

Factors controlling the disposition of primary nutrients

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In the steady-state an organism maintains a dynamic balance between the dietary intake and metabolism of each of the primary nutrients, carbohydrate (CHO), fat and protein. However, such a steady-state, while generally true in adults in the long term, is never achieved during short periods (i.e. within 1 d) because of the discontinuous nature of food intake. The fact that we consume our food as meals, with postabsorptive periods in between, means that short-term storage in glycogen, adipose stores and tissue protein is necessary. Thus, the capacity to control the partition of nutrients after meals between immediate metabolism and storage, and then the mobilization of the stores during fasting, is essential for maintaining balance over the longer term.

Fuel utilization

Indirect calorimetry is a useful technique for assessing the utilization and storage of primary nutrients. Although in past years there has been some resistance to its use in the fed state rather than in fasting (Brody, 1945), a number of workers have now shown that the technique is valid and useful in fed subjects (Clugston, 1981; Frayn, 1983; Acheson *et al.* 1984). However, the traditional formulas for calculating fat and CHO utilization (Lusk, 1921) use factors derived by assuming that the composition of oxidized nutrients is the same as body stores. When dietary nutrients are metabolized these factors are not appropriate and we have shown how more-accurate values may be obtained for use in fed subjects when the chemical composition (in terms of carbon, hydrogen, oxygen, nitrogen and sulphur) of the dietary nutrients are known (Garlick, 1987). The error incurred by not correcting for dietary composition is increased with some clinically-used synthetic diets containing either large amounts of unusual fats (e.g. medium-chain triglycerides) or amino acid mixtures.

Calculation of nutrient utilization rates from respiratory exchange also requires an estimate of the rate of protein oxidation, which is usually determined from N excretion. Because acute changes in N excretion are obscured by the body urea pool, we have used the rate of leucine oxidation measured by primed continuous infusion of [$1\text{-}^{13}\text{C}$]leucine as a rapidly responsive index of protein oxidation. Leucine oxidation is assessed from the rate of production of $^{13}\text{CO}_2$ in the breath and the isotopic enrichment of the keto acid derivative of leucine in the plasma (Garlick *et al.* 1987*b*). The quantification of $^{13}\text{CO}_2$ from leucine requires correction for incomplete recovery of $^{13}\text{CO}_2$ (by infusion of labelled bicarbonate) and for changes in labelling arising from differences in natural enrichment of ^{13}C between body stores and dietary sources.

Fig. 1 shows CHO, fat and protein utilization in normal human volunteers when fasting and feeding (Garlick *et al.* 1987*b*). The total [^{13}C]leucine infusion time of 8 h was divided into 4 h fasting (after an overnight fast) followed by 4 h of regular small meals, and respiratory gases were analysed throughout using a ventilated hood system. In the fasting state the main fuel was fat: CHO and protein together contributed only 35% of total energy. On commencement of feeding there was a rapid rise in respiratory quotient (RQ), which stabilized to a new value between 2 and 4 h of feeding corresponding to a shift to CHO as the dominant fuel. It is also possible to calculate the overall balance

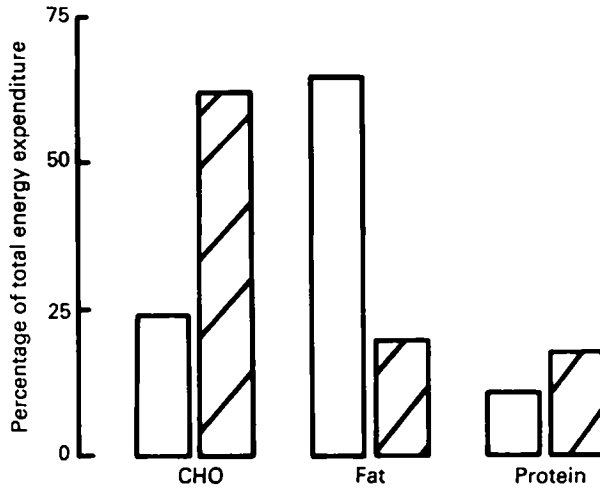


Fig. 1. Percentage of total energy expenditure from carbohydrate (CHO), fat and protein in post-absorptive subjects during 4 h of fasting (□) followed by 4 h of feeding (▨). In the feeding period meals were given hourly at the rate of three times the estimated basal metabolic rate (BMR). (From Garlick *et al.* 1987b.)

between utilization and intake, reflected in changes in body stores. In fasting all nutrients must have come from stores, but during feeding storage occurred for each nutrient by an amount equal to the excess of intake over utilization. Thus, fat and CHO were stored in approximately equal amounts and together contributed 91% of the total energy stored.

