Theoretical considerations for partitioning nutrients between muscle and adipose tissue

G. C. WAGHORN

Applied Biochemistry Division DSIR, Palmerston North

J. E. WOLFF

Ruakura Animal Research Station
Ministry of Agriculture and Fisheries, Hamilton

ABSTRACT

To apportion the intake of metabolisable energy (MEI) used for synthesis of lean and fat tissue, current knowledge of substrate oxidation and substrate utilisation for biosynthetic reactions has been combined with estimates of fat and protein turnover to develop models of energy metabolism and nutrient fluxes in a lean 20 kg male and a fat 40 kg female sheep, each gaining 200 g/d. When the most efficient pathways and lowest turnover rates are employed the biosynthetic reactions account for 38 and 34% of MEI in the lean and fat animals. These values rise to 45 and 43% of MEI respectively when higher estimates of turnover and less efficient pathways are used in the calculations. Hormonal regulation of metabolite utilisation is believed responsible for such differences but hitherto, only the glucose-insulin system has been quantitated. Two techniques used for its quantitation are described to provide examples of the methods needed to elucidate the control of nutrient utilisation and ultimately body composition in ruminants.

INTRODUCTION

Increasing the growth rate of lean tissue at the expense of fat requires changes in partitioning of absorbed nutrients. It is now widely anticipated that a manipulation of metabolism at a molecular or cellular level can effect desired changes in body composition but these have yet to be realised.

Our approach in this paper is to illustrate the value of current knowledge of metabolism for examining and quantifying the importance of metabolic control at various sites. In the first part we have examined the rates of fat and protein turnover in sheep, then calculated the relative efficiencies with which ATP is generated from different absorbed nutrients. This information is combined with the energetic costs of fat and protein turnover to develop models of energy expenditure in a 20 kg and a 40 kg lamb each gaining 200 g/d. These examples illustrate the importance of metabolic control.

In the latter part of the paper, the methods of investigating metabolic control are considered with particular reference to the glucose-insulin system. This particular system has been chosen because there is information relating its control to productive functions in ruminants (see Wolff, 1982) and because the techniques now being applied to its analysis are far ahead of those used for any other hormone system. We believe a coordination of these 2 approaches provide the surest way of identifying important animal factors responsible for control of body composition.

FAT AND PROTEIN TURNOVER

The rate of triglyceride (TG) synthesis may be estimated as the sum of TG gain, and hydrolysis. Some of the released fatty acids (FA) are oxidised while some are re-esterified, but there do not appear to be any quantitative estimates of these separate fluxes in the adipose tissue of sheep or cattle. Measurements on the rates of lipogenesis in tissue slices are too low to be realistic and cannot be extrapolated to the whole animal. Rates of adipose tissue TG turnover can, however, be inferred from measured turnover rates of free fatty acids (FFA) and glycerol, the products of TG hydrolysis. The *in vitro* release of glycerol from sheep subcutaneous adipose tissue is 12 nmoles/min/g (Vernon, 1981), with a 3 to 4-fold increase from catecholamine stimulation. This is equivalent to a release of 14 to 56 mg/d of long chain FA per g of adipose tissue and is in reasonable agreement with measured FFA entry rates of 1.4 mg/min per kg of body weight in fed sheep (Leat and Ford, 1966). This latter estimate converts to FFA entry rates of 40 and 80 g/d in fed sheep of 20 and 40 kg respectively and it is these data which are used for the estimates of TG turnover in Fig. 1.

TG are synthesised from long chain fatty acids (LCFA) of dietary origin and via *de novo* synthesis, primarily from acetate with over 90% occurring in adipose tissue of ruminants (Ingle *et al.*, 1972). In ruminants absorption of LCFA is confined primarily to FA of C_{18} and shorter chain length (Grace and

Body, 1981) so that FA enter intestinal lymph as chylomicron or very low density lipoprotein TG. Estimates of absorption in sheep fed 800 g of organic matter per day are approximately 15, 20 and 45 g FA/d for white clover, pelleted grass and fresh ryegrass respectively (Scott *et al.*, 1969; Ulyatt and MacRae, 1974; Grace and Body, 1981).

