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Carbohydrate balance and body-weight regulation

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Carbohydrates generally provide 40–60% of the total food energy, and daily carbohydrate intakes in adults consuming mixed diets range from 200 g in relatively small and sedentary individuals to more than 500 g in physically-active adults. The body's glycogen reserves (Björntorp & Sjöström, 1978) can be presumed to be not much greater than the amount of carbohydrate consumed and oxidized during a typical day. Given the small size of this fuel reserve in relation to its turnover, maintenance of appropriate glycogen stores and blood glucose levels presents a considerable challenge to metabolic regulation, but even large variations in carbohydrate intake can be accommodated without noticeable stress and while keeping up usual functions and activities (Stubbs *et al.* 1995*a*). Evidently, glucose oxidation adjusts itself to carbohydrate availability quite effectively. Maintenance of appropriate glycogen levels under conditions of *ad lib.* food intake would be facilitated if, in addition to adjustment of glucose oxidation to carbohydrate intake, food intake were to be regulated in a manner helping to sustain the balance between the use of glucose and the influx of dietary carbohydrate (Flatt, 1987). Making these considerations at a time when the existence of specific glucose receptors in the brain had just been revealed, Mayer (1955) developed the concept of 'glucostatic regulation' of food intake, in which it is presumed that monitoring of blood glucose levels by the brain generates signals for the regulation of food intake by the central nervous system (CNS). Indeed, it is now known that transient small declines in blood glucose levels tend to elicit feeding (LeMagnen, 1981; Campfield & Smith, 1990). However, blood glucose levels vary greatly during the day, and there is much overlap between the values that prevail under very different nutritional and metabolic conditions. Subsequently, it was considered, therefore, that changes in liver glycogen levels (Russek, 1981), or in liver substrate oxidation rates (Schärrer & Langhans, 1986; Friedman, 1990) would be more suitable or more likely to provide feedback signals to the CNS, because this input could be based on some integrated variable of substrate and carbohydrate utilization and availability. In the following discussion I will attempt to delineate how the need to maintain carbohydrate balance contributes to the regulation of food intake and thereby to the control of body weight.

BODY-WEIGHT REGULATION

In view of the high, and still increasing incidence of obesity (Kuczmarski *et al.* 1994; Seidell, 1995), the factors influencing the regulation of body weight have been and continue to be under intensive investigation. Considering the complexity of the factors involved (genetic (Bouchard & Pérusse, 1993), dietary (Lissner & Heitmann, 1995), lifestyle, socio-economic (Sobal & Stunckard, 1989), etc.), it is helpful to realize that the problem of body-weight regulation boils down to two central issues (Flatt, 1993, 1995*c*): (1) what are the factors responsible for the maintenance of a stable body composition

during long periods of most individuals' lives? (2) what are the factors responsible for the fact that the plateau of weight maintenance may occur at widely-differing degrees of adiposity?

Energy expenditure has been extensively investigated, particularly in man, where this endeavour is facilitated by the fact that human subjects are cooperative, willing to lie still, to eat, or to exercise as requested. The precise measurements which this has made possible have shown that differences in the rates at which energy is expended in the fat-free mass (FFM) are relatively small, so that their effects on overall energy expenditure are offset by minor weight gains or losses. Total energy expenditure is thus primarily determined by body size and composition, and by physical activity (Schoeller & Field, 1991). Changes in resting energy expenditure can only slightly attenuate, but not reverse, the impact on the energy balance of short-term variations in energy intake or of overfeeding (Diaz *et al.* 1992). Corrections of errors committed in maintaining an equilibrated energy balance, therefore, can be brought about only by appropriate increases or decreases in food intake (Flatt, 1987). Maintenance of stable body weight when food is freely available is thus dependent either on the spontaneous adjustment of food intake to energy expenditure, or on consciously-imposed control over the amounts of food consumed.

FOOD INTAKE REGULATION

A multitude of physiological phenomena have been recognized which are capable of influencing food consumption, but their relative importance with regard to weight maintenance and body composition under free-living conditions has been very difficult to determine. This is due in part to the fact that there appears to be considerable redundancy in the control of food intake. Inactivation or stimulation at one control site may thus lead to temporary disruptions, but weight maintenance often tends to become re-established subsequently, although generally at a different plateau.

The roles which various types of regulatory effects play can be evaluated by considering the response time and duration of their influences (Fig. 1). The acute hunger elicited when depletion of the glycogen reserves threatens to cause hypoglycaemia lasts only until food consumption has allowed blood glucose to be restored. It should probably be regarded as an emergency response rather than as a component habitually involved in the regulation of food intake, since hypoglycaemia, or an imminent threat of hypoglycaemia, is usually avoided (Russek, 1981). The ability to regulate food intake within a given day has been investigated by assessing the extent to which consumption of high or low carbohydrate 'preloads' may elicit compensations during subsequent meals. This phenomenon operates best in so-called 'unrestrained eaters', who are generally lean, young adults, whereas in overweight or 'restrained' eaters such compensations are very inaccurate (Rolls *et al.* 1992). The influence of manipulation of the carbohydrate content of preloads is generally greater than that of altering its fat content, indicating that carbohydrate-related feedback is more manifest than fat-metabolism-related feedback (Blundell *et al.* 1994; Rolls *et al.* 1994). The influence of food consumption on subsequent eating is mediated in part by the impact of nutrient ingestion on the secretion of intestinal peptides. For example, enterostatin, a peptide released during the activation of pancreatic lipase (*EC* 3.1.1.3), provides the organism with a signal through which recent fat intake can inhibit further fat ingestion (Erlanson-Albertsson, 1992). This is

