SUPPRESSED LIPOLYSIS IN GENETICALLY FAT PIGS

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1. Introduction

Pig adipose tissue has been regarded as unresponsive to the lipolytic effect of both catecholamines and pituitary lipolytic polypeptides [1]. Evidence has been presented that this unresponsiveness may be due to inactivation of lipolytic hormones by enzymes present in adipose tissue itself [1], or in serum [2, 3]. Furthermore a hormone antagonist that inhibited the ability of lipolytic hormones to increase adenosine cyclic 3′, 5′-monophosphate levels has been partially purified from adipocytes [4].

Specific lipolytic substances (lipotropins) have been prepared from ovine [5, 6], bovine [7], porcine [8] and human [9, 10] pituitary glands. The mobilization of depot fat seems to be regulated by the action of lipolytic hormones and their antagonists moderating the hormone response.

We have now shown that fat tissue obtained from pigs selected for leanness was responsive to the *in vitro* lipolytic effect of epinephrine as well as pituitary lipotropins of porcine and human origin. Fat pads from pigs selected for fatness showed some although reduced responsiveness. Furthermore, serum from fat pigs suppressed the *in vivo* lipolytic activity of pituitary lipotropin in rabbits. The results support the view that genetic factors may be involved in the control of fat metabolism.

2. Materials and methods

Preparations of pituitary lipotropins of human and porcine origin were obtained according to a method described [9].

All chemicals were reagent grade.

The pigs studied were from two lines selected for fatness or leanness for four generations. The selection criterion was the thickness of the backfat of the pig determined by ultrasonic equipment [11]. As controls a group of unselected pigs was used. The mean backfat thickness in mm was 24.1 ± 0.34 for the lean pigs, 27.8 ± 0.43 for the controls, and 36.6 ± 0.34 for the fat pigs. Castrated male pigs with live body weights of 90 kg were used.

The animals were fed at 7:30 a.m. and slaughtered by electric stunning and exsanguination at 1–2 p.m.. Subcutaneous fat samples were obtained from the stab wound in the neck after exsanguination and sampling of blood. One half of each carcass was dissected in order to estimate the amount of fat and muscle tissues.

All pigs were fed a B-standard diet [12]. In order to study if the amount of food consumed influenced the lipolytic response, pigs from the lean and fat groups were fed at different levels from 25 to 90 kg of body weight. On the ad libitum level of feeding the pigs had free access to food all day, the "regular feeding" involved ad libitum eating twice a day, whereas the "reduced diet" involved feeding twice daily at a level 20% less than the "regular feeding". The animals on ad libitum and reduced feeding were selected ran-

Table 1

The *in vitro* release of non-esterified fatty acids (NEFA) in fat pads sampled from control pigs and pigs selected for fatness or leanness, and the effect on lipolysis of a lipid-mobilizing factor prepared from porcine pituitary glands (p. LMF).

	Number of pigs	Weight of half carcass (kg)	Age at slaughter (days)	Composition of carcass muscle (%)	fat (%)	Mean release of NEFA (μmole/100 mg/3 hr, ± SE) Fat pads blank	1 μg p. LMF
Controls	10	31.43 ± 0.28	173.1 ± 3.5	48.1 ± 0.5	25.7 ± 0.7	0.46 ± 0.03	1.29 ± 0.06
Fat pigs	35	31.81 ± 0.33	185.2 ± 4.3	46.0 ± 0.5*	30.0 ± 0.6*	0.58 ± 0.04	0.98 ± 0.04*
Lean pigs	31	31.17 ± 0.31	169.4 ± 3.1	52.0 ± 0.4*	21.5 ± 0.5*	0.42 ± 0.02	1.58 ± 0.18*

* = $P < 0.01$

All pigs were slaughtered at a live body weight of 90 kg.

Table 2

The influence of the level of feeding on the *in vitro* lipolytic effect of a lipid-mobilizing factor prepared from human pituitaries [9] (h. LMF, 1 μg/1.1 ml) and epinephrine (10 μg/1.1 ml) on fat pads obtained from pigs selected for fatness or leanness.

Level of feeding		Number of pigs	Age at slaughter (days)	Fat content of carcass (%)	Mean net release of NEFA (μmole/100 mg/3 hr ± SE) h.LMF	Epinephrine
Ad libitum	Fat	11	180.2 ± 9.9	30.6 ± 1.2	0.54 ± 0.05	2.22 ± 0.11
	Lean	10	155.2 ± 3.1	22.9 ± 1.2	1.30 ± 0.07	5.13 ± 0.29
Regular	Fat	35	185.2 ± 4.3	30.0 ± 0.6	0.53 ± 0.06	2.27 ± 0.15
	Lean	31	169.4 ± 3.1	21.5 ± 0.5	1.41 ± 0.05	5.00 ± 0.20
Reduced diet	Fat	11	205.1 ± 6.8	27.5 ± 1.3	0.46 ± 0.04	2.19 ± 0.13
	Lean	10	188.9 ± 10.5	20.4 ± 1.0	1.38 ± 0.11	4.97 ± 0.39

The pigs were fed a B-standard diet [12] at different levels from 25 kg until attainment of live body weight of 90 kg, when the pigs were slaughtered. The feeding levels were: ad libitum, regular (e.e. ad libitum eating twice a day) and reduced, which was 20% less than the regular feeding.

domly from the same litters of pigs.

