Effect of insulin on glucose transport and metabolism in isolated fat-cells of gonadal adipose tissue from mature age-matched male and female rats

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Insulin action on glucose transport and metabolism was studied in paraovarian adipocytes from 3-month-old female rats and compared with insulin action in epididymal adipocytes from closely age-matched males. At maximal insulin concentrations the stimulations of 2-deoxyglucose uptake (4-fold the basal value) and of [U-¹⁴C]glucose incorporation into CO₂ and total lipids (3- and 2-fold the basal values respectively) were similar in adipocytes from rats of both sexes. At sub-maximal insulin concentrations (<0.2 nM) the ability of paraovarian adipocytes to transport and to metabolize glucose was higher than that of epididymal adipocytes; accordingly an increase in insulin binding was observed in paraovarian adipocytes as compared with epididymal adipocytes. These results show that paraovarian adipocytes from mature female rats were highly responsive to insulin, and exhibited a higher sensitivity to the hormone than did epididymal adipocytes from male rats of the same age.

The high responsiveness of glucose metabolism to insulin in adipocytes isolated from epididymal fat-pads of young adult rats is well documented. In epididymal adipocytes the maximal insulin stimulation is reported to be achieved within the physiological concentration range of the hormone, with a several-fold increase in the rates of glucose conversion into lipids or CO₂ (Olefsky, 1976; Olefsky & Saekow, 1978; Lavau *et al.*, 1979; Kobayashi & Olefsky, 1979*a,b*; Foley *et al.*, 1980*a*; Salans *et al.*, 1981; Fried *et al.*, 1982). In contrast, few studies have been performed on insulin action in adipocytes from female rats, and the results are somewhat conflicting. Whereas some authors, studying glucose conversion into CO₂ or lipids in parametrial or paraovarian adipocytes, report maximal insulin effect of a magnitude comparable with that routinely observed in epididymal fat-cells (Czech *et al.*, 1980; Craig *et al.*, 1981; Olefsky *et al.*, 1982; Wardzala *et al.*, 1982), others, using the same gonadal fat-cells, report maximal insulin effects less than 40% over the basal rates (Flint *et al.*, 1979; Sutter-Dub *et al.*, 1981, 1983), conveying the notion that glucose metabolism in adipocytes from

female rats is poorly responsive to insulin. Moreover, insulin was observed to be maximally active either within the physiological concentration range of the hormone (Craig *et al.*, 1981; Wardzala *et al.*, 1982; Olefsky *et al.*, 1982) or at concentrations 10 times higher (Czech *et al.*, 1980).

Therefore the present work was undertaken to examine both the sensitivity and the responsiveness to insulin of glucose transport and metabolism in paraovarian adipocytes and to compare them with epididymal fat-cells. As age has been shown to play a major role in the magnitude of the response to insulin in epididymal adipocytes (Di Girolamo & Rudman, 1968; Lavau *et al.*, 1972; Di Girolamo *et al.*, 1974), this comparison was carried out in male and female rats carefully matched for age. A preliminary account of part of this work has been presented (Guerre-Millo *et al.*, 1983).

Experimental

Animals

Age-matched male and female Wistar rats were obtained from CERJ (Le Genest, France) at the age of 70 ± 2 days. Rats were fed *ad libitum* on standard pellets [65% carbohydrate, 11% fat, 24%

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protein (% of total energy)]. Animals were housed at $23 \pm 1^\circ\text{C}$ with light from 07:00 to 19:00h. They were killed between 90 and 100 days unless otherwise stated.