In contrast to measurements of fat synthesis and turnover, rates of protein synthesis are usually measured *in vivo*. Different techniques have produced

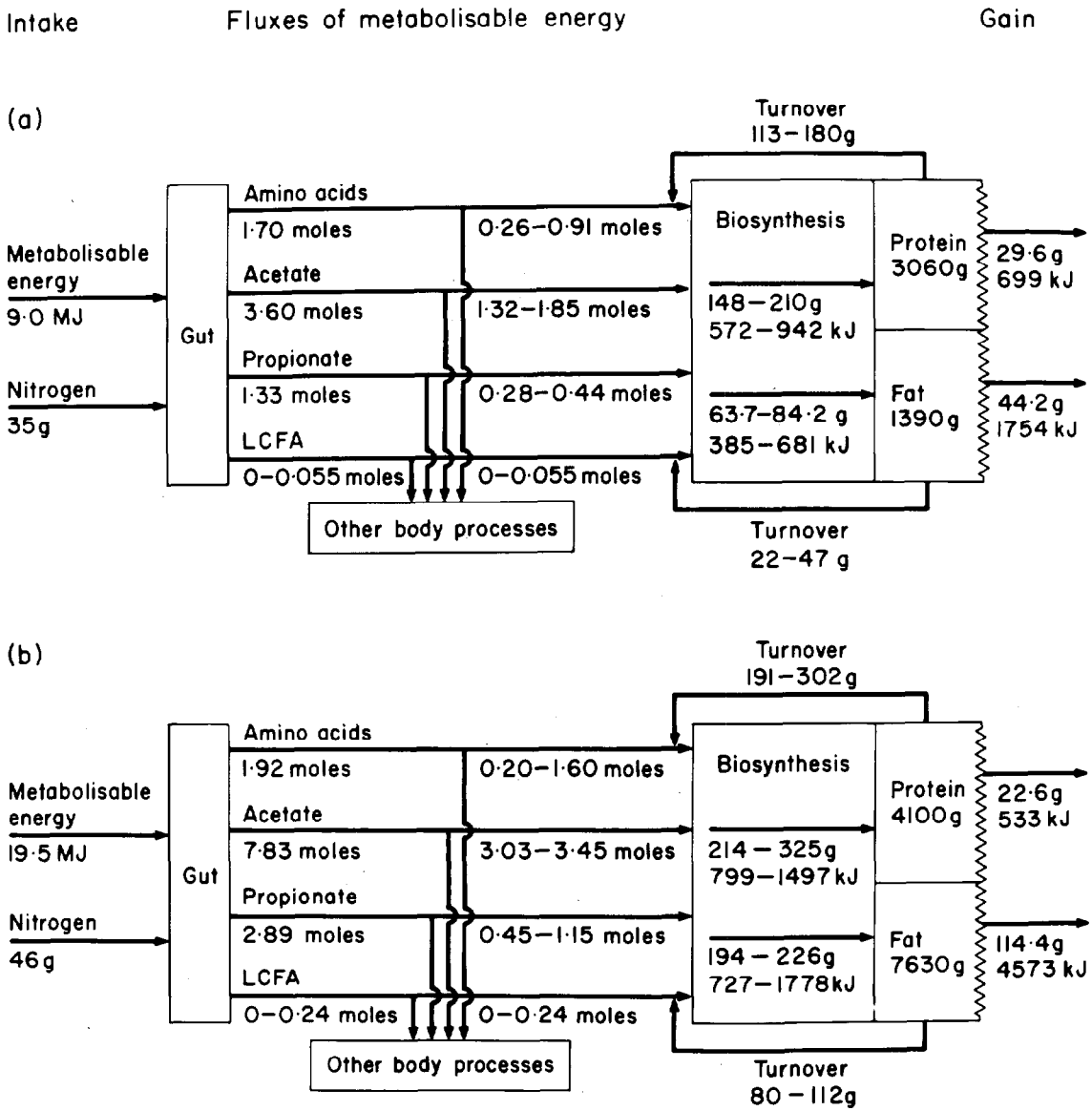


FIG. 1 Model energy and nutrient expenditures in a 20 kg male (a) and 40 kg female (b) sheep each gaining 200 g/d and fed white clover and ryegrass/clover diets respectively. All fluxes are expressed on a per day basis. Ranges in nutrient utilisation and energy expenditure indicate high and low energetic efficiencies of nutrient utilisation and turnover rates. Origin of data: sheep body composition from Ørskov *et al.* (1971); intakes from MacRae and Ulyatt (1974), Ulyatt *et al.* (1980), ARC (1980); volatile fatty acid absorption from Waghorn (1982); composition of gain from ARC (1980); protein turnover rates from Reeds and Lobley (1980); fat turnover from Vernon (1981, p. 313), Leat and Ford (1966); efficiencies of protein and fat synthesis calculated according to text.

a wide range of values and there is still ambiguity in the interpretation of data (e.g., Lobley *et al.*, 1980; Davis *et al.*, 1981). Reeds and Lobley (1980) consider protein synthesis in growing animals and in adults in energy balance to be 12 to 19 g/d per $BW^{0.75}$ and to account for about 15% of energy expenditure and it is these values which have been used for the models of energy expenditure (Fig. 1).

It is important to realise that rates of fat and protein synthesis are influenced by both age and diet so that estimates of energy costs are unlikely to remain static for any length of time. The decline in protein fractional synthetic rate (FSR) with age is illustrated by the decline in FSR of skeletal muscle protein from between 23 and 30% per day in newborn lambs (Soltesz *et al.*, 1973; Ferrara *et al.*, 1977) to between 2.5 and 4.0 at weaning (about 10 weeks) (Ferrara *et al.