A similar pattern has been observed by Flatt *et al.* (1985), who used classical indirect calorimetry, with urinary N output, to study the effect of single large meals. Our method, involving regular small meals to achieve a constant rate of absorption, was developed to simplify interpretation by avoiding the non-steady-state during the prolonged absorptive period after single meals.

Repeat measurements of fasting RQ in the normal subjects shown in Fig. 1 showed significant variations between individuals, suggesting that inter-individual differences in the capacity to mobilize stored nutrients might occur. When the relative contributions of fat, CHO and protein to total energy expenditure were compared in obese and lean subjects, however, no differences were observed (Fig. 2(a)), in spite of higher fasting energy expenditures in the obese (see also Bessard *et al.* 1983). The response to feeding in obese and lean volunteers is shown in Fig. 2(b). When the two groups were given the same intake relative to their ideal body-weights, the obese utilized less CHO and protein and more fat than the lean. However, the obese had higher total energy expenditures in relation to their ideal body-weights, and so were relatively underfed. Experiments with the lean subjects were therefore repeated with the same intakes as the obese relative to their measured fasting energy expenditures. Even when the lean subjects were given this lower intake the pattern of nutrient utilization was still different from that of the obese, with more protein and less fat providing the energy. The significance of these differences is not yet clear, but the lower rate of protein oxidation, and hence higher protein retention, by the obese may reflect their higher lean body mass. Flatt (1978) has suggested that a lower efficiency of storage would occur if CHO were converted to fat before storage. This could, in theory, account for a greater metabolic efficiency in the obese if they synthesized more fat, but this did not appear to occur in the present experiment as there was no net lipogenesis during feeding in either lean or obese subjects.

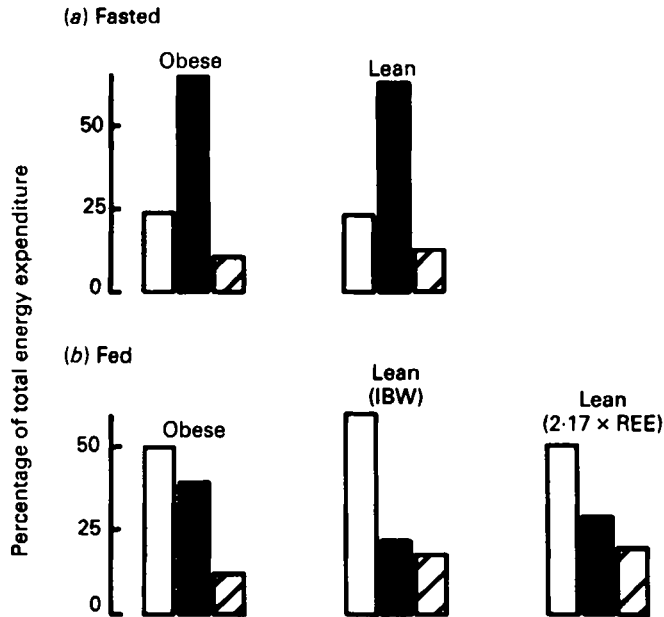


Fig. 2. Percentage of total energy expenditure from carbohydrate (□), fat (■) and protein (▨) in obese and lean subjects, (a) in the fasted state and (b) during feeding. Obese subjects were given hourly meals at a rate of three times their estimated basal metabolic rate (BMR) calculated from their ideal body-weights (IBW). On average this provided 2.17 times their resting energy expenditures ($2.17 \times \text{REE}$) measured in the fasting period. Lean subjects were given hourly meals at a rate of either three \times BMR (lean (IBW)) or $2.17 \times \text{REE}$ (lean ($2.17 \times \text{REE}$)). (From Bruce *et al.* 1988.)

