An assessment of the rate of fat accumulation in ruminant adipose tissue *in vitro*

K.J. LEWIS AND P.C. MOLAN

Department of Biological Sciences
University of Waikato, Hamilton

J.J. BASS

Ruakura Agricultural Research Centre
Ministry of Agriculture and Fisheries, Hamilton

ABSTRACT

A method for the evaluation of the rate of fatty acid accumulation and recycling in adipose tissue *in vitro* is described. The influence of insulin on fatty acid accumulation in adipose tissue is shown to be greater than its influence on fatty acid manufacture. This was achieved by the ability of the hormone to reduce the rate at which fatty acid was mobilised from the adipose tissue. It is proposed that studies of the total fatty acid balance in this tissue will provide a better understanding of the effects of hormones on overfatness in meat animals than a study which simply addresses the rate of fatty acid manufacture.

Keywords Fatty acid metabolism; insulin; overfatness; adipose tissue.

INTRODUCTION

The process of fat metabolism in ruminants is influenced by many factors. These include the quantity and quality of feed, exercise levels, the physiological state and sex of the animal together with other genetically determined susceptibilities associated with both the breed and the individual within a breed. These factors all influence the quantity of raw materials made available to the fat cell, its ability to manufacture and retain fat and its ability to subsequently break down and release fat. The body exerts control over these simultaneous processes using hormones. The hormones act on the cell either by controlling the amounts of raw materials available to it or by altering the quantities or activities of enzymes required for the processes. The hormones can act either directly on the fat cell itself or indirectly by altering the level of another more effective hormone.

The complexity of the situation *in vivo* has prompted this study of adipose tissue in a controlled environment and to measure the effect of a hormone on the ability of the tissue to accumulate fat.

MATERIALS AND METHOD

Wether lambs less than 1 year old which had been pasture-fed *ad lib.* up to and immediately prior to slaughter were used for the experiment. Explants of subcutaneous adipose tissue from the lower dorsal region near the base of the tail were incubated at 39°C for 4 h in Difco Medium 199 containing 1% w/v bovine serum albumin, 25 mmol/*l* Hepes buffer and 1.1 mmol/*l* sodium acetate at pH 7.4.

The rate of fatty acid manufacture in the cell was measured using the incorporation of ^{14}C-acetate into long chain fatty acids (FA), assuming all fatty acids manufactured had 16 carbon atoms. The rate of lipolysis within the tissue was estimated by measuring the rate of release of glycerol into the incubation medium, using the method described by Galletti (1967). It was assumed that for every molecule of glycerol in the medium, 3 molecules of fatty acid were released within the tissue. The rate at which free fatty acid (FFA) was mobilised and passed out of the tissue and into the incubation medium was also measured, using the method described by Crane and Lane (1977).

From these 3 measurements, the amount of fatty acid that accumulated within the tissue during the 4 h incubation was calculated using the formula:

moles FA accumulated = moles FA manufactured — moles FFA mobilised

Also an estimate of the amount of fatty acid retained and presumably recycled back to triacylglycerol by the adipose tissue was obtained using the formulae:

moles FA retained = 3 (moles glycerol released) − moles FFA mobilized

$$\% \text{ recycling of FA} = \frac{100 \text{ (moles FA retained)}}{3 \text{ moles glycerol released}}$$

Insulin at a concentration of 2.6 ng/ml was added to the medium of some incubations and its effects of the rates of fatty acid accumulation and recycling in the adipose tissue observed. This was compared with the rates in tissue in the absence of hormone (referred to as the basal rates).

Results are expressed as mean and standard error of the mean for triplicate observations of tissue from one animal. Statistical analyses were carried out on log-transformed data using the Student's *t*-test.

RESULTS

Two experiments were performed, each on tissue from a different animal. Changes in the measured rates of fatty acid manufacture, lipolysis and fatty acid mobilisation from adipose tissue are given in Table 1, together with the calculated values of fatty acid retained by the tissue during the 4 h incubation. With tissue from Sheep A, insulin increased the rate of fatty acid manufacture by 92% ($P<0.01$), and the rate of fatty acid accumulation in the tissue by 132% ($P<0.001$). Ninety one percent of the fatty acid released in the tissue by the process of lipolysis was recycled within the tissue when no hormone was present. This increased to 97% in the presence of insulin. Insulin had little or no effect on the rate of lipolysis. Although there was an increase of 10% in the amount of fatty acid which was retained by the tissue in the presence of insulin in this sheep, the difference was not significant. However, insulin was seen to decrease the rate of fatty acid mobilisation from the tissue by 61% ($P<0.001$).

TABLE 1 The effect of insulin (2.6 ng/ml) on the balance of fatty acid (FA) in ovine adipose tissue *in vitro*.

