Determinants of Human Adipose Tissue Lipoprotein Lipase

EFFECT OF DIABETES AND OBESITY ON BASAL- AND DIET-INDUCED ACTIVITY

OLAVI J. PYKÄLISTÖ,† PHILLIP H. SMITH, and JOHN D. BRUNZELL

From the Department of Medicine, Veterans Administration Hospital, and the University of Washington School of Medicine, Seattle, Washington 98108

ABSTRACT The role of insulin in the regulation of human adipose tissue lipoprotein lipase was evaluated. Adipose tissue heparin-releasable lipoprotein lipase (thought to be related to peripheral clearance of plasma triglycerides) was low in insulin-deficient, untreated hyperglycemic diabetic subjects ($P < 0.001$) and treatment of hyperglycemia returned the activity to normal. In chronic hyperinsulinism, represented by obesity, heparin-releasable activity among control subjects was correlated to percent of ideal body weight ($r = 0.53$, $P < 0.05$) and to fat cell size ($r = 0.61$, $P < 0.02$). Acetone-ether powder lipoprotein lipase activity (presumed to reflect total tissue enzyme) was also related to percent of ideal body weight ($r = 0.76$, $P < 0.001$ for controls; $r = 0.67$, $P < 0.05$ for diabetics) and to fat cell size ($r = 0.71$, $P < 0.01$ for controls; $r = 0.85$, $P < 0.01$ for diabetics. Postprandial-stimulated insulin secretion was related to diet-induced changes in lipoprotein lipase in control subjects; both were dependent upon the amount of dietary carbohydrate. In contrast, the diabetic patients with low insulin responses, failed to increase lipoprotein lipase activity with feeding. The changes in heparin-releasable ($r = 0.66$, $P < 0.01$) and acetone-ether powder ($r = 0.69$, $P < 0.01$) activity during feeding were related to the percent increase in plasma insulin.

Thus, insulin appears to be important in the regulation of human adipose tissue lipoprotein lipase activity. Elevated insulin levels in obesity and increased insulin secretion after eating were associated with increased lipoprotein lipase activity. Defects in insulin secretion, both in postabsorptive and postprandial states, are associated with low adipose tissue lipoprotein lipase and may lead to hypertriglyceridemia in diabetic man.

This study represents a part of the clinical investigation by the late Dr. Olavi J. Pykälistö during a fellowship at the Veterans Administration Hospital in Seattle, Wash.

Received for publication 28 February 1975 and in revised form 21 July 1975.

INTRODUCTION

Lipoprotein lipase (LPL),[1] present mainly in adipose tissue and to some extent in other tissues (1–3), is the rate-limiting enzyme for tissue uptake of plasma triglycerides (TG) (4–7). In a number of animal studies, adipose tissue LPL has been shown to be sensitive to caloric and hormonal balance (8-11) and particularly dependent on availability of glucose and insulin (11-13). Normal adipose tissue LPL levels have been found in treated human diabetic patients (14). However, the activity of this enzyme in human tissues has usually ben estimated only indirectly as postheparin plasma lipolytic activity (PHLA). In most, but not all instances, low PHLA has been associated with impaired TG removal and hypertriglyceridemia (15-18).

Hypertriglyceridemia of varying degrees is frequently seen in untreated diabetes mellitus; it is almost the rule in severe insulin deficiency with ketoacidosis (19-22). Treatment with insulin or oral antidiabetic agents returns elevated plasma TG to or toward normal indicating that absolute or relative lack of endogenous insulin may be a cause of diabetic hypertriglyceridemia. However, studies of plasma TG removal in diabetes using PHLA as an index, have been inconclusive. Thus, PHLA, measured after a small heparin dose, was low only in some uncontrolled severely diabetic patients (23), whereas most diabetics had normal activity (24). Recent studies using high-dose prolonged heparin infusion have demonstrated an abnormality in PHLA in all untreated hyperglycemic subjects examined (25).