*, 1977; Davis *et al.*, 1981; Bryant and Smith, 1982; Bickerstaffe and Chambers, 1983). Protein FSR is further reduced in older sheep when fed a reduced plane of nutrition (Bryant and Smith, 1982). Rates of acetate incorporation into LCFA, and esterification of LCFA also declined in older ruminants but there do not appear to be any reductions in lipoprotein lipase activity or rates of lipolysis with age (particularly when stimulated by catecholamines) (Vernon, 1981). Restricted feeding reduced fat synthesis in steers and sheep (Vernon, 1981; G. C. Waghorn, unpublished), as did low protein diets fed to sheep (G. C. Waghorn, unpublished).

EFFICIENCIES OF ATP GENERATION

The principle energy currency of living cells is the pyrophosphate bond of ATP.

$$ATP \rightarrow ADP + Pi \quad \Delta G^\circ = -30.5 \text{ kJ/mole}$$

The change in free energy represents the energy available for doing work. Substrates (nutrients) are oxidised by the living cell in an orderly, coupled system so that free energy is conserved as ATP (~P). ATP hydrolysis is then coupled to energy-requiring processes e.g., synthesis, muscular contractions, nervous excitation or active transport. Heat is released at all stages of this energy interchange. The efficiencies with which nutrients are oxidised to yield ATP, and the heat production during turnover or synthesis of protein and lipid by ruminants can be calculated on a biochemical basis using heats of combustion (ΔH) rather than the Gibbs free energy (ΔG°), with minimal loss of accuracy.

For example: The ΔH of glucose is 2813 kJ/mole.

Oxidation of glucose by a tissue proceeds via glycolysis and the TCA cycle to produce CO_2, H_20 and 38 moles of ATP after oxidation of $NADH_2$ and $FADH_2$.

$\therefore$ ΔH trapped by each mole of ATP = 2813/38 = 74 kJ/mole.

