Time-course changes in macronutrient metabolism induced by a nutritionally balanced low-calorie diet in obese women

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The use of low-calorie diets is a common strategy for body-weight reduction purposes, but the time-course of the metabolic changes induced by moderately energy-restricted, otherwise balanced, diets is still poorly known. The aim of this nutritional intervention design was to study in obese women the effect of a balanced low-calorie diet on the metabolic rate, and metabolic fuel utilization changes during the weight loss process through the application of breath tests with stable isotope-labeled tracers.

Seven obese (body mass index > 30 kg/m²) women were assigned to a 10-week dietary hypoenergetic intervention regime supplying 55% of energy as carbohydrate, 30% as fat and 15% as protein. Metabolic rate and substrate utilization were evaluated for 6 h in separate occasions during the weight loss program by indirect calorimetry and after ¹³C-labeled glucose, triolein and leucine administration. Body weight loss after 10 weeks was 4.2 ± 1.1 kg, while the percent body fat decrease was about 5%. Slimming was accompanied by a marked decrease in fasting leptin (about 25%). Postprandial carbohydrate utilization after the administration of a test meal with the same macronutrient distribution as the experimental low-energy diet was decreased (24.1%, P < 0.05) as a consequence of the dietary restriction, which was associated with lower insulin plasma levels (P < 0.05). Although protein and lipid oxidation were not significantly different after weight reduction (day 1 versus day 70), the metabolic utilization of these substrates tended to increase. Moreover, marginally significant indications obtained on days 15 and 45 suggest that the weight and body composition changes are attributable to a shift in endogenous and exogenous glucose utilization in favor of lipid burning. The breath tests determinations, which were performed on different occasions along the experimental trial, confirmed that the cumulative ¹³C output decreased for labeled tracers with time, being only statistically significant for the glucose utilization between days 15 and 45. In summary, the weight and fat mass losses were associated with a lower carbohydrate oxidation, which were probably compensated by an increase in lipid oxidation without major changes in protein mobilization.
Introduction

Obesity is characterized by an increase in fat mass, which is generally associated with inadequate dietary and lifestyle habits (Martinez, 2000; Moreno et al., 2000). Furthermore, it has been shown that an excessive fat or carbohydrate intake can be stored in the adipose tissue (Fricke et al., 1989; Labayen et al., 1999) and that carbohydrate and lipid metabolism are closely inter-related (Whitley et al., 1997; Blundell et al., 2002). On the contrary, the fat balance does not appear to be acutely regulated in humans as compared with the carbohydrate balance (Schutz, 1999).

Moreover, current recommendations to prevent obesity state that fat should be no more than 30% of the energy intake and that carbohydrate should supply about 50–60% of the daily calories provided by the diet (National Research Council, 2001). In this context, the effects of variations in carbohydrate, protein and fat content of the diet upon weight loss have been surveyed following different dietary approaches with diverse outcomes. Thus, similar weight losses were achieved in adult obese women receiving three different 1200 kcal diets containing 25%, 45% or 75% carbohydrate with variations in fat and proteins (Alford et al., 1990), while low-energy food combining or balanced diets contributed likewise to weight reductions (Golay et al., 2000). Also, hypoenergetic diets based on high-complex carbohydrate foods that produce a low glycemic response appear to enhance weight control because they promote satiety, minimize postprandial insulin secretion, and maintain insulin sensitivity (Brand-Miller et al., 2002). Other reports, in experimental animals, challenge the assumption that ‘a calorie is a calorie’ based upon studies where energy and macronutrient intakes were precisely controlled (Simon et al., 2001). A reduction of the fat consumption with a concomitant increase in carbohydrate intake in isocaloric low-carbohydrate diets resulted in a modest but significantly different decrease of body fatness (Saris et al., 2000). Another randomized dietary intervention study showed that replacement of some dietary carbohydrate by protein in hypoenergetic balanced diets improved the weight reduction (Labayen et al., 2001), while ad libitum fat-reduced rich-in-protein diets increased the proportion of obese subjects achieving a clinically relevant weight loss (Skov et al., 1999). In contrast, other studies suggest that dietary protein content is positively associated with body fatness (Buemann et al., 1995). Therefore, the study of the metabolic fate of dietary macronutrients in obese subjects is essential in understanding this disease and in providing diet-based therapies (Harvey-Berino, 1998; Astrup, 1999) through measurements of exogenous, endogenous and total substrate oxidation at different experimental stages (Tissot et al., 1990; Binnert et al., 1998; Raguso et al., 1999).

