EXPERIMENTAL STUDIES ON THE REGULATION OF MYOCARDIAL AND ADIPOSE TISSUE LIPOPROTEIN LIPASE ACTIVITIES IN RAT

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SUMMARY : Lipoprotein lipase (LPL) activity was studied in rat epididymal fat and heart. In adipose tissue, diet LPL induction lowered with age and was inhibited during cold exposure. Nicotinic acid, glucagon and norethindrolone propionate enhanced LPL activity and estrogens showed an opposite effect. In heart, starvation and cold exposure induced LPL activation ; glucose and nicotinic acid decreased LPL activity, but hormones had no significant effect. To some extent, AMP_c seems to be a mediator regulating LPL activity in adipose tissue. In heart the regulation mechanisms are still controversial.

Lipoprotein lipase (LPL) plays an important role in triglyceride (TG) removal from the circulation. As reported previously (1, 2), this enzyme is present in most tissues and there exists a nutritionnally regulated balance between the enzymatic activity of fatty acids utilizing tissues and that of adipose tissue.

The first studies about the regulation of tissue LPL activities were performed "in vitro" (3, 4) and our purpose was to investigate the "in vivo" variations of LPL activity in rat epididymal fat and heart in relation with age, nutritionnal conditions, cold exposure and injection of hormonal or pharmacodynamic factors wich affect the regulation of TG metabolism.

MATERIALS AND METHODS : Tissue LPL activity (5) - Tissues were homogenized in acetone ; the tissue powder was delipidated and dried by acetone and ether. The substrate was obtained in preincubating 1 part of Lipiphysan (EGIC : TG 10 %) with 6 parts of human serum. The enzyme extract was prepared by homogenizing

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the acetone powder in 0,025 M NH_4OH - NH_4Cl (pH 8,5). The incubation medium contained TRIS - HCl buffer (pH 8,5), bovine albumin, heparin and substrate. The assay medium was incubated at + 37° C ; aliquots were removed at different times for the automatically F.F.A. analysis.

Experiments - These studies were conducted in male Wistar rats.

The effect of age was studied in rats 6, 8 and 15 weeks old over 15 h fasting and in normal fed state (8 animals per group). The influence of particular nutritionnal conditions was examined in groups of 9 rats (140 - 160 g), fasting over 20 h ; LPL assay was performed 3 h after force fed 4 ml of glucose solution (132 g %), 4 ml of peanut oil and 4 ml of saline.

The effects of temperature was determined in 4 groups of 8 rats (140 - 160 g) : 2 groups housed 48 h at + 22° C, the two others 48 h at + 4° C. In each case, one group was sacrified after fasting period (20 h) and the other in normal fed state.

The effect of hormones and pharmacodynamic factors was studied as follows : 8 rats (140 - 160 g) fasting over 4 h were given an intraperitoneal injection (250 mg/kg) of nicotinic acid (NIACIN - UCB) ; samples were drawn 2 h after.

- Groups of 7 rats (120 - 140 g) starving for a night were injected intramusculary with saline or glucagon (NOVO) (25 µg/rat) 3 times at hourly intervals ; 2 other groups (9 rats), at the time of the first injection, were force fed with 3 ml of glucose solution (60 % w/v) or 3 ml of saline ; all animals were killed 3 h after the initial injection.
- Groups of 8 over night fasting animals (140 - 160 g) were given 2 intramusculary injections of saline or Estradiol hexahydrobenzoate (2,5 mg/rat) with a 3 days interval ; animals were killed on the 7th day.
- Groups of 9 rats (150 - 170 g) which had been starved for a night were administered 3 intramuscular injections of saline or Norethindrolone propionate (25 mg/rat) ; the samples were drawn on the 7th day.

RESULTS : Effect of age and nutritionnal state - LPL activity in heart and adipose tissue declines with age in both starved and fed animals (table I) ; this decrease is significant after 15 weeks. In the epididymal fat the lowest rates were found in fasting rats and the induction by the diet was so much the lower as the animal was older. On the contrary, the highest myocardial activities were found in fasting rats and diet induced activity decreases were in the same range for different ages. Glucose and oil induced a high LPL activity in adipose tissue (table II) ; by contrast, myocardial LPL activity was low in the fed state and high in starvation ; differences were less significant.

TABLE I

Effect of age and nutritionnal state on LPL activity

	AGE (weeks)			P	
	6	8	15	8 - 6	15 - 6
HEART (μ mol/h/g ± S.D.)					
A	182±29	173±31	137±13	N.S.	< 0,001
B	135±26	139±30	89±34	N.S.	< 0,01
P	<0,01	<0,05	<0,01		
ADIPOSE TISSUE (μ mol/h/g ± S.D.)					
A	17±6	10±4	8±1	< 0,01	< 0,001
B	65±16	16±4	10±3	< 0,001	< 0,001

A : 8 rats fasted for 20 h ; B : 8 fed rats ; P : student's t test

Response to cold exposure (table III) - Cold exposure induced a considerable increase of LPL activity in heart of fed or starved rats ; in adipose tissue, the LPL activity decreased only in fed animals.

