Incorporation of Glucose into Lipid of Perirenal and Subcutaneous Adipocytes of Rats and Sheep: Influence of Insulin

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Abstract

Adipocytes were prepared by collagenase digestion of perirenal and subcutaneous fat from rats and sheep and were incubated in vitro with various concentrations of glucose and insulin. The lipogenic rate of perirenal and subcutaneous adipocytes of rats showed a quadratic response to glucose concentration. The addition of 10 nm insulin increased lipogenesis, especially at low lipogenic rates. At constant glucose concentrations, insulin concentrations up to 50 nm stimulated lipogenesis a similar amount in adipocytes from both depots. The rate of lipogenesis increased relative to cell volume in perirenal adipocytes only. The lipogenic rate of perirenal and subcutaneous adipocytes of sheep showed a positive linear response to glucose concentration, but insulin did not affect the rate of lipogenesis in adipocytes from either depot. In both rats and sheep, the rate of lipogenesis was higher in the perirenal adipocytes. It was concluded that insulin is unlikely to be the agent responsible for the differential growth rates of subcutaneous and perirenal fat depots in rats or sheep.

Introduction

Most of the triacylglycerol stored in the body of an animal is deposited in identifiable fat depots. These fat depots grow at different rates as the animal ages and increases in weight (McCarthy 1979; Broad and Davies 1980). Insulin is known to accelerate fat deposition and stimulate the growth of adipose tissues in rodents and man (Hausberger 1965; Mendel 1980). This anabolic action results from the increased synthesis of glycogen, protein and lipid, and the inhibition of degradation of these substances (Czech 1977) in adipocytes and other cells.

In this study, we measured the rate of insulin-stimulated lipogenesis in adipocytes prepared from subcutaneous and perirenal fat depots in rats and sheep to see if differences in lipogenic rates could account for the reported differences in relative growth rates of the respective depots.

Materials and Methods

Materials

Collagenase from Clostridium histolyticum (type II, Lot 70F-6804), bovine serum albumin (fraction V) and bovine insulin were obtained from the Sigma Chemical Company, St Louis, Mo., U.S.A. D-[U-14C]glucose (114·7 MBq mmol⁻¹) was purchased from the Radiochemical Centre, Amersham, U.K.
Experimental Animals

Male Sprague–Dawley rats weighing 400–500 g were fed a standard diet *ad libitum* from weaning, and housed in a room at 20 ± 1°C with a 12-h light–12-h dark cycle. The composition of the diet was 400 g kg⁻¹ barley, 150 g kg⁻¹ maize, 20 g kg⁻¹ lucerne, 10 g kg⁻¹ lime, 35 g kg⁻¹ blood meal, 200 g kg⁻¹ whole milk powder, 180 g kg⁻¹ lupin meal and 5 g kg⁻¹ vitamin and salt mixture (James and Treloar 1981). The proximate analysis of the diet was: 21·8% crude protein, 3·8% crude fibre, 4·8% ether-extracted fat and 5·1% ash.

The sheep were obtained from two sources: Southdown rams aged 1–2 years with a carcass weight of 35–45 kg were obtained from Massey University, Palmerston North, N.Z.; wethers of mixed breed with carcass weight also of 35–45 kg were obtained from a local abattoir. All animals had been free-grazing pasture and were killed during mid–late summer (January–March).

Fat samples were taken from all animals as soon as possible after death. The rats were killed with diethyl ether, and the sheep by stunning with a captive bolt pistol or by electric shock, followed by bleeding. The whole perirenal and subcutaneous abdominal depots were dissected from rats. Samples (5–10 g) of the perirenal fat, and subcutaneous fat from the base of the tail, were taken from the right-hand side of the sheep. All samples were immediately placed in 0·9% (w/v) NaCl.

Preparation of Adipocytes

Sheep adipocytes, especially from the perirenal fat, are disrupted by even a short exposure (1–2 min) to temperatures below 35°C, so care was taken to maintain the temperature at 39°C for sheep and 37°C for rats throughout the whole period of fat collection and adipocyte preparation.

