

## Sessions 7/8 Interactions between fat and protein metabolism

# A comparison of lipolysis and lipogenesis in sheep and rats

S.L. Campbell and J.G. van der Walt

Rumen Biochemistry, Animal and Dairy Science Research Institute, Irene 1675, Republic of South Africa

Hormone sensitive lipase is thought to be the rate-limiting enzyme of lipolysis; phosphatidate phosphohydrolase that of lipogenesis. Both these enzyme systems were studied *in vitro* with the addition of epinephrine. The results show that:

- hormone sensitive lipase is four times more active in rat epididymal fat than in sheep tail fat and
- sheep omental fat is twice as active as tail fat.
- Phosphatidate phosphohydrolase is twice as active in rat fat as in tail fat, which is half as active as omental fat.
- Epinephrine had a stimulatory effect on hormone sensitive lipase and an inhibitory effect on phosphatidate phosphohydrolase, the peaks of both overlapping at  $4 \times 10^{-1} \mu\text{g/ml}$  in the case of sheep. This would indicate that the two processes occur simultaneously and suggests a very sensitive hormonally-regulated system.

Hormoon-sensitiewe lipase is vermoedelik die tempo-beperkende ensiem van lipolise; fosfatidaat-fosfohidrolase die van lipogense. Beide die ensiemstelsels is *in vitro* bestudeer met byvoeging van epinefrien. Die resultate toon aan dat:

- Hormoon-sensitiewe lipase is vier maal meer aktief in epididimale vet van rotte as in skaapstertvet en
- skaap omentumvet is twee keer so aktief as stertvet.
- Fosfatidaat-fosfohidrolase is twee maal aktiewer in rotvet as in stertvet, wat die helfte so aktief is as omentumvet.
- Epinefrien stimuleer hormoon-sensitiewe lipase en inhibeer fosfatidaat-fosfohidrolase, terwyl die pieke van beide by  $4 \times 10^{-1} \mu\text{g/ml}$  oorvleuel in die geval van skape. Dit sou impliseer dat die twee prosesse gelyktydig plaasvind en sugereer 'n baie sensitiewe hormoonbeheerde stelsel.

**Keywords:** Sheep, rats, epinephrine, adipose tissue, rate-limiting enzymes, hormone sensitive lipase, phosphatidate phosphohydrolase

### Introduction

Hormone sensitive lipase is thought to be the rate-limiting enzyme in lipolysis and is activated by a cyclic AMP-dependent protein kinase cascade system (Bauman, 1976). Catecholamines have been shown to affect lipolysis in sheep (Vernon, 1981), but less markedly so than in non-ruminants. There is evidence that phosphatidate phosphohydrolase is the rate-limiting enzyme of lipogenesis (Cheng & Sagger-son, 1978) and that it is influenced by the fatty acid concentration in the blood and the glucose availability, but the role of catecholamines in the process is unknown (Vernon, 1981). Vernon (1981) suggests that lipolysis and lipogenesis occur simultaneously in the sheep with some of the fatty acids from lipolysis being recycled into the pool of fatty acids being esterified. Such a cycle would be under very sensitive control. The aim of this work was to determine the effect

of catecholamines on both processes and whether they occur simultaneously under the same experimental conditions.

A comparison between rat epididymal fat and sheep omental and tail fat revealed significant differences in the rates of lipolysis and lipogenesis and in their control.

### Methods

#### Animals

Male laboratory white rats aged 2–3 months and fed high roughage laboratory chow were decapitated and the epididymal fat was removed for analysis. Karakul fat tail ewes aged 1–2 years and fed 1 300 g lucerne hay daily were sampled for tail fat under local Lignocaine anaesthesia and for omental fat via a paracostal laparotomy after a Lignocaine spinal block.

#### Laboratory Analyses

Hormone sensitive lipase and phosphatidate phosphohydrolase assays were carried out using similar protocols. Fat (0.5–1.0 g) was weighed into 8 vials containing 5 ml Earle's buffer each and were incubated under 95%O<sub>2</sub>:5%CO<sub>2</sub> for varying lengths of time (0–150 min), with and without differing concentrations of epinephrine. One sample was immediately transferred to the buffer for tissue enzyme activity analysis (sample A). Another was transferred after 90 min (B) and the remaining 6 after 150 min (C–H). Epinephrine was added to samples D–H after 90 min to give final concentrations of  $4 \times 10^{-4}$  to  $4 \times 10^1 \mu\text{g/ml}$ . These tissue enzyme activity analyses were carried out after the fat samples had been homogenized and then incubated at 39°C. Subsamples of 1 ml were taken at 0, 20 and 40 min for glycerol or phosphate analyses (lipase and phosphatase activities respectively).

