12.6$   $\mu$ U/mL and  $21206.3 \pm 9492.4$   $\mu$ U/mL $\cdot$ 180 min, respectively) than in whites ( $31.7 \pm 7.9$   $\mu$ U/mL and  $14696.5 \pm 6113.5$   $\mu$ U/mL $\cdot$ 180 min, respectively) both before and after adjustment for sex, age, and %BF ( $P = 0.01$  and  $P = 0.002$ , respectively). After adjustment for body weight and sex, there were no racial differences in DEI ( $4546 \pm 1371$  kcal/d for whites compared with  $4272 \pm 1154$  kcal/d for Pima Indians) or in %EN-WM ( $158 \pm 42\%$  for whites compared with  $154 \pm 41\%$  for Pima Indians). In contrast, both DEI and %EN-WM were higher in men than in women; this was

also true after adjustment for race and body weight ( $P < 0.0001$  and  $P = 0.003$ , respectively), as previously reported in a smaller sample of the same population (26).

There were no significant differences in DEI across the 3-d ad libitum period ( $\bar{x} \pm SD$ : day 1,  $4456 \pm 1436$ ; day 2,  $4331 \pm 1415$ ; and day 3,  $4256 \pm 1423$  kcal/24 h;  $P = 0.4$ , analysis of variance). Therefore, energy intake variables are expressed as means  $\pm$  SDs of the 3 d. Body weight was significantly correlated with ad libitum energy intake expressed as DEI ( $r = 0.21$ ,  $P = 0.02$ ) but not as %EN-WM ( $r = -0.05$ ,  $P = 0.6$ ). Body weight was also significantly correlated with daily ad libitum fat ( $r = 0.29$ ,  $P = 0.002$ ) and protein ( $r = 0.24$ ,  $P = 0.01$ ) intakes, but not with carbohydrate intake ( $r = 0.12$ ,  $P = 0.2$ ). Owing to the observed effect of body size on energy intake variables and the established effect of body size on energy metabolism and substrate oxidation rates (6,18,30), the associations between these variables were all adjusted for body size. Correlation analyses of substrate oxidation rates in the respiratory chamber with energy intake variables during the subsequent 3-d ad libitum period are reported in Table 2 and Figure 1. The adjusted 24-h RQ and 24-h CHO-Ox in the respiratory chamber were positively associated with DEI, %EN-WM, and daily carbohydrate, fat, and protein intakes during the ad libitum period. Further adjustment for weight changes before or during the chamber stay did not change these results (data not shown). Consistently, the 24-h carbohydrate balance (24-h CHO-Bal) in the respiratory chamber was negatively associated with DEI and %EN-WM (Figure 2). Neither the 24-h Fat-Ox nor the 24-h Fat-Bal was associated with either DEI or %EN-WM.

When 24-h CHO-Ox and 24-h Fat-Ox in the respiratory chamber were both entered in a general linear regression model (Table 3) to determine significant predictors of DEI or %EN-WM during the ad libitum period, 24-h CHO-Ox, but not 24-h Fat-Ox, was an independent determinant of the dependent variable; this was also true after adjustment for race, sex, age, FM, FFM, and 24-h energy balance. With the use of a stepwise multiple regression approach (data not shown), the 24-h CHO-Ox explained the largest amount of variance in DEI (ie, 32%) and in %EN-WM (ie, 15%).

During the 3 d of ad libitum feeding, subjects gained  $1.0 \pm 1.1$  kg (range:  $-1.2$ – $4.9$  kg; Figure 3 and Figure 4), which corresponds to a relative weight change of  $1.2 \pm 1.2\%$  (range:  $-1.5\%$ – $5.4\%$ ; Figure 3 and Figure 4). Both the absolute change and percentage change in body weight over the 3-d ad libitum period were positively associated with the adjusted 24-h CHO-Ox in the respiratory chamber (Figure 3) and were negatively associated with the 24-h CHO-Bal in the respiratory chamber (Figure 4). These associations were not significant after adjustment for DEI or %EN-WM (data not shown).