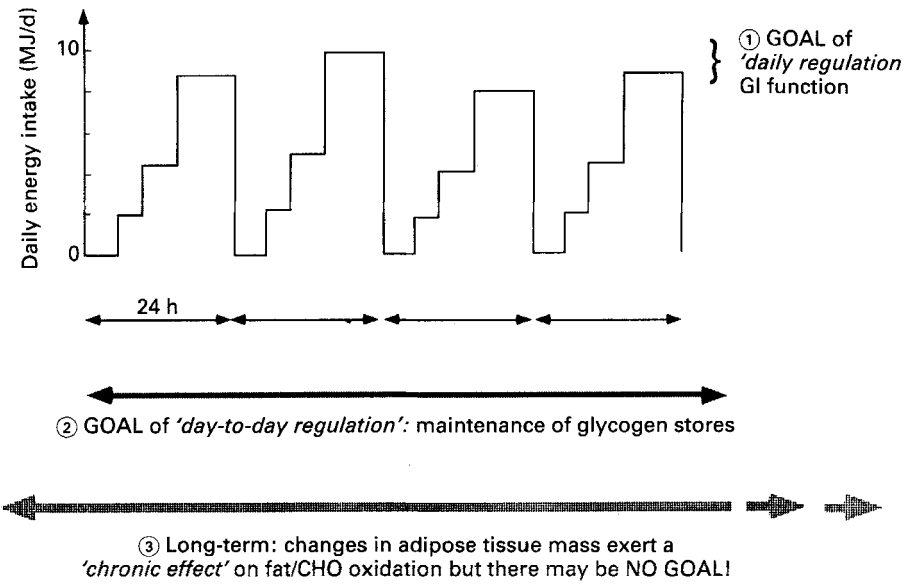

Fig. 1. Three types of food intake regulation. Cumulative daily energy intakes resulting from the consumption of breakfast, lunch and dinner are shown as a function of time of day, over consecutive 24 h periods. Food intake regulation serves to achieve three types of goals: (1) to restrain daily variations in food intake. The evolutionary purpose of this 'regulation of daily food intake' may have been to prevent the threat of hypoglycaemia and to avoid gastrointestinal (GI) overloads (Russek, 1981); (2) over periods of a few days, the primary goal of the interactions between metabolic regulation and food intake regulation appears to be maintenance of adequate glycogen levels, which implies the operation of 'corrective responses' to offset deviations from carbohydrate balance during previous days; (3), over the long-term, changes in the size of the adipose tissue mass ultimately bring about the oxidation of a fuel mix in which glucose and fatty acids are oxidized in the same proportions as supplied by the diet. The achievement of this condition may be incidental, rather than targeted. When it is achieved, however, regulation of food intake in a manner serving to maintain carbohydrate balance also results in maintenance of the energy balance.

particularly important considering that ingestion of fat affects metabolism only minimally (Flatt *et al.* 1985; Griffiths *et al.* 1994). The impact of the regulatory responses elicited by nutritional and metabolic events during a particular day are largely attenuated after the overnight fast. It seems reasonable, therefore, to consider that these mechanisms mainly help to control food intake in a manner placing some limits on the variability in daily nutrient ingestion and in preventing overloads that could lead to nausea or to other intestinal side-effects (Russek, 1981; Fig. 1).

In spite of the numerous phenomena which have been recognized to contribute to the regulation of food intake (Kissileff & Van Itallie, 1982; Nicholl *et al.* 1985; Westerterp-Platenga *et al.* 1993), variations in daily food consumption are large (SD 23%; Bingham *et al.* 1994) and very substantial deviations from energy balance can occur from day to day (Edholm *et al.* 1955). The stability of body composition which is so commonly observed implies that 'corrective responses' are elicited by gains or losses of protein, carbohydrate and fat. These responses include adjustments in the composition of the fuel mix oxidized, which serve primarily to minimize changes in the body's protein and glycogen contents (Abbott *et al.* 1988; Shetty *et al.* 1994; Stubbs *et al.* 1995*b*). Such

adjustments are possible because the large fat stores in adipose tissue readily provide additional fuel when needed, or accept fat for storage when the energy balance is positive. Changes in fuel composition, however, do not by themselves elicit compensation for deviations in the fat or overall energy balances. Such compensations depend on adjustments in food consumption (Flatt, 1987).

In view of the small storage capacity for glycogen, gains or losses of glycogen content are small compared with the amounts of glucose turned over and, when considered over a few days, glucose oxidation is thus essentially equal to carbohydrate ingestion (plus the comparatively small amounts of glucose produced via gluconeogenesis from protein and from triacylglycerol). When food and/or carbohydrate intakes are imposed, glycogen levels are stabilized by adjustment of oxidation to intake (Shetty *et al.* 1994; Stubbs *et al.* 1995*b*). A key question is, whether or not under conditions of unrestricted food availability, carbohydrate balance is achieved only by adjustment of oxidation to intake, or whether in addition food consumption is influenced in a manner contributing to the preservation of stable glycogen levels, and to which extent (Flatt, 1987).

The decreases or increases in food intake which occur from day to day when food is freely available have been found to be negatively correlated with the carbohydrate balances on the previous day in mice (Flatt, 1987, 1993), and recently in human subjects as well (Stubbs *et al.* 1995*b*). Changes in food intake from day to day thus appear to reflect a type of regulation that operates over periods of several consecutive days to help in maintaining the body's glycogen reserves (and possibly its protein reserves as well (Stubbs *et al.* 1995*b*); Fig. 1).

