

## Symposium 2

### Defects in substrate oxidation involved in the predisposition to obesity

BY ARNE ASTRUP, BENJAMIN BUEMANN, SØREN TOUBRO  
AND ANNE RABEN

*Research Department of Human Nutrition and KVL Centre for Food Research, The Royal  
Veterinary and Agricultural University, Rolighedsvej 30, 1958 Frederiksberg C,  
Copenhagen, Denmark*

Obesity is a highly prevalent condition, both in industrialized countries and in many developing countries. The prevalence of obesity has increased dramatically and its complications are among the leading causes of premature mortality, such as coronary heart disease, stroke, type 2 diabetes, and certain cancers (Bouchard & Bray, 1996). Obesity develops as an interaction between a genetic predisposition and certain environmental factors, such as high-fat diets and a low habitual energy expenditure caused by low levels of physical activity. There is evidence to suggest that only subjects with a genetic predisposition to obesity gain weight when exposed to a high-fat diet over extended periods of time (Heitmann *et al.* 1995). It is a matter for speculation whether the reason for the less-effective suppression of appetite caused by fat than by carbohydrate is a result of the higher energy density of fat compared with carbohydrate, or whether the effect is due to unrelated fat-specific properties (Astrup & Raben, 1996a). These properties are difficult to dissociate because foods rich in fat have a high energy density and vice versa. There is, however, accumulating evidence to suggest that fat oxidation capacity has a metabolically-driven, genetically-determined component which, in the form of a low fat-oxidation rate, may play an important role in overconsumption of energy in predisposed individuals consuming a high-fat diet. We, therefore, find it pertinent to seek an in-depth understanding of the physiological regulation of fat oxidation by identifying the factors determining its rate; to elucidate to what extent the fat oxidation rate is influenced by genetic determination, and whether the genes express their effect indirectly through food choices or by control of fat transport and  $\beta$ -oxidation via the regulation of enzyme activities, and levels of and sensitivity to hormones.

#### THE PRINCIPLE OF MACRONUTRIENT BALANCE

The traditional concept of the energy balance equation, which describes weight gain as an excessive positive energy imbalance, can usefully be replaced by a series of macronutrient balance equations in which gains in body fat stores are viewed specifically as an imbalance of fat. The rationale for this replacement is that energy is not equal in its metabolic effects; interconversion between the four macronutrients is negligible and an oxidative hierarchy operates in inverse proportion to the size of available stores for each macronutrient (Fig. 1). Amino acid, glucose and alcohol oxidation adjust readily to protein, carbohydrate, and alcohol intakes. Seen over a period of a few days, regulation appears to be geared primarily to maintenance of appropriate glycogen reserves.

Alcohol is most readily oxidized because it cannot be stored. Oxidation of carbohydrate

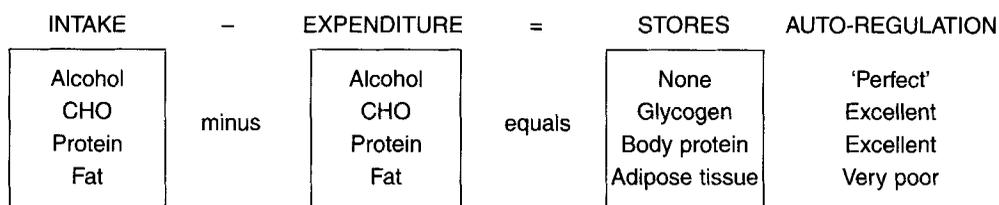


Fig. 1. The replacement of energy balance by the hierarchy of macronutrient balances and their regulations. CHO, carbohydrate. (Adapted from Prentice, 1995.)