The fat samples (1 g) were incubated for 1 h in a shaking water bath in a ‘digestion solution’ (3 ml) of Krebs–Ringer–HEPES* buffer (pH adjusted to 7·4 with 1 M NaOH) containing bovine serum albumin (20 mg ml⁻¹) (Kono and Barham 1971) and collagenase (1 mg ml⁻¹). Tissue fragments were removed from the incubation mixture by filtration through surgical lint and the isolated adipocytes were washed three times in the incubation buffer (the ‘digestion solution’ without collagenase) by gently shaking the adipocytes in a 40-ml volume of the buffer and waiting for the adipocytes to float to the surface, and then carefully removing the infranatant by suction with a siliconized glass pipette. The adipocytes were then suspended in 10–30 ml of incubation buffer. The dry weight of adipocytes was estimated in 200-µl aliquots of the suspension pipetted onto filters (Upton et al. 1979) and the mean diameter of the cells determined by phase-contrast light microscopy (Hirsh and Gallian 1968). Adipocyte preparations from different animals were not pooled.

Assay of Rate of Lipogenesis

The effect of varying glucose concentration at constant insulin concentrations was measured by incubating isolated adipocytes for 1 h at 39°C for sheep, and 37°C for rats, with gentle shaking in incubation buffer containing 7·4 kBq d-[14C]glucose, various concentrations of ‘cold’ glucose (0·04–4·44 mM) and insulin (0 or 10 nM), in a final volume of 1·25 ml. All incubations were duplicated.

The effect of varying insulin concentration at a constant glucose concentration was measured by incubating isolated adipocytes from rats and sheep for 1 h, as before, but with insulin concentrations ranging from 0 to 50 nM, in an incubation buffer containing [U-14C]glucose and 0·88 mM ‘cold’ glucose. All incubations were duplicated.

The dry weight of adipocytes used per incubation in different series of incubations ranged from 6 to 70 mg. The rate of lipogenesis was linear with time up to 2 h, and linear relative to the number of adipocytes per incubation. In preliminary incubations, it was found that cells disrupted by chilling on ice incorporated negligible amounts of [¹⁴C]glucose into lipid. Also, since it was found that the rates of lipogenesis were similar when the albumin concentration was varied from 1 to 3%, a concentration of 2% albumin was used throughout.

At the end of the incubation period, the total lipid was extracted by the addition of 10 ml of toluene containing Omnifluor scintillation powder (New England Nuclear, Boston, U.S.A.) (4 mg ml⁻¹) (Moody et al. 1974). Aliquots (3 ml) of the toluene phase were counted in a liquid

* Krebs–Ringer–HEPES buffer: 1·2 M NaCl, 4·8 mM KCl, 1·2 mM KH₂PO₄, 1·2 mM MgSO₄, 7H₂O, 25 mM HEPES.
scintillation spectrometer (Searle Isocap 300) at an efficiency of 75–80%. Quenching was determined by the external standard ratio procedure.

The efficiency of lipid extraction, checked with the Folch procedure (Kates 1972), was essentially quantitative. Aliquots of the Folch-extracted lipid were saponified (Kates 1972) and the non-saponifiable lipid and free fatty acids sequentially extracted leaving the water-soluble fraction, which presumably contained the glycerol moiety of the lipids. Samples of each of these fractions were subject to liquid scintillation counting, the lipid material in the toluene scintillation mixture, and the aqueous material in Triton–toluene scintillation mixture (1:2 v/v).

Calculations and Statistical Analysis

The number of adipocytes per incubation was calculated from the mean diameter and the dry weight of cells per incubation (Kono and Barham 1971).

The amount of glucose incorporated into lipid was calculated from the specific activity of \(^{14}\text{C}\)glucose per incubation and the total lipid radioactivity. This amount of glucose was then adjusted to be used on a standard number of \(10^9\) adipocytes per incubation, as better linear model (larger \(R^2\) values) (Snedecor and Cochran 1967) estimates were obtained than when the data were expressed in terms of a standard weight of adipocytes per incubation.

Logarithmic transformation (base e) of the lipogenic-rate data was necessary to reduce the large variation between animals and to stabilize the variance, which increased as the glucose and insulin concentrations increased. The logarithms of the lipogenic rates were plotted against the logarithm of the glucose concentration or against insulin concentrations.