Preparation of isolated adipocytes

In each experiment, two females and one male closely matched for age were used. They were killed between 09:00 and 10:00h by cervical dislocation. The total epididymal and paraovarian adipose tissues were removed. The two paraovarian tissues were pooled. After the tissues were minced with scissors, the fat-cells were isolated by digestion in Krebs–Ringer (Krebs & Heinseleit, 1932) bicarbonate buffer, pH 7.4, containing 3% bovine serum albumin (Sigma), 5 mM-glucose, half the recommended Ca^{2+} concentration (1.25 mM) and 1 mg of collagenase (Boehringer)/ml, by a modification of the method of Rodbell (1964). After 1 h at 37°C , fat-cells were filtered through a $190\mu\text{m}$ nylon screen, washed three times by allowing the cells to float and aspirating away the infranatant buffer, and suspended in either Krebs–Ringer bicarbonate buffer, for use in metabolic studies, or Krebs–Ringer phosphate buffer (see below), for use in glucose-transport and insulin-binding studies. Mean fat-cell diameter was measured by a photomicrographic method previously described (Lavau *et al.*, 1977).

Glucose-transport studies

Glucose transport was assessed by using labelled 2-deoxyglucose (Livingston & Lockwood, 1974; Olefsky, 1978; Kobayashi & Olefsky, 1978; Lavau *et al.*, 1979; Fried *et al.*, 1982). Foley *et al.* (1980b) have shown that the uptake of 2-deoxyglucose was a measurement of glucose-transport activity in adipose cells provided that the assay was performed at low substrate concentration and at low temperature. Therefore the assays were carried out at 24°C with 0.3 mM-2-deoxyglucose. Under these conditions the uptake of the tracer was linear with time for at least 4 min. Triplicate samples (0.475 ml) of the fat-cell suspensions in Krebs–Ringer (Krebs & Henseleit, 1932) phosphate buffer, pH 7.4, containing 2% serum albumin and 2 mM-pyruvate, were preincubated for 45 min at 37°C , without or with insulin at concentrations in the range 0.06–2.5 nM. Incubations, performed under magnetic stirring, were started by the addition of 2-deoxy[1- ^{14}C]glucose (sp. radioactivity 52 Ci/mol; Amersham International, Amersham, Bucks., U.K.). Then 3 min later, the assay was terminated by subjecting 300 μl samples of the incubation mixture to the oil-centrifugation procedure (Gammeltoft & Gliemann, 1973). The radioactivity in the cell layer was corrected for sugar

trapped in the extracellular water by using [^3H]-inulin (sp. radioactivity 3 Ci/mmol; Amersham International) in triplicate samples of each cell suspension.

Glucose-metabolism studies

The two major pathways of glucose metabolism in adipocytes (i.e. to CO_2 and incorporation into total lipids) were studied as previously described (Lavau *et al.*, 1979). In brief, triplicate samples (0.5 ml) of adipocytes suspended in Krebs–Ringer bicarbonate buffer, pH 7.4, containing 3% serum albumin and 5 mM-glucose were incubated at 37°C in plastic vials containing 2 ml of the same buffer, [$\text{U-}^{14}\text{C}$]glucose (sp. radioactivity 300 Ci/mol; CEA, Saclay, France) and insulin concentrations in the range 0–2.5 nM. After 2 h, the incubation was terminated by addition of 0.5 ml of 3 M- H_2SO_4 into the flasks, and $^{14}\text{CO}_2$ was collected in 0.3 ml of Hyamine added to a centre well. Then the contents of the incubation flasks were extracted with heptane/propan-2-ol mixture (Dole & Meinertz, 1960) and used for the measurement of ^{14}C incorporation into total lipids.

Insulin-binding studies

Samples (0.6 ml) of the fat-cell suspensions in Krebs–Ringer phosphate buffer, pH 7.4, containing 2% serum albumin and 5 mM-glucose, were incubated for 1 h at 37°C with mono[^{125}I]iodo-insulin with the labelled iodine in tyrosine-A14 (CEA, Saclay, France; sp. radioactivity 500 mCi/mg), with or without the addition of unlabelled insulin at final concentrations in the range 0.018–17 nM, in a final volume of 0.8 ml. The incubations were terminated by the oil-centrifugation procedure (Gammeltoft & Gliemann, 1973). Data are reported as specific binding, which was obtained by subtracting from total binding the amount of radioactivity that remained bound to the cells in the presence of a large excess of unlabelled insulin (15 μM). Non-specific binding averaged 5–15% of total binding, without any difference between male and female rats. The radioactivity was measured in a gamma spectrometer (Beckman Gamma 4000).