CARBOHYDRATE BALANCE AND 'MODULATION OF FOOD INTAKE'

Except in the presence of some cases of an inherited enzyme defect, the mechanisms serving to maintain carbohydrate balance work effectively in all individuals; be they lean or obese, sedentary or athletic. This does not necessarily, however, assure maintenance of the energy balance. Indeed, large errors in the daily fat and energy balances are accommodated without giving rise to significant metabolic alterations and/or shifts in glucose utilization (Dallosso & James, 1984; Schutz *et al.* 1989). However, over periods of weeks or months, cumulative gains or losses of fat can become sufficient to markedly alter the size of the adipose tissue mass. This affects the amount of free fatty acids (FFA) released into the circulation from adipose tissue, and thereby FFA levels and turnover (Björntorp *et al.* 1969; Campbell *et al.* 1994), as well as the contribution made by fat to the composition of the fuel mix oxidized (Randle *et al.* 1963). Since the size of the fat mass does not change rapidly, the impact of the adipose tissue mass on fat oxidation is of a 'chronic' nature, rather than being a response to recent errors in the fat balance. The impact of fat accumulation is enhanced by the fact that substantial expansion of the adipose tissue mass induces a type of insulin resistance which promotes FFA oxidation and inhibits glucose oxidation (Flatt, 1988; Zurlo *et al.* 1990; Eckel, 1992; Seidell *et al.* 1992). Thanks to these long-term effects, a situation will be reached by most individuals in which the average fat oxidation becomes at least approximately commensurate with habitual fat intake.

When the average composition of the fuel mix is equivalent to the nutrient distribution in the diet, regulation of food intake in a manner serving to maintain the balance of one of the macronutrients will also bring about overall energy balance. For the reasons cited

previously (Mayer, 1955; Russek, 1981; Flatt, 1988), regulation of food intake serving primarily the maintenance of the carbohydrate balance appears to be the most likely phenomenon, and the one presently best supported by experimental evidence (Flatt, 1993; Stubbs *et al.* 1995*b*). The fact that carbohydrate influx affects food consumption is documented by many observations, be it by the marked suppression of food intake induced by infusion of glucose-containing solutions (Giner *et al.* 1992), or by the decrease (or increase) in total energy intake which takes place when dietary carbohydrate content is raised (or lowered) (Lissner *et al.* 1987; Kendall *et al.* 1991). The changes in food intake which occur from day to day in *ad lib.*-fed mice show that deviations from carbohydrate balance, and to a lesser extent deviations from an even fat balance, lead to compensatory increases or decreases in food intake during the following day (Flatt, 1993).

Animal studies

Data illustrating these effects were obtained by determining daily food consumption and respiratory exchanges of individual adult mice. To allow these measurements and the establishment of daily carbohydrate and fat balances, their cages were placed into 55 gall drums, hermetically sealable with Plexiglas lids (Flatt, 1991*a*). The mice were on a 12 h dark–light cycle, with lights turning on at 07.00 hours. The measurements covered about 23 h of each day and were extrapolated to 24 h. They were ended and restarted in the early afternoon (after the drums had been vented), at a time when food consumption could be expected to have been low for several hours, so that changes in the intestinal nutrient content would contribute the least to the observed 24 h carbohydrate and fat balances. By studying ten animals concurrently during many consecutive days, large data pools were generated to allow multiple-regression analysis. The data in Table 1 were obtained in five normal-weight and five obese male CD1 mice. They were provided at different times with diets providing either 41, 27, or 13% of total energy as fat, 19% of total energy as protein (casein), with the balance as starch–sucrose (1:1). Energy expenditure was the same in the two groups, the lower spontaneous running activity of the obese mice being compensated by the effect of their enlarged body size (Fig. 2). The errors committed in maintaining daily energy balance were also very similar, as indicated by the fact that the standard deviations about the means (0·81 (SD 13·0) and 0·55 (SD 12·8) kJ/d) were the same. Changes in food consumption from day to day were also essentially identical.

The results of multiple-regression analysis (Table 1) show that energy intakes are correlated positively with the previous day's intakes, but negatively with the carbohydrate and fat balances which prevailed during the previous day. Previous energy intake is the most significant predictor of intake (accounting for 20–40% of the observed variance), the carbohydrate balance being a more potent modifier than the fat balance (10 *v.* 3% of the observed variances). When one examines the energy balance, one sees that the changes in food intake induced by errors in the carbohydrate balance tend to induce a reversal of the energy balance trend, since energy balances are negatively correlated with the previous day's carbohydrate balances. By contrast, energy balances are positively correlated with the previous day's fat balances. The decrease in food intake elicited by positive fat balances (see above) thus appears to be too weak to reverse a prevailing trend. Furthermore, the relatively powerful correlation between the energy

