Effects of insulin *in vitro* on lipogenesis and fatty acid uptake in white adipose tissue of Zucker rats aged 10 days

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Adipose-tissue lipogenesis and fatty acid uptake *in vitro* were higher in obese than in lean Zucker rats aged 10 days. On average, insulin stimulated each of these two metabolic pathways to the same extent in both genotypes. However, in *fa/fa* pups, we observed that insulin stimulation decreased when adipose-tissue weight increased.

Adipose-tissue lipogenesis and fatty acid uptake have been shown to be higher per total tissue in obese (*fa/fa*) than in lean (*Fa/fa*) rats aged 10 days (de Waziers & Planche, 1983). These metabolic pathways are responsive to insulin (Godbole & York, 1978; Bally *et al.*, 1960). Although at 10 days there was shown to be no detectable difference in serum insulin concentration between lean and obese rats (Bazin & Lavau, 1982), it is possible that the response to insulin is different in *fa/fa* rats from that in *Fa/fa* rats. The purpose of the present study was to compare in both genotypes the insulin-responsiveness *in vitro* of lipogenesis and fatty acid uptake in white adipose tissue.

Materials and methods

Animals used in this study were 10-day-old obese Zucker rats (*fa/fa*) and their lean littermates (*Fa/fa*) resulting from male *fa/fa* and female *Fa/fa* crosses. Pups (8–12 per litter) and their mothers were housed in polypropylene cages in a temperature-controlled room (22°C) where lights were on from 07:00 to 19:00 h. Metabolic studies were performed from 09:00 to 11:00 h on pups in a fed state. Because of the small amount of tissue obtained from individual rats, the effect of insulin on either fatty acid synthesis or exogenous fatty acid uptake in white adipose tissue was studied in two independent series of animals. Pups were numbered and the genotype was visually identified at 5 weeks of age. The entire right inguinal fat-pad was surgically removed as described by Bazin & Lavau (1982), weighed and divided into two parts. Each part of the inguinal fat-pad was incubated at 37°C in 1.5 ml of gassed Krebs–Ringer bicarbonate buffer (pH 7.4), containing glucose (5.5 mM), palmitic acid (2 mM), defatted bovine serum albumin (2 g/100 ml) and either $^3\text{H}_2\text{O}$ (500 $\mu\text{Ci/ml}$)

or [^{14}C]palmitic acid (0.33 $\mu\text{Ci/ml}$), as previously described by de Waziers & Planche (1983). These incubations were performed with or without insulin (0.1 unit/ml). Preliminary experiments had shown that this insulin concentration induced, in both genotypes, a maximal stimulation of lipogenesis. Fatty acid synthesis was expressed as nmol of fatty acid synthesized (Windmueller & Spaeth, 1966) and incorporation of palmitic acid as nmol incorporated into the whole pad. Results were analysed by variance analysis.

Results and discussion

At 10 days of age, body weights were not significantly different between the *fa/fa* and the *Fa/fa* rats, as shown in Table 1. Conversely, the inguinal fat-pad weight was significantly increased in obese rats compared with lean rats. As previously described (de Waziers & Planche, 1983) we observed an increase in lipogenesis and fatty acid uptake in obese-rat adipose tissue. Moreover, our data show that, on average, insulin stimulated lipogenesis by 80–85% in both genotypes, whereas fatty acid uptake was increased by only 15% in *Fa/fa* and 23% in *fa/fa* rats.

Figs. 1 and 2 show the relationship between fat-pad weight and either lipogenesis or fatty acid uptake with or without insulin. In the basal state, the regression-line slopes differed significantly between *fa/fa* and *Fa/fa* rats for lipogenesis (0.15 ± 0.01 and 0.08 ± 0.02 respectively; $P < 0.01$), but not for fatty acid uptake (3.96 ± 0.85 and 3.12 ± 0.79 respectively; not significant). In the presence of insulin, the values of the regression-line slopes for the *fa/fa* and *Fa/fa* pups were respectively 0.12 ± 0.03 and 0.16 ± 0.03 (not significant) for lipogenesis and 1.82 ± 0.7 and 6.18 ± 1.6 ($P < 0.05$) for fatty acid uptake. The effects of

Table 1. *Fatty acid synthesis and palmitic acid incorporation in inguinal adipose tissue: effect of insulin*
Effects of insulin (0.1 unit/ml) on lipogenesis and fatty acid uptake was studied *in vitro* on adipose tissue of 10-day-old Zucker rats. Results are means $\pm$ S.E.M. for the numbers of pups shown in parentheses: * $P < 0.01$ between lean and obese rats; † $P < 0.05$ and ‡ $P < 0.01$ for insulin-treated compared with control.