and protein are also under tight auto-regulatory feedback control; oxidation increases in direct response to intake. In contrast, there is virtually no acute feedback between fat intake and fat oxidation. Fat oxidation is primarily a function of the gap between total energy expenditure and the amounts of alcohol, protein and carbohydrate energy consumed (Astrup & Flatt, 1996), which results in a much less accurately maintained fat balance. Although regulatory responses serving to achieve fat balance exist, their effectiveness in inhibiting the expansion of the fat mass seems to be limited. Leptin, a hormone secreted from adipose tissue at an increased rate when the fat mass is expanded, is supposed to inhibit food intake and increase sympathetic nervous system activity through a central action (Weigle *et al.* 1995; Considine *et al.* 1996). The finding that obese subjects remain obese in spite of 10-fold higher circulating leptin concentrations suggests that lipostatic mechanisms are insufficient to restrict energy intake in this category of individuals. However, long-term fat and energy balances tend to remain close to zero over prolonged periods once a weight-maintenance plateau has been reached (i.e. less than 2–3% error relative to energy turnover when considered over 1 year; Eckel *et al.* 1996).

In conclusion, protein, carbohydrate and ethanol oxidation rates relate well to the respective intake of these nutrients. Dietary fat oxidation, however, relates poorly to daily variations in fat consumption (Flatt, 1987). This is a probable explanation as to why obesity develops particularly among individuals with a genetically-determined low fat-oxidation capacity, when the diet is high in fat and when physical activity is limited (Astrup & Flatt, 1996).

#### DETERMINANTS OF FAT OXIDATION

The fat oxidation rate and the composition of the fuel mix oxidized is mainly determined by energy requirements, energy balance and the dietary macronutrient composition. Overall, substrate oxidation is dictated by the body's need to regenerate the ATP used in performing its metabolic functions, in maintaining body temperature, and in moving at rates depending on an individual's size and physical activity. The absolute rate of total oxidation equals energy expenditure and energy requirements, which is mainly determined by the size of the fat-free mass, the amount of physical activity and, to a smaller extent, by the size of the fat mass, by plasma concentrations of triiodothyronine (T<sub>3</sub>) and androstenedione, and sympathetic nervous system activity (Toubro *et al.* 1996). Thus, on a standardized diet with fixed macronutrient composition, subjects with a large fat-free mass and a high level of physical activity will oxidize greater amounts of all macronutrients expressed in g/d (or kJ/d) than an individual with a lower fat-free mass or physical activity level (Fig. 2). Oxidation rates in individuals with different metabolically active fat-free

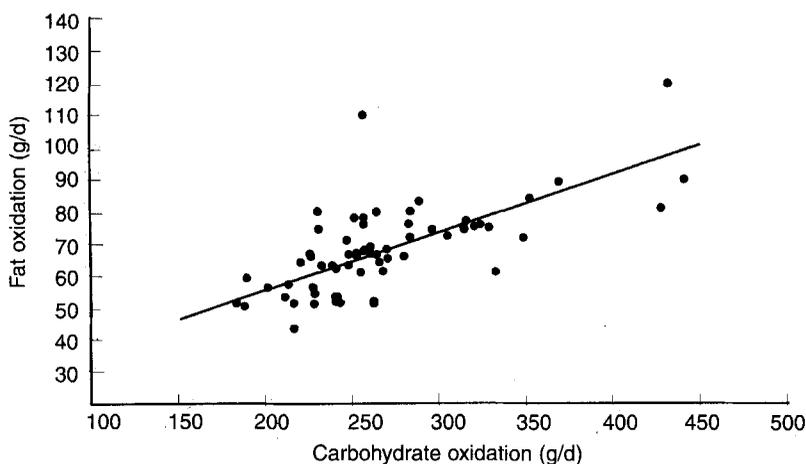


Fig. 2. Relationship between carbohydrate and fat oxidation (g/d) for sixty-two healthy subjects (Astrup *et al.* 1994b). The subjects consumed a diet with identical macronutrient composition, and most of the variation in oxidation rates between subjects is entirely due to differences in energy requirements, i.e. an individual with a high energy requirement consumes greater amounts of all nutrients than an individual with a low energy requirement. A significant proportion of the residual variation in fat and carbohydrate oxidation can be explained by differences in macronutrient composition of the habitual, previous diet.  $r\ 0.70$ ,  $P < 0.00001$ .

mass can be compared when the oxidation rates are expressed as a proportion of total oxidation (i.e. proportion or percentage), or if adjustment is made for differences in total energy expenditure. These proportional rates are referred to as the composition of the fuel mix.