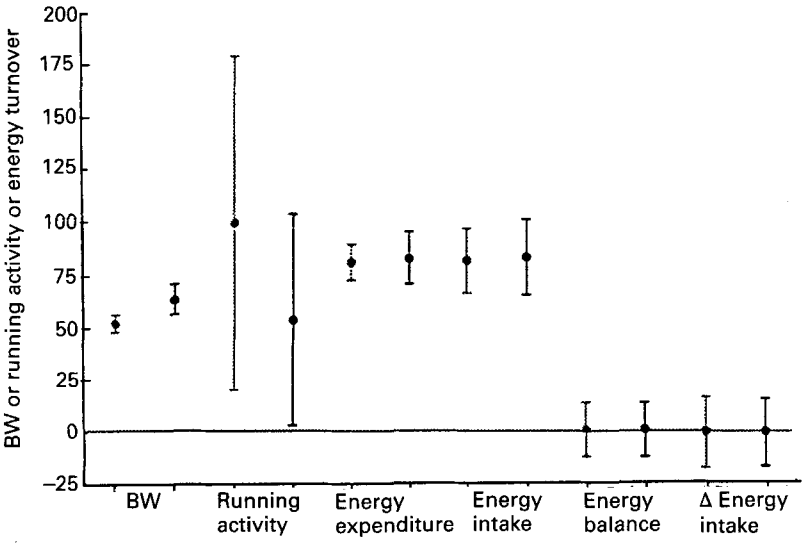

Fig. 2. Average body weights (BW; g), spontaneous running activity (rev./d ×100) and energy turnover (kJ/d) in two groups of five male CD1 mice ((⊢—●—⊣), control; (——●——), obese) selected for having either typical weights or for being the heaviest among a batch of twenty-five mice. Average initial BW (g) were: 46·4 (SD 0·5) in the control group and 58 (SD 3·3) in the obese group. They were maintained on diets containing at different times either 41, 27, or 13% of dietary energy as fat, 19% of dietary energy as protein, and the balance as starch–sucrose (1:1). Energy expenditures and substrate balances were established during 345 consecutive days by combining daily food intake and 24 h gas exchange measurements (as described in Flatt (1991*a*)). Days during which food was withheld, on which the diet's composition was changed, or during which the running wheel was blocked, and the subsequent 3 d were not included. No. of observations during undisturbed *ad lib.* feeding days were 1214–1221, and 1128–1135 in the control and obese mice respectively. Points represent average values and standard deviations are represented by vertical bars.

balance and previous fat balances (10–15% of the variance) indicates the significance of such trends and that responses to alterations in the diet's fat content can be expected to be sluggish, as previously observed (Thomas *et al.* 1992; Astrup *et al.* 1994).

The manner in which the carbohydrate and fat balances modulate food intake becomes more strikingly apparent when one examines changes in food consumption and changes in the energy balance from day to day (Table 1). The contributions to the observed variations, as well as the numerical values of the coefficients, show again that carbohydrate balances exert a substantially greater influence than fat balances (accounting for 30 *v.* 5–10% of the variance respectively). The data in Table 1 and in Fig. 2 are also of interest in demonstrating that the manner in which modulation of food intake contributes to weight maintenance is very similar in the two groups of mice, except for the difference in the body fat contents at which weight maintenance tends to occur. The forces tending to stabilize body weight by up and down modulation of food intake thus appear to be comparable in normal and obese mice.

Since control of the carbohydrate balance deserves high priority under all circumstances, up and down modulation of food intake should be related to deviations from the carbohydrate balance even during periods of changing body weight. This issue was evaluated by examining data obtained in *ad lib.*-fed female CD1 mice during periods of weight gain induced by subcutaneous implantation of corticosterone-releasing pellets (25 mg; Innovative Research of America, Sarasota, FL, USA), as well as during the subsequent periods of spontaneous weight loss (Fig. 3; cf. Flatt, 1993).

Table 1. *Multiple regression analysis of data from food intake regulation studies in male normal-weight and obese CD1 mice*†

(No. of observations during undisturbed *ad lib.* feeding days were 1215 and 1124 in the control and obese mice respectively)

	kJ/d	Previous day's energy intake	Previous day's carbohydrate balance	Previous day's fat balance	R^2
Control group:					
Energy intake =	16·9	+0·80* (SE 0·04)	−1·11* (SE 0·08)	−0·34* (SE 0·05)	0·30
% Variance†		18	10	2	
Obese group:					
Energy intake =	4·3	+0·95* (SE 0·03)	−1·18* (SE 0·07)	−0·38 (SE 0·05)	0·53
% Variance†		40	11	3	
Control group:					
Energy balance =	−1·5	−0·02 (SE 0·04)	−0·34* (SE 0·07)	+0·41 (SE 0·05)	0·13
% Variance‡		NS	3	10	
Obese group:					
Energy balance =	−7·3	−0·03 (SE 0·03)	−0·34* (SE 0·07)	+0·49 (SE 0·04)	0·17
% Variance‡		NS	3	14	
Control group:					
Δ Energy intake =	15·9	−0·19* (SE 0·04)	−1·11* (SE 0·08)	−0·34* (SE 0·05)	0·41
% Variance‡		3	32	6	
Obese group:					
Δ Energy intake =	4·3	−0·05 (SE 0·03)	−1·18* (SE 0·07)	−0·38* (SE 0·05)	0·37
% Variance‡		NS	30	7	
Control group:					
Δ Energy balance =	−1·5	−0·02 (SE 0·04)	−1·34* (SE 0·07)	−0·59* (SE 0·05)	0·46
% Variance‡		NS	33	13	
Obese group:					
Δ Energy balance =	−2·4	−0·03 (SE 0·03)	−1·33* (SE 0·07)	−0·50* (SE 0·05)	0·42
% Variance‡		NS	32	10	

*$P \leq 0{\cdot}0001$.
† For details of procedures, see Fig. 3.
‡ Percentage of variance accounted for in the multiple correlation.

The negative correlation between energy intakes and changes in the energy balance and the previous day's carbohydrate balances are manifest not only during the periods of approximate weight maintenance (during which weight increases slowly with age), but also during the acute weight-gaining and weight-losing periods induced by corticosterone administration (Table 2). By contrast, the correlations between energy intakes and changes in the energy balance from day to day with the previous day's fat balance are weaker, the coefficients describing the effect are more variable, and the phenomenon is not recognizable during some of the weight-gaining or weight-losing episodes. The corrective responses through which short-term errors in substrate balances are compensated for thus appear to be more specifically dedicated to the preservation of adequate glycogen reserves, than to the maintenance of the overall energy balance (cf. Fig. 1).

