*J. Physiol.* (1972), **221**, *pp.* 85–92
*With* 1 *text-figure*
*Printed in Great Britain*



# THE ACTION OF INSULIN ON BROWN ADIPOSE TISSUE *IN VIVO*

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(*Received 2 August 1971*)

SUMMARY

1. The action of insulin on the net exchange of glucose, free fatty acids and glycerol by brown adipose tissue of young rabbits was investigated.

2. Infusion of insulin (10,000 μu./kg.min I.V. for 10 min) caused a large increase in the uptake of glucose by brown adipose tissue of fed week-old rabbits. The release of fatty acids fell but glycerol release was unchanged.

3. The brown adipose tissue of 9-day-old rabbits fasted in a warm environment had a high initial rate of fatty acid and glycerol release and low rates of glucose uptake. Insulin infusions (10,000 and 100 μu./kg.min) greatly reduced fatty acid release but had little or no effect on glucose uptake.

4. The brown adipose tissue of 9-day-old rabbits fasted in a cold environment was depleted of fat and took up free fatty acid as well as glucose from the circulation. Infusion of insulin (10,000 μu./kg.min) caused a large increase in glucose uptake accompanied by a reduction in fatty acid uptake.

5. The experiments support the view that insulin has a direct effect on fatty acid transport across the cell membrane.

## INTRODUCTION

When adipose tissue, white or brown, is incubated *in vitro*, addition of insulin to the medium causes an increase in the rate of glucose uptake and glucose oxidation. Lipid stores enlarge due to an increase in the rate of lipogenesis from glucose (Ball, 1970; Shackney & Joel, 1966). At the same time the rate of fatty acid release falls (Fain, Reed & Saperstein, 1967). On the basis of studies *in vitro* it has been suggested that this latter effect is due to the inhibition of lipolysis by insulin. However, interpretation of fatty acid exchange in isolated tissues is difficult because so much depends on the characteristics of the surrounding medium. There have been relatively few studies on the effect of insulin on the metabolism of

white adipose tissue *in vivo* (Spitzer & Hohenleitner, 1961) and none on brown adipose tissue. In this paper experiments are described which show that *in vivo* insulin has a rapid and direct effect on fatty acid release by brown adipose tissue which appears to be independent of any action insulin might have on the rate of lipolysis. A preliminary report of some of these findings has been given (Hardman & Hull, 1971).

## METHODS

Three groups of young rabbits were studied. All were reared from birth in a thermoneutral environment (36° C) and had fed well and grown satisfactorily. Group I were 7 days old and they were studied when their next feed was due. Group II were treated similarly but they were kept unfed for a further 48 hr in a thermoneutral environment (9 days old). Group III was also unfed for 48 hr but they were kept, during this period, in a cool environment (20° C). The animals were given water during their fast.

Preliminary investigations were made on eight rabbits in Group I. They were anaesthetized with urethane (1 g/kg body wt. I.P.) and infusions were given via a polyethylene catheter inserted into a branch of the right external jugular vein. Blood samples were taken from a second catheter in the right cartoid artery. Bovine insulin 10,000 $\mu$u./kg.min was infused for 1 hr and blood samples were taken at 0, 30 and 60 min and analysed for glucose, free fatty acids and glycerol.

In all the other experiments the arteriovenous difference of these metabolites across brown adipose tissue was measured. On the day before the investigation the rabbits were given phenindione (3 mg/kg) orally to avoid clotting in the flow catheter. The rabbits were anaesthetized with urethane (1 g/kg body wt. I.P.) and a catheter was placed in the left external jugular vein so that the rate of venous outflow from the lateral vein of brown adipose tissue could be measured and venous blood collected (for details see Hardman & Hull, 1970). Arterial blood samples were collected from a catheter in the right carotid artery. Blood flow was measured and arterial and venous blood samples collected before and at the tenth minute of the insulin infusion.

Infusions were given via a catheter in a branch of the right jugular vein. Insulin was infused by a motor-driven pump at a rate of 10,000 $\mu$u./kg.min in all three groups of rabbits. Insulin in smaller doses (100 $\mu$u./kg.min) and saline were also infused into rabbits in Group II. The environmental temperature during the experiments was 35° C.

The blood glucose concentration was estimated by the method of Huggett & Nixon (1957), plasma-free fatty acids by the method of Novák (1965), and the plasma glycerol by the micro-method of Boehringer (Biochemica Test Combination). The S.D. of the mean of replicate estimations of glucose was $\pm 1{\cdot}0$ mg/100 ml., of free fatty acids was $\pm 0{\cdot}035$ m-equiv/l. and of glycerol was $\pm 0{\cdot}003$ m-mole/l.