Liquid-scintillation counting

^{14}C and ^3H were measured in a liquid-scintillation spectrometer with stored quench-calibration curves and automatic d.p.m. calculation (Beckman LS 6800). Liquid scintillators were MP or NA Ready-Solv mixtures (Beckman).

Statistical analysis

Results are expressed as means $\pm$ s.e.m.. All *P* values were obtained by using non-paired or paired (as indicated) Student's *t* test.

Results

Female rats (3 months old) weighed 100g less than age-matched male rats [192 ± 2 g (24) versus 290 ± 4 g (12); $P < 0.01$]. Paraovarian adipocytes were slightly, but not significantly, larger than epididymal adipocytes: 0.178 ± 0.01 (12) versus 0.161 ± 0.01 (12) μ g of lipids per cell.

To examine insulin-sensitivity and responsiveness of isolated adipocytes, 2-deoxyglucose uptake, glucose oxidation and glucose incorporation into total lipids were studied at various insulin concentrations. The results reported in Fig. 1(a) show that the rate of 2-deoxyglucose uptake was similar in paraovarian and epididymal adipocytes under both basal (i.e. without insulin) and maximally insulin-stimulated conditions, with an average increase of 4-fold the basal value in the two groups. However, at 0.06 nM-insulin, 2-deoxyglucose uptake was significantly higher in paraovarian than in epididymal fat-cells, suggesting that the sen-

sitivity of 2-deoxyglucose uptake to insulin was higher in female than in male adipocytes. This was further examined by setting the maximal response in each cell suspension to 100% and expressing the effects of smaller insulin concentrations as a percentage of maximal response (Fig. 1b). With this mode of expression, the insulin-dose-response curve from paraovarian adipocytes was significantly displaced to the left as compared with the curve for epididymal fat-cells, clearly demonstrating an increased sensitivity.

The insulin-dose-response curves for glucose conversion into CO_2 and lipids in paraovarian and epididymal adipocytes are illustrated in Fig. 2. The basal rates of [$\text{U-}^{14}\text{C}$]glucose oxidation and incorporation into lipids were not significantly different for the two types of fat-cells. At maximally effective insulin concentrations, the rates of these two metabolic pathways were slightly, but not significantly, higher in paraovarian than in epididymal adipocytes. The effect of insulin, expressed as percentage increase over basal values, was similar in female and male rats for both glucose oxidation ($185 \pm 31\%$ versus $150 \pm 17\%$) and glucose conversion into lipids ($91 \pm 21\%$ versus $64 \pm 4\%$). At sub-maximal insulin concentrations (0.06 and 0.18 nM) the rates of [$\text{U-}^{14}\text{C}$]glucose oxidation and conversion into lipids were significantly higher in paraovarian than in epididymal fat-cells. The lowest concentration of insulin tested (0.06 nM) produced an increment in [$\text{U-}^{14}\text{C}$]glucose metabolism (oxidation + conversion into lipids) of 191 ± 36 nmol/2h per 10^6 cells in paraovarian adipocytes, compared with only 55 ± 5 nmol/2h per 10^6 cells ($P < 0.01$) in epididymal adipocytes. Furthermore, when the data were plotted as a percentage of the maximal insulin effect (Fig. 2b), the same feature was observed as for glucose transport, i.e. a shift to the left of the insulin-dose-response curves from paraovarian adipocytes as compared with the curves from epididymal fat-cells. Thus paraovarian adipocytes exhibited a higher sensitivity to insulin than did epididymal fat-cells, with respect to both glucose transport and glucose metabolism.