Human studies

The applicability of these concepts to the regulation of food intake in man was recently examined at the Dunn Nutrition Research Centre, whose team of investigators is the first to undertake the challenging task of trying to link food intake responses in human subjects to cumulative carbohydrate, fat and protein balances, as determined by

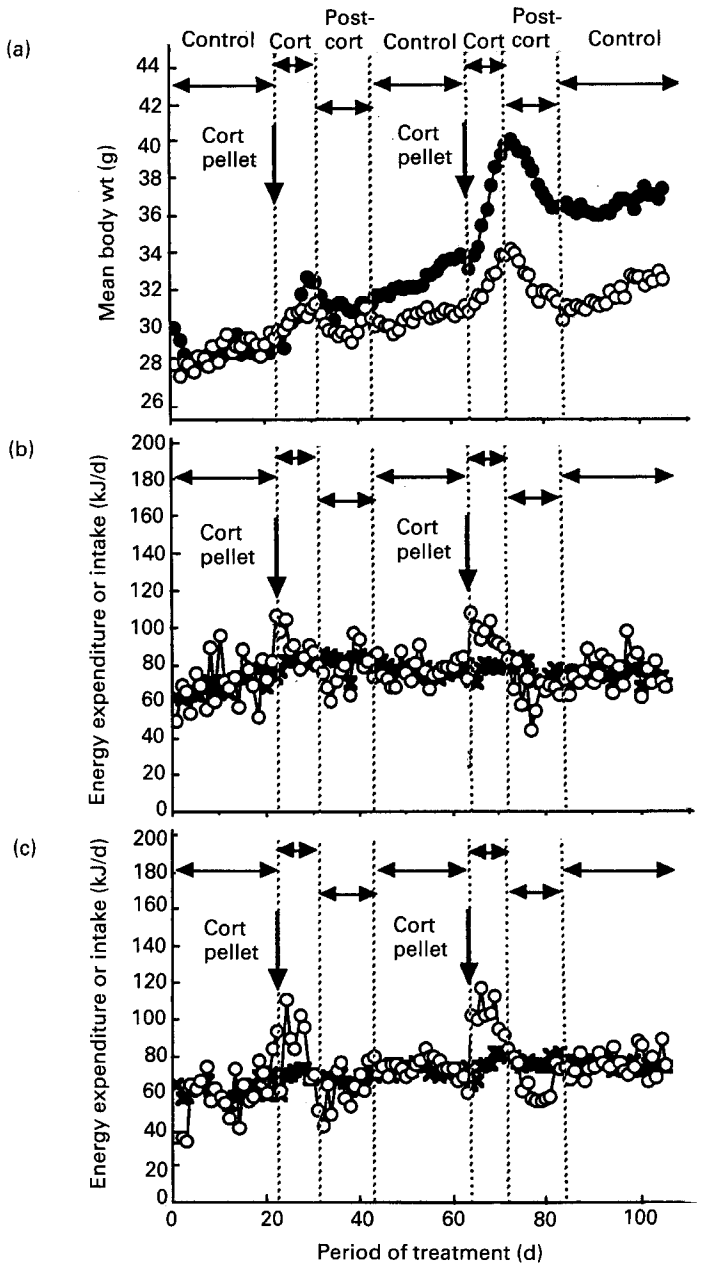

Fig. 3. (a) Average body weights in two groups of five female CD1 mice maintained *ad lib.* on diets containing either 27 (○) or 55 (●) % of dietary energy as fat, 19% of dietary energy as protein, and the balance as starch–sucrose (1:1). (b,c) Average energy intakes (○) and expenditures (**X**) on the diets containing 27 (b) and 55 (c) % of dietary energy as fat. Energy expenditure and substrate balances were established by combining daily 24 h food intake and gas exchange measurements (Flatt, 1991*a*). Pellets containing 25 mg corticosterone (cort) were implanted subcutaneously on days 22 and 64, which led to periods of weight gain, followed by periods of weight loss.

Table 2. *Multiple regression analysis of data from food intake regulation studies in female CD1 mice before, during, and after administration of corticosterone**

(Mean values and standard deviations)

Experimental periods†	Δ Body wt (g/d) Mean	SD		kJ/d	Previous day's energy intake	Previous day's carbohydrate balance	Previous day's fat balance	*n*	R^2
Baseline	0·05	0·84	Energy intake =	32·7	+0·53 (SE 0·08)	−1·32 (SE 0·16)	−0·34 (SE 0·11)	582	0·12
			P		0·0001	0·0001	0·0016		
			% Variance‡		4	7	1		
Corticosterone	0·38	1·00	Energy intake =	39·3	+0·62 (SE 0·17)	−1·11 (SE 0·31)	−0·28 (SE 0·22)	180	0·13
			P		0·0004	0·0004	0·2		
			% Variance‡		6	6	–		
Post-corticosterone	−0·14	0·90	Energy intake =	57·9	+0·12 (SE 0·15)	−0·50 (SE 0·26)	+0·30 (SE 0·17)	260	0·12
			P		0·42	0·053	0·078		
			% Variance‡			(6)	(5)		
Baseline	0·05	0·84	Δ Energy balance =	12·3	−0·17 (SE 0·08)	−1·50 (SE 0·4)	−0·63 (SE 0·10)	580	0·54
			P		0·03	0·0001	0·0001		
			% Variance‡		2	38	14		
Corticosterone	0·38	1·00	Δ Energy balance =	21·7	−0·11 (SE 0·16)	−1·21 (SE 0·29)	−0·61 (SE 0·21)	180	0·44
			P		0·49	0·0001	0·0004		
			% Variance‡		–	29	15		
Post-corticosterone	−0·14	0·90	Δ Energy balance =	30·7	−0·50 (SE 0·14)	−0·83 (SE 0·25)	−0·11 (SE 0·17)	260	0·43
			P		0·0006	0·0011	0·51		
			% Variance‡		21	19	–		

ΔBW, Average body weight changes during the particular period considered.