## RESULTS

The effect of infusions of insulin (10,000 $\mu$u./kg.min) on the circulating concentrations of glucose, free fatty acids and glycerol in 7-day old, well-nourished rabbits, just before their next feed (Group I), are shown in

Fig. 1. The blood sugar tended to fall but the differences did not reach statistical significance. The free fatty acid concentration fell significantly in the first 30 min ($P > 0{\cdot}05$) and then returned to the initial level. Glycerol concentrations fell steadily during the 60 min infusion.

The effect of insulin infusion (10,000 $\mu$u./kg.min for 10 min) on the net exchanges of metabolites across brown adipose tissue in six rabbits in Group I are shown in Table 1. Initially brown adipose tissue took up glucose and released fatty acids and glycerol. The effect of insulin was to greatly increase the rate of glucose uptake and to significantly decrease the rate of fatty acids release ($P < 0{\cdot}01$). It did not alter the rate of glycerol release.

The results of infusions of insulin (10,000 $\mu$u./kg.min) on seven unfed rabbits kept warm (Group II) is shown in Table 2. At rest the tissue took up little or no glucose but released far greater amounts of fatty acids and glycerol than the tissue in Group I rabbits. Insulin caused an increase in the uptake of glucose and a large fall in the rate of fatty acid release. Again it did not significantly alter the rate of glycerol release.

In another series of experiments, a smaller dose of insulin (100 $\mu$u./kg.min) was given to nine rabbits in Group II. It caused a small but not significant increase in the rate of glucose uptake. There was again a marked fall in the rate of fatty acid release in seven out of nine experiments, but no change in glycerol release (Table 3).

Infusions of saline were given in a third series of experiments on rabbits in Group II. The brown adipose tissue of these rabbits in the initial state,

TABLE 1. The arterial concentrations and arteriovenous differences of glucose, free fatty acids and glycerol before and at the tenth min of insulin infusions (10,000 $\mu$u./kg.min) into 1-week-old rabbits (group I). Body weight $109 \pm 5{\cdot}6$ g. The net weight of the lateral lobe and the upper half of the posterior lobe of brown adipose tissue drained by the lateral vein was $0{\cdot}56 \pm 0{\cdot}07$ g. The figures shown in this and the subsequent tables are the mean $\pm$ S.E. of mean

| | Initial | | After 10 min infusion | |
|---|---|---|---|---|
| | Arterial concentration | Arterio-venous difference | Arterial concentration | Arterio-venous difference |
| Blood glucose (mg/100 ml.) | $170 \pm 11{\cdot}8$ | $-5{\cdot}3 \pm 1{\cdot}9$ | $169 \pm 12{\cdot}6$ | $-13{\cdot}8 \pm 2{\cdot}2$ |
| Plasma-free fatty acids (m-equiv/l.) | $0{\cdot}65 \pm 0{\cdot}04$ | $+0{\cdot}16 \pm 0{\cdot}02$ | $0{\cdot}53 \pm 0{\cdot}07$ | $+0{\cdot}07 \pm 0{\cdot}01$ |
| Plasma glycerol (m-mole/l.) | $0{\cdot}14 \pm 0{\cdot}008$ | $+0{\cdot}05 \pm 0{\cdot}007$ | $0{\cdot}12 \pm 0{\cdot}007$ | $+0{\cdot}05 \pm 0{\cdot}001$ |
| Blood flow from the lateral vein (ml./min) | $0{\cdot}41 \pm 0{\cdot}07$ | | $0{\cdot}34 \pm 0{\cdot}06$ | |

was not taking up glucose but after the infusion there was a small uptake of glucose. The net release of fatty acid and glycerol remained unchanged (Table 4).

The brown adipose tissue of unfed rabbits kept in the cold (Group III) took up fatty acids as well as glucose from the circulation. Insulin infusion

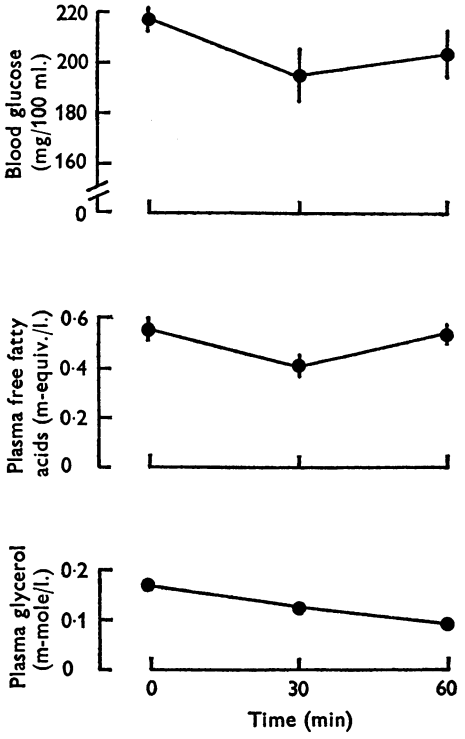


Fig. 1. The mean arterial concentrations of glucose, free fatty acids and glycerol in 1-week-old rabbits (Group I) before and during insulin infusion for 1 hr (10,000 $\mu$u./kg.min I.V.). The vertical lines indicate the S.E. of mean.