The composition of the fuel mix oxidized to drive oxidative phosphorylation varies considerably during the day, and from day to day, mainly due to the influence of energy balance and, to a minor degree, the composition of the meals (Toubro *et al.* 1995).

#### *Energy balance and diet composition*

The body's energy balance changes all the time and never reaches a steady-state, except when viewed over a longer period, e.g. 1 week. This is due to the fact that energy is expended continuously whereas energy intake takes place intermittently, i.e. at meals. A positive energy balance increases RQ due to an increase in carbohydrate oxidation and a decrease in fat oxidation. Conversely, a negative energy balance causes a decrease in RQ due to suppression of glucose oxidation and an increase in fatty acid oxidation. Meal ingestion may be regarded as an acutely invoked positive energy balance, and even meals with a high fat and a very low carbohydrate and protein content increase glucose oxidation and, hence, RQ. In the resting post-absorptive phase, energy balance becomes negative and RQ decreases due to inhibition of glucose oxidation and stimulation of lipolysis and increased free fatty acid (FFA) oxidation. The same relationship between energy balance and RQ applies when viewed over 24 h. When measurements are carried out in a respiratory chamber a positive relationship between 24 h RQ and 24 h energy balance is found (Fig. 3) (Toubro *et al.* 1996). If the macronutrient composition of the diet is fixed, and exactly matched to the subject's energy requirements, the body will eventually oxidize macronutrients in the same proportions as in the diet. Calorimetry studies (Bumann *et al.*

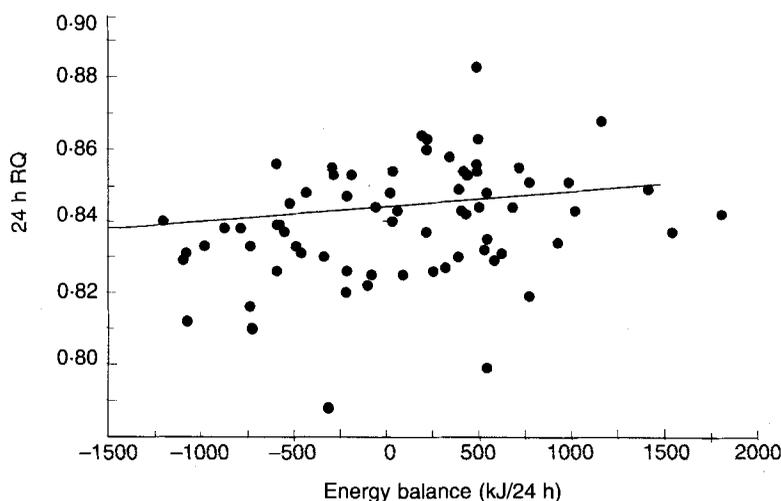


Fig. 3. Relationship between 24 h RQ and 24 h energy balance measured during a stay in a calorimeter. The seventy-one healthy subjects consumed a diet with a fixed macronutrient composition.  $r$  0.31,  $P < 0.01$ .

1994) suggest that it takes normal individuals 3–4 d to reach macronutrient balance (RQ equals food quotient (FQ;  $\text{CO}_2$  produced: $\text{O}_2$  consumed during the biological oxidation of a representative sample of the diet, derived by calculation rather than direct measurement of gas exchange) under these conditions. Real life, however, is more complex. Diet composition and energy balance vary considerably from day to day, and the metabolic demand for various substrates influences appetite. Differences in physical fitness and constitutional differences in muscle lipoprotein lipase (*EC* 3.1.1.34; LPL) activity and oxidative pathways cause fundamental changes in substrate use, which in turn influence energy intake and macronutrient preferences (Klausen *et al.* 1996). In the experimental setup, however, it is crucial to acknowledge that the previous diet exerts an important impact on the subsequent days' RQ. The diurnal pattern in fat oxidation is shown in Fig. 4.