Correlations of glucose and insulin AUCs during an OGTT (performed before the chamber stay) with the substrate oxidation rates (measured in the respiratory chamber) and with the energy intake variables (evaluated during the subsequent 3-d ad libitum period) are shown in Table 4. The glucose  $AUC_{OGTT}$  was negatively associated with the adjusted 24-h RQ and 24-h CHO-Ox in the respiratory chamber; this was also true after further adjustment for fasting plasma concentrations of glucose and insulin (partial  $r = -0.24$ ,  $P = 0.01$ ) and insulin  $AUC_{OGTT}$  (partial  $r = -0.30$ ,  $P = 0.002$ ). No association was found between glucose  $AUC_{OGTT}$  and 24-h Fat-Ox in the respiratory chamber. With the use of a general linear regression model adjusted for race, sex, age, %BF, 24-h energy balance, fasting plasma glucose and insulin concentrations, and insulin  $AUC_{OGTT}$  (model  $R^2 = 0.39$ ,  $P < 0.0001$ ), the 24-h CHO-Ox in the respiratory chamber was a negative, independent determinant of glucose  $AUC_{OGTT}$  ( $P = 0.002$ ). Glucose  $AUC_{OGTT}$  was negatively associated with DEI ( $r = -0.23$ ,  $P = 0.02$ ) and %EN-WM ( $r = -0.26$ ,  $P = 0.006$ ) during the ad libitum period. After adjustment for race, sex, age, %BF, and insulin  $AUC_{OGTT}$ , the significance of these associations was attenuated (DEI: partial  $r = -0.18$ ,  $P = 0.08$ ; %EN-WM: partial  $r = -0.16$ ,  $P = 0.11$ ). Notably,

the associations of glucose AUC<sub>OGTT</sub> with DEI and with %EN-WM during the ad libitum period were no longer significant after further adjustment for 24-h CHO-Ox in the respiratory chamber (partial  $r = -0.04$  and  $-0.05$ , respectively,  $P = 0.6$ ).

## DISCUSSION

The present study tested the hypothesis that substrate oxidation rates, as measured during a weight maintenance diet in a respiratory chamber, predict subsequent ad libitum food intake, as measured by a previously validated automated food-selection system, used in our laboratory (21, 24–26). The 24-h RQ in the respiratory chamber was a positive predictor of subsequent energy intake during the 3-d ad libitum period, independent of race, sex, age, %BF, and 24-h energy balance, ie, the major determinants of 24-h RQ (18, 30). This result is consistent with the finding that a relatively high RQ is associated with weight gain in long-term follow-up (5, 6). This observation was interpreted as an indication that subjects with a low ratio of fat to carbohydrate oxidation may tend to store more energy in their fat depots and, therefore, gain more weight over time (5, 6). Interestingly, a high 24-h RQ was correlated with subsequent gain in body weight independent of 24-h energy expenditure (5). In light of this observation, the results of the present study raise the possibility that the positive relation between RQ and weight change can be a consequence of the effects of the macronutrient oxidation ratio on energy intake.

The 24-h RQ reflects the overall ratio between 24-h CHO-Ox and 24-h Fat-Ox. Some evidence points to a role for fat oxidation in the regulation of energy intake. The inhibition of fat oxidation by etomoxir stimulated food intake in men (31), and an increase in fat oxidation by diglyceride-rich oil decreased appetite in women (32). When using 24-h CHO-Ox and 24-h Fat-Ox in place of 24-h RQ in the regression analyses with energy intake, 24-h CHO-Ox in the respiratory chamber, but not 24-h Fat-Ox, was a strong, independent predictor of subsequent DEI and % EN-WM during the 3-d ad libitum period. This finding is consistent with a recent report showing that 24-h CHO-Bal was a negative predictor of long-term gains in weight and fat mass (7). Our data indicate that the relation between 24-h CHO-Bal and weight change is mediated by the effects of carbohydrate balance on food intake (7). The change in body weight over the 3-d ad libitum period in the present analysis was positively correlated with 24-h CHO-Ox and was negatively associated with 24-h CHO-Bal in the respiratory chamber. These associations were no longer significant after adjustment for DEI or for %EN-WM, which indicates that the effects of carbohydrate balance on short-term weight change are mediated by the effects of carbohydrate balance on food intake.

