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Adrenaline Responsiveness of Glucose Metabolism in Insulin-Resistant Adipose Tissue of Rats Fed a High-Fat Diet

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The effects of adrenaline (0.5 μM) and the combination of adrenaline and insulin (1.7 nM) on [6-^{14}C]glucose metabolism were assessed in epididymal fat-pads from rats fed either a low- or high-fat diet. The response of lipolysis to adrenaline was clearly diminished in fat-fed rats. Insulin added to adrenaline inhibited the lipolysis by 50% regardless of the diet. Glucose utilization in adipose tissue of fat-fed rats was markedly stimulated by adrenaline (glucose uptake was increased 3-fold and the production of CO_2 and the glycerol moiety of acylglycerol was increased 4-fold). However, adipose tissue from fat-fed rats was resistant to the effect of insulin to produce a further increase in adrenaline-stimulated glucose uptake. The intracellular capacity of lipogenesis on the one hand, and the production of CO_2 and the glycerol moiety of acylglycerol on the other, are of prime importance in the action of insulin and adrenaline on glucose utilization in this model.

The response of adipose tissue to insulin in terms of glucose utilization is severely decreased in rats given a high-fat diet (Lavau *et al.*, 1972; Susini & Lavau, 1978). The interaction of the hormone with the cell membrane is not the rate-limiting factor in the expression of insulin action in this model (Lavau *et al.*, 1979). Neither insulin binding nor the sensitivity of 2-deoxyglucose uptake to insulin are altered in the adipocytes of rats given a high-fat diet, compared with rats given a low-fat diet. The underlying mechanism of this resistance to insulin has been shown to be the drastic decrease in the intracellular enzymic capacity of fatty acid synthesis, the target pathway for insulin action in rat adipose tissue. In marked contrast with the rate of lipogenesis, the rate of tricarboxylic acid-cycle CO_2 production and the rate of synthesis of the glycerol moiety of acylglycerol, relative to the amounts of pyruvate or glucose taken up by the tissue, were not diminished by giving the rats a high-fat diet (Lavau *et al.*, 1970, 1972). This finding led us to postulate that glucose utilization in adipose tissue of fat-fed rats might remain fully responsive to adrenaline, since this hormone has been reported to preferentially stimulate those pathways (Cahill *et al.*, 1960). The present work was undertaken to examine the validity of this hypothesis.

Materials and Methods

Animals and diets

Wistar rats were purchased at weaning, divided into two groups and fed *ad libitum* either a low- or a high-fat diet for 6 weeks. The low-fat diet consisted of the following (%, w/w): casein, 12.1; DL-methionine, 0.4; lard, 1.1; wheat flour, 77.1; salt mixture, 4.0; vitamin mixture and essential fatty acids, 2.2; bran, 3.0; water, 0.2. As a percentage of total joules, this diet was: proteins, 18%; carbohydrates, 73%; and fat, 9%. The high-fat diet consisted of the following (%, w/w): casein, 31.8; lard, 41.5; wheat flour, 7.4; salt mixture, 6.0; vitamin mixture, 3.3; bran, 4.5; water, 5.5. As a percentage of total joules, this diet was: proteins, 18%; carbohydrates, 10%; fat, 72%. The diets were formulated to provide the same amount of protein, vitamins, and salt per joule. The rats had access to food until they were killed.

Studies in vitro

Rats were decapitated at 10:00h and bled. Epididymal fat-pads were quickly removed. They were weighed, cut into small pieces (30–50mg), and carefully pooled (so as to ensure that each incubation performed on 300–400mg of tissue had an equal representation of proximal and distal parts of the pads). Tissue sections were blotted, weighed, and placed in flasks containing 3ml of Krebs–Ringer bicarbonate buffer, pH7.4, with 3% 48h-dialysed bovine serum albumin (fraction V), 10mM-[6-^{14}C]-glucose (1 μCi/flask) with or without hormones. Final concentrations were 0.5 μM for adrenaline and 250 μ-units of insulin/ml, i.e. 10ng/ml or 1.7nM. The flasks were gassed for 45s with O_2/CO_2 (19:1, v/v) and capped with a centre-well-equipped stopper (Kontes Glass). Incubations were carried out for 2h at 37°C in a Dubnoff incubator. At the end of the