For study of *in vitro* lipolysis samples of fat weighing 40 ± 4 mg were sliced and weighed on a torsion balance. The fat pads were incubated in triplicate in vials containing 1 ml of Krebs – Ringer bicarbonate buffer, pH 7.4, containing 4% bovine serum albumin, Sigma Fraction V (lot 100 c-3070). Lipolytic preparations were added in small aliquots (0.1 ml) at the start of the incubation. All vials were placed in a metabolic shaker at 37° with 95% O_2 and 5% CO_2 as gas phase. After 3 hrs of incubation, the vials were immersed in ice water. The adipose tissue was homogenized in the medium and Dole's extraction mixture for determination of total non-esterified fatty acids (NEFA) [13] was added. The means of the triplicate determinations are expressed as micromoles of NEFA per 100 mg adipose tissue per 3 hr (μmole/100 mg/3 hr).

The suppression by pig serum of the lipolytic effect of pituitary lipotropin was studied by an *in vivo* technique [2]. Rabbits were given a subcutaneous injection of 0.1 mg of human lipotropin in 3 ml of pig serum. The concentration of serum NEFA (meq./l) in the rabbits was determined before and 90 min after the injection.

The investigations on *in vitro* lipolysis and serum suppression of *in vivo* lipolysis were done as "blind studies". The samples of fat and blood given successive numbers were obtained by two of us (E.V. and N.S.) at slaughter of the pigs and the studies on lipolysis were done by the others at the research institute.

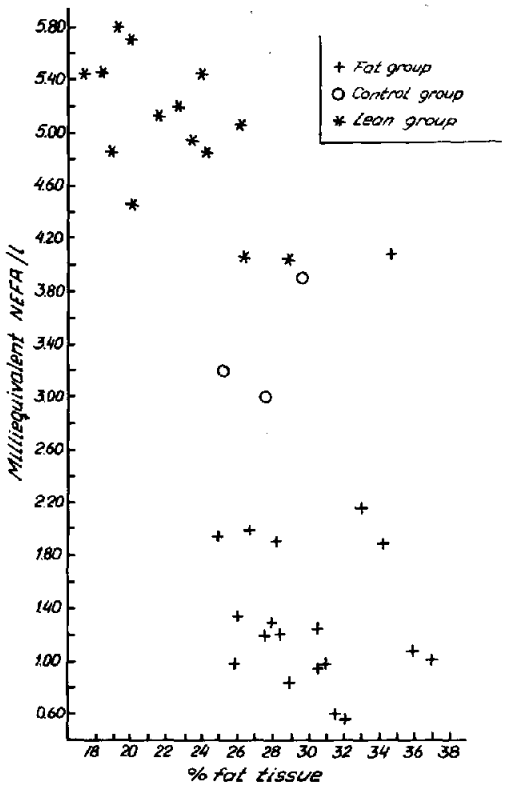

Fig. 1. The increase of serum non-esterified fatty acids (NEFA) in rabbits following subcutaneous injection of 0.1 mg of a lipid-mobilizing factor prepared from human pituitary glands [9] in 3 ml of serum obtained from pigs selected for fatness (n = 19). The content of fat tissue in carcass at slaughter after attainment of a live body weight of 90 kg is given in percentage

3. Results and discussion

The carcasses of pigs selected for fatness contained 40% more fat than those of pigs selected for leanness, table 1. The *in vitro* lipolysis in fat pads obtained from fat pigs, without additions (fat pads blanks) was significantly higher than in fat pads from lean pigs ($P < 0.001$). However, compared to unselected controls the increased mean release of NEFA in the fat pads blanks was significant only at the 10% level. When 1 μg porcine lipotropin was added to the vials there appeared a marked difference in lipolysis (table 1) with a mean net increase of NEFA (μmole/100 mg/3 hr) of 1.16 for the lean group, 0.83 for the controls and 0.40 for the fat group. The marked reduction in lipolysis in fat pads obtained from fat pigs was confirmed by the addition of 1 μg human lipotropin or 10 μg epinephrine (table 2).

The level of feeding influenced more markedly the growth rate of the pigs, as measured by the time required to attain 90 kg of live body weight, than the accumulation of body fat (table 2). In all instances the lean pigs grew faster than the fat ones. The *in vitro* lipolysis in pig fat induced by human lipotropin or epinephrine was not influenced by the level of feeding, suggesting that the lipolytic response is a genetic rather than an acquired characteristic.

The inhibition by pig serum of *in vivo* lipolysis induced by human lipotropin in rabbits is demonstrated in fig. 1. The increase of serum NEFA was negatively correlated to the fatness of the pig from which the serum was sampled. The mean concentrations were 5.03 ± 0.15, 3.25 ± 0.27 and 1.29 ± 0.11 meq./l, for serum obtained from the lean group, the controls and the fat group, respectively. The differences are highly significant ($P < 0.001$). The data indicate that serum from the fat pigs contained a factor which inhibited the activity of lipotropin. The correlation between the two studies on lipid metabolism, the suppression by pig serum of *in vivo* lipolysis in rabbits, and the *in vitro* lipolysis in pig fat pads is good (r = 0.86). Thus, the control of lipid metabolism differs in pigs selected for fatness and leanness.

Preliminary studies on suppression of lipotropic activity *in vivo* by serum obtained from the two selected lines of pigs at 5–6 weeks of age, before appearance of demonstrable differences in fat depots, gave in principle the same results as for adult pigs. This supports the view that serum suppression of lipotropin induced lipolysis is a genetic and not an acquired characteristic.

Similar studies are in progress with human fat and human serum, and they support the observations here reported. Subcutaneous fat pads obtained from persons considered to have a "metabolic" obesity show reduced responsiveness to lipolysis induced by epinephrine or pituitary lipotropin. Furthermore, a serum factor isolated from patients with "metabolic" obesity has been found to abolish the lipolytic effect of a pituitary lipid-mobilizing peptide [2, 3]. Altogether these results provide evidence that genetic factors may be involved in the control of fat metabolism.

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