However, Eckel et al (7) examined the predictive role of substrate balance in a respiratory chamber on long-term weight and fat changes by feeding the study subjects an isocaloric diet that provided 55% of the calories as carbohydrate. In the present study, the predictive role of substrate balance on subsequent, short-term ad libitum food intake was tested, and the subjects were fed a diet that provided 50% of the calories as carbohydrate and 80% of EN-WM as overall calories to account for the reduced physical activity in the respiratory chamber. Although our results support the prospective observations of Eckel et al (7), these differences in study design and purposes must be acknowledged.

It must also be acknowledged that the lack of an association of fat oxidation and balance with ad libitum food intake and short-term weight change may have been due to the short observation period and to the fact that the energy balance and, consequently, the fat balance were somewhat clamped in the respiratory chamber, which may have dampened the possibility of finding a significant effect of fat oxidation and balance on either subsequent ad libitum food intake or short-term weight change. Further studies are needed to appropriately address this point.

The physiologic mechanism responsible for the association of carbohydrate balance with food intake may be related to the glycogenostatic model, developed by Flatt (16,17), which is based on the negative relation between carbohydrate balance on one day and food intake on the following day in mice fed a mixed diet ad libitum. According to this model, food intake is stimulated by low glycogen stores to produce a carbohydrate intake that will maintain or replenish glycogen stores (16,17,33). Therefore, a higher 24-h CHO-Ox would lead to a greater tendency to deplete glycogen stores, thereby prompting the ingestion of more total energy. On the other hand, short-term studies conducted in humans with dietary manipulation of muscle glycogen stores have produced inconsistent, mostly negative, effects on food intake (34,35). A comprehensive reconciliation between these inconsistent results is difficult and beyond the scope of this work. The above studies that failed to report an association between glycogen stores and food intake were conducted by manipulating the muscle glycogen content. It may well be that it is changes in liver glycogen content, as opposed to muscle glycogen content, that play the central role in the regulation of food intake. In addition, the energy intake on day 2 was inversely related to the carbohydrate balance on day 1 also in humans (34–36), which confirmed the findings from animal studies (16,17) and indicated that a higher carbohydrate oxidation relative to intake prompts subsequent food intake, possibly with the aim of reestablishing adequate glycogen stores.

Other mechanisms may have been operating to explain the positive association of 24-h RQ and 24-h CHO-Ox in the respiratory chamber with subsequent ad libitum food intake. Several central and peripheral peptides involved in the regulation of food intake also have an effect on substrate oxidation. For example, fasting plasma concentrations of glucagon-like peptide-1, a satiety-inducing gut peptide, were negatively correlated with fasting RQ in adult patients (37), and it has been proposed that an increase in RQ is a pivotal mediator of the orexigenic action of neuropeptide Y (38).

One surprising finding in our study was the negative association between fat mass and DEI in the full model in Table 3. The explanation for this is not clear, but perhaps fat mass, after other metabolic variables are accounted for (fat-free mass in particular), acts as a brake on increased food intake.

The glucose AUC<sub>OGTT</sub>, which was recently shown to be a negative predictor of long-term changes in body weight (13), was negatively associated with both energy intake during the 3-d ad libitum period and with carbohydrate oxidation in the respiratory chamber. The correlation between glucose AUC<sub>OGTT</sub> and ad libitum energy intake was no longer significant after adjustment for carbohydrate oxidation in the respiratory chamber. Conversely, the association between 24-h CHO-Ox in the respiratory chamber and subsequent DEI (or %EN-WM) during the 3-d ad libitum period was still significant after adjustment for glucose AUC<sub>OGTT</sub> (data not shown). This indicates that carbohydrate oxidation may mediate the relation between the postload glucose response and food intake, thus explaining the reported role of the postload glucose response as a negative predictor of long-term weight changes (13).

The results of the present analysis are to be considered in the context of some potential limitations. When offered unlimited access to a variety of palatable and familiar foods for 3 d, most subjects overfed themselves by 55% above EN-WM (mean %EN-WM: 155%; range: 54–250%). This observation is consistent with previous reports (19,24,25) and has been named “opportunistic voracity” (39). This consideration warrants some caution in viewing food intake under these experimental conditions as mirroring the actual eating behavior in free-living conditions. On the other hand, the automated food-selection system is a useful tool that affords accurate and reliable measurements of food intake on a metabolic ward, with a much higher degree of accuracy than that afforded by techniques based on self-report, as extensively discussed (40,41). Moreover, the within-person reliability of this method across multiple visits

was highly significant (intraclass correlation coefficient for both DEI and %EN-WM = 0.9), which indicates that this model is valid and reliable for assessing energy intake when food is abundant and freely available (27). Nonetheless, these observations must be interpreted with caution owing to the massive overfeeding seen in the study subjects, and replication and confirmation in a more physiologic setting are required.