The aim of this investigation was to evaluate the influence in obese women of a balanced but energy-restricted diet followed for 10 weeks, on energy expenditure and metabolic fuel utilization changes during the weight loss process. This purpose was evaluated using whole-body indirect calorimetry and 13C-labeled glucose, leucine and triolein tracers, to respectively estimate endogenous and exogenous substrate utilization.

Subjects and methods

Subjects

A group of healthy obese females aged 23–57 years were recruited (body mass index > 30 kg/m²), but only seven women with a regular menstrual cycle (aged 23–43 years) completed the study. For this reason, we analyzed and presented only the results concerning these subjects.

Before participating in the study, subjects underwent a complete physical examination as well as a medical history, and they were interviewed by a dietitian to assess their eating habits. None of the volunteers had any apparent hepatic, renal or metabolic dysfunction. Females were not pregnant and were excluded if they were smokers,
anemic, or suffered from hyperlipidemia. The local Ethics Committee approved the protocol of the study and an informed consent was obtained from all participant subjects.

**Experimental design**

The study was conducted as a dietary intervention for 10 weeks, controlled in terms of macronutrient composition and energy content, and was carried out in the Metabolic Room of the University of Navarra. During at least 3 months preceding the test, none of the participants was following a hypoenergetic diet and they did not perform any intense regular physical activity, as well as during the nutritional study.

The group received a nutrient-balanced and hypoenergetic diet (55% energy as carbohydrate, 15% energy as protein and 30% energy as fat) based on their food preferences under the supervision of an accredited diettitian. The energy content of the moderately restricted diet was initially 500 kcal less than calculated individual daily energy requirements from appropriate equations (Shetty et al., 1996). To achieve the energy restriction during this program, all subjects attended a 2-weekly nutrition counseling session, and they were weighed and asked about compliance. If volunteers had lost less than 0.5 kg during this period and this was not due to non-compliance on their part, the energy restriction was adjusted to insure a progressive weight loss throughout the duration of the program.

At the beginning of the metabolic study (day 1 (D1)) and 10 weeks after the energy restriction program (day 70 (D70)), each subject received a test meal containing 1 mg/kg L[1-13C]leucine (Euriso-top, Saint-Aubin, France), whose excretion reflected exogenous amino acid oxidation (Raguso et al., 1999), while on day 15 (D15) and day 45 (D45) they were administered 550 mg D[1-13C]glucose (Euriso-top), to assess endogenous and exogenous glucose utilization (Tissot et al., 1990) and on day 30 (D30) and day 60 (D60), the volunteers ingested 150 mg [1,1,1-13C3]triolein (Euriso-top), to measure exogenous lipid utilization (Binnert et al., 1996; Demmelmair et al., 1997).

On the test days (D1, D15, D30, D45, D60 and D70) the women arrived by car or bus at the university at 8:00 h in the postabsorptive state (after 12 h of fasting), and for the duration of the study they were in a comfortable temperature environment. The exploratory study lasted for 6 h and during this period the women were under medical supervision. Blood samples were collected through a venous catheter from an antecubital vein, in the morning after a 12 h fast just before and at 30, 60, 90, 120, 180, 240, 300 and 360 min after the test meal intake to build the area under the curve (AUC) of glucose, leptin and insulin plasma levels. Blood was immediately centrifuged and plasma was stored at −20°C until analyzed. Respiratory exchange measurements were used to estimate total lipid and carbohydrate oxidation rate as well as the energy expenditure rate at baseline, 30 min after test meal intake and every 60 min for the 6 h postprandial period as previously described (Labayen et al., 1999). Urine was collected in the postabsorptive state and during the whole postprandial study to determine nitrogen excretion. A sample of expired gas was obtained at the basal state to determine the 13CO2 background, and expired gas samples were collected every 30 min as described previously (Burstein et al., 1996). Anthropometrical measurements were carried out in the fasting state. Body weight was measured using a digital scale accurate (SECA), and height was measured to the nearest 0.01 m. Body fat mass and percentage body fat were calculated by air displacement plethysmography (Kyle et al., 2002) on D1 and D70.