To analyse the data, a linear model was assumed, of the form

\[ y_{ij} = \mu + bx_i + a_j + e_{ij}; \quad \sum a_j = 0, \]

where \(\mu\) is the mean, \(y_{ij}\) is the log(lipogenic rate), \(x_i\) is the \(i\)th level of the medium concentration [i.e. log(glucose concentration) or insulin concentration], and \(a_j\) is the constant effect of the \(j\)th animal. Where marked curvature was noticed, the model

\[ y_{ij} = \mu + bx_i + cx_i^2 + a_j + e_{ij}; \quad \sum a_j = 0, \]

was fitted. Least-squares estimates of parameters were computed by the use of 'MINITAB' (Minitab Project, University Park, Pa., U.S.A.).

In the figures, the lines drawn represent \(y_i = \mu + bx_i\), or \(y_i = \mu + bx_i + cx_i^2\) where appropriate and the points plotted are \(y_{ij} = y_i + e_{ij}\), so that the constant (and quite large) differences between animals are eliminated to reveal the average trends implied by the data.

Results

Effect of Glucose and Insulin Concentration on Lipogenic Rate in Rats

Fig. 1 shows that the lipogenic rates in perirenal and subcutaneous adipocytes of rats are approximately described by quadratic curves over the range of concentrations of glucose in the incubation medium (0·04–4·44 mM). The maximum rate of lipogenesis implied by the curves occurred at a glucose concentration of about 0·88 mM. A closer look at the points on Fig. 1 suggests that the response could be more closely patterned by an asymmetric curve that rises to a maximum of about 2 mM and then sharply declines as the glucose concentration increases to 4·4 mM. The lipogenic rate of perirenal adipocytes was significantly greater (\(P < 0·001\)) than that of subcutaneous adipocytes at all concentrations of glucose, both in the absence and presence of 10 nM insulin.

In rats, 10 nM insulin significantly increased (\(P < 0·001\)) the average rate of lipogenesis in adipocytes from both depots (Fig. 1). However, the lipogenic rate of adipocytes from both depots in the presence of 10 nM insulin was less affected by glucose concentration than when insulin was absent. This effect is shown by the 'flatter' lines on the graph.
When the glucose concentration was optimal for lipogenesis (0·88 mM), increasing the concentration of insulin from 0 to 50 nM elicited a constant, linear increase in the lipogenic rate of adipocytes from both depots of rats (Fig. 2). The slopes of the regressions did not differ significantly, indicating that the lipogenic response to insulin was similar in both depots. However, the intercept value for the perirenal adipocytes (2·15) was greater (P < 0·001) than that for the subcutaneous adipocytes (1·49), demonstrating that the difference between depots, noted in the absence of insulin, persisted when insulin was added.
**Effect of Glucose and Insulin Concentration on Lipogenic Rate in Sheep**

In marked contrast to the data for rats, the data for sheep (Fig. 3) show considerable variability, even for adipocytes from the same depot in the one animal. A measure of the variability is that the multiple regression coefficient, $R^2$, is much less for sheep (0.67) than for rats (0.93).

![Graph](image1)

**Fig. 3.** Effect of glucose concentration on the lipogenic rate of perirenal and subcutaneous adipocytes of sheep. The units of lipogenic rate are nmoles of glucose incorporated into lipid per hour per $10^5$ adipocytes. The data points and regression equations for the perirenal adipocytes in the absence ($\triangle$, ---) and presence ($\Delta$, ---) of $10 \text{ nM}$ insulin are: $y = 1.24 + 0.29x$ and $y = 1.08 + 0.41x$, respectively, where $y$ is log (lipogenic rate) and $x$ is log (glucose concentration, mM). The data points and regression equations for subcutaneous adipocytes in the absence ($\bigcirc$, ----) and presence ($\bullet$, ----) of $10 \text{ nM}$ insulin are: $y = 0.72 + 0.50x$ and $y = 0.69 + 0.21x$, respectively. The multiple correlation coefficients, $R^2$, were 0.67 for both the perirenal and subcutaneous data.

![Graph](image2)

**Fig. 4.** Effect of insulin concentration on the lipogenic rate of perirenal and subcutaneous adipocytes of sheep, at a constant glucose concentration of 0.88 mM in the incubation medium. The units of lipogenic rate are nmoles of glucose incorporated into lipid per hour per $10^5$ adipocytes. The regression equations for the perirenal ($\Delta$, ---) and subcutaneous ($\bullet$, ----) adipocytes are: $y = 1.504 + 0.004x$ and $y = 1.49 + 0.006x$, respectively, where $y$ is log (lipogenic rate) and $x$ is insulin concentration (nM). The intercepts of the regression lines for the two tissues are significantly different ($P < 0.01$), but the slopes of both regression are not significantly different from zero ($P > 0.05$). The multiple correlation coefficients, $R^2$, were 0.89 and 0.72 for the perirenal and subcutaneous data, respectively.