* For details of procedures, see p. 453.

† Subcutaneous implantation of corticosterone (25 mg)-releasing pellets on days 22 and 64 led to periods of weight gain (9 d; corticosterone), followed by periods of weight loss (13 d; post-corticosterone). The remaining 61 d are the baseline.

‡ Percentage of variance accounted for in the multiple correlations.

continuous respiratory-exchange measurements during consecutive days. In a first set of experiments, subjects were provided with foods in portions designed to maintain energy balance, but containing either the usual, or greatly increased or decreased proportions of carbohydrates and fat during 1 d (Stubbs *et al.* 1993) or 2 d (Shetty *et al.* 1994). These experiments provide a quantitative description of the changes in carbohydrate oxidation which occur in response to such dietary manipulations, revealing that these changes are not sufficient to prevent significant gains or losses of glycogen. Thus, the consumption of a weight-maintaining diet providing 581 g carbohydrate/d for two consecutive days led to a gain of 208 g glycogen, whereas an isoenergetic diet providing only 66 g carbohydrate daily led to a loss of 121 g glycogen in 2 d (Shetty *et al.* 1994). In spite of these large changes in the subjects' glycogen reserves, food intake was not measurably altered when

ad lib. consumption of the usual mixed diet was re-instituted, so that the authors concluded that: 'We believe that if carbohydrate status is not exerting an influence on voluntary food intake under such conditions, it is unlikely to be a very important regulatory factor in normal life.' However, the fact that resumption of *ad lib.* feeding occurred simultaneously with a change in the composition of the diet was a confounding factor in this study (for discussion, see Flatt, 1995*b*; Prentice *et al.* 1995). This problem was avoided in a subsequent set of experiments, during which subjects were studied while consuming diets of constant composition for seven consecutive days (Stubbs *et al.* 1995*b*). Under these conditions negative correlations between changes in the energy balance from day to day and the previous day's carbohydrate and protein balances were observed, accounting for 5–10% of the variance in the subjects' energy balances. Since physical activity was approximately constant during the days spent in the respiratory chamber, this negative correlation also applies to the relationship between food consumption and previous carbohydrate and protein balances. On the other hand, energy balances (and food intakes) were found to be correlated positively with the previous day's fat balances (accounting for 20% of the variance). The fat content of the diet thus markedly influenced energy intake and balance, without eliciting counter-regulatory influences on food intake which could help to preserve weight stability.

The impact of deviations from the carbohydrate balances on food intake observed by Stubbs *et al.* (1995*b*) is comparable with that seen in *ad lib.*-fed mice (Tables 1 and 2). It is rather weak, but this should not come as a surprise considering the large variations in daily food intake which occur in *ad lib.*-fed subjects, the considerable inaccuracies with which daily energy balances are maintained, and the ease with which these are tolerated. The fact that deviations from the fat balance did not alter food intake noticeably in these studies, although such an effect could be recognized in mice (see Tables 1 and 2), is quite understandable, since human fat reserves are much larger in relation to energy turnover and, hence, less likely to elicit feedback responses perceptible over periods of a few days.

At any rate, the experiments of Stubbs *et al.* (1995*b*) now provide support for the concept that changes in food intake from day to day are influenced to some small, but systematic, extent by negative feedback exerted by previous carbohydrate, but not (or much less) by previous fat imbalances. An important issue about the biological significance of these findings is to what extent one may assume that variations in food intake are otherwise essentially random, once a particular lifestyle has become established in a constant environment. If this were the case, one could anticipate that a relatively small, but systematic, influence on food intake may indeed be sufficient to exert a dominant long-term influence in the control of the energy balance. The relative weakness of the long-term steering effects elicited in response to deviations in substrate balances in opposing the influence of the numerous factors capable of altering food intake is well recognized (Thomas *et al.* 1992; Astrup *et al.* 1994). It is important, therefore, to realize that experiments seeking to define the role of the metabolically-based corrective responses in the regulation of food intake may readily fail to achieve statistical significance in documenting such effects.

HABITUAL GLYCOGEN LEVELS AND BODY WEIGHT

Regulation of food intake in a manner that helps to maintain carbohydrate balance provides a means by which body-weight stability may be achieved, but only when the

particular situation has been reached at which the composition of the fuel mix oxidized matches the nutrient distribution in the diet (i.e. when the average RQ is equal to the diet's food quotient (CO_2 produced:O_2 consumed during biological oxidation of a representative sample of the diet); Flatt, 1987). However, in terms of body-weight regulation, the issue of practical importance is less the mechanisms involved in maintaining weight, but rather the factors which determine the body fat content at which this tends to occur.

Glycogen levels are spontaneously maintained at levels sufficient to prevent hypoglycaemia and to carry out habitual physical tasks, and in adults who consume mixed diets, they naturally remain far below the level at which rapid conversion of carbohydrate into fat is induced (Acheson *et al.* 1984; Hellerstein *et al.* 1991). An expansion of the glycogen stores by at least 200–300 g appears to be necessary before the non-protein RQ reaches and exceeds the value of 1·0, which indicates that fat synthesis exceeds concomitant fat oxidation (Acheson *et al.* 1988). Glycogen levels in adults can thus be estimated to lie between a lower limit of 150–200 g and an upper limit of 500–600 g. Glycogen levels are known to be higher on high-carbohydrate diets than on low-carbohydrate diets (Hultman & Nilsson, 1975). They are not likely, therefore, to vary over the entire range when conditions are stable, but to oscillate within a much narrower band. Since neither the lower nor the upper limit are rigidly determined, the range within which glycogen levels are habitually maintained can differ among individuals and be affected by circumstances as well.