Genotype	<i>fa/fa</i>	<i>Fa/fa</i>
Body wt. (g)	14.5 $\pm$ 0.4 (15)	14.3 $\pm$ 0.5 (14)
Fat-pad wt. (mg)	39.1 $\pm$ 5.2*	28.6 $\pm$ 3.3
Fatty acid synthesized (nmol/h per pad)		
Control	4.18 $\pm$ 0.81*	2.74 $\pm$ 0.35
+ insulin	6.50 $\pm$ 0.82*†	4.93 $\pm$ 0.70‡
Body wt. (g)	14.1 $\pm$ 0.4 (12)	14.6 $\pm$ 0.3 (19)
Fat-pad wt. (mg)	35.5 $\pm$ 4.0*	27.9 $\pm$ 1.5
Palmitic acid incorporated (nmol/h per pad)		
Control	180.3 $\pm$ 19.2*	147.9 $\pm$ 6.8
+ insulin	210.3 $\pm$ 14.9*†	173.3 $\pm$ 14.2‡

Fig. 1. Relationship between fat-pad weight and lipogenesis in white adipose tissue of obese and lean rats

Inguinal adipose tissue of (a) obese ($n = 15$) and (b) lean ($n = 14$) Zucker rats aged 10 days was incubated without (—, ●) and with (---, ○) insulin (0.1 unit/ml) in the presence of $^3\text{H}_2\text{O}$ (see the Materials and methods section). The correlation coefficient r was 0.97 without insulin and 0.74 with insulin for the obese rats, and 0.76 without insulin and 0.75 with insulin for the lean rats.

Fig. 2. Relationship between fat-pad weight and fatty acid uptake in white adipose tissue of obese and lean rats

Inguinal adipose tissue of (a) obese ($n = 12$) and (b) lean ($n = 19$) Zucker rats aged 10 days was incubated without and with insulin (0.1 unit/ml) in the presence of $[1-^{14}\text{C}]$ palmitic acid (see the Materials and methods section). The correlation coefficient r was 0.83 without insulin and 0.48 with insulin for the obese rats, and 0.70 without insulin and 0.66 with insulin for the lean rats. Symbols are as in Fig. 1.

insulin on lipogenesis and fatty acid uptake differed according to the genotype. In *Fa/fa* pups, the percentage stimulation of lipogenesis and fatty acid uptake was nearly constant whatever the fat-

pad weight. The correlation coefficient were $r = 0.03$ for lipogenesis and $r = 0.002$ for fatty acid uptake.

In contrast, in *fa/fa* pups, this percentage stimulation of both metabolic pathways decreased when the fat-pad weight increased. We found a significant correlation ($r = 0.71$) between fat-pad weight and the percentage stimulation of lipogenesis, with a negative regression-line slope of -2.90 ± 0.8 . For fatty acid uptake, we also obtained similar results, with a negative slope (-1.93 ± 1.26); however, the correlation coefficient was not significant ($r = 0.48$), since the percentage stimulations were small and more dispersed. Thus in obese pups the small pads seemed more responsive and large pads less responsive to insulin than in the corresponding lean pups at 10 days of age. Zucker & Antoniades (1972) observed in *fa/fa* rats an impaired response to insulin at 20 weeks, but not at 5–6 weeks of age. On the other hand, Goldstein & Johnson (1980) reported, for foetal hepatocyte cultures, a significantly lower stimulation of lipogenesis by insulin in *fa/fa* than in *Fa/fa* rats. It thus appears that responsiveness to insulin differs with age in the *fa/fa* rats.

Whatever the case, our results show that in 10-day-old *fa/fa* pups the increases in adipose-tissue lipogenesis and fatty acid uptake cannot be explained by an increase in the response to insulin.

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References

- Bally, P. R., Cahill, G. F., Leboeuf, B. & Renold, A. E. (1960) *J. Biol. Chem.* **235**, 333–336
- Bazin, R. & Lavau, M. (1982) *J. Lipid Res.* **23**, 839–849
- de Waziers, I. & Planche, E. (1983) *Horm. Metab. Res.* **15**, 564–565
- Godbole, V. & York, D. A. (1978) *Diabetologia* **14**, 191–197
- Goldstein, A. L. & Johnson, P. R. (1980) *In Vitro* **16**, 288–296
- Windmueller, H. G. & Spaeth, A. E. (1966) *J. Biol. Chem.* **241**, 2891–2899
- Zucker, L. M. & Antoniades, H. N. (1972) *Endocrinology* **90**, 1320–1330