**Test meal and macronutrient balance**

The composition of the test meal was calculated to provide the same macronutrient distribution as the experimental intervention diet and was administered on the designed days as a liquid formula, which was always isoenergetic (1672 kJ) and isovolumetric (200 ml). The substrate balance was calculated as the net difference between the macronutrient intake from the test meal minus total (exogenous+endogenous) oxidation for a given macronutrient (Schutz, 1995), while endogenous oxidation was estimated as the
difference from indirect calorimetry values (total) and exogenous oxidation as assessed from $^{13}$C breath output data.

**Gas exchange measurements**

Energy expenditure was measured by indirect calorimetry (Deltatrac; Datex-Ohmeda, Helsinki, Finland) using a computerized ventilated canopy analyzer system for 20 min, which was calibrated every two measurements. The first and final 5 min of each set were routinely discarded and the mean value of the remaining 10 min used in the calculations, if steady-state conditions were obtained. The resting metabolic rate and the thermic effect of food were calculated according to previous works (Weir, 1949; Livesey & Elia, 1988) and expressed as J/kg/minute or kJ/kg/6 h, respectively. Protein oxidation was obtained from the urinary excretion of nitrogen values, while carbohydrate and fat oxidation were calculated as described elsewhere (Ferranini, 1988).

**Analytical procedures**

Serum glucose, glycerol, triglycerides (TG), free fatty acids, $\beta$-hydroxybutyric acid and urine urea concentrations were determined enzymatically on a Cobas Mira (ABX Roche, Geneva, Switzerland). Plasma leptin and insulin concentrations were made by commercially available radioimmunoassays (Diagnostic Products Co, Los Angeles, CA, USA; and DSL-23100, Austin, Texas, respectively). AUCs were estimated by the trapezoidal rule.

**Assessment of exogenous glucose, amino acid and lipid oxidation**

The isotopic $^{13}$C/$^{12}$C ratio in breath samples was measured using isotopic ratio mass spectrometry (BreathMAT$^\text{plus}$; Finnigan, Bremen, Germany) and the $\delta^{13}$C$_{CO_2}$ values were calculated. These measured $\delta^{13}$C$_{CO_2}$/O$_2$ data were transformed to $^{13}$C atom percent as previously described (Tissot et al., 1990). The CO$_2$ production was assumed to be 300 mmol/m$^2$ per h, and the body surface area was calculated according to the validated equations (Haycock et al., 1978). The oxidation of the $^{13}$C substrate load was calculated by using previously described formulas (Binnert et al., 1996, 1998) and macronutrient oxidation was expressed as percentage of $^{13}$CO$_2$ cumulative excretion in the breath (Tissot et al., 1990; El Khoury et al., 1995; Raguso et al., 1999).

**Statistical analysis**

Because of the sample size ($n = 7$), the non-parametric Wilcoxon-paired or Mann–Whitney $U$ tests were applied to detect differences before and after weight loss in the experimental group. Given the homogeneous distribution of all data after a careful recruitment of the volunteers, the expression of the results by the mean ± standard error of the mean is statistically allowed. All statistical analyses were performed by using the SPSS 7.5 version for WINDOWS.

**Results and discussion**

Obesity is defined as an excessive lipid storage in adipose tissue resulting from a positive energy balance maintained during a period of time (Martinez, 2000). Therefore, different low-calorie diets and nutritional approaches affecting the energy equation are being investigated in order to reduce body weight in obese subjects (Hill et al., 1993; Astrup, 1999). Furthermore, studies in animal models or humans with different dietary macronutrient distribution might distinctively affect fat accumulation and lipolytic capacity despite the fact that macronutrient and energy intake were precisely controlled (Simon et al., 2001; Brand-Miller et al., 2002).