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incubation 0.3ml of 1M-Hyamine hydroxide and 0.5ml of 6M-H_2SO_4 were injected through the stopper into the centre well and the medium respectively. The CO_2 was collected during slow shaking for an additional 90min. The centre wells were then dropped into counting vials containing 10ml of toluene/ethanol (4:1, v/v) with 0.4% 2,5-diphenyloxazole and 0.015% 1,4-bis-(5-phenyloxazol-2-yl)-benzene. The fragments of tissue were removed from the flasks, rinsed three times in iso-osmotic saline, gently blotted on filter paper, and processed for determination of radioactively labelled lipids as previously described (Lavau *et al.*, 1970). Glucose and glycerol concentrations were determined in triplicate on the deproteinized and neutralized incubation media by the methods of Bergmeyer & Bernt (1965) and Wieland (1965) respectively. Blanks were run for all the metabolic parameters by incubating tissue in the presence of acid (0.5ml of 6M-H_2SO_4), and values were corrected accordingly.

Results and Discussion

When the rats were given a high-fat diet for 6 weeks a 2-fold enlargement of the epididymal fat-pads compared with the rats given a low-fat diet (4.1g against 2.2g; $P<0.01$) was observed. Therefore we decided to express the results with respect to total tissue.

The pattern of glucose metabolism in adipose tissue of rats given a high-fat diet, shown in Table 1, was consistent with previous reports (Lavau *et al.*, 1972; Susini & Lavau, 1978), i.e., an uptake of glucose much lower than that in adipose tissue of rats given a low-fat diet; an increased channeling of the glucose taken up into the glycerol moiety of acylglycerol (13% against 7%); a marked decrease in fatty acid synthesis (7% against 62%); and an unaltered efficiency of glucose oxidation through the tricarboxylic acid cycle (about 5% in both groups).

In agreement with the results of Leboeuf *et al.* (1959), Table 1 shows that the addition of adrenaline to adipose tissue removed from rats given a low-fat diet resulted in a marked increase in the uptake of glucose. As reported by Cahill *et al.* (1960), adrenaline caused a strong stimulation of CO_2 and labelling of the glycerol moiety of acylglycerol, together with a substantial decrease in the rate of fatty acid synthesis. The response of glucose metabolism to adrenaline in adipose tissue of rats given a high-fat diet has never been assessed. The present work shows (Table 1) that rats given a high-fat diet did not alter the responsiveness to this hormone, which caused a nearly 3-fold increase in glucose uptake. As in the epididymal fat-pads from rats fed on a low-fat diet, adrenaline inhibited fatty acid synthesis in fat-pads from rats fed on the high-fat diet and preferentially diverted the glucose taken up into CO_2 and the

Table 1. *Effect of adrenaline (0.5μM) without or with insulin (250μ-units/ml or 1.7nM) on [6-^{14}C]glucose metabolism and glycerol output in epididymal adipose tissue of rats given a low- or a high-fat diet*

Results are expressed as means ± S.E.M. for eight rats.

Diet	Incubation	Glucose uptake (μmol/total tissue per 2h)	Incorporation (μmol/total tissue per 2h) of ^{14}C from [6-^{14}C]glucose into:				Glycerol output (μmol/total tissue per 2h)
			CO_2	Lipids	Fatty acids	Glycerol	
Low-fat	No hormone	71.3 ± 6.98	3.2 ± 0.46	51.1 ± 6.07	43.6 ± 5.14	4.8 ± 0.41	5.0 ± 0.66
	Adrenaline	94.4 ± 10.11†	11.8 ± 1.56†	41.7 ± 5.07†	28.8 ± 3.92*	12.1 ± 0.99†	28.2 ± 2.04†
	Adrenaline+insulin	121.7 ± 13.0‡	9.8 ± 1.03	73.7 ± 6.34‡	58.8 ± 6.09‡	13.2 ± 1.09	13.1 ± 1.68‡
High-fat	No hormone	22.2 ± 3.24*	1.2 ± 0.03*	4.6 ± 0.17*	1.6 ± 0.16*	2.9 ± 0.10*	5.3 ± 0.63
	Adrenaline	59.0 ± 5.29*†	5.9 ± 0.46*†	11.6 ± 0.85*†	0.9 ± 0.27*†	10.4 ± 0.71†	16.2 ± 1.19*†
	Adrenaline+insulin	63.3 ± 4.85*	5.2 ± 0.63*	10.6 ± 1.12*	1.4 ± 0.15*‡	9.2 ± 0.95	8.3 ± 1.10‡