There is an extensive literature on the control of metabolism of individual nutrients (e.g. blood glucose, adipose tissue lipolysis) by hormone and metabolite concentrations, but relatively little is known of how the disposition of nutrients from mixed meals is regulated. As insulin is well recognized to be of primary importance in the control of CHO, fat and protein metabolism, we have chosen to investigate its role in the response of nutrient utilization to food intake by suppressing its secretion with somatostatin. Insulin suppression was studied in normal volunteers with 8 h infusions of [$1\text{-}^{13}\text{C}$] leucine, including 4 h of fasting followed by 4 h of feeding as in Fig. 1. Somatostatin (Stilamin®) was infused throughout at a rate of $250 \mu\text{g/h}$ and on a separate day controls were run on each subject without somatostatin. During the fasting period somatostatin caused a fall in plasma insulin concentration to low levels in all subjects. There was also a fall in plasma glucose, probably as a result of inhibition of glucagon secretion as well as insulin (Cherrington *et al.* 1976). However, the degree of insulin suppression varied. In four subjects insulin was barely detectable ($<20\%$ of starting value), while in two subjects insulin remained detectable at about 50% of the starting value. The two subgroups were termed 'fully suppressed' and 'partially suppressed'. The fasting pattern of nutrient utilization was no different from control values in the partially suppressed group, but was altered towards more use of fat and less of CHO in the fully suppressed subjects (Fig. 3(a)). On commencement of feeding, all subjects had pronounced hypoinsulinaemia and hyperglycaemia. Full suppression (insulin $<10\%$ of fed control value) occurred in two subjects and partial suppression (insulin $17\text{--}33\%$ of fed control values) in four subjects. In the partially suppressed subjects there was no detectable

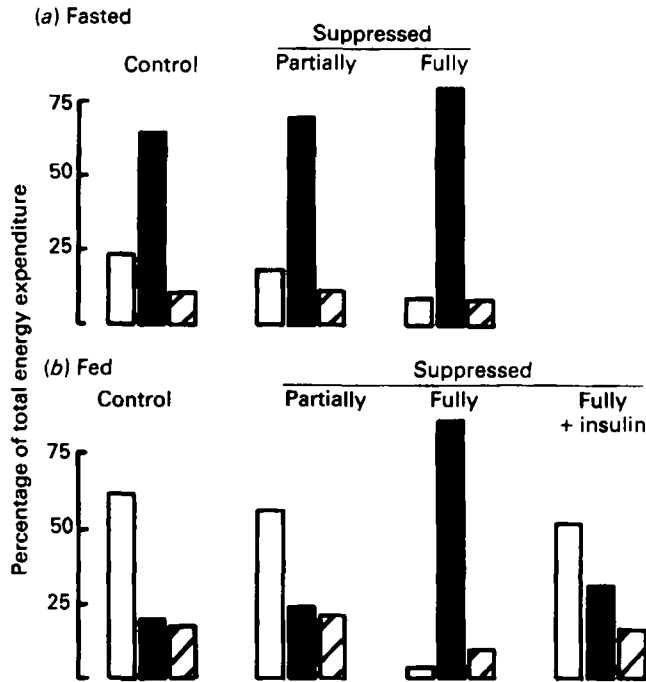


Fig. 3. Percentage of total energy expenditure from carbohydrate (□), fat (■) and protein (▨) with and without infusion of somatostatin, (a) in the fasted state and (b) whilst feeding, as in Fig. 1. 'Fully suppressed', plasma insulin barely detectable during somatostatin infusion; 'partially suppressed', plasma insulin lowered by somatostatin infusion, but still detectable; 'fully suppressed + insulin', subjects from fully suppressed group repeated with somatostatin plus insulin infusion to give plasma insulin concentrations similar to those in the 'partially suppressed' group.

difference in nutrient utilization from the pattern seen on the control day, in spite of the lower concentrations of insulin. However, in the fully suppressed subjects the normal switch of metabolism on feeding failed to occur. They continued to use mainly fat and little CHO, as might be expected from the sensitivity of glucose uptake and lipolysis (De Fronzo *et al.* 1985) to insulin. The ability of small amounts of insulin to induce the switch to the fed pattern of nutrient utilization was further demonstrated by infusing sufficient insulin into the fully suppressed subjects to raise the plasma insulin to 25% of the concentration achieved on the control day. As shown in Fig. 3(b), this resulted in a normal switch in metabolism on commencement of feeding.