Because fat oxidation (%) and glucose oxidation (%) are inversely related it is of interest to know how the former influences the latter and vice versa. In the classical view, fat regulates glucose metabolism. This concept has, however, recently been challenged (Dyck *et al.* 1993) and it is now proposed that the opposite is the case. There is at present no evidence to support the existence of the so-called 'glucose–fatty acid cycle' *in vivo* (Randle *et al.* 1963). Instead, there are indications that carbohydrate may regulate fat metabolism, possibly through malonyl-CoA, which is involved in the regulation of FFA transportation into the mitochondria. Carbohydrate ingestion increases plasma glucose and decreases fat utilization by inhibiting both plasma FFA oxidation and intramuscular triacylglycerol utilization. This effect is induced by insulin and by a muscle effect, probably via malonyl-CoA. Consequently, the carbohydrate content of the diet and overall energy balance are crucial for the rate of fat oxidation and, thus, for fat balance. This normal physiological regulatory mechanism does not exclude the susceptibility of fat oxidation to influence by the activity of muscle LPL activity and by 3-hydroxyacyl-CoA dehydrogenase (*EC* 1.1.1.35; HADH; Ferraro *et al.* 1993; Zurlo *et al.* 1994), and by sympathetic nervous system activity (Tremblay, 1995).

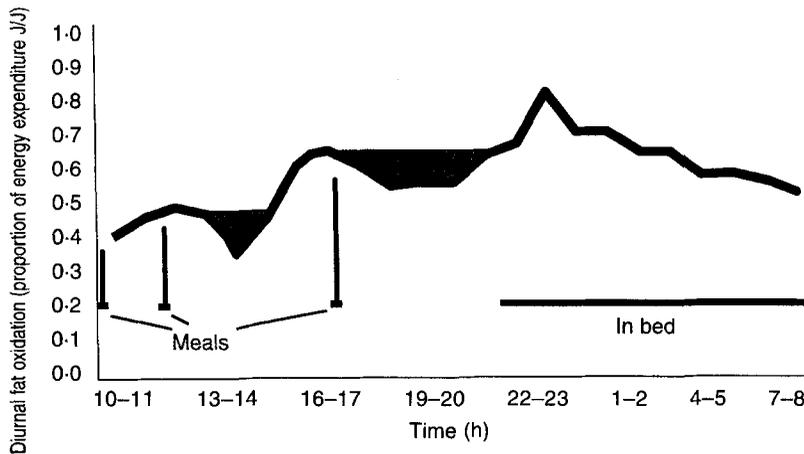


Fig. 4. Oxidation profiles (24 h) in healthy subjects on a high-fat diet (55% energy from fat). Note that fat oxidation is decreased postprandially even following these high-fat meals. (■), Postprandial suppression. (Data modified from Buemann *et al.* 1994.)