Measurement	Basal rate (μmoles/4 h/10^6 cells)	Change with insulin (%)	Signif.
Sheep A			
FA manufactured	0.62±0.06	+ 92	**
FA released	1.50±0.04	+ 3	NS
FA mobilised	0.13±0.01	− 61	***
FA retained	1.37±0.04	+ 10	NS
FA accumulated	0.49±0.06	+ 132	***
FA recycled (%)	91	97	
Sheep B			
FA manufactured	1.82±0.14	+ 34	*
FA released	2.63±0.08	− 12	*
FA mobilised	0.29±0.04	− 52	*
FA retained	2.34±0.05	− 7	NS
FA accumulated	1.53±0.15	+ 50	*
FA recycled (%)	89	94	

With tissue from Sheep B, insulin stimulated the rate at which fatty acid was manufactured by 34% ($P<0.05$) and increased the rate of fatty acid accumulation in the tissue by 50% ($P<0.05$). In this case insulin slightly decreased ($P<0.05$) the rate of lipolysis but the decrease in the rate of fatty acid retention by the tissue was not significant. On the other hand, insulin significantly decreased the rate of fatty acid mobilisation from the tissue by 52% ($P<0.05$). Insulin increased the rate of fatty acid recycling from 89% to 94%.

DISCUSSION

In both these experiments, by far the major part of the fatty acid released within the adipose tissue by the process of lipolysis was retained within the cells and was presumably recycled back to triacylglycerol. It is apparent that the rate of fatty acid accumulation in the adipose tissue is dependent not only on the rate at which the tissue manufactured fat, but also on the amounts of fatty acid mobilised or retained. Insulin appears to be able to influence all 3: it can decrease fatty acid mobilisation from the tissue while at the same time it can increase the rate of fatty acid manufacture and recycling. Because the percentage of fatty acid recycling in the tissue was already high, insulin did not have to change the percentage by much to accommodate any increase in the amount of fatty acid retained by the tissue. It also appears that small, non-significant changes in the rates can influence the overall rate of fat accumulation in the tissue. In the the second experiment (Sheep B), insulin also decreased the rate of lipolysis. It is apparent in both experiments, however, that by increasing the amount of fatty acid retained in the tissue, the overall effect of insulin is to significantly increase the amount of fatty acid being accumulated there.

From these observations it can be seen that the effects of insulin on the adipose tissue would have been underestimated if only the rate of fatty acid manufacture had been observed. Clearly, any study which observes only the rate of fatty acid manufacture (Vernon, 1978, 1979, 1982; Vernon *et al.*, 1981; Yang and Baldwin, 1973; Etherton and Evock, 1986; Walton and Etherton, 1986) will not necessarily give an accurate estimate of the rate of fatty acid accumulation within the tissue. Since many studies of adipose tissue *in vitro* are prompted by concerns of overfatness in meat-producing animals, it would appear that it is the rate of fatty acid accumulation which should be of prime concern. Thus an evaluation of the total balance of fatty acid in adipose tissue *in vitro* and the effects of outside factors such as hormones on the rate at which fatty acid accumulates could lead to a better understanding of the cases of overfatness *in vivo*.

REFERENCES

Crane B.; Lane C. 1977. Modified semi-automated method

for free fatty acids in serum. *Journal of clinical pathology* **30:** 754-757.

Etherton T.D.; Evock C.M. 1986. Stimulation of lipogenesis in bovine adipose tissue by insulin and insulin-like growth factor. *Journal of animal science* **62:** 357-362.

Galletti F. 1967. An improved colorimetric micromethod for the determination of serum glycerides. *Clinica chimica acta* **15:** 184-186.

Vernon R.G. 1978. Lipogenesis in sheep adipose tissue maintained in tissue culture: effects of insulin and growth hormone. *Biochemical Society Transactions* **6:** 988-993.

Vernon R.G. 1979. Metabolism in ovine adipose tissue in tissue culture. *International journal of biochemistry* **10:** 57-60.

Vernon R.G. 1982. Effects of growth hormone on fatty acid synthesis in sheep adipose tissue. *International journal of biochemistry* **14:** 255-258.

Vernon R.G.; Clegg R.A.; Flint D.J. 1981. Metabolism of sheep adipose tissue during pregnancy and lactation: adaptation and regulation. *Biochemical journal* **200:** 307-314.

Walton P.E.; Etherton T.D. 1986. Stimulation of lipogenesis by insulin in swine adipose tissue: antagonism by porcine growth hormone. *Journal of animal science* **62:** 1584-1595.

Yang Y.T.; Baldwin R.L. 1973. Preparation and metabolism of isolated cells from bovine adipose tissue. *Journal of dairy science* **56:** 350-365.