In the perirenal adipocytes, there was a highly significant ($P < 0.001$) response in lipogenesis to increasing glucose concentration in both insulin treatments, but there was no significant difference between insulin treatments, the response lines being not
significantly different either in vertical displacement or in angle of slope. Therefore, no inference can be drawn from the apparent depressive effect that insulin has on perirenal lipogenesis, particularly at low concentrations of glucose.

In the subcutaneous adipocytes, there was a highly significant response ($P < 0.001$) in lipogenesis to increasing glucose concentration in the absence of insulin, and a significant ($P < 0.05$) response in the presence of 10 nM insulin. The slopes were significantly different ($P < 0.05$) but there was no significant mean overall difference due to insulin.

No significant differences were detected between tissues either for overall rate of lipogenesis, or for response to increasing glucose concentration.

When the glucose concentration was held constant at 0.88 mM, i.e. the concentration that supported nearly maximum rate of lipogenesis, increasing insulin concentrations from 0 to 50 nM had no significant effect on the lipogenic rate of either perirenal or subcutaneous adipocytes of sheep (Fig. 4), even though the lipogenic rate of perirenal adipocytes at zero insulin was significantly higher than that for subcutaneous adipocytes.

![Fig. 5. Effect of adipocyte volume on lipogenic rate of perirenal adipocytes of rats, at a constant glucose concentration of 0.88 mM in the incubation medium. The units of lipogenic rate are nmoles of glucose incorporated into lipid per hour per 10^3 adipocytes. The regression equations for the adipocytes incubated with 10 nM insulin (●) and without insulin (○) are: $y = -22.30 + 0.16x$ and $y = -13.52 + 0.09x$, respectively, where $y$ is lipogenic rate and $x$ is adipocyte volume.](image)

The absence of significant differences in lipogenesis of adipocytes from the two tissues, studied at two insulin concentrations over a range of glucose concentrations as shown in Fig. 3, contrasts with the significant difference in the latter experiment. This anomaly reflects the high variability of the sample of adipocytes prepared for the first part of this experiment, and is shown in the respective $R^2$ values: 0.67 for the first part and 0.72–0.89 for the second part.

**Effect of Cell Volume on Lipogenic Rate**

The mean volumes ($±$ s.e.) of the perirenal and subcutaneous adipocytes of rats were $(3.42±0.82) \times 10^5$ and $(1.94±0.21) \times 10^5 \mu m^3$ respectively; those of the
perirenal and subcutaneous adipocytes of sheep were \((2.75 \pm 0.31) \times 10^5\) and \((2.85 \pm 0.27) \times 10^5\) \(\mu\)m\(^3\) respectively. None of these volumes differed significantly \((P > 0.05)\) from each other.

The rate of lipogenesis in perirenal adipocytes of rats increased as a linear function of their volume, both in the absence and presence of 10 nM insulin (Fig. 5). The slope in the presence of insulin \((0.16)\) was significantly greater \((P < 0.01)\) than that in the absence of insulin \((0.09)\). There was no significant linear or curvilinear relationship between the rate of lipogenesis and cell volume in the other tested depots of rats or sheep.

[Graph showing distribution of fat depots of black and white hooded rats relative to body weight (Harris 1980). The depots are epididymal (●), perirenal (■), subcutaneous abdominal (△) and scapular (□). The line fitting the data for epididymal adipose tissue also fits the data for perirenal tissue.]

**Distribution of Label in Lipid**

In rat and sheep adipocytes, approximately 75% of the radioactivity was present in the putative 'glycerol' fraction of the lipids after saponification, the remainder being fatty acids. Negligible radioactivity was present in non-saponifiable lipids, i.e. sterols. There was no significant effect of glucose concentration, insulin concentration, or source of adipocytes on this pattern of distribution of radioactivity.