Similar calculations for other substrates are summarised in Table 1 and show that the efficiency with which energy is conserved as ATP varied from 106 to 74 kJ/mole. For proteins and amino acids (AA) the energy costs of urea synthesis are taken into account with the result that oxidation of 100 kJ of AA is 43% less efficient in terms of energy conservation than the oxidation of 100 kJ of glucose.

EFFICIENCIES OF ENERGY STORAGE

To illustrate how a knowledge of biochemical pathways combined with heats of combusion is used to calculate the efficiencies of nutrient storage (Baldwin, 1968; Baldwin and Smith, 1974) we examine the synthesis of 1 mole of distearyl-monopalmityl triglyceride that is typical of ruminant adipose tissue. Acetate is oxidised as a source of energy (~P) and is also used as the source of carbon for FA synthesis. Propionate is required to make glucose which is then used for $NADPH_2$ production and the synthesis of glycerol by adipose tissue.

Palmitate synthesis:

8 acetate + 23 ~P + 14 $NADPH_2$ → Palmityl-CoA + 14 NADP

TABLE 1 Heat of combustion, net ~P yield and efficiency of energy conservation as ~P from oxidation of several substrates (adapted from Milligan, 1971).

Substrate	ΔH (kJ/mole)	Net yield of ATP/mole	ΔH/~P (kJ/ mole)	Efficiency of conservation† (%)
Glucose	2 813	38	74	41
Stearate	11 336	146	77	39
Propionate	1 534	18	85	36
Acetate	873	10	87	35
Casein‡ (115 g)	2 461	23.2	106	29
Butyrate	2 190	27	81	38

† Based on free energy of hydrolysis of ATP to ADP (30.5 kJ/mole).

‡ ΔH for 115 g casein (equivalent to 1 mole AA) is corrected for ΔH of urea resulting from catabolism. Urea synthesis also costs 4 ~P/mole urea.

Stearate synthesis:

18 acetate + 50 ~ P + 16 $NADPH_2$ → 2 Stearyl-CoA + 32 NADP

Formation of $NADPH_2$ from propionate for FA synthesis:

7.68 propionate + 15.36 ~ P → 3.84 glucose (liver)

3.84 glucose + 3.84 ~ P → 3.84 glucose-6-P (adipose tissue)

3.84 G-6-P + 46 NADP → 46 $NADPH_2$ + CO_2 (adipose tissue)

Triglyceride formation:

Propionate + 2 ~ P → 0.5 glucose

0.5 glucose + 0.5 ~ P + $NADH_2$ → α-glycerol-P + NAD

α-glycerol-P + palmityl-CoA + 2 stearyl-CoA → TG

Δ H of the distearyl-palmityl TG = 34,384 kJ/mole

Calculation of efficiency:

26 acetate @ 873 kJ/mole + 97.7 ~ P @ 87 kJ/mole + 8.68 propionate @ 1534 kJ/mole = 44,509 kJ

$\therefore$ Efficiency = 34384/44509 × 100 = 77.2%

This calculation assumes the supply of high energy phosphate to arise from acetate oxidation costing 87 kJ/mole (Table 1).

Similar calculations enable the energy costs of storage and turnover of other metabolites to be determined. For example, lipolysis and re-esterification of TG would take 2 ~ P for the acylation of each FA and 5 ~ P to convert glycerol to glucose (liver) for regeneration of α-glycerol-P (adipose tissue), totalling 11 ~ P (about 960 kJ) for the turnover of 1 mole (863 g) of TG. Protein synthesis from preformed amino acids costs at least 5 ~ P per peptide bond, which can be obtained from the oxidation of 0.5 mole of acetate with the release of 437 kJ as heat for every 115 g turned over.