In this context, the 10-week dietary intervention with a macronutrient-balanced but energy-restricted diet markedly reduced total body weight, body mass index and percent fat mass (Table 1). Despite the shifts due to limitations and variability in measuring body composition in obese individuals in slimming programs (Kyle et al., 2002), the determinations of fat depletion indirectly assessed by plethysmography (about 5%) suggest that adipose tissue accounted for most of the weight loss ($4.2 \pm 1.1$ kg). However, the observed reductions were lower than those obtained
Table 1. Changes in body weight, body mass index, fat mass and blood measurements from obese women in response to the weight loss induced by a balanced and moderately energy-restricted diet before (day 1) and after the 10-week nutritional intervention (day 70)

<table>
<thead>
<tr>
<th>Diet implementation</th>
<th>Before (day 1)</th>
<th>After (day 70)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>87.3 ± 1.0</td>
<td>83.1 ± 1.8**</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>38.6 ± 1.9</td>
<td>33.4 ± 1.3*</td>
</tr>
<tr>
<td>Fat mass (%)</td>
<td>47.8 ± 1.9</td>
<td>41.9 ± 2.0**</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>102 ± 6</td>
<td>88 ± 4*</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>0.68 ± 0.10</td>
<td>0.90 ± 0.11**</td>
</tr>
<tr>
<td>Free fatty acids (mmol/l)</td>
<td>0.5 ± 0.2</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>β-hydroxybutyrate (mmol/l)</td>
<td>0.08 ± 0.01</td>
<td>0.10 ± 0.03</td>
</tr>
<tr>
<td>Glycerol (mmol/l)</td>
<td>81.0 ± 16.7</td>
<td>106.2 ± 22.7</td>
</tr>
<tr>
<td>Insulin (μu/ml)</td>
<td>13.34 ± 4.20</td>
<td>11.38 ± 2.41</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>45.03 ± 12.39</td>
<td>33.42 ± 8.01</td>
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Values presented as mean ± standard error of the mean. * P < 0.05, ** P < 0.10.

with a isonenergetic, moderately high protein, diet (Labayen et al., 2001).

The impact of the experimental diet and the weight loss on fasting blood glucose, TG, free fatty acids, glycerol and β-hydroxybutyrate (Table 1) was only statistically significant (P < 0.05) for plasma glucose and marginally significant for TG (P < 0.10). Additionally, weight loss reduced post-absorptive leptin levels (about 25%, P < 0.05) and produced a slight decrease in insulin values (15%, P < 0.05) at the end of the experimental period (Table 1). The TG data and the other lipid-related determinations were similar to values obtained with other slimming dietary approaches (Alford et al., 1990; Saris et al., 2000). All these plasma values may be explained by the fact that the dynamic mobilization of lipid stores to be used as fuel induced by the caloric restriction would increase the disposal of adipose tissue TG, producing an increment in glycerol and fat free acids (Table 1), and the trend to increase the plasma levels of the ketone body β-hydroxybutyrate after dieting was probably due to this utilization of lipids (Table 1).

The glucose, insulin and leptin measurements which were reduced after the weight loss could be related to the observed decrease in the fat mass induced by the energy restriction diet (Marti et al., 1999), benefitting insulin sensitivity (Golay et al., 2000; Willett et al., 2002) as suggested by the lower postprandial AUC for insulin (Table 2). Obese subjects usually have a larger absolute fat oxidation than lean subjects, as evidenced by a lower respiratory quotient (Kunz et al., 2002). The implementation of the low calorie-diet produced no statistical changes on the measured resting metabolic rate (Table 2) expressed on a weight basis (2.8% increment), despite the obese reducing their body weights by about 4–5 kg (4.8% reduction), which could suggest that this component of the energy expenditure may play some role in the weight loss induced by the energy-restricted diet (Dulloo & Jacquet, 1998).