Insulin binding was then studied to determine whether the difference in insulin sensitivity between paraovarian and epididymal adipocytes could be related to a difference in the amount of insulin bound to the cells. Fig. 3 shows that at low insulin concentration (< 0.09 nM) paraovarian adipocytes bound significantly more insulin than did epididymal adipocytes. However, at higher insulin concentrations, the ability of adipocytes from both sexes to bind insulin was not significantly different, suggesting that the increased insulin binding by paraovarian adipocytes was due to an increased affinity of the receptors for insulin rather

Fig. 1. Effect of insulin on 2-deoxyglucose uptake by paraovarian (○) and epididymal (●) adipocytes from age-matched mature female and male rats: (a) absolute rates; (b) relative rates (expressed as a percentage of the maximal insulin effect)

Data represent means $\pm$ S.E.M. for four experiments performed on fat-cells from one male and two female rats, 95 $\pm$ 2 days old. 2-Deoxyglucose concentration was 0.3 mM. * $P < 0.05$, and ** $P < 0.01$, compared with epididymal adipocytes.

Fig. 2. Effect of insulin on $[U-^{14}C]$ glucose metabolism in paraovarian (O) and epididymal (●) adipocytes from age-matched mature female and male rats: (a) absolute rates; (b) relative rates (expressed as a percentage of the maximal insulin effect). Data represent means $\pm$ S.E.M., when large enough to record, for four experiments performed on fat-cells from one male and two female rats, 95 ± 2 days old. Glucose concentration was 5 mM. * $P < 0.05$, and ** $P < 0.01$, compared with epididymal adipocytes.

Fig. 3. Insulin binding to paraovarian (O) and epididymal (●) adipocytes from age-matched mature female and male rats. Data represent means $\pm$ S.E.M. for four experiments performed on fat-cells from one male and two female rats, 95 ± 2 days old. They were corrected for non-specific binding by subtracting the amount of radioactivity remaining bound at an insulin concentration of 15 μ M from the radioactivity in the cell pellet at all other insulin concentrations. Note the expanded scales in the insert. * $P < 0.05$ compared with epididymal adipocytes.

Table 1. Rates of 2-deoxyglucose uptake and [$U\text{-}^{14}\text{C}$]glucose conversion into CO_2 and total lipids, without insulin and in presence of 2.5 nM-insulin in adipocytes from paraovarian and parametrial adipose tissue from mature female rats. Data represent the means $\pm$ S.E.M. for three experiments on 2-deoxyglucose uptake and four experiments on [$U\text{-}^{14}\text{C}$]glucose metabolism, each done on paraovarian and parametrial adipocytes from one female rat. Rats were 105 ± 2 days old. * $P < 0.05$ compared with paraovarian adipocytes (paired Student's t test).

Adipose-tissue site	Insulin	2-Deoxyglucose uptake (nmol/min per 10^6 cells)	[$U\text{-}^{14}\text{C}$]Glucose conversion ($\mu\text{mol}/2\text{h}$ per 10^6 cells) into	
			CO_2	Total lipids
Paraovarian	—	0.79 ± 0.19	0.10 ± 0.02	0.16 ± 0.02
	+	2.4 ± 0.28	0.25 ± 0.08	0.33 ± 0.06
Parametrial	—	0.65 ± 0.15	0.12 ± 0.01	0.24 ± 0.01
	+	2.1 ± 0.75	0.30 ± 0.02	0.45 ± 0.05

than to an increased number of receptors per cell. The insulin-binding curves were not further analysed, since their ultimate interpretation must await a better understanding of the insulin-binding process (internalization, negative co-operativity, several types of binding sites etc.).

In female rats, the gonadal adipose tissue extends to the uterus via the parametrial fat depot, which is used in most of the studies dealing with female adipocytes. Therefore we performed additional experiments to compare the insulin-responsiveness of adipocytes from the two parts of the tissue. Table 1 shows that, with the exception of a slight increase in lipid labelling in the basal state in parametrial as compared with paraovarian adipocytes, there was no significant difference in the rates of any of the metabolic pathways assessed here under either basal or maximally stimulated conditions. This demonstrates that insulin-responsiveness was the same in adipocytes from the two locations of the gonadal adipose tissue.