**Discussion**

**Lipogenic Rates of Rat and Sheep Adipocytes**

Three clear species differences have emerged in this study. First, the rate of lipogenesis in the perirenal and subcutaneous adipocytes of the rat was curvilinear with respect to the external glucose concentration, whereas in the sheep the lipogenic rate increased linearly relative to glucose concentration (cf. Figs 1 and 3). Crofford (1968) and Gliemann (1965) reported a similar curvilinear response of rat adipocytes
to increasing glucose concentrations. The data of Yang and Baldwin (1973) replotted on log–log axes show that the rate of lipid synthesis in bovine thoracic adipocytes increased linearly with respect to glucose concentration. Second, the lipogenic response to insulin differed in rats and sheep. In rats, insulin stimulated lipogenesis in adipocytes of both depots, especially when the external glucose concentrations were low, an effect also noted by Crofford (1968). In sheep, insulin did not affect the lipogenic rate in either depot when the external concentration of glucose was 0·88 mM (Fig. 4). Insulin has less effect on adipose tissue of ruminants than on that of other species (Bauman 1976). That observation is substantiated by this study, and by Vernon (1979) who noted the insulin insensitivity of fresh ovine perirenal and omental adipose tissue. Third, the rate of lipogenesis from glucose was greater in rat adipocytes than in sheep adipocytes. Hanson and Ballard (1967) reported that the rates of fatty acid synthesis from glucose in slices of rat epididymal fat were seven times that in slices of sheep mesenteric adipose tissue, per gram weight of tissue. The volumes of adipocytes in the tissue slices used in that study were not reported so it is not known whether differences in adipocyte volume or number contributed to that apparent difference between species. Although Ballard et al. (1969) concluded that ruminant tissues were less able than rat tissues to use glucose as a source of carbon for lipogenesis, the in vivo infusion studies by Armstrong et al. (1966), the in vitro studies of Vernon (1979) and those reported here suggest that the rates of lipogenesis from glucose in sheep are appreciable. Thus, the lipogenic rates of perirenal adipocytes from rats and sheep in the absence of insulin, and at a glucose concentration of 0·88 mM (calculated from Figs 2 and 4) are 8·6 and 4·5 nmoles of glucose incorporated into lipid per hour per 10³ adipocytes, respectively.

Lipogenic Rates of Perirenal and Subcutaneous Adipocytes

Perirenal adipocytes of both rats and sheep have a higher rate of lipogenesis than subcutaneous adipocytes. Jamdar et al. (1981) reported that homogenates of perirenal adipocytes of rats had higher activities of three triacylglycerol-synthesizing enzymes than homogenates of subcutaneous adipocytes. In that study, the perirenal adipocytes were larger than the subcutaneous cells, but this was not the case in the present study.

The rate of lipogenesis of perirenal adipocytes from rats only increased as a function of cell volume (Fig. 5), both in the presence and absence of insulin. Cell volume and lipogenic rates are related in rat epididymal fat and human adipose tissue (Ballard et al. 1969). Hood and Thornton (1980) found an approximately linear relationship between the volumes of subcutaneous adipocytes and the rate of lipogenesis from acetate in sheep fed a restricted diet late in their growth.

Comparison of Growth Rate and Lipogenic Rates of the Depots

Data, originally published by Harris (1980), of the relative growth rates of the rat fat depots, have been re-expressed in Fig. 6. This shows that perirenal fat grows faster relative to body weight than subcutaneous fat. In sheep, the reverse is found (Fourie et al. 1970; Broad and Davies 1980).

In rats, the rate of lipogenesis of the perirenal adipocytes was higher than that of the subcutaneous adipocytes, corresponding to the relative growth rates of the tissues. In sheep, however, the perirenal adipocytes had a high relative rate of lipo-
genesis but the depot had a slow relative growth rate compared to the subcutaneous depot. This observation is consistent with the finding of Broad and Ulyatt (1980) that in sheep infused with $^{14}C$acetate the more slowly growing perirenal fat was relatively more highly labelled than the faster growing subcutaneous depots.

At a glucose concentration supporting a nearly maximal rate of lipogenesis, insulin stimulated the rate of lipogenesis in adipocytes from the two fat depots of rat to a similar extent, but had no stimulatory effect in adipocytes from either of the sheep depots. It would seem, therefore, that as insulin does not appear to differentiate between perirenal and subcutaneous adipocytes for its effect on the lipogenic activities in sheep or rats, its effect on lipogenesis is unlikely to explain the different growth rates of the fat depots.

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**References**


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