These findings show that insulin is an essential hormone without which the normal response of nutrient utilization to food intake does not occur. It also shows that the effect of the hormone during absorption of a mixed meal is not simply related to its plasma level and that the level required to bring about the feeding response is much lower than the normal plasma concentration. However, sensitivity to insulin might have been influenced by the absence of the counter-regulatory hormones, glucagon and growth hormone, which are also suppressed by somatostatin. Further studies will be needed to delineate the interactions of the various regulatory influences more clearly.

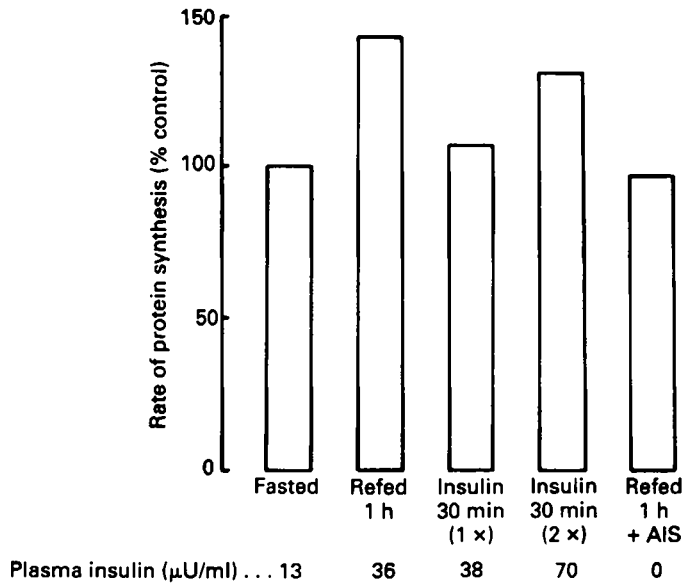


Fig. 4. Rates of protein synthesis in skeletal muscle of young male rats that were fasted for 12 h before the experiment and then either refed or given intravenous infusions of insulin. AIS, anti-insulin serum injected intravenously just before refeeding. Values from Garlick *et al.* (1983) and Preedy & Garlick (1986).

Protein metabolism

The effect of food intake on whole-body protein turnover rates has been measured in man by continuous intravenous infusion of [1-¹⁴C] leucine. Volunteers were infused for 24 h periods, including 12 h with regular small meals (daytime) followed by 12 h of fasting (night-time). Rates of whole-body protein synthesis and oxidation and leucine oxidation were calculated from the plasma leucine specific radioactivity and the rate of production of respiratory ¹⁴CO₂ when these had reached plateau values during the last 6 h of each 12 h period. Both protein synthesis and leucine oxidation were higher while subjects were feeding, and degradation of protein was lower (Clugston & Garlick, 1982a). Similar responses to the intake of a protein-containing diet have been reported in other studies using similar techniques (Clugston & Garlick, 1982b; Rennie *et al.* 1982; Clague *et al.* 1983; Hoffer *et al.* 1985), and they result in storage of protein in the tissues during the fed phase and mobilization of the stores during fasting.

Because skeletal muscle has been shown to be particularly sensitive to food intake both in young rats and in adult man (McNurlan *et al.* 1982; Rennie *et al.* 1982), this tissue may be a major contributor to the feeding response observed in the whole body. There are many published studies of factors that control protein turnover in muscles that have been incubated or perfused in synthetic media *in vitro*, but relatively few studies of the factors involved in its control *in vivo*. We have measured rates of protein synthesis in skeletal muscle of young male rats *in vivo* by injection of a flooding dose of [³H] phenylalanine in an effort to elucidate the role of circulating hormone and metabolite concentrations in the response of protein turnover to feeding.