[1] *Abbreviations used in this paper:* LPL, lipoprotein lipase; PHLA, postheparin plasma lipolytic activity; TG, triglycerides.

This suggests that decreased adipose tissue LPL availability may be a possibility in untreated human diabetes. The normal PHLA in diabetics receiving low-dose heparin may also be related to the complexity of PHLA; in addition to adipose tissue LPL, heparin releases a TG-splitting activity from liver (26, 27) and possibly LPL from muscle and other tissues into the circulation. Thus, total PHLA may not be an accurate index of adipose tissue LPL, which is the major determinant of peripheral TG removal (28).

In the present study, adipose tissue LPL was measured directly from human subcutaneous fat tissue, and the importance of insulin in the regulation of this enzyme activity was determined. Since adipose tissue LPL may exist in several forms and multiple compartments (2, 29), measurements of enzyme were made both in acetone-ether powder preparations (thought to represent the total tissue activity [2]), and after release from tissue by heparin (presumed to be the readily releasable fraction related to PHLA and plasma TG clearance [2, 30]). Because there is a close relationship among obesity, insulin and glucose metabolism (31), and hypertriglyceridemia (32), the role of obesity and the effect of feeding on adipose tissue LPL were evaluated by measurement of the enzyme activity after overnight fasting and after feeding with diets containing different amounts of carbohydrate. Results indicate that variability in insulin secretion, as seen in obesity, diabetes, and after feeding, may be an important determinant of human adipose tissue LPL.

METHODS

Subjects. Untreated diabetic subjects for this study consisted of eight patients with varying degrees of hyperglycemia, hypertriglyceridemia, and obesity (Table I). Four of these subjects had been treated previously with oral sulfonylurea agents, the other four had never been treated. Three of them had a concomitant familial form of hypertriglyceridemia as indicated by the presence of

TABLE I

Basic Clinical and Laboratory Data of Control and Diabetic Subjects

Subject	Age/Sex	IBW*	Plasma				Fat cell size	Adipose tissue LPL				Family‡ history hyper-TG
								Heparin releasable		Acetone-ether powder		
			Glucose	Insulin	TG	Cholesterol						
			mg/dl	*μU/ml*	*mg/dl*	*mg/dl*	*μg TG/cell*	*/g*	*/10⁶ cells*	*/g*	*/10⁶ cells*	
Control subjects												
P. R.	35, M	114	110	8	54	146	0.372	8.6	3.2	4.2	1.6	
R. C.	38, M	108	82	9	61	172	0.340	5.4	1.8	2.2	0.8	
O. P.	32, M	115	100	7	64	180	0.412	4.1	1.7	1.7	0.7	
J. H.	29, M	96	95	9	68	163	0.279	10.0	2.8	1.8	0.5	
J. B.	37, M	113	95	4	94	218	0.420	11.2	4.7	3.9	1.6	
E. L.	44, M	155	96	17	101	142	0.561	5.1	2.8	2.1	1.2	
S. W.	32, M	97	96	7	104	160	0.302	5.2	1.6	1.3	0.4	
P. S.	29, M	149	104	31	160	167	0.623	6.3	4.0	3.6	2.3	NS
A. G.	29, M	123	105	14	164	204	0.563	5.7	3.2	1.2	0.7	NS
T. H.	23, M	102	93	12	186	169	0.406	4.7	1.9	1.2	0.5	NS
G. S.	23, M	175	81	71	192	205	0.539	8.8	4.5	4.9	2.6	NS
S. K.	39, M	118	92	13	230	228	0.499	6.0	3.0	4.2	2.1	+
B. H.	46, M	122	84	19	559	220	0.451	1.2	0.5	0.3	0.1	+
R. R.	44, M	171	100	22	704	288	0.710	4.7	3.3	3.4	2.4	+
D. H.	53, M	110	96	30	900	292	0.222	3.4	0.7	2.7	0.6	+
Mean	36	125	95	18	243	197	0.446	6.0	2.7	2.6	1.2	
±SD	9	26	8	16	261	46	0.130	2.6	1.2	1.4	0.8	
Diabetic subjects												
W. G.	51, M	157	279	29	208	294	0.341	4.7	1.6	2.1	0.7	NS
C. S.	53, M	157	192	52	216	154	0.600	1.5	1.0	2.0	1.2	−
S. S.	30, F	175	224	63	356	206	0.600	3.0	1.8	2.6	1.6	−
A. K.	53, M	118	392	—	719	281	0.303	2.3	0.7	0.7	0.2	NS
J. S.	47, M	127	320	—	875	281	0.429	1.2	0.5	0.7	0.3	−
D. N.	40, M	115	137	26	1,125	324	0.331	2.4	0.8	1.5	0.5	+
C. G.	47, M	156	255	35	2,330	526	0.528	1.1	0.8	3.0	1.6	+
J. V.	42, M	176	250		5,470	456	0.358	1.6	0.6	1.6	0.6	+
Mean	45	147	256	41	1,412	315	0.436	2.2	1.0	1.8	0.8	
±SD	8	24	77	16	1,779	122	0.118	1.2	0.5	0.8	0.5	
$P <$§	0.02	0.05	0.001	0.02			NS	0.001	0.001	NS	NS	