Both spontaneous (24) and experimental (42) overfeeding are associated with increased carbohydrate oxidation rates. Hence, the hypothesis that higher food intake may be the cause rather than the consequence of higher 24-h carbohydrate oxidation rates cannot be positively ruled out. However, this hypothesis is less likely for several reasons. The pretest conditions were highly controlled, and, although the subjects had been in slightly negative energy balance ( $\approx -188$  kcal/d) on the day of substrate oxidation assessment in the respiratory chamber and had experienced small changes in body weight during the weight maintenance period, neither this small weight change between the time of admission and the testing days ( $-0.02 \pm 1.3$  kg) nor the time spent on the metabolic ward before testing ( $5 \pm 3$  d) was correlated with the adjusted 24-h substrate oxidation rates in the respiratory chamber ( $P = 0.99$  and  $P = 0.13$  for 24-h CHO-Ox;  $P = 0.74$  and  $P = 0.49$  for 24-h Fat-Ox), which indicates that the effect of the change from free-living conditions to the metabolic ward was negligible. Therefore, any overeating before admission is unlikely to have influenced the substrate oxidation rates in the present study. Finally, measurements of energy metabolism and substrate oxidation in the respiratory chamber were always performed before the 3-d ad libitum food intake period in all subjects.

In conclusion, the present study showed that carbohydrate oxidation and balance, as measured in a respiratory chamber, are strong predictors of subsequent ad libitum food intake and short-term changes in body weight, which lends support to Flatt's (17) hypothesis that food intake is driven primarily to maintain carbohydrate balance. Whether diet composition, via its effects on substrate oxidation, can, in turn, affect food intake could not be addressed by the present study. The mechanisms responsible for the observed effects and the possibility of regulating food intake by changing substrate oxidation should be explored.

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The authors' responsibilities were as follows—NP: planned the study, analyzed the data, and wrote the report; ADS: planned the study, collected and analyzed the data, and wrote the report; EO: analyzed the data and wrote the report; CAV: collected and analyzed the data and wrote the report; and CB and JK: planned the study, analyzed the data, and wrote the report. None of the authors had any conflicts of interest.