Insulin stimulates synthesis and inhibits degradation of protein in muscle *in vitro* (Fulks *et al.* 1975; Jefferson *et al.* 1977) and is able to elevate the rate of synthesis in perfused rat hind-limb preparations from fasted rats to values similar to those in preparations from fed rats (Preedy & Garlick, 1983). Furthermore, in diabetic rats *in vivo* there is a drop in muscle protein synthesis (Pain *et al.* 1983). To investigate the role

of insulin in non-diabetic animals in vivo, rats were fasted for 12 h before being refeed or infused with insulin. Fig. 4 shows that 1 h after refeeding, the rate of muscle synthesis was stimulated and the plasma insulin concentration had risen to 36 $\mu\text{U/ml}$. Intravenous infusion of insulin sufficient to give a similar insulin concentration (38 $\mu\text{U/ml}$) did not, however, have any effect on protein synthesis. Only when the insulin was infused at a higher rate was an increase in synthesis observed. Insulin was essential, since refeeding after injection of anti-insulin serum did not result in any stimulation of protein synthesis.

From these results it appears that although insulin is essential, it is not the only mediator of the response to feeding in muscle. Amino acids have also been shown to stimulate protein synthesis in vitro, particularly the branched-chain amino acids (Buse & Reid, 1975; Fulks *et al.* 1975). Measurements on rats in vivo have shown that infusion of a mixture of amino acids does not stimulate muscle protein synthesis except when glucose is also infused (Preedy & Garlick, 1986). The additional glucose gave rise to elevated insulin in plasma, although at a concentration that was too low to stimulate protein synthesis on its own. Our conclusion was that the amino acids increase the sensitivity of protein synthesis to insulin, and experiments with insulin infusion have confirmed this (P. J. Garlick and I. Grant, unpublished results). The values in Fig. 5 show the effect of infusing various mixtures of amino acids in combination with glucose. The findings suggest that the essential, but not the non-essential, amino acids are as effective as the complete mixture. Indeed, the effect of branched-chain amino acids was almost as great as that of the complete mixture.

A variety of hormones in addition to insulin has been shown to influence rates of protein turnover in muscle, but their role in the response to food intake is less clear. Glucagon is a hormone that has a number of actions that oppose those of insulin and its concentration rises during fasting. Although traditionally its actions have been thought to involve responses in the liver but not skeletal muscle, it has been possible to show that administration of this hormone inhibits muscle protein synthesis both in the perfused rat

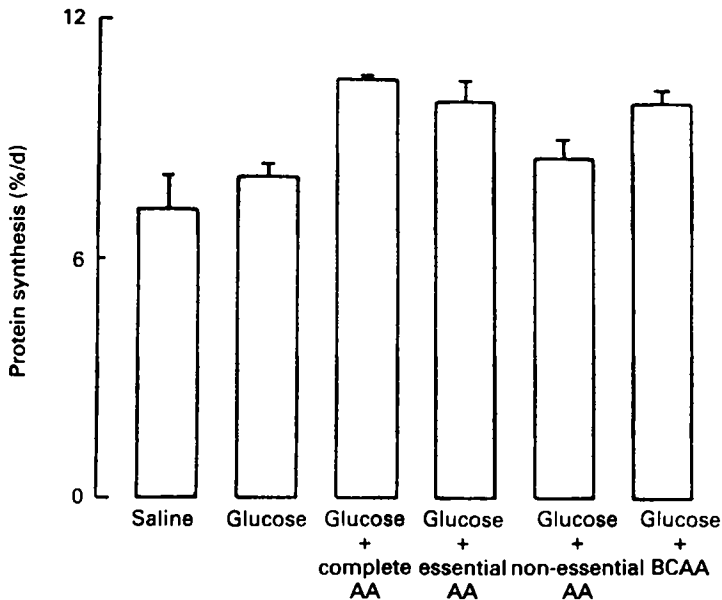


Fig. 5. Rates of protein synthesis in skeletal muscle of young male rats that were fasted for 12 h before infusion for 1 h with saline (9 g sodium chloride/l) or glucose solution plus complete, essential or non-essential amino acids (AA) or branched-chain AA (BCAA). Values from P. J. Garlick and I. Grant (unpublished results).