Hormone sensitive lipase activity was estimated from the production rate of glycerol by the crude homogenate (Wieland, 1965), and phosphatidate phosphohydrolase activity from the formation of inorganic phosphate (Ames, 1966).

### Results and Discussion

#### Lipolysis

The basal rate of lipolysis in rat adipose tissue was four times higher than that of sheep tail and omental fat (Figure 1). The degree of activation above baseline in response to epinephrine is greatest in the rat adipose tissue, and omental fat was activated to a greater extent than tail fat. While these results confirm Vernon's (1981) findings they also show that optimal activation is achieved at a 10 times lower concentration in the case of sheep adipose tissue enzyme.

These observations are in agreement with hypothesis that fat tail sheep have evolved in a hot arid environment where laying down of subcutaneous and omental fat would be disadvantageous to the animal with regard to temperature regulation (H.S. Hofmeyr, pers. comm.). However, in these regions, the ratio of protein to carbohydrate in the diet is

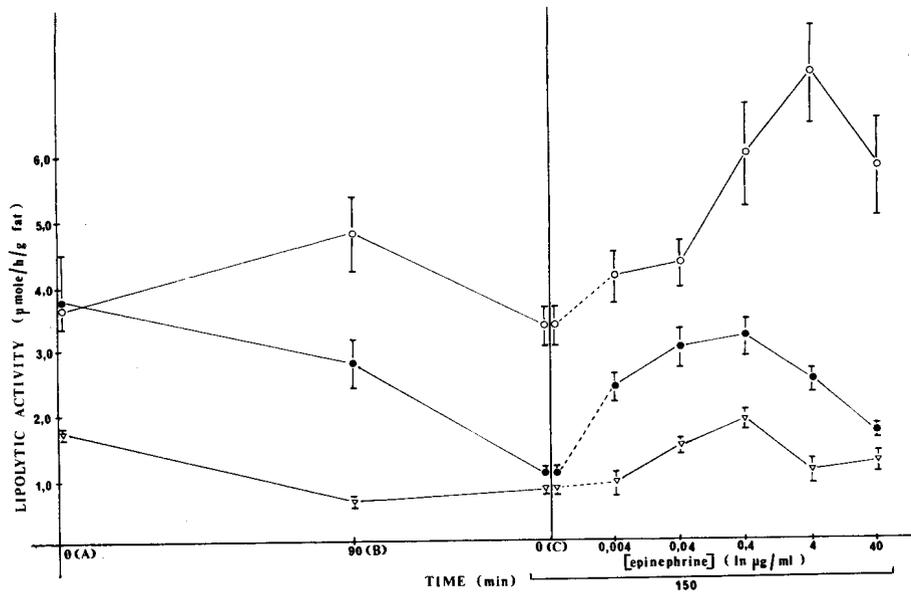


Figure 1 Effect of epinephrine on lipolysis in rat and sheep adipose tissue. (○ rat, ● sheep omental, ▽ sheep tail)