(10,000 $\mu$u./kg.min) greatly increased the glucose uptake and *reduced* the uptake of fatty acids. The tissue did not release glycerol at rest or in response to insulin infusion (Table 5).

There was a small fall in blood flow through brown adipose tissue during the course of the experiments. It may have been secondary to the serial blood sampling.

## DISCUSSION

Before discussing the interpretation of these experiments one limitation of technique must be mentioned. The blood glucose concentrations varied in these rabbits from 100 to 200 mg/100 ml. Small arteriovenous differences across brown adipose tissue of less than 2 mg/100 ml. would not be measured accurately for they are close to the reproducibility of the chemical

TABLE 2. The arterial concentrations and arteriovenous differences of glucose, free fatty acids and glycerol before and at the tenth min of insulin infusions (10,000 μu./kg.min) into 1-week-old rabbits (Group II). Body weight 115 ± 4·3 g. The wet weight of the lateral lobe and the upper half of the posterior lobe of brown adipose tissue drained by the lateral vein was 0·50 ± 0·02 g

| | Initial | | After 10 min infusion | |
|---|---|---|---|---|
| | Arterial concentration | Arterio-venous difference | Arterial concentration | Arterio-venous difference |
| Blood glucose (mg/100 ml.) | 141 ± 5·7 | −2·0 ± 1·2 | 146 ± 3·2 | −6·6 ± 1·5 |
| Plasma-free fatty acids (m-equiv/l.) | 0·50 ± 0·08 | +0·44 ± 0·07 | 0·33 ± 0·04 | +0·16 ± 0·06 |
| Plasma glycerol (m-mole/l.) | 0·17 ± 0·008 | +0·08 ± 0·02 | 0·13 ± 0·01 | +0·10 ± 0·03 |
| Blood flow from the lateral vein (ml./min) | 0·62 ± 0·05 | | 0·56 ± 0·08 | |

TABLE 3. The arterial concentrations and arteriovenous differences of glucose, free fatty acids and glycerol before and at the tenth min of insulin infusions (100 μu./kg.min) into 1-week-old rabbits (Group II). Body weight 114 ± 8·6 g. The wet weight of the lateral lobe and the upper half of the posterior lobe of brown adipose tissue drained by the lateral vein was 0·49 ± 0·07

| | Initial | | After 10 min infusion | |
|---|---|---|---|---|
| | Arterial concentration | Arterio-venous difference | Arterial concentration | Arterio-venous difference |
| Blood glucose (mg/100 ml.) | 141 ± 9·1 | −4·0 ± 2·4 | 157 ± 9·2 | −6·1 ± 1·3 |
| Plasma-free fatty acids (m-equiv/l.) | 0·35 ± 0·05 | +0·29 ± 0·05 | 0·38 ± 0·04 | +0·18 ± 0·06 |
| Plasma glycerol (m-mole/l.) | 0·18 ± 0·02 | +0·09 ± 0·03 | 0·20 ± 0·03 | +0·08 ± 0·02 |
| Blood flow from the lateral vein (ml./min) | 0·71 ± 0·10 | | 0·57 ± 0·08 | |

method used. Thus small changes with respect to glucose uptake would pass undetected. On the other hand, free fatty acid concentrations varied between 0·3 and 0·7 m-equiv/l. and small arteriovenous differences of the order of 0·07 m-equiv/l. could be measured.

The dose of insulin used in most of the experiments was large (10,000 $\mu$u./kg.min for 10 min), however the smaller dose produced similar effects when infused into Group II rabbits (100 $\mu$u./kg.min for 10 min). With this rate the total dose given to a 100 g rabbit would be 100 $\mu$u. Even if all