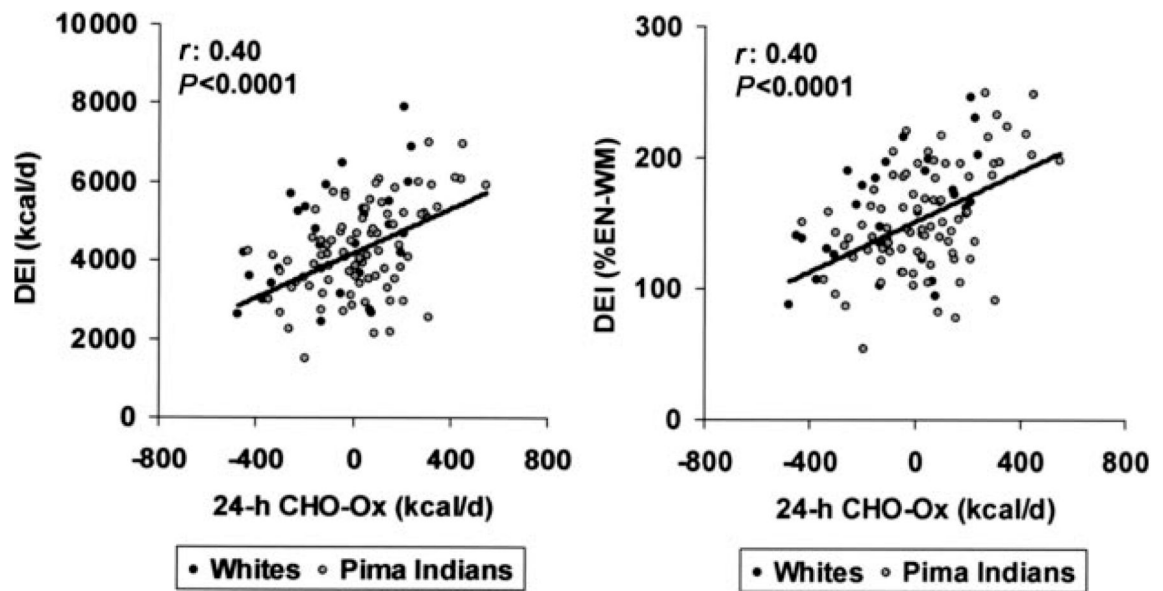
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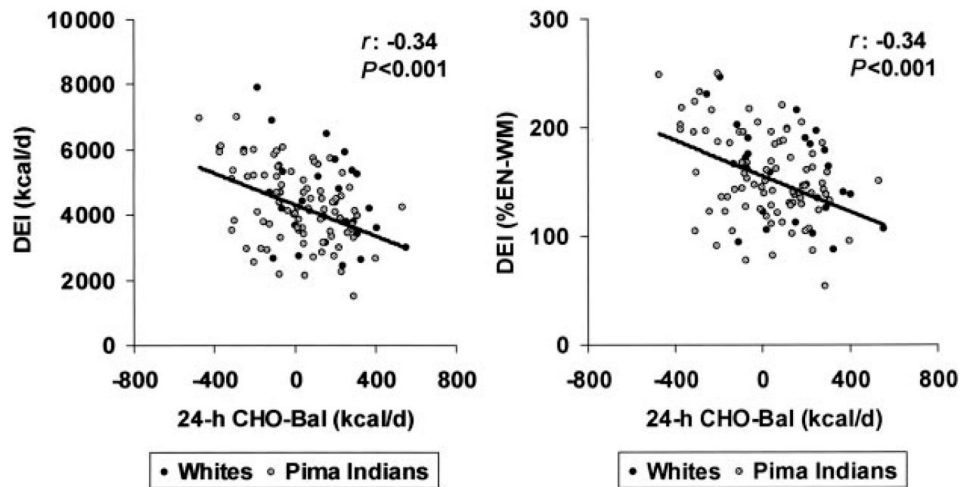
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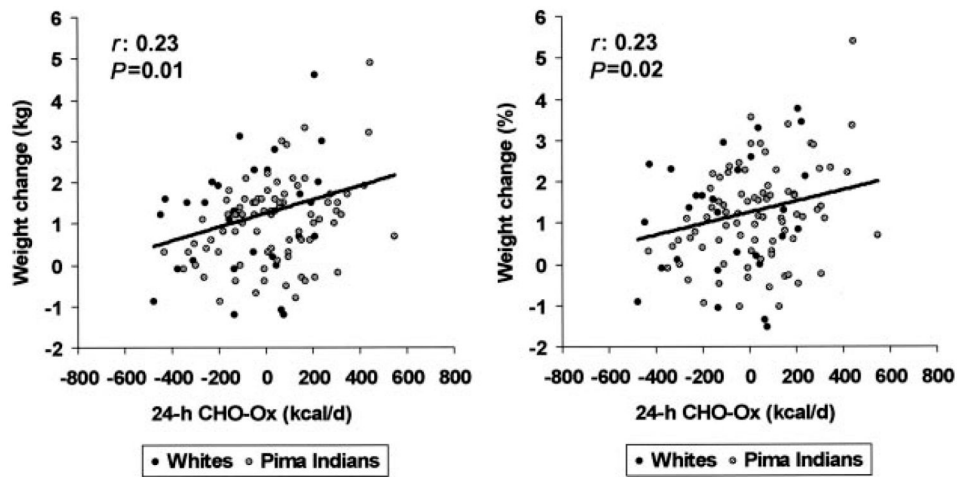
**FIGURE 1.**

Daily energy intake (DEI) expressed as kcal/d and as a percentage of weight maintenance energy needs (%EN-WM) in the study population ( $n = 112$ ) during the 3-d ad libitum period as a function of the 24-h carbohydrate oxidation (24-h CHO-Ox) rate in the respiratory chamber. 24-h CHO-Ox values were adjusted for race, sex, age, fat-free mass, fat mass, and energy balance by using general linear regression.