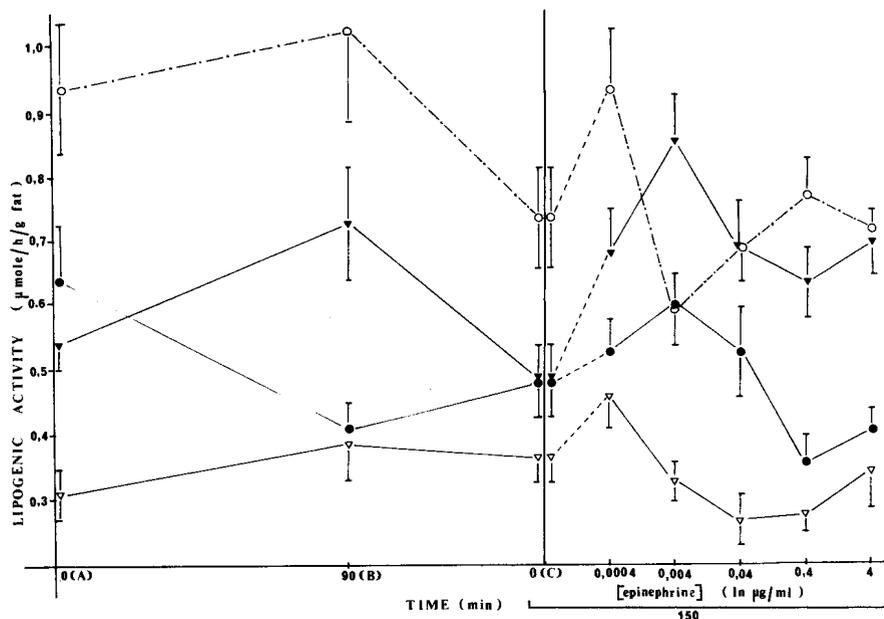


Figure 2 Effect of epinephrine on lipogenesis in rat and sheep adipose tissue. (○ rat, ● sheep omental, ▽ sheep tail A, ▼ sheep tail B)

generally low, and since the animal tends to feed to its protein requirement, an excess intake of carbohydrate results. This is stored in the tail as fat where it will not adversely affect temperature regulation in the animal. Thus omental and subcutaneous fat would be more metabolically active and hence more responsive to hormonal control (e.g. epinephrine) than tail fat.

### Lipogenesis

Rat epididymal fat was once again more active than sheep tail or omental fat. However, there appeared to be different activities of the enzyme in the two groups of sheep tail fat, one group being half as active as the other group (Figure 2). Enzyme rates in omental fat fell between the two tail fat groups.

Cheng & Saggerson (1980) postulate the generation of a temperature-sensitive inhibitory factor in crude homogenates without epinephrine. As a result, the control samples (zero concentration of epinephrine) are not comparable with the epinephrine-treated samples and the  $4 \times 10^{-4} \mu\text{g/ml}$  concentration of epinephrine was used as the reference value.

From the samples treated with this lowest concentration of epinephrine, there is a steady decrease in enzyme activity in all cases and then a tendency to stabilize or even to increase in activity at the higher concentrations of epinephrine, which agrees with the findings of Cheng & Saggerson (1978).

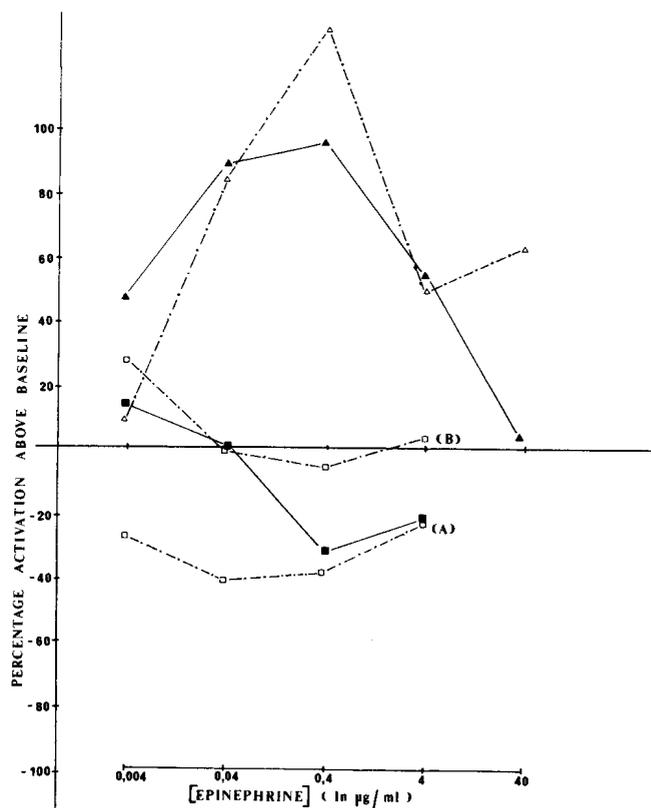
Generally, the rat adipose tissue enzyme appears to be more sensitive to the inhibitory effects of epinephrine than that of the sheep, in that the inhibition of the enzyme occurred at a 10 times lower concentration of epinephrine.

The fact that omental fat shows a more marked inhibition to the effects of epinephrine than does tail fat may be of significance in that lipogenesis would occur more readily in tail fat than in omental fat. This is to be expected, as it is more advantageous in terms of temperature regulation for the animal to store fat in the tail than in the omentum.