#### Genetics of fat oxidation

Direct evidence for a genetic influence on RQ was delivered by Dériaz *et al.* (1994) who studied the relationship between DNA variation at the genes coding for the Na,K-ATPase (*EC* 3.6.1.37) peptides, RQ, and body fat. Post-absorptive RQ was found to be associated with the  $\alpha_2$  gene and linked with the  $\beta$  gene of the Na,K-ATPase, which suggests that these, or neighbouring genes, influence RQ. Twin studies also support heritability of RQ. Bouchard *et al.* (1989) found greater similarity in RQ during exercise among monozygotic twins than among dizygotic twins. Moreover, in the Quebec Family Study involving 300 individuals from seventy-five nuclear families, the genetic heritability of RQ was 20% (Bouchard *et al.* 1994). It cannot be ruled out, however, that the genetic effect is indirect, i.e. mediated through food preferences, which in turn influence RQ. Other studies where diet composition and energy intake have been rigorously controlled have provided evidence that disallows this theory. Bouchard *et al.* (1990) conducted a 100 d controlled overfeeding study on identical twins, where significant within-pair resemblance was found in changes in RQ, both in the post-absorptive and postprandial state. RQ studies (24 h) also suggest that the oxidation pattern is partially under a genetic influence which is independent of dietary macronutrient selection. Using calorimeters Zurlo *et al.* (1990) measured 24 h RQ in Pima Indian siblings receiving a controlled weight-maintenance diet while staying in a metabolic ward. After adjustments for previous change in body weight, 24 h energy balance, gender and body fat, it was shown that 24 h RQ was a family trait where family membership explained 28% of the variation between individuals. In the Copenhagen Sibling Study 24 h RQ was measured in seventy-one adult subjects from thirty-two nuclear families on a standardized diet without control of previous food composition (Toubro *et al.* 1996). The habitual diet, however, was assessed by a 7 d food record during the period immediately before the measurement. The theoretically calculated RQ of their habitual diet (the so-called FQ) was found to be a family trait with a heritability of 45% (S. Toubro, C. Simonsen, T. I. A. Sørensen and A. Astrup, unpublished results). Similarly 24 h RQ,

adjusted for age, gender and 24 h energy balance, also aggregated within families, with a heritability of 32%. FQ and RQ were also positively correlated. However, when 24 h RQ was adjusted for differences in habitual FQ the heritability estimate did not change. This suggests that RQ is a family trait, probably genetically determined, and that the familial resemblance cannot be attributed to shared dietary composition.

#### *Associations between fat oxidation and weight gain and obesity*

Genetically-predisposed individuals gain weight when exposed to a high-fat diet over extended periods of time. Weight gain after a 6-year follow-up for all subjects was found to be only weakly associated with dietary fat content at baseline, but the association was seven times greater in overweight and obese subjects, and fifteen times greater in those at familial risk (Heitmann *et al.* 1995). Rolls *et al.* (1994) have demonstrated that intake of high-fat foods suppresses subsequent intake less in obesity-prone restrained eaters than in normal unrestrained subjects. The physiological mechanisms behind this susceptibility in predisposed individuals may relate to their lower ability to oxidize fat. This hypothesis is supported by a study which demonstrated that restrained eaters managed to oxidize the fat content of the diet following exposure to low-fat and medium-fat diets, but that their 24 h fat oxidation was impaired as compared with unrestrained subjects while on a high-fat diet (50% fat energy; Verboeket-van de Venne *et al.* 1994).

There are also prospective studies to suggest that there is a combination of a deficient fat oxidation and an enhanced fat deposition in subjects with a predisposition to weight gain and obesity. In a study of Pima Indians, 24 h RQ was measured at baseline after intake of a controlled weight-maintenance diet during a stay in a metabolic ward. Those with a low fat oxidation (high RQ) were more likely to gain weight over a 3-year period than those with a low RQ (high fat oxidation; Zurlo *et al.* 1990). Seidell *et al.* (1992) analysed data from the Baltimore Longitudinal Study on Aging, and found a significant positive association between resting RQ and subsequent weight gain. The adjusted relative risk of gaining >5 kg in initially non-obese men with a RQ >0.85 was 2.42 (95% CI 1.10–5.32) compared with those with a RQ <0.76. Energy balance and previous diet composition were much less rigorously controlled than in the study of Zurlo *et al.* (1990), which poses the risk that the high RQ were mainly produced by subjects who were already gaining weight and that their positive energy balance confounded the interpretation of the high RQ values (see Fig. 3). Taken together, however, the studies on 'pre-obese' subjects support the hypothesis that a low rate of fat oxidation is a risk factor for weight gain in those exposed to high-fat diets accompanied by low levels of physical fitness. The importance of the gene–environment interaction is emphasized by comparing body fatness of genetically comparable Pima Indians living in very different environments in Mexico and Arizona. In Pima Indians matched for age and gender those living in the USA were 25 kg heavier than their Mexican relatives (Ravussin, 1995).