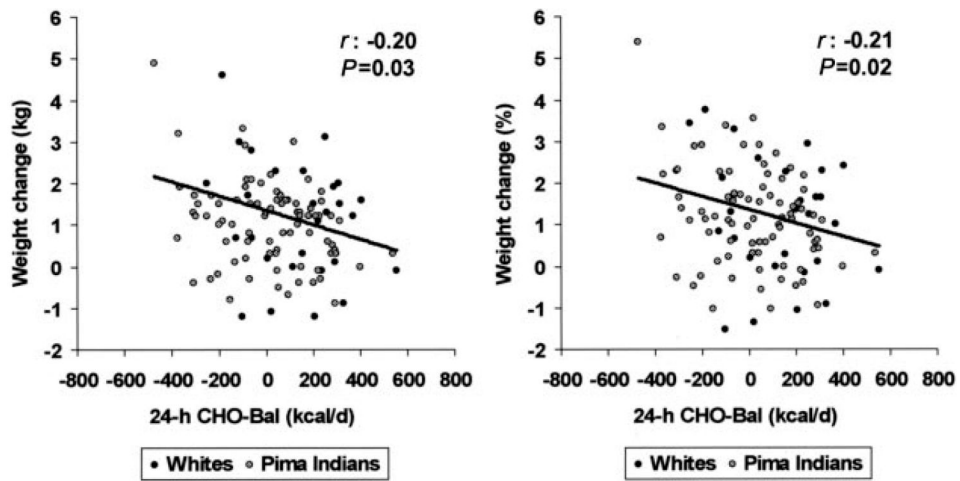
**FIGURE 2.**

Daily energy intake (DEI) expressed as kcal/d and as a percentage of weight maintenance energy needs (%EN-WM) in the study population ( $n = 112$ ) during the 3-d ad libitum period as a function of the 24-h carbohydrate balance (24-h CHO-Bal) in the respiratory chamber. Relations between variables were assessed with Spearman's correlation analysis.



**FIGURE 3.**

Absolute weight change and percentage weight change in the study population ( $n = 112$ ) during the 3-d ad libitum period as a function of the 24-h carbohydrate oxidation (24-h CHO-Ox) rate in the respiratory chamber. 24-h CHO-Ox values were adjusted for race, sex, age, fat-free mass, fat mass, and energy balance by using general linear regression.



**FIGURE 4.**

Absolute weight change and percentage weight change in the study population ( $n = 112$ ) during the 3-d ad libitum period as a function of the 24-h carbohydrate balance (24-h CHO-Bal) in the respiratory chamber. Relations between variables were assessed with Spearman's correlation analysis.

TABLE 1

General, anthropometric, metabolic, and energy intake and expenditure characteristics of the study population<sup>1</sup>