TABLE 4. The arterial concentrations and arteriovenous differences of glucose, free fatty acids and glycerol before and at the tenth min of saline infusions into 1-week-old rabbits (Group II). Body weight 96 ± 6·2 g. The wet weight of the lateral lobe and the upper half of the posterior lobe of brown adipose tissue drained by the lateral vein was 0·49 ± 0·7 g

| | Initial | | After 10 min infusion | |
|---|---|---|---|---|
| | Arterial concentration | Arterio-venous difference | Arterial concentration | Arterio-venous difference |
| Blood glucose (mg/100 ml.) | 110 ± 10·6 | + 1·0 ± 1·8 | 120 ± 11·1 | − 4·0 ± 2·8 |
| Plasma-free fatty acids (m-equiv/l.) | 0·38 ± 0·07 | + 0·39 ± 0·07 | 0·36 ± 0·06 | + 0·35 ± 0·06 |
| Plasma glycerol (m-mole/l.) | 0·16 ± 0·02 | + 0·12 ± 0·02 | 0·15 ± 0·01 | + 0·12 ± 0·03 |
| Blood flow from the lateral vein (ml./min) | 0·65 ± 0·11 | | 0·54 ± 0·09 | |

TABLE 5. The arterial concentrations and arteriovenous differences of glucose, free fatty acids and glycerol before and at the tenth min of insulin infusions (10,000 $\mu$u./kg.min) into 1-week-old rabbits (Group III). Body weight 111 ± 3·8 g. The wet weight of the lateral lobe and the upper half of the posterior lobe of brown adipose tissue drained by the lateral vein was 0·31 ± 0·02 g

| | Initial | | After 10 min infusion | |
|---|---|---|---|---|
| | Arterial concentration | Arterio-venous difference | Arterial concentration | Arterio-venous difference |
| Blood glucose (mg/100 ml.) | 143 ± 4·5 | + 2·5 ± 0·7 | 138 ± 5·1 | + 10·2 ± 2·8 |
| Plasma-free fatty acids (m-equiv/l.) | 0·37 ± 0·06 | − 0·18 ± 0·07 | 0·15 ± 0·02 | − 0·04 ± 0·02 |
| Plasma glycerol (m-mole/l.) | 0·11 ± 0·02 | 0 | 0·09 ± 0·001 | 0 |
| Blood flow from the lateral vein (ml./min) | 0·53 ± 0·09 | | 0·41 ± 0·08 | |

the insulin remained active and solely in the vascular compartment during the infusion, this would produce changes in circulating concentrations not greater than 20 $\mu$u./ml. blood. This is well within the physiological range (Hardman, Hull & Milner, 1971).

Brown adipose tissue has been shown to have two major biological activities. The first, which is present at birth but which gradually disappears with increasing age, is the production of extra heat during cold exposure. The second, which does not operate immediately after birth but which is its major activity in adult life, is the storage and modulated supply fatty acids. In the young rabbits studied in this investigation brown adipose tissue is actively involved in both processes. However, when the tissue is depleted of fat it can no longer act as an organ of supply and indeed, at this time, it draws free fatty acids from the circulation for its own energy requirements. It was perhaps not surprising, therefore, to find that insulin had the same effects on the tissue in this state as it does on muscle, namely it increased the rate of glucose and decreased the rate of fatty acid uptake (Carlsten, Haligren, Jagenburg, Svanborg & Werko, 1966; Zierler & Rabinowitz, 1964).

There is a considerable body of evidence to show that insulin has a direct action on glucose transport across cell membranes (Reiser, 1967) and it has been argued that if insulin increased glucose uptake then by making more glucose available for cellular energy it would decrease the need and therefore the uptake of free fatty acids. Thus the effect of insulin on free fatty acid exchange would be secondary to its action on glucose transport. However, if this were the case then insulin would be expected to increase the rate of release of fatty acids from brown adipose tissue containing fat. In fact, it did not, in contrast it greatly reduced the rate of fatty acid release. This fall in release was not secondary to a fall in the rate of triglyceride hydrolysis for the rate of glycerol release did not change. It has been suggested that insulin reduces fatty acid release from adipose tissue by providing more glucose to form glycerol-$\alpha$ phosphate and thus increasing the rate of fatty acid re-esterification (Ball, 1970). However the amount required for this purpose is small compared with the resting glucose uptake and thus it is difficult to imagine that it plays a rate limiting role. Furthermore the effect of insulin on free fatty acid release was greatest when its effects on glucose uptake was least (Group II).

The obvious explanation for the different effects of insulin on brown adipose tissue in the three different states is that it directly inhibits the transport of fatty acids both in and out of the cell. Zierler & Rabinowitz (1964) reached the same conclusion with respect to muscle on the basis of their studies on the arteriovenous differences across the human forearm during insulin infusion. It is a conclusion which has important clinical as

well as physiological implications for the effects of insulin on fatty acid transport are greatest when its effects on glucose metabolism are least, namely, in the fasting state.

We acknowledge with gratitude financial support from the Medical Research Council and thank Mrs J. E. J. Oyesiku for her technical assistance.

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