#### *Studies in post-obese subjects*

The importance of studying fat oxidation in subjects with normal-size fat stores is underlined by the fact that obesity brings about an increased proportion of fat utilized as fuel, both in the fasting state and on a 24 h basis (Astrup *et al.* 1992). Whether the higher

proportion of energy expenditure due to fat oxidation in the obese state is caused by the habitual consumption of a high-fat diet, or whether it is determined by the increased supply of fat substrates provided by the enlarged fat mass is not entirely clear. Nevertheless, the finding makes obese subjects less suitable for causative studies of fat oxidation. Apart from larger prospective studies linking RQ with subsequent weight gain, and experiments using normal-weight restrained eaters, formerly obese subjects who have intentionally reduced body weight and composition to normal size (so-called post-obese) are used as a more appropriate model (Eckel *et al.* 1996). The first study reporting that post-obese subjects had impaired fat oxidation in response to high-fat diets was carried out by Lean & James (1988). They reported striking differences in substrate handling by obese, post-obese and control subjects. While mean 24 h RQ was similar in the obese group and in the control group, it was significantly higher in the post-obese group, both during fasting and following a high-fat diet. This indicates that the post-obese group utilized relatively less fat and relatively more carbohydrate than the control group during fasting and on the high-fat days. In a more-rigorously-controlled dietary study Astrup *et al.* (1994a) examined the ability of post-obese women to adjust macronutrient oxidation in response to three isoenergetic diets: a low-fat (20%), a medium-fat (30%) and a high-fat diet (50% fat-energy), using 24 h calorimetry. No differences were found between groups on the low-fat and medium-fat diets. On the high-fat diet, however, the post-obese women failed to increase fat:carbohydrate oxidation appropriately, which caused a positive fat balance. The preferential storage of fat in the post-obese group on the high-fat diet was caused by a failure to increase fat oxidation sufficiently to match the amount of fat consumed. It is possible that the accompanying negative carbohydrate balance would tend to reduce glycogen stores, which is thought to be a signal for decreased satiety and increased hunger. The phenomenon of an altered substrate pattern has also been found after a single high-fat meal (Raben *et al.* 1994). Whereas the thermic effect of the meal was found to be similar in post-obese and controls, postprandial fat oxidation was 2.5 times lower in the post-obese group. The lower fat oxidation following high-fat meals and diets observed in these studies may have been subject to a confounding effect of differences in habitual diets between post-obese and control subjects. If the post-obese subjects had consumed a diet with lower fat and higher carbohydrate content in the days or weeks before the measurements, this may have led to a more imprecise auto-regulation of fat oxidation, which is likely to be seen only following high-fat challenges. There is evidence to suggest that post-obese subjects do indeed choose low-fat, high-carbohydrate diets compared with never-obese, otherwise healthy subjects (Hansen *et al.* 1995). To avoid the confounding effect of previous diet composition we undertook a 5 d calorimetry study where diet and energy balance were strictly controlled (Bumann *et al.* 1994). On days 0 and 1 post-obese and matched control subjects consumed a diet providing 30% energy from fat, 55% energy from carbohydrate and 15% energy from protein. On days 2, 3 and 4 they consumed a high-fat diet (55% energy from fat, 30% energy from carbohydrate and 15% energy from protein). Changes in 24 h RQ were similar in the post-obese and control subjects after introduction of the high-fat diet, but the postprandial fat oxidation was consistently suppressed in the post-obese group (Bumann *et al.* 1994). This study confirms that postprandial fat oxidation is particularly suppressed in post-obese subjects, but it does not exclude the possibility that the adjustment of 24 h RQ following introduction of the high-fat diet would have been impaired if the post-obese subjects were allowed to eat an unrestricted diet.