* IBW = percent of ideal body weight.

‡ Family history of hypertriglyceridemia determined by measurement of cholesterol and TG levels in adult relatives. + = nondiabetic hypertriglyceridemic first-degree relatives present; − = no hyperlipidemic relatives found, at least four adult first-degree relatives studied; NS = family not studied or no relatives available for study.

§ $P <$ = statistical significance between control and diabetic subjects.

nondiabetic hypertriglyceridemic first-degree relatives in their families. Seven nondiabetic normolipidemic and eight nondiabetic hypertriglyceridemic subjects served as controls (Table I). Of the eight hypertriglyceridemic control subjects, four had a familial form of hypertriglyceridemia; the families of the remaining hypertriglyceridemic subjects have not been studied.

Experimental design. To assess the relationship among obesity, fat cell size, fasting plasma insulin, and adipose tissue LPL, the diabetic and control subjects were studied as outpatients during periods of weight stability. Blood was drawn for determinations of plasma glucose, immunoreactive insulin, TG, and cholesterol after an overnight 12-h fast. A sample of subcutaneous buttock adipose tissue was taken by needle aspiration to determine LPL activity and fat cell size. Four of the diabetic patients were restudied 1 wk after institution of therapy with an oral sulphonylurea and five of them after 10–12 wk of therapy with insulin or an oral sulphonylurea to test the effect of treatment of hyperglycemia on adipose tissue LPL.

Five control (E. L., S. K., B. H., R. R., and D. H.: Table I) and three diabetic (J. S., C. G., and C. S.) subjects were admitted to a metabolic ward for dietary studies. For 2 wk the control subjects were fed a weight-maintaining, constant composition liquid formula diet containing 40% of calories as fat (half corn oil, half butter fat), 45% carbohydrates (dextrose), and 15% protein (skim milk powder), and four of these subjects were subsequently fed a fat-free high-carbohydrate liquid formula diet (85% carbohydrate, 15% protein) for 2 wk. The diabetic patients were studied only on the fat-free formula diet. The feedings were divided into five equal parts and served at 8 a.m., 11 a.m., 2 p.m., 5 p.m., and 8 p.m. At the end of each dietary period, adipose tissue LPL was measured after a 12-h overnight fast and after ingestion of the formula diet (Table II). A similar study of the effect of feeding with fat-free formula on adipose tissue LPL was performed with two control (T. H. and G. S.) and two diabetic (D. N. and S. S.) subjects as outpatients on ad lib. diets. The subjects were kept in bed during the feeding studies; smoking and all intake except water and formula was prohibited.