VARIATIONS IN EFFICIENCIES OF METABOLISM

The efficiencies of energy conservation for several metabolite interconversions for storage or turnover are summarised in Table 2. These have been based on a Δ H/ ~ P of 87 kJ/mole (Table1) from the oxidation of acetate—one of the main end products of rumen fermentation and a major precursor of CO_2 production in the body (Pethick *et al.*, 1981). Use of different substrates for energy will result in varying costs for the synthesis and turnover of body constituents. For example, oxidising stearate to produce energy will produce 385 kJ of heat during the synthesis of 115 g of protein while oxidising AA will produce 540 kJ of heat to make the same product.

It must be emphasised that these calculations have assumed no cost incurred for protein degradation or transport into or out of cells. Active transport across membranes is likely to cost at least 1 ~ P/mole, and whilst this may be a relatively small cost in relation to absorption, any recycling of metabolites will increase the energy cost of the transformation.

TABLE 2 Energetic efficiencies of substrate/product interactions.

Storage or turnover reaction	Efficiency of energy conservation† (%)
Glucose storage as glycogen	94
Propionate storage as glycogen	80
Glucose → lactate → glucose	88
Blood AA → protein (max. efficiency)	84
Blood AA → fat	65
Plasma FA → triglyceride	97
Acetate → triglyceride	77

† Calculations are based on ~ P generation to 'cost' 87 KJ/mole from the oxidation of acetate.

Usually the supply of nutrients to portal blood is not constant, especially under intermittent feeding regimes. Any surplus of absorbed nutrient can either be stored as fat or oxidised. Acetate may be stored as fat or oxidised with maximum efficiencies of 77% and 35% respectively. Amino acids cannot be stored as such; AA surplus to the immediate needs for protein synthesis may be converted to fat or oxidised with net efficiencies of energy conservation of 65% and 28% respectively.

The importance of feeding frequency has been indicated in feeding trials with cattle (Gibson, 1981) where increased frequency resulted in an 18% increase in efficiency of feed utilisation. Differences in utilisation of a single diet have also been demonstrated in sheep and cattle treated with growth promotants (e.g., Galbraith, 1980; Griffiths, 1982). The calculations shown above indicate that these changes in the efficiency of feed utilisation could have arisen through the animal making better metabolic decisions on the fate of absorbed nutrients. It is interesting to observe that the changes in efficiency have been accompanied by increases in net gains of lean tissue.

MODELS OF METABOLITE UTILISATION BY GROWING SHEEP

Calculations of energy expenditure associated with gain in a 20 kg lean and a 40 kg fat sheep are shown in Fig. 1 and require a knowledge of body composition, rates and costs of triglyceride and protein synthesis, the efficiency with which ATP is generated, and which absorbed nutrients are sequestered into tissue. The variability of estimates of fat and protein synthesis, especially in relation to turnover enable a realistic yet wide range of turnover rates to be postulated in the models (Fig. 1). These, coupled with alternative substrates for oxidation, suggest 200 g gain would cost between 3.4 and 4.1 MJ of ME in the young male, and between 6.6 and 8.7 MJ of ME in the older female sheep (Fig. 1).

The difference in energy cost of gain between sheep is due primarily to the composition of the gain (Fig. 1), but in both models the synthetic costs associated with gain and turnover range from between 8 and 10% to 18% of MEI. In both sheep costs of TG turnover are approximately only 10% of protein turnover, so that even quite large changes in TG turnover (say +30%) would have less effect on the efficiency of nutrient utilisation than a much smaller change in protein turnover. On the other hand, both synthetic costs and the energy stored as TG greatly exceed that for protein.

The calculations involving alternative substrates and energy sources (Fig. 1) have been determined with regard to nutrient availability. Only absorbed, available nutrients were used in model formulations, so that solutions presented in Fig. 1 are feasible from both physiological and nutritional viewpoints.