## MECHANISMS RESPONSIBLE FOR A LOW RATE OF FAT OXIDATION

*Impaired lipolysis or oxidation?*

The impaired fat oxidation in the pre-obese and post-obese state may be due to a number of different mechanisms in fat and glucose metabolism, and it is possible that the disorder may be heterogeneous. We have undertaken studies designed to assess whether fat mobilization from the adipose tissue stores through lipolysis is a limiting factor for fat oxidation in post-obese subjects during exercise, or whether it is rather uptake and oxidation in skeletal muscle which is impaired. Lipolysis was measured by a combination of a Xe-clearance technique and microdialysis in the abdominal subcutaneous adipose tissue (Astrup *et al.* 1994c). Post-obese and control subjects were matched for body composition, age, gender and aerobic capacity. During a 60 min submaximal exercise test the increase in lipolysis was intact in the post-obese subjects, but fat oxidation was lower in the post-obese subjects during rest and recovery, and it was also lower during exercise if fat oxidation was adjusted for differences in plasma FFA. This study clearly demonstrates that the impaired fat oxidation was localized in skeletal muscle.

*Fat oxidation in skeletal muscle*

Skeletal muscle accounts for 20–30% of the body's energy expenditure at rest and up to 90% during exercise. At rest more than 80% of the muscle oxidation is accounted for by fat. Accumulating evidence points to skeletal muscle as the site of the impaired capacity for uptake and oxidation of fat substrates, and a number of possible mechanisms have been studied. The major fat substrates for skeletal muscle oxidation are plasma FFA, and triacylglycerols in lipoproteins, i.e. VLDL and chylomicrons, which require hydrolysis by the rate-limiting enzyme LPL at the endothelial or luminal interface of muscle capillaries. A low muscle LPL activity, therefore, may represent a mechanism responsible for a lower fat oxidation in predisposed individuals. In a cross-sectional study Ferraro *et al.* (1993) found skeletal muscle LPL activity to be inversely correlated with 24 h RQ ( $r = -0.57$ ) in subjects who had been on a controlled diet for at least 3 d. So far, however, it remains to be determined whether a low LPL activity is a risk factor for weight gain. In post-obese subjects muscle LPL activity was not found to be lower than that in matched controls (A. Raben, B. Saltin and A. Astrup, unpublished results).

Similarly, skeletal-muscle insulin sensitivity is another important variable influencing the local partitioning between fat and glucose substrates. While insulin insensitivity is a feature of obesity, Swinburn *et al.* (1991) showed that insulin-sensitive subjects were more likely to gain weight over 3.5 years than were insulin-resistant subjects (7.6 v. 3.1 kg). Insulin sensitivity has also been evaluated in post-obese subjects. Using the insulin-glucose clamp technique Toubro *et al.* (1994) were unable to detect any significant difference in insulin sensitivity and glucose oxidation in post-obese and well-matched control subjects. By contrast, after controlling for previous diet Astrup *et al.* (1994c) and Raben *et al.* (1996) found greater insulin sensitivity in post-obese than in control subjects. The studies in post-obese subjects should not be regarded as conflicting; an enhanced insulin sensitivity should rather be viewed as a risk factor, not present in all predisposed subjects, but a mechanism which may promote uptake and oxidation of glucose at the expense of fat.

It is a commonly-held belief that type I muscle fibres have a higher capacity for substrate

oxidation than type II muscle fibres, and it has been suggested that a lower value for type I: type II fibres may be responsible for a low fat oxidation and propensity to obesity. Based on a very small number of subjects Wade *et al.* (1990) found an inverse relationship between body fatness and the proportion of type I fibres of *vastus lateralis* muscle, which had been suggested previously in a larger study by Lillioja *et al.* (1987). In a better controlled and larger study no significant relationship was found between muscle fibre type and body fatness (Simoneau & Bouchard, 1995).