Materials. Fatty acid-poor bovine serum albumin was obtained from Pentex Biochemical, Kankakee, Ill.; unlabeled triolein from Hormel Institute, Austin, Minn.; and glyceryl [1-^{14}C]triolein from the Radiochemical Centre, Amersham, England. The purity of triolein was over 99% as judged by thin-layer chromatography on silica gel. Purified egg lecithin was a gift from William C. Vogel.

TABLE II
Schedule for Feeding Studies

Samples	Fasting	During Feeding, *h* 1	2	3	4	5	6
Adipose tissue	x			x			x
Plasma glucose	x	x	x	x	x	x	x
Plasma insulin	x	x	x	x	x	x	x
Plasma TG	x			x			x
Plasma cholesterol	x			x			x
Feeding (20% of daily calories each time)	Δ		Δ			Δ	

Assay of adipose tissue LPL activity. The tissue yield from the subcutaneous adipose tissue biopsy, performed by the method of Hirsch and Goldrick (33), was usually 80–120 mg. After rinsing in approximately 100 ml of Krebs-Ringer phosphate buffer, pH 7.4, the tissue pieces were divided into three parts. One part (5–10 mg) was used for measurement of fat cell diameter from a formaldehyde-fixed frozen section according to Sjöström et al. (34). Fat cell volume was calculated from fat cell diameter according to Goldrick (35) and fat cell size (weight) by multiplying the cell volume by the specific gravity of triolein (34). The other parts of tissue (40–50 mg each) were used to measure the LPL activity in two different ways: (*a*) as heparin-releasable LPL, and (*b*) as LPL in ammonium hydroxide extract of acetone-ether powder. One piece of tissue was incubated for 45 min in Krebs-Ringer phosphate buffer at 37°C in the presence of heparin (2 U/ml). After extraction of the final adipose tissue piece, the enzyme was extracted with 0.05 M NH_4OH-NaCl without added heparin. The enzyme activities of the incubation medium and the acetone powder extract were assayed as previously described using triolein emulsified with lecithin as the substrate (36). Strict adherence to the procedure of sonication of the substrate minimized day-to-day variability in substrate. Heating during sonication was avoided by keeping the tube in ice and sonicating intermittently (10 s sonication, 10 s pause, repeated 10 times). To evaluate interassay variability, a pooled postheparin plasma sample was measured in parallel with each adipose tissue LPL assay: 10 μl of postheparin plasma and 200 μl of the substrate were incubated and free fatty acids (FFA) extracted. PHLA values usually differed by less than 10%. On two occasions, when this limit was exceeded, the results were corrected to the corresponding mean pool PHLA values. LPL activity was expressed both on a per gram (neq FFA/g per min) and on a per cell (neq FFA/10^6 fat cells per min) basis (36).

Other methods. Plasma glucose was measured by the Technicon autoanalyzer ferricyanide method (Technicon Instruments Corp., Tarrytown, N. Y.), and plasma TG[2] and cholesterol as previously described (37). Plasma immunoreactive insulin was measured by a modification of the double antibody method of Morgan and Lazarow (38).

Obesity was estimated from the percent of ideal body weight, calculated from the mean ideal body weight for height from Metropolitan Life Insurance tables. Statistical evaluation of data was performed by analysis of variance, by least squares regression, and covariance analysis (39).

RESULTS

Effect of diabetes on adipose tissue LPL. The heparin-releasable LPL activity of adipose tissue in control subjects (2.7±1.2 neq FFA/10^6 cells per min; mean ±SD) was significantly higher than in untreated diabetic subjects (1.0±0.5 neq FFA/10^6 cells per min; $P < 0.001$; Table I). This difference was also statistically significant when the activity was expressed on a per gram basis ($P < 0.001$) or when diabetics (with and without concomitant famalial forms of hypertriglyceridemia) were compared to normolipidemic

[2] Normal age adjusted levels for plasma TG in this laboratory are below 165 mg/dl for a 45-yr-old male or female.