INVESTIGATION OF A REGULATORY MECHANISM

The concepts of control theory needed to understand the behaviour of a regulated system are now well described in several texts (Milhorn, 1966; Riggs, 1970; Auslander *et al.*, 1974) but until recently they have found only limited application to metabolic or endocrine systems. This is now changing rapidly with the development of an artificial pancreas for an insulin dependant diabetic patient and a clearer perception of the role of modelling for investigating the sensitivity of metabolism to hormonal release. In order to illustrate the significance of control theory in relation to metabolic regulation and nutrient partitioning, 2 recent techniques for quantifying the action of insulin are discussed in relation to glucose metabolism in ruminants. These are the 'glucose clamp' and the minimal models of the glucose-insulin system.

RUMINANT GLUCOSE METABOLISM

In ruminants fed forage diets, nearly all glucose originates from the liver with a small contribution from the kidneys. Absorption from the gastrointestinal tract is negligible unless a high grain diet is fed. Many tissues of the body utilise glucose for both oxidative and synthetic needs as shown in Fig. 2. Many of the fluxes shown in Fig. 2 have been well determined either by measurements of arteriovenous differences and blood flows and/or by the intravenous infusion of isotopically labelled glucose (see Leng, 1970; Bergman, 1973; Lindsay, 1979; Weekes, 1979; Wolff and Meschia, 1982). Some fluxes, however, have not been so well estimated and this is particularly true for the separate contributions of fat, muscle, skin and other connective tissues.

Also shown in Fig. 2 are 4 hormones which have some role in maintaining glucose homeostasis. Of these, the 2 pancreatic hormones, insulin and glucagon are the most important. Although modulated by many

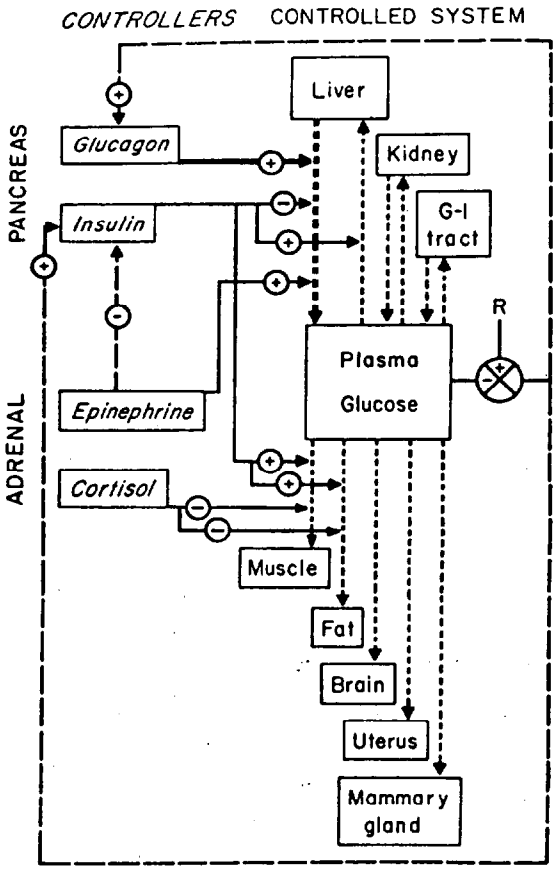


FIG. 2 Glucose metabolism and its control in the ruminant. Fluxes between plasma and the various tissues producing and utilising glucose are shown on the right. Hormones known to be responsible for controlling flux rates are shown on the left with a + sign indicating stimulation of action and a − indicating inhibition. Feedback arcs are shown between the sensed changes in plasma glucose concentration and the release of glucagon or insulin. The inhibitory effect of epinephrine on insulin release is also shown.

factors, their release is principally determined by the sensed changes in plasma glucose concentration — a rise causing the release of insulin and a fall the release of glucagon. As the principal actions of insulin are an inhibition of hepatic glucose production and an acceleration of glucose uptake by insulin sensitive tissues, while that of glucagon is to promote hepatic glucose production, both hormones constitute negative feedback mechanisms for glucose homeostasis. Two other hormones, cortisol and epinephrine, are less important for glucose homeostasis but they do affect the fluxes of glucose as shown in Fig. 2.