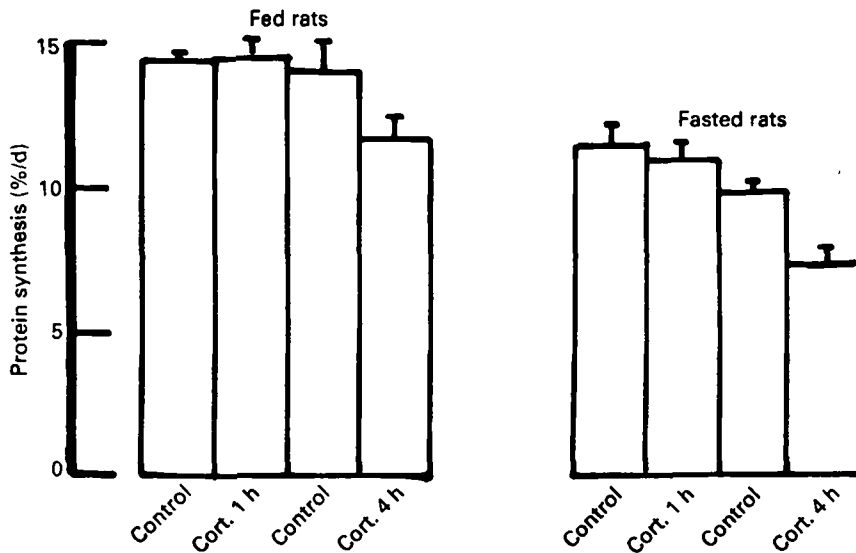


Fig. 6. Rates of muscle protein synthesis in fed and 12 h fasted rats at 1 h or 4 h after injection of corticosterone (2.5 mg; cort.). Values from Garlick *et al.* (1987a).

hind-limb *in vitro* (Preedy *et al.* 1980) and *in vivo* (Preedy & Garlick, 1985). However, the length of time and the unphysiological concentrations required to elicit a response (Preedy & Garlick, 1985) would suggest that glucagon is not involved in the acute response to food. Glucocorticoid hormones are also known to be protein catabolic and to inhibit protein synthesis in muscle *in vitro* (McGrath & Goldspink, 1982; Reeds & Palmer, 1984) and *in vivo* (Odedra & Millward, 1982). It has been suggested that the rise in corticosterone concentration after feeding of rats is in part responsible for the rise in muscle protein synthesis (Millward *et al.* 1983). However, this does not appear to be possible. The effects of corticosteroids that have been demonstrated in muscle *in vitro* have not occurred until 3–4 h of incubation (McGrath & Goldspink, 1982; Reeds & Palmer, 1984). Fig. 6 shows the effect of corticosterone injection on muscle protein synthesis in fed and fasted rats *in vivo*. In agreement with the *in vitro* experiments there was no effect of the hormone after 1 h, but an inhibition was apparent in both fed and fasted animals after 4 h. It therefore seems unlikely that corticosteroids have a role in the immediate effects of food intake on protein synthesis in muscle, and they are probably involved in the longer-term regulation, for example after stress or injury.

Conclusions

Feeding results in a switch in the pattern of utilization of energy substrates so that CHO replaces fat as the dominant energy source. This change is dependent on the presence of insulin, but the amount required to restore the normal pattern of nutrient utilization appears to be much lower than the plasma concentration normally found in fed subjects, perhaps because of interactions with other hormones suppressed by somatostatin infusion. On feeding there is also a switch from utilization to replenishment of stores of each of the primary nutrients. Storage of protein is brought about by an increase in synthesis and a decrease in degradation and studies in animals have suggested that insulin may be important also for this response. The observation that the action of insulin on muscle protein synthesis can be amplified by the provision of amino acids is

another illustration of the need to consider not only the individual hormones, but also their interactions with other factors in controlling the disposition of nutrients from mixed meals.

The authors are grateful to Serono Laboratories (UK) Ltd for the gift of Stilamin, and to the Medical Research Council and the Scottish Hospitals Endowments Research Trust for financial support.

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