Food-induced thermogenesis is the other component of total energy expenditure, which constitutes a functional index of the net efficiency of energy utilization during the postprandial phase (Schutz, 1995). Thus, a nutritional trial demonstrated the high reproducibility of macronutrient oxidation rate values obtained after a single buffet-type meal in healthy male subjects (Arvaniti et al., 2000). Furthermore, the use of an acute dietary load with a known composition of energy-yielding substrates is a reliable method for the assessment and prediction of the macronutrient utilization in lean and obese individuals (Labayen et al., 1999). The validity of a single measurement of food-induced thermogenesis as a marker of the habitual fuel mixture oxidized was further validated in other study, where the energy balance over the days prior to the measurements was the most important factor influencing postabsorptive respiratory quotient (Goris & Westerterp, 2000).

Of course, an important issue is whether a blunted ability to stimulate endogenous or/and exogenous fat, glucose or protein oxidation in response to a single meal might affect the outcome of a body-weight reducing strategy. However, the quantitative importance of the postprandial thermogenesis seems to be limited and not all obese patients have a blunted meal-induced thermogenesis (Schutz, 1995; Toubro & Astrup, 1997).

The test meal induced non-statistically significant increases in the thermic effect of food after the dietary intervention in the obese participants in the trial when expressed
on a relative basis (kJ/kg per 6 h) and compared with baseline values (Table 2). Indeed, our data support the motion that a low-calorie diet may have a small effect on this component of the energy expenditure, since the values were slightly higher (3%) after losing 5% body weight. A reduced thermic effect of food in obesity is generally attributed to insulin resistance (Schutz, 1995), which was not seen in this design following a balanced hypoenergetic diet as estimated by the AUC insulin values (Table 2).

The macronutrient distribution and nutritional balance of an energy-restricted regime may be of importance in losing weight (Burstein et al., 1996; Skov et al., 1999; Labayen et al., 2001). Thus, there is a hierarchy in the extent to which macronutrients are used, stored and transformed within the body, which may influence fat deposition and utilization in some cases (Prentice, 1998). Although it is sometimes claimed that macronutrients oxidation can be considered separately, one should realize that fat and CHO oxidation are not mutually independent, but that there is an intimate inverse inter-relationship between them (Whitley et al., 1997). On the contrary, the sympathoadrenal system may play an important role in the regulation of both energy intake and expenditure in the slimming process (Astrup, 1995). Thus, it has been suggested that a reduced ability to oxidize fat may be involved in the blunted lipolytic response to fasting found in obese women, in which the neuroendocrine regulation would be involved (Buijs et al., 2003). Furthermore, there is substantial evidence that human obesity is characterized by abnormalities in sympathetic cardiovascular control that are related with the leptin and the insulin function (Dobbins et al., 2003), while blood pressure, as a marker of the sympathetic activity, is usually reduced during slimming (Corry & Tuck, 1999). Thus, the extent of substrate oxidation depends upon body composition, the activity of the sympathetic nervous system and genetic factors, in addition to the size and the composition of the meal ingested (Tremblay, 1995; Schutz, 1999).

Weight loss in our experimental design was associated with a statistically significant decrease in postprandial carbohydrate oxidation (Table 2), which was accompanied by increases in fat utilization (12%) after 70 days of dietary treatment. Despite the lack of statistical significance in overall lipid oxidized, measured by indirect calorimetry at the end of the experimental trial, the fat balance and fat oxidation values measured at D15 and D45 showed statistically marginal differences ($P < 0.10$) between these two test days (Table 3). This metabolic outcome at D15 and D45 was associated with a higher carbohydrate balance ($P < 0.05$) and with a lower glucose oxidation, which should be ascribed to a decrease in both endogenous and exogenous glucose utilization as assessed