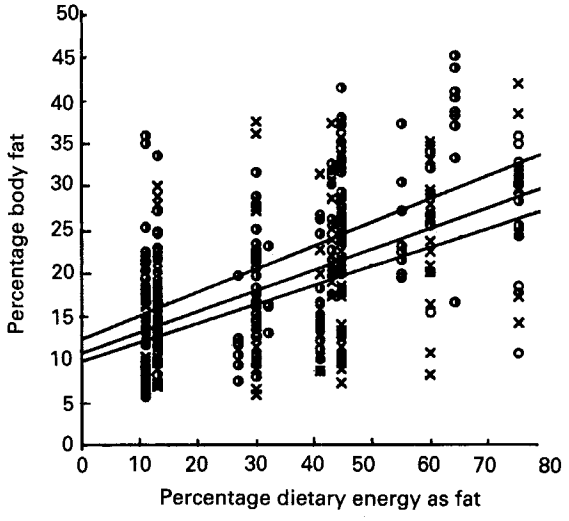

Fig. 4. Dose-dependent relationship between body fat content and dietary fat content in male and female CD1 mice maintained from 150–280 d old *ad lib.* on diets containing different percentages of total energy in the form of fat, 19% of total energy as protein, and the balance as starch–sucrose (1:1). Correlations are shown for mice whose liver glycogen levels at the time of death were greater than 4% (●), between 2 and 4% (×), or less than 2% (○). Body fat content was determined by carcass analysis. The multiple regression equation and the significance of the effects were:

Body fat (%) = 8·7 + 0·23 dietary fat (%) + 0·78 liver glycogen (%); R^2 0·28, $P<0·0001$, n 296.
(SD 0·02) (SD 0·32)
($P<0·0001$) ($P=0·015$)

(Adapted from Flatt, 1996).

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Since the regulation of metabolic fuel utilization is poised to minimize gains or losses of glycogen and proteins, and given the lack of mechanisms serving to adjust fat oxidation to fat intake (Flatt *et al.* 1985; Schutz *et al.* 1989), fat oxidation is determined primarily by the gap between overall energy expenditure and the amount of energy consumed in the form of carbohydrates and proteins (Flatt, 1991*b*). The achievement of a balance between fat oxidation and fat intake depends on the changes in FFA levels and in insulin sensitivity brought about by long-term changes in the adipose tissue mass. Body composition thus drifts toward the degree of adiposity for which the average rate of fat oxidation becomes commensurate with fat intake. Since fat oxidation is inhibited when glycogen levels are high (Shetty *et al.* 1994; Stubbs *et al.* 1995*b*), one would expect that the fat mass would have to reach a greater expansion to overcome this inhibition when relatively high glycogen reserves are maintained.

It is well established that increases in the proportion of fat in the diet tend to raise the degree of adiposity for which weight stability is approached (Lissner & Heitmann, 1995). Experiments in *ad lib.*-fed mice show this to occur in a dose-dependent manner (Flatt, 1987). Their body fat contents were correlated not only with dietary fat contents, but also with the liver glycogen levels that prevailed at the time of death (Fig. 4). As seen by multiple-regression analysis, the correlation with liver glycogen levels was substantially weaker than that with dietary fat. This can be attributed in part to the fact that liver glycogen levels tend to vary from day to day, introducing a random variability that does not affect the body fat data which do not change so rapidly. These findings support the expectation that differences in the range within which glycogen levels are habitually maintained amount to a potentially-important factor in determining 'how fat one has to be, to burn as much fat as one eats' (Flatt, 1995*c*).

DIETARY FAT CONTENT, GLYCOGEN LEVELS AND OBESITY

Many factors affecting weight maintenance can be illustrated with the help of a two-compartment model comprising a small and a large reservoir, representing the human body's limited capacity for storing glycogen and its large capacity for fat storage respectively (Fig. 5). The small turbine represents the exclusive use of glucose by the brain. The relative proportions of glucose and of fatty acids used by the rest of the body (i.e. the large turbine) are assumed to be influenced by the availability of glucose and FFA, which may be thought to be proportional to the levels to which the two reservoirs are filled at a given time. This reproduces qualitatively the features of the Randle *et al.* (1963) glucose–fatty acid cycle. Replenishment of the body's glycogen and fat stores occurs from time to time by consumption of meals (illustrated by the portions in the small containers shown above the reservoirs). The proportion delivered into the large reservoir corresponds with the diet's fat content. Food consumption is determined by the habitual pattern of meal consumption, complemented when necessary by physiological regulatory mechanisms which assure that glycogen reserves are sufficient to avoid hypoglycaemia, while also preventing their build-up to levels at which *de novo* lipogenesis would be induced (i.e. through the conduit from the small to the large reservoir). Additions of fuel to the large reservoir cause only insignificant changes in its level, whereas marked changes in the content of the small reservoir occur. This causes the contribution made by the small reservoir to increase after meals, reproducing the postprandial rise in the RQ. As the glycogen reserves decline after the meal, the use of glucose by the large turbine