At the present time, it is only possible to show the signs of hormonal effects in Fig. 2. The more important information is the partial differential which express

the sensitivity of each flux rate to the change in each hormone concentration when all other factors are held constant. Recently techniques have been developed for measuring the *in vivo* sensitivity of glucose metabolism to insulin. These techniques have been extensively applied to dogs and humans but hardly used for ruminants.

THE GLUCOSE CLAMP

The 'glucose clamp' was originally developed by R. Andres and his co-workers at the National Institutes of Health in Bethesda, Maryland (Sherwin *et al.*, 1974; DeFronzo *et al.*, 1979). In the euglycaemic clamp, an insulin infusion is commenced and followed by a variable intravenous infusion of glucose so that the plasma concentration of glucose does not change. It is conducted by drawing samples of blood every 5 or 10 minutes, measuring the plasma glucose concentration within 3 or 4 mins and making an appropriate adjustment to the infusion rate of glucose. Experience has shown that after several hours the glucose infusion rate approaches a plateau and is a measure of the effectiveness of insulin in promoting glucose utilisation by all body tissues. In a different version of the protocol, called the hyperglycaemic clamp, the plasma concentration is elevated to 220 mg/100 ml and held at that level by the clamping procedure already described. This elevation causes the pancreas to release insulin. From the rise in circulating insulin concentrations, the pancreatic sensitivity of insulin release to glucose can be estimated.

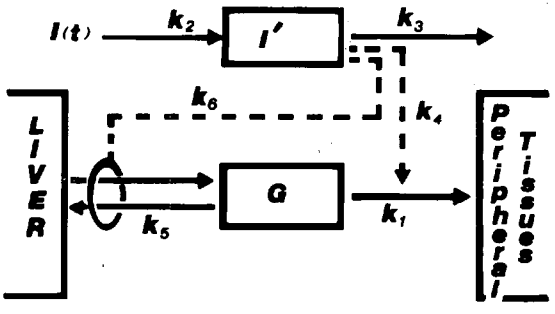
A major advantage of the glucose clamp technique is that it breaks the feedback loop. This makes it possible to estimate the effects of insulin on glucose metabolism separately from the effects of changes in glucose concentration on glucose metabolism. Some recent work by Ferrannini *et al.* (1982) showed that a change in plasma glucose concentrations from 85 to 110 mg/100 ml enhanced the effect of insulin in suppressing hepatic glucose production. Similar interactions between different elements of the system also feature in the integrated model of human glucose metabolism recently published by Cobelli *et al.* (1982).

MINIMAL MODELS OF THE INSULIN-GLUCOSE SYSTEM

The other technique which has provided an improved interpretation of the effects of insulin on glucose metabolism and of the effects of glucose on insulin release is work undertaken by Richard Bergman and his colleagues in constructing and testing minimal models of the insulin-glucose system in the dog (Bergman *et al.*, 1979; Toffolo *et al.*, 1980). For this approach the glucose insulin system is divided into 2 parts. One part is the pancreas which is stimulated to release insulin by the raised concentrations of plasma glucose during an intravenous glucose tolerance test (IVGTT). The response is a 2-phase increase in plasma insulin concentrations. Two phases of insulin release have been recognised. The sensitivity of the first phase release has been determined from the relative magnitude of the first insulin peak to the increment in plasma glucose concentration seen immediately after injection.

The second phase is dependent on the sensitivity of insulin release to glucose and includes a threshold or set point for insulin release. Toffolo *et al.* (1980) developed and validated equations for these 2 phases of insulin release in dogs. All parameters of the model were identifiable from the data obtained during an IVGTT.