|                                       | Whole population    | Men (n = 67) | Women (n = 45) | P <sup>2</sup> |
|---------------------------------------|---------------------|--------------|----------------|----------------|
| Race (n)                              |                     |              |                |                |
| Pima Indians                          | 83                  | 47           | 36             | NS             |
| Whites                                | 29                  | 20           | 9              | NS             |
| Age (y)                               | 33 ± 8 <sup>3</sup> | 33 ± 8       | 33 ± 8         | NS             |
| Body weight (kg)                      | 94 ± 24             | 97 ± 25      | 89 ± 20        | NS             |
| Body fat (%)                          | 31 ± 8              | 27 ± 6       | 38 ± 5         | < 0.0001       |
| Fasting plasma glucose (mg/dL)        | 89 ± 8              | 88 ± 8       | 91 ± 9         | NS             |
| 2-h Plasma glucose (mg/dL)            | 121 ± 30            | 117 ± 27     | 128 ± 34       | NS             |
| 24-h Energy expenditure (kcal/d)      | 2422 ± 397          | 2598 ± 342   | 2160 ± 322     | < 0.0001       |
| 24-h Sleeping metabolic rate (kcal/d) | 1738 ± 288          | 1845 ± 277   | 1580 ± 227     | < 0.0001       |
| 24-h Respiratory quotient             | 0.84 ± 0.03         | 0.85 ± 0.03  | 0.84 ± 0.03    | NS             |
| 24-h Carbohydrate oxidation (kcal/d)  | 1069 ± 276          | 1173 ± 278   | 915 ± 189      | < 0.0001       |
| 24-h Fat oxidation (kcal/d)           | 1032 ± 321          | 1098 ± 297   | 933 ± 333      | < 0.01         |
| 24-h Energy balance (kcal/d)          | -188 ± 268          | -201 ± 252   | -169 ± 292     | NS             |
| EN-WM (kcal/d)                        | 2800 ± 270          | 2919 ± 251   | 2622 ± 187     | < 0.0001       |
| DEI                                   |                     |              |                |                |
| (kcal/d)                              | 4343 ± 1213         | 4776 ± 1162  | 3698 ± 988     | < 0.0001       |
| %EN-WM (%)                            | 155 ± 41            | 164 ± 39     | 142 ± 41       | 0.005          |
| 24-h Carbohydrate intake (kcal/d)     | 2162 ± 604          | 2366 ± 573   | 1859 ± 521     | NS             |
| 24-h Fat intake (kcal/d)              | 1698 ± 542          | 1875 ± 509   | 1436 ± 483     | NS             |
| 24-h Protein intake (kcal/d)          | 606 ± 188           | 662 ± 184    | 521 ± 163      | NS             |

<sup>1</sup> EN-WM, weight maintenance energy needs; DEI, ad libitum daily energy intake; %EN-WM, an expression of DEI as a percentage of EN-WM.

<sup>2</sup> Sex differences were evaluated by Student's *t* test for independent samples except race (chi-square analysis).

<sup>3</sup>  $\bar{x} \pm SD$  (all such values).

Spearman's correlation analyses of substrate oxidation and energy expenditure rates in the respiratory chamber with energy intake variables during the 3-d ad libitum period ( $n = 112$ )<sup>1</sup>

TABLE 2

|                          | DEI                | %EN-WM             | Daily carbohydrate intake | Daily fat intake   | Daily protein intake |
|--------------------------|--------------------|--------------------|---------------------------|--------------------|----------------------|
| 24-h RQ <sup>2</sup>     | 0.28 <sup>3</sup>  | 0.27 <sup>3</sup>  | 0.22 <sup>4</sup>         | 0.24 <sup>4</sup>  | 0.24 <sup>4</sup>    |
| 24-h CHO-Ox <sup>5</sup> | 0.40 <sup>6</sup>  | 0.40 <sup>6</sup>  | 0.34 <sup>7</sup>         | 0.34 <sup>7</sup>  | 0.29 <sup>3</sup>    |
| 24-h CHO-Bal             | -0.34 <sup>7</sup> | -0.34 <sup>7</sup> | -0.29 <sup>3</sup>        | -0.28 <sup>3</sup> | -0.22 <sup>4</sup>   |
| 24-h Fat-Ox <sup>5</sup> | -0.14              | -0.11              | -0.09                     | -0.12              | -0.14                |
| 24-h Fat-Bal             | 0.01               | 0.08               | -0.001                    | -0.04              | 0.06                 |
| 24-h EE <sup>8</sup>     | 0.26 <sup>9</sup>  | 0.34 <sup>7</sup>  | 0.26 <sup>9</sup>         | 0.23 <sup>4</sup>  | 0.21 <sup>4</sup>    |
| 24-h SMR <sup>8</sup>    | 0.001              | 0.07               | 0.05                      | 0.02               | 0.01                 |

<sup>1</sup> DEI, ad libitum daily energy intake; %EN-WM, DEI as a percentage of weight maintenance energy needs; RQ, respiratory quotient; CHO-Ox, carbohydrate oxidation; CHO-Bal, carbohydrate balance; Fat-Ox, fat oxidation; Fat-Bal, fat balance; EE, energy expenditure; SMR, sleeping metabolic rate.

<sup>2</sup> Adjusted for race, sex, age, percentage body fat, and 24-h energy balance.

<sup>3</sup>  $P < 0.005$ .

<sup>4</sup>  $P < 0.05$ .