The variability in fat oxidation among subjects may be due to differences in activities of key enzymes in the  $\beta$ -oxidation pathway. In a number of studies biochemical characteristics of skeletal muscle have been assessed in the *vastus lateralis* muscle by muscle fibre histochemistry and by measuring activities of six key enzymes involved in the energy-generating pathways. Zurlo *et al.* (1994) found 24 h RQ correlated negatively with HADH, and more weakly with adenylokinase and creatine kinase (EC 2.7.3.2). Another interesting enzyme is malate dehydrogenase (EC 1.1.1.37; MDH) which is an activity marker of enzymes of the Krebs cycle. Inverse correlations have been found between body fatness and MDH activity. It has also been found that fatter men have lower MDH activities than lean controls matched for physical fitness (Simoneau & Bouchard, 1995). More convincing, however, was the finding that those individuals with a low MDH activity gained more body fat than those with high levels during 100 d of 4.2 MJ/d overfeeding (Simoneau *et al.* 1994). Muscle histochemistry and biochemistry in post-obese subjects has recently been studied and compared with subjects also matched with respect to aerobic capacity. The distribution of muscle fibre types and LPL activity did not differ between post-obese and control subjects (A. Raben, B. Saltin and A. Astrup, unpublished results), but HADH activity was 20% lower in post-obese subjects than in controls. So it is quite possible that several different mechanisms are involved in the lower rates of fat oxidation of skeletal muscle in predisposed individuals. This suggests that some neuro-hormonal influence may be responsible, such as a lower thyroid hormone status (Astrup *et al.* 1996), and a lower sympathetic nervous system activity (Spraul *et al.* 1993). There are now studies showing that both a low free  $T_3$  and low sympathetic activity are risk factors for weight gain, and both systems could be responsible for lower fat oxidation capacities in skeletal muscle (S. Toubro, T. I. A. Sørensen and A. Astrup, unpublished results).

#### *Do obese subjects consume a diet higher in fat?*

There is reliable evidence that obese subjects habitually consume a diet with a higher fat content than normal-weight subjects. The taste preference for fat was found to be increased in obese and post-obese individuals (Drewnowski *et al.* 1985), and this observation has been confirmed recently (Drewnowski *et al.* 1991). This could be the mechanism that precipitates the gene expression which is seen physiologically as an inappropriately low fat oxidation, which in turn stimulates appetite via an impaired postprandial fat oxidation. It is, therefore, of interest to assess cross-sectional and longitudinal studies which have examined the relationship between dietary fat:carbohydrate and body fatness. In the valid cross-sectional studies, case-control analysis of dietary composition in obese *v.* non-obese subjects consistently shows that obese individuals consume a diet with a higher fat and a lower carbohydrate content than non-obese subjects (Astrup & Raben, 1996b). The diet of the obese groups has been found to have 5–8% more fat energy than that of the control

groups. Clearly, there is a need for a biological marker of habitual macronutrient intake. A proxy for dietary macronutrient composition can be obtained indirectly by measurement of substrate oxidations, as the oxidative pattern seems to be relatively undisturbed by changes in dietary fat content in the first 24 h. Using 24 h calorimetry Astrup *et al.* (1994b) found oxidative fat energy in overweight and obese subjects to be higher than that in normal-weight controls (40.2 v. 36.0%,  $P < 0.02$ ). This suggests that obese subjects consume a diet characterized by a higher fat content than non-obese subjects, but it cannot be ruled out that the higher oxidative fat energy is stimulated by the enlarged body fat stores. It clearly indicates, however, that studies aimed at providing an insight into the physiological and biochemical phenotypic expressions of the obesity genes and how they interact with dietary fat should be conducted with caution. Indeed, they should probably be avoided as the confounding effect of the increased body fat stores and their impact on glucose and fat metabolism renders results extremely difficult to interpret.

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