Role of nicotinic acid and hormones (table IV) - Nicotinic acid enhanced LPL activity in epididymal fat of fasting animals within 4 h of administering drug and induced heart LPL activity decrease. In adipose tissue, estrogens made the LPL activity lower consistently though norethindrolone propionate made it enhance ; after glucagon injection into starved or fed animals, a meaning full activation was obtained. In tests on the myocardial tissue with hormones, no appreciable increase was revealed.

DISCUSSION : In rat tissues, LPL activity decreased with age when compared with the tissue weight ; in adipose tissue, this decrease might be correlated with an increase of the adipocyte

TABLE II

Effect of particular nutritionnal conditions on LPL activity

	GROUPS OF 9 RATS			P	
	A	B	C	B - A	C - A
HEART	150±26	105±23	142±40	< 0,01	N.S.
ADIPOSE TISSUE	23±4	40±6	37±5	< 0,001	< 0,001

μ mol/g/h ± S.D. (A : control ; B : glucose ; C : oil)
P : student's t test

TABLE III

Effect of cold exposure on LPL activity (μ mol/h/g ± S.D.)
(8 rats/group)

	STARVED			FED		
	+22°C	+4°C	P	+22°C	+4°C	P
HEART	70±24	149±18	< 0,001	72±15	152±28	< 0,001
ADIPOSE TISSUE	14±5	13,8±5	N.S.	43±4	22±4	< 0,001

P : student's t test

size, then it is recognized that the activity varied in the opposite direction (6). Like others (1) we noticed that starvation enhanced LPL activity in heart and reduced that of adipose tissue for which the induction by the diet was important but was lower as the age increased. We also show that this induction might be given by both oil and glucose ; our results are supported by the fact that fats are known to induce, like carbohydrates, a reactionnal hyperinsulinism (7) which, in turn, induces

LPL activity in adipose tissue (8). On the other hand, since insulin decreased cellular concentrations of AMP_c (9), activator of "hormone-sensitive lipase", and dibutyryl AMP_c inhibited the induction of LPL by glucose and insulin (4), it would appear that, "in vivo", AMP_c might be a negative regulator of LPL activity.

In accordance with other investigators (10, 11), it has been found that LPL activity of adipose tissue in fed rats was lower at + 4° C than at + 22° C ; furthermore, the difference did not appear when animals were starved. Thus, cold exposure inhibited the LPL induction by diet ; on the other hand, since it enhanced the circulating level of catecholamines (12) producing AMP_c, it could be thought that this rise was related to low LPL activities elicited at + 4° C in the fed animals. Then nicotinic acid which decreased the AMP_c rate (13) enhanced LPL activity in adipose tissue ; similar results have been reported (14).

Finally glucagon increased LPL activity in this tissue ; thought it is an AMP_c secretion activator (15), its administration was followed normally by an insulin secretion (16) which could secondarily induce the LPL activation. Our results "in vivo" were in agreement with those "in vitro" (3) and it appears that AMP_c regulated to some extent the LPL activity in adipose tissue. However, an increase in plasma TG was observed in pregnancy (17) and by oral contraceptive therapy (18, 19) ; the rise might be due to an epuration defect by extra hepatic tissues since it was shown that extended synthetical estrogenic administration decreased the LPL activity in adipose tissue and some authors (20) had noted the decrease of post-heparin LPL activity by estrogens. Unlike these, some synthetical progesta-

tives diminish plasma TG (21) ; this decrease might be due to the activation of the same process of clearing since we found that injection of an analogue among these progestatives enhanced LPL activity. Then, another way of regulation could be suggested.

It was demonstrated that LPL activity in the heart change in a direction opposite to that of epididymal fat and it was of interest to note that it is glucose and not fat which made the LPL activity decrease ; insulin had no effect on the regulation (8). Cold exposure and nicotinic acid induced, in our animals, variations opposite to those observed in adipose tissue whereas variations induced by glucagon were not significant ; the important role which other authors (22) have supposed glucagon to play in myocardial enzyme regulation has to be sustained. Synthesis estrogens and norethindrolone propionate have no influence on heart LPL activity.

TABLE IV

Effect of hormones and pharmacodynamic factors on LPL activity

	A (8)	B (9)	C (8)	D (7:Starved)	D (9:Fed)
HEART (μ mol/h/g $\pm$ S.D.)					
Control	157±21	124±40	157±21	135±30	91±17
Experiment	98±36	136±24	131±35	154±53	81±11
P	< 0,01	N.S.	N.S.	N.S.	N.S.
ADIPOSE TISSUE (μ mol/h/g $\pm$ S.D.)					
Control	16±5	21±6	24±4	25±11	55±18
Experiment	46±21	30±8	13±4	47±8	142±44
P	< 0,01	< 0,01	<0,001	<0,01	< 0,001

A : nicotinic acid ; B : Norethindrolone propionate ; C : Estradiol hexahydrobenzoate ; D : glucagon ; ( ) : animals ;
P : student's t test

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