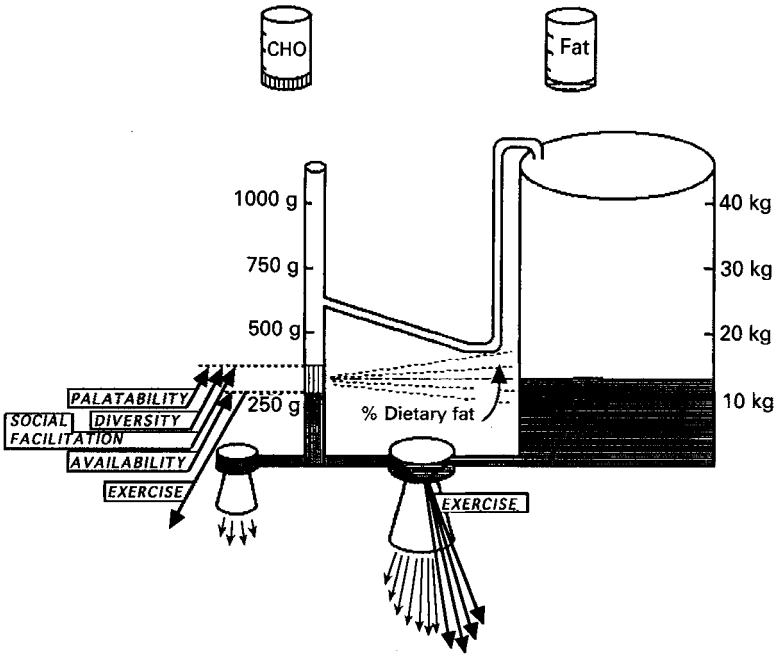

Fig. 5. Two-compartment model illustrating the impact of circumstantial, lifestyle and genetic factors on adiposity (for explanations, see p. 460; adapted from Flatt, 1995*c*). CHO, carbohydrate.

decreases, adjusting itself to glycogen availability. The interplay between the factors controlling food intake and these shifts in fuel composition causes glycogen levels to oscillate up and down within a particular operating range. When outflow from the large reservoir (fat oxidation) is not commensurate with inflow (fat intake), its content (adipose tissue mass) will change slowly over time, until it is filled to the level that causes the contribution made by the large reservoir to the flow through the large turbine to be equal on average to the amount of fuel added to it. A 'steady-state' is then reached, which corresponds to the situation for which fat oxidation is equal (or nearly equal) to fat intake over time.

To induce an outflow commensurate with the inflow when the proportion of fat in the diet is raised requires an increase in the content of the large reservoir, or an appropriate decline in the level maintained in the small reservoir. Such shifts may need to be quite substantial if the diet's fat content rises to the point where fat contributes a major proportion of total intake. In many cases, the steady-state is in effect re-established by a combination of such changes. The leverages exerted by various other circumstantial factors on weight maintenance are also shown. The availability of appetizing foods and their diversity (Rolls *et al.* 1981), as well as good company (de Castro & Brewer, 1992) tend to increase the amounts of food consumed, thereby raising the upper limit of the range over which habitual glycogen levels vary. Also illustrated is that the constant availability of foods and snacks may promote food consumption between meals, attenuating glycogen depletion and raising the lower limit of the range over which habitual glycogen levels vary. The combined effects of these 'environmental factors' in raising habitual glycogen levels require the content of the fat reservoir to increase to

higher levels before fat contributes enough to the fuel mix oxidized to equal fat intake. Regular physical activity, on the other hand, is a factor limiting excessive body fat accumulation. This can be attributed to two effects: (1) to an increase in substrate oxidation in skeletal muscle (i.e. flux through the large turbine), which uses both glucose and FFA, thereby reducing the impact of the tissues that preferentially use glucose on the composition of the fuel mix used; (2) to an increase in the amounts of substrate oxidized between meals, which has the effect of lengthening the meal-to-meal interval (if energy expended, rather than hours elapsed are considered), causing greater glycogen depletion and facilitating fat oxidation.

The impact of inheritance on body fat distribution and on the susceptibility to develop obesity can be extremely powerful (Sorensen *et al.* 1992; Bouchard *et al.* 1993; Zhang *et al.* 1994). Such genetic characteristics have in common the fact that they affect the manner in which the organism regulates the use of glucose and of FFA, since they alter the degree of expansion of the adipose-tissue mass necessary to induce a rate of fat oxidation commensurate with fat intake (although this situation may never be reached in extreme cases of obesity). Such effects can be mimicked in the model by expanding the diameter of the large reservoir. This can lead to considerable differences in the amount of body fat needed to achieve (or approach) balance between fat inflow and outflow, although without necessarily preventing the usual weight-maintaining phenomena from operating, or the leverage of various circumstantial variables on weight maintenance.

CONCLUSIONS

Weight maintenance is achieved by adjustment of food intake to match energy expenditure. Modulation of food intake in a way that helps to maintain stable glycogen reserves has now been recognized in animals and human subjects. With regard to this mechanism of food intake regulation, it matters little whether these adjustments result in exact energy balances or in slow rates of gain or loss of fat over time.

While carbohydrate balance is effectively maintained in all individuals, the range within which glycogen levels are maintained may differ. The diversity and high quality of the foods now offered in affluent societies, as well as their ubiquitous availability between meals, tend to raise the range within which glycogen levels are habitually maintained. A greater expansion of the fat mass may then be required for fat oxidation to become commensurate with fat intake. Because glycogen levels are not presently measurable in human subjects, the role played by differences in habitual glycogen levels in the development of obesity has not generally been taken into consideration, even though shifts in this range can be assumed to have considerable influence on fat oxidation. It seems likely that the increases in the incidence of obesity in affluent societies during the last decade (Kuczmarski *et al.* 1994; Seidell, 1995), during which the fat content in Western diets was relatively stable (Lissner & Heitmann, 1995), may be due in large part to the influence of circumstantial variables which have raised the range over which glycogen levels are habitually maintained (Flatt, 1995*c*).

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