* $P<0.01$ against the corresponding value for the low-fat diet.
† Effect of addition of adrenaline significant at the level $P<0.01$ (paired t test).
‡ Effect of addition of insulin + adrenaline significant at the level $P<0.01$ (paired t test).

glycerol moiety of acylglycerol. In contrast the response of lipolysis (glycerol output) to adrenaline was clearly diminished in adipose tissue of rats given a high-fat diet, compared with rats given the low-fat diet (Table 1). These findings are relevant when compared with those by Gorman *et al.* (1973), who reported an unimpaired adrenaline binding and a loss of adrenaline-stimulated adenyl cyclase activity in adipocyte plasma membranes in rats given a high-fat diet.

The combined effects of insulin and adrenaline on glucose utilization in adipose tissue was dependent on the fat-content of the diet given to the rats. When the tissue was removed from rats given a low-fat diet, the combined addition of insulin and adrenaline resulted in a further increase in glucose uptake that correlated closely with the enhancement of fatty acid synthesis. On the other hand when tissue was removed from rats given a high-fat diet, addition of insulin + adrenaline did not cause any detectable increase in glucose uptake, but only restored fatty acid synthesis to the low basal value. Insulin inhibited the lipolysis elicited by adrenaline by 50% (Table 1), a result in keeping with several reports providing evidence that insulin was able to antagonize lipolysis at low ($< 10^{-5}$ M) adrenaline concentrations (Jungas & Ball, 1963; Miller & Allen, 1973). This inhibitory effect of insulin was observed whatever the fat-content of the diet. Taken together these results provide evidence of the pathway specificity of the resistance to hormones exhibited by the adipose tissue of fat-fed rats.

The responsiveness of glucose utilization to adrenaline in adipose tissue of fat-fed rats is marked in comparison with the negligible action of insulin. These findings strongly suggest that adrenaline prevails over insulin in the regulation of adipose-tissue glucose metabolism in fat-fed rats. This may have some relevance to the physiology of human adipose tissue, since the diet in developed Western countries is a high-fat diet.

In conclusion adipose tissue adapts to high-fat diet by drastic changes in the pattern of glucose metabolism. Moreover the present work clearly shows that under such dietary conditions adipose tissue is resistant to insulin and fully responsive to adrenaline in terms of glucose utilization. The results support the premise that the critical factors in the expression of hormone actions in this model are the intracellular capacities of the preferential pathways of the hormones.

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References

Bergmeyer, H. U. & Bernt, E. (1965) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed), pp. 123–130, Academic Press, New York

Cahill, G. F., Leboeuf, B. & Flinn, R. B. (1960) *J. Biol. Chem.* **235**, 1246–1250

Gorman, R. R., Tepperman, H. M. & Tepperman, J. (1973) *J. Lipid Res.* **14**, 279–285

Jungas, R. L. & Ball, E. G. (1963) *Biochemistry* **2**, 383–388

Lavau, M., Nadeau, M., Griglio, S., de Gasquet, P. & Randall, R. J. (1970) *Bull. Soc. Chim. Biol.* **52**, 1363–1379

Lavau, M., Nadeau, M. & Susini, C. (1972) *Biochimie* **54**, 1057–1067

Lavau, M., Fried, S. K., Susini, C. & Freychet, P. (1979) *J. Lipid Res.* **20**, 8–16

Leboeuf, B., Flinn, R. B. & Cahill, G. F. (1959) *Proc. Soc. Exp. Med.* **102**, 527–530

Miller, E. A. & Allen, D. O. (1973) *J. Lipid Res.* **14**, 331–336

Susini, C. & Lavau, M. (1978) *Diabetes* **27**, 114–120

Wieland, O. (1965) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 211–214, Academic Press, New York