The different responses found in tail fat from the two groups of sheep may be explained by postulating different forms of the same enzyme, of which one may be more sensitive to epinephrine than the other. Karakul sheep have a mixed genetic background and this may account for the two forms of the enzyme being present.

In sheep, in both tail and omental fat, the peaks of the lipolytic and lipogenic curves coincide at  $4 \times 10^{-1} \mu\text{g/ml}$ . In the case of omental fat, at low concentrations of epinephrine, there would be a low rate of lipolysis and a high rate of lipogenesis, resulting in low blood levels of both glycerol and FFA. This is owing in part to the re-esterification (lipogenesis) of the FFA from lipolysis. At higher epinephrine concentrations, the rate of lipolysis increases and that of lipogenesis decreases, resulting in a peak in the FFA levels in the blood. At still higher concentrations of epinephrine the lipolytic rate drops and the lipogenic rate increases again (Figure 3), resulting in an autoregulatory limitation to the high concentrations of FFA. In the case of tail fat a similar trend is observed, but in the B group of sheep, the lipogenic rate appears to be inhibited from a higher basal rate (Figure 3).

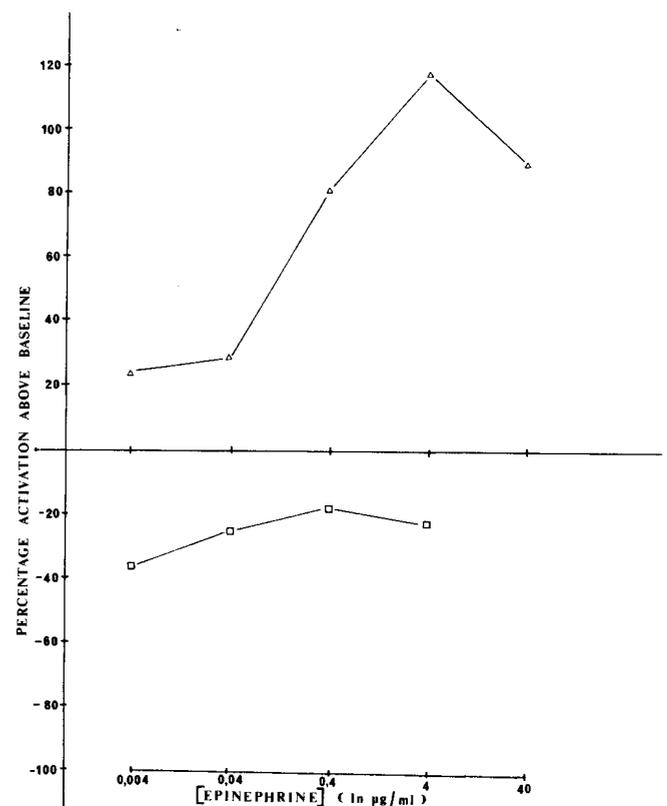
In rat epididymal fat the peaks of lipolysis and lipogenesis do not appear to coincide, that of lipogenesis being at  $4 \times$



**Figure 3** Effect of epinephrine on lipolysis and lipogenesis in sheep omental and tail fat. (lipolysis —  $\Delta$  tail,  $\blacktriangle$  omental; lipogenesis —  $\square$  tail A & B,  $\blacksquare$  omental)

$10^{-3} \mu\text{g/ml}$  and that of lipolysis at  $4 \mu\text{g/ml}$ . As a result, at a low epinephrine concentration, there will be a low rate of both lipolysis and lipogenesis causing a moderate level of FFA and glycerol in the blood. At higher concentrations, lipolysis will be greatly increased and lipogenesis partially de-inhibited, resulting in slightly lower levels of FFA in the blood than expected (Figure 4).

In conclusion, one may postulate that epinephrine has a greater effect on lipolysis than on lipogenesis and that norepinephrine may have a greater effect on lipogenesis than on lipolysis. Thus lipolysis would be predominantly under systemic control and lipogenesis under neuronal control. The results tend to support the theory that the two processes operate simultaneously in the animal, and suggest that there are two hormones involved in the regulation, which would make it a very sensitive control mechanism indeed.



**Figure 4** Effect of epinephrine on lipolysis and lipogenesis in rat epididymal fat. ( $\Delta$  lipolysis,  $\square$  lipogenesis)

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