Discussion

The aim of this work was to clarify whether gonadal adipocytes of female rats were responsive to insulin, as are those of male rats. The data presented here, which are the first to document a comparison between rat paraovarian and epididymal adipocytes, clearly show that female gonadal fat-cells were as responsive to insulin as male gonadal fat-cells with respect to both glucose transport and glucose metabolism. At maximal stimulation, a 4-fold increase in glucose transport, a 3-fold increase in glucose oxidation and a 2-fold increase in glucose conversion into lipids was observed in the two types of adipocytes. When compared with paraovarian adipocytes from the same rat, parametrial fat-cells exhibited a similar responsiveness to insulin. These observations, of a high insulin-responsiveness of paraovarian and parametrial adipocytes from mature female rats,

are in agreement with the data reported by Czech *et al.* (1980), Wardzala *et al.* (1982) and Olefsky *et al.* (1982), but differ from those of Sutter-Dub *et al.* (1981, 1983) and Flint *et al.* (1979), who observed a poor responsiveness of glucose metabolism to insulin in parametrial adipocytes from mature female rats. The reason for this discrepancy is unclear, but could be due to modest differences in the age of the rats. Indeed, our conclusion that paraovarian adipocytes are as responsive to insulin as are epididymal fat-cells is valid only for male and female rats carefully matched for age. In a preliminary study, performed on rats matched for body weight (200 g), we found a significantly higher insulin effect on glucose metabolism in epididymal than in paraovarian adipocytes (results not shown), probably because the male rats were actually 1 month younger than the females. A previous study from this laboratory has reported large variations in the lipogenic capacity of adipocytes from various tissue sites in male rats between 7 and 12 weeks of age (Karaoghlanian, 1983). This observation indicates that variations of adipocyte insulin-responsiveness with age might be substantial during the first 3 months of age, since the lipogenic capacity has been shown to play a major role in the modulation of insulin action on fat-cells (Lavau *et al.*, 1972, 1979; Fried *et al.*, 1981). In female rats, an effect of age on the responsiveness of parametrial adipocytes to insulin has also been reported (Craig *et al.*, 1981).

The main finding of the present study is the high insulin-sensitivity of paraovarian as compared with epididymal adipocytes. The insulin-dose-response curves for female rats were shifted to the left, as compared with those for male rats, for glucose transport as well as for glucose metabolism to CO_2 and lipids. Half-maximal stimulation of glucose transport and metabolism was achieved at an insulin concentration of 0.05 nM in paraovarian fat-cells and only 0.13 nM ($P < 0.05$) in epididymal adipocytes. Consistent with the increased sensi-

tivity of glucose transport and metabolism, we observed an increased insulin binding to para-ovarian fat-cells, at low insulin concentration, that could be related to a greater apparent affinity of the receptors for the hormone. The high sensitivity of paraovarian adipocytes to insulin reported here is in agreement with the data of Craig *et al.* (1981), Olefsky *et al.* (1982) and Wardzala *et al.* (1982), but in marked contrast with the observations of Czech *et al.* (1980) that the insulin dose eliciting half-maximal stimulation was approx. 1 nM-insulin. As only rat weight (250 g), but not age, was shown in the paper of Czech *et al.* (1980), those authors probably used older female rats, which could explain the low sensitivity of adipocytes to insulin.

Our results suggest that, in rats, adipocytes from females might be more sensitive to insulin than are adipocytes from males, as reported for humans by Foley *et al.* (1984). However, as the fat-cells compared in the present paper were not removed from identical sites in male and female rats, the difference in insulin-sensitivity could be due to a site effect. Further comparisons between adipocytes obtained from the same fat depot in both sexes are necessary to determine whether or not sex differences in adipocyte sensitivity to insulin exist in rats.

This work shows that gonadal adipocytes from mature virgin female rats are highly sensitive and highly responsive to insulin. Therefore these cells could be a useful model in which to study the changes in insulin action in physiological conditions specific to females, such as pregnancy and lactation, when a state of insulin resistance develops (Leturque *et al.*, 1980, 1984; Burnol *et al.*, 1983).

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