The second part of the system shown in Fig. 3 comprises distribution kinetics of the released insulin, glucose metabolism by liver and peripheral tissues, and the action of insulin on glucose metabolism. The equations and the meanings of the model parameters $k_1 - k_5$ are also shown in Fig. 3. Again, Bergman *et al.* (1979) have shown that the parameters can be estimated from the data of an IVGTT and that the parameters are amenable to physiological interpretation. Most important is the measure of insulin sensitivity



$$\frac{dG}{dt} = (p_1 - X)G + p_4$$

$$\frac{dX}{dt} = p_2 X + p_3 I(t)$$

$$X = (k_4 + k_6) I' \qquad p_3 = k_2 (k_4 + k_6)$$

$$p_1 = -(k_1 + k_5) \qquad p_4 = B_0$$

$$p_2 = -k_3 \qquad p_5 = G(0)$$

FIG. 3 Minimal model for calculating the effect of insulin on glucose disappearance. I(t) is the time course of plasma insulin concentration, I^1 is insulin an in 'extravascular' compartment and G is the plasma glucose concentration. Glucose uptake by peripheral tissues and net hepatic glucose balance are both considered to be non-linear functions of glucose and the concentration of insulin in an extravascular compartment; $k_1 - k_5$ are rate constants for the processes shown and B_0 is the (extrapolated) hepatic glucose production at zero plasma glucose concentration. (Redrawn from Bergman *et al.* (1979) with the authors' permission).

(S_I) which is obtained as a ratio of 2 model parameters ($-P_3/P_2$) and has the units of min^{-1} per (μU/ml) or fractional clearance rate of glucose per unit of insulin concentration. It can also be defined as the effect of insulin to augment the effectiveness of glucose in enhancing the rate of its own disappearance.

In humans, these models of glucose control by insulin have been used to show that glucose intolerance had a different aetiology between lean and obese subjects (Bergman *et al.*, 1981). In the lean group, pancreatic insufficiency was primarily responsible for the glucose intolerance with the second phase sensitivity being 77% lower than in controls. In the obese group with glucose intolerance, however, the second phase sensitivity was unchanged but S_I (the sensitivity of glucose metabolism to insulin) was reduced by 60%, when compared with obese subjects having a normal tolerance. This work has shown that the parameters of the model are amenable to physiological interpretation. If phenotypic differences like these can be observed between genetically lean and fat farm animals and a casual role for the hormone on body composition established, then improved selection criteria for the control of body composition should become possible.

CONCLUSIONS

The information presented in this review should be considered as a tentative first step toward a more detailed understanding of nutrient partitioning and metabolic control in the growing lamb. Our examples of modelling, drawn mainly from the labours of R. L. Baldwin and R. N. Bergman illustrate the many benefits that accrue when modelling is used as an adjunct to experimentation.

A major benefit from the above analysis was the identification of serious deficiencies in our knowledge on rates of lipogenesis in the whole animal. This information should be obtainable by measuring the incorporation of $2-{}^{14}C$ acetate or ^3H_2O into the lipids of various tissues and undertaking a compartmental analysis of tracer movement. Modelling also ensures that data from different techniques and from different parts of the system are consistent with one another.

A second benefit is that it illustrates how variations in the efficiency of productive processes can so easily arise from changes in the supply of absorbed nutrients or from changes in the substrates oxidised for energy. These predictions are derived solely from current knowledge of metabolic pathways and some careful bookkeeping of the ATP ledger. As Baldwin suggested in 1968, this material is an invaluable teaching aid for bridging the chasm that too easily develops between the disciplines of biochemistry and nutrition.

A third benefit is that it could improve our understanding of the way hormones operate to influence metabolism in different tissues. As the equations for biological control systems are nearly always non-linear and therefore difficult to solve analytically, this is clearly a most difficult aspect. But given the apparently tight regulation of body composition that is no doubt achieved via a multitude of different feedback arcs, a rigorous modelling effort appears to be one of the few techniques capable of measuring the separate effects of many different hormones. The development of challenge tests like that described above for the insulin-glucose system is imperative for identifying metabolically superior animals while a knowledge of metabolic sensitivity to different hormones is essential for predicting the consequences of hormonal therapy on different genotypes under various feeding regimes.

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