<table>
<thead>
<tr>
<th>Diet implementation</th>
<th>Before (day 1)</th>
<th>After (day 70)</th>
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<tbody>
<tr>
<td>Resting metabolic rate (J/kg × min)</td>
<td>46.12 ± 1.29</td>
<td>47.43 ± 1.00</td>
</tr>
<tr>
<td>Thermic effect of food (kJ/kg × 6 h)</td>
<td>17.02 ± 0.56</td>
<td>17.45 ± 0.34</td>
</tr>
<tr>
<td>Postprandial substrate utilization (mg/kg × 6 h)</td>
<td>406.96 ± 29.71</td>
<td>308.76 ± 42.67*</td>
</tr>
<tr>
<td>Glucose oxidation</td>
<td>102.93 ± 22.78</td>
<td>114.09 ± 25.82</td>
</tr>
<tr>
<td>Lipid oxidation</td>
<td>328.07 ± 54.87</td>
<td>414.49 ± 59.24</td>
</tr>
<tr>
<td>Postprandial blood measurements (areas under the curve)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>−90.0 ± 1771.3</td>
<td>−2535.0 ± 654.3</td>
</tr>
<tr>
<td>Leptin</td>
<td>−2880.4 ± 744.5</td>
<td>−1476.5 ± 1514.9</td>
</tr>
<tr>
<td>Insulin</td>
<td>7379.6 ± 2130.4</td>
<td>2937.8 ± 679.7*</td>
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Values presented as mean ± standard error of the mean. * $P < 0.05$. 

Table 2. Changes in resting metabolic rate and thermic effect of food as well as postprandial substrate utilization and area under the curve measurements on obese women in response to weight loss induced by a balanced and moderately energy-restricted diet before (day 1) and after the ten weeks nutritional intervention (day 70).
by the \textsuperscript{1-13C} glucose breath test on those days (Table 3). These data suggest that the effects on lipid oxidation induced by the dietary restriction may adapt (decrease) with time as previously described (Dulloo & Jacquet, 1998).

Postprandial (exogenous) lipid and leucine oxidation during 6 h did not change under the energy restriction program (Figure 1), as measured by \textsuperscript{13}CO\textsubscript{2} cumulative excretion from \textsuperscript{13}C-labeled triolein consumed at D30 and D60, and the \textsuperscript{13}C-leucine received at D1 and D70, respectively. The fractional oxidation rate from exogenous sources, as assessed by labeling the substrate with stable isotopes on D30 and D60, also showed that only a very small percentage of exogenous fat is apparently oxidized in the postprandial phase (3–6\%).

The moderate effect on weight loss induced by this restricted diet with a balanced macronutrient distribution confirms previous reports indicating that, in the metabolic day-to-day, the habitual macronutrient intake affects basal and postprandial macronutrient oxidation as well as body weight regulation (Stubbs et al., 1996; Kunz et al., 2002). Also, a close inter-relationship between carbohydrate and fat metabolism following isoenergetic meals has been previously described (Whitley et al., 1997), since re-adjustments in fuel utilization seem to be an important mechanism for re-establishing the macronutrient balance. (Stubbs et al., 1993), in which endocrine counter-regulatory mechanisms

<table>
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<tr>
<th>Table 3. Carbohydrate and fat utilization in obese women after 15 or 45 days receiving a balanced moderately energy restricted diet</th>
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<tr>
<td></td>
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<tr>
<td>Total glucose oxidation (mg/kg \times 6 h)</td>
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<tr>
<td>Exogenous glucose oxidation (g)</td>
</tr>
<tr>
<td>Endogenous glucose oxidation (g)</td>
</tr>
<tr>
<td>Carbohydrate balance (g)</td>
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<tr>
<td>Lipid oxidation (mg/kg \times 6 h)</td>
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<td>Lipid balance (g)</td>
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Values presented as mean ± standard error of the mean. * \(P < 0.05\), ** \(P < 0.10\).

Figure 1. Changes in cumulative \textsuperscript{13}C-labeled elimination (percentage administered dose) from different tracers (\textsuperscript{13}C-glucose, \textsuperscript{13}C-triolein and \textsuperscript{13}C-leucine) measuring exogenous substrate utilization in response to the weight loss induced by a balanced and moderately energy-restricted diet across the 10-week nutritional intervention. D, day.
may be involved (Vicennati et al., 2002; Willett et al., 2002). Taking all measurements together, they suggest that the weight loss should be associated with a higher fat oxidation and accompanied by an inverse interaction between carbohydrate and fat oxidation following the energy-restricted diet, which may be time dependent.

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References


Labayen I, Forga L & Martinez JA (1999): Nutrient oxidation and metabolic fate as affected by meals contain-