<sup>5</sup> Adjusted for race, sex, age, fat-free mass, fat mass, and 24-h energy balance.

<sup>6</sup>  $P < 0.0001$ .

<sup>7</sup>  $P < 0.001$ .

<sup>8</sup> Adjusted for race, sex, age, fat-free mass, and fat mass.

<sup>9</sup>  $P < 0.01$ .



**TABLE 3**

Determinants of absolute ad libitum daily energy intake (DEI) and ad libitum DEI as a percentage of 24-h weight maintenance energy needs (%EN-WM) in the study subjects ( $n = 112$ )<sup>1</sup>

|                                      | Dependent variable |          |                     |          |
|--------------------------------------|--------------------|----------|---------------------|----------|
|                                      | DEI <sup>2</sup>   |          | %EN-WM <sup>3</sup> |          |
|                                      | $\beta$            | <i>P</i> | $\beta$             | <i>P</i> |
| Intercept                            | 1329.4             | 0.08     | 109.2               | 0.0001   |
| Race (white)                         | 342.2              | 0.13     | 9.5                 | 0.26     |
| Sex (male)                           | -360.5             | 0.42     | -22.8               | 0.16     |
| Age (y)                              | -17.2              | 0.14     | -0.7                | 0.11     |
| Fat-free mass (kg)                   | 13.6               | 0.48     | 0.03                | 0.96     |
| Fat mass (kg)                        | -39.8              | 0.02     | -1.8                | 0.005    |
| 24-h Energy balance (kcal/d)         | -0.3               | 0.48     | -0.01               | 0.42     |
| 24-h Fat oxidation (kcal/d)          | 0.8                | 0.21     | 0.02                | 0.32     |
| 24-h Carbohydrate oxidation (kcal/d) | 3.0                | < 0.0001 | 0.1                 | < 0.0001 |

<sup>1</sup> Determinants evaluated by using general linear regression analysis.

<sup>2</sup> Model  $R^2 = 0.46$ ,  $P < 0.0001$ .

<sup>3</sup> Model  $R^2 = 0.35$ ,  $P < 0.0001$ .

**TABLE 4**

Spearman's correlation analyses of glucose and insulin areas under the curve during the 3-h oral-glucose-tolerance test (AUC<sub>OGTT</sub>) with the energy expenditure and intake variables in the study subjects ( $n = 112$ )<sup>1</sup>

|                          | Glucose AUC <sub>OGTT</sub> | Insulin AUC <sub>OGTT</sub> |
|--------------------------|-----------------------------|-----------------------------|
| 24-h RQ <sup>2</sup>     | -0.21 <sup>3</sup>          | 0.04                        |
| 24-h CHO-Ox <sup>4</sup> | -0.27 <sup>5</sup>          | -0.01                       |
| 24-h Fat-Ox <sup>4</sup> | 0.08                        | -0.11                       |
| 24-h EE <sup>6</sup>     | -0.05                       | -0.22 <sup>3</sup>          |
| 24-h SMR <sup>6</sup>    | 0.09                        | -0.09                       |
| DEI                      | -0.23 <sup>3</sup>          | -0.10                       |
| %EN-WM                   | -0.26 <sup>7</sup>          | -0.18                       |
| Carbohydrate intake      | -0.25 <sup>7</sup>          | -0.15                       |
| Fat intake               | -0.16                       | -0.04                       |
| Protein intake           | -0.21 <sup>3</sup>          | -0.20 <sup>3</sup>          |

<sup>1</sup> RQ, respiratory quotient; CHO-Ox, carbohydrate oxidation; Fat-Ox, fat oxidation; EE, energy expenditure; SMR, sleeping metabolic rate; DEI, ad libitum daily energy intake; %EN-WM, ad libitum DEI as a percentage of weight maintenance energy needs.

<sup>2</sup> Adjusted for race, sex, age, percentage body fat, and 24-h energy balance.

<sup>3</sup>  $P < 0.05$ .

<sup>4</sup> Adjusted for race, sex, age, fat-free mass, fat mass, and 24-h energy balance.

<sup>5</sup>  $P < 0.005$ .

<sup>6</sup> Adjusted for race, sex, age, fat-free mass, and fat mass.

<sup>7</sup>  $P < 0.01$ .