($P < 0.02$) or to hyperlipidemic control subjects ($P < 0.02$). The acetone-ether powder LPL activity tended to be lower in the diabetic patients than in the control subjects, but this difference was not statistically significant (Table I). The heparin-releasable LPL activity was a function of the activity in acetone-ether powder in nondiabetic individuals ($r = 0.78$; $P < 0.001$; Fig. 1). All the untreated diabetics fell below this regression line for nondiabetic controls due to the disproportionate decrease in heparin-releasable activity in diabetes (Fig. 1).

Short-term treatment of hyperglycemia (1 wk) in the diabetics was associated with a 35% decrease in plasma glucose ($P < 0.02$), but did not affect the heparin-releasable LPL or acetone-ether powder LPL. The changes in plasma TG were variable; in two very lipemic patients they decreased related to the simultaneous initiation of a fat-free diet, which was necessary for relief of abdominal pain in these patients. In contrast, long-term treatment of diabetes (10-12 wk) with insulin or chlorpropamide consistently increased adipose tissue heparin-releasable LPL activity (Table III). The increase of acetone-ether powder LPL did not reach statistical significance. Plasma glucose and TG decreased significantly in all patients, while weight increased slightly.

Effect of obesity on adipose tissue LPL. When acetone-ether powder LPL activity, expressed per 10^6 cells, in nondiabetic and untreated diabetic subjects was compared to two parameters of obesity, percent of ideal body weight and fat cell size, there was a significant correlation between these indices of obesity and LPL activity among both controls and diabetics (Fig. 2). The regression lines for diabetics were parallel to those for controls (identical slopes). In the case of percent of ideal body weight, the regression line for diabetics fell significantly below that for controls (Fig. 2A), indicating that for a given degree of obesity, diabetics have lower acetone-ether powder LPL. The LPL activity per 10^6 cells was significantly related to fasting insulin levels ($r = 0.59$; $P < 0.02$). If the LPL activities in acetone-ether powder were expressed per gram of wet weight of adipose tissue instead of per cell, the above correlations were not found.

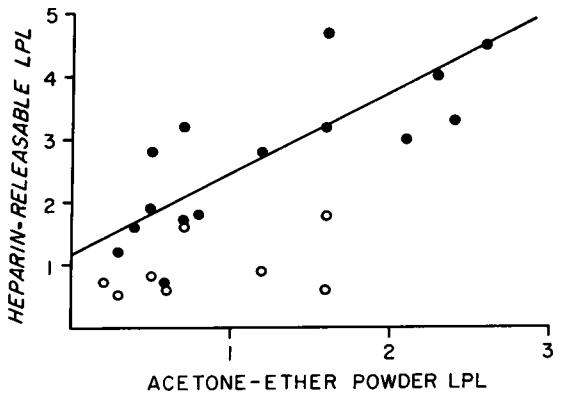

FIGURE 1 Relationship between acetone-ether powder LPL and heparin-releasable LPL among control and untreated diabetic subjects. Controls (●); Diabetics (○). The activities are expressed as neq FFA/10^6 cells per min. The slope $y = 1.21x - 1.18$ ($r = 0.78$; $P < 0.001$) is for control subjects.

TABLE III

Effect of Long-Term (10–12 wk) Treatment of Diabetes on Adipose Tissue LPL, Plasma Glucose, and Plasma TG

	Adipose tissue LPL, *neq FFA/10^6 cells/min*				Plasma glucose, *mg/dl*		Plasma TG, *mg/dl*	
	Heparin releasable		Acetone-ether powder					
Subject	Before	After	Before	After	Before	After	Before	After
C. S.	1.0	1.6	1.2	1.7	192	120	216	121
S. S.	1.8	8.7	1.6	2.6	224	162	356	226
J. S.	0.5	1.7	0.3	1.0	320	162	875	168
C. G.	0.8	2.0	1.6	1.4	225	164	2,330	2,080
J. V.	0.6	3.3	0.6	1.3	250	91	5,470	825
Mean±SD	0.9±0.5	3.5±2.9	1.1±0.6	1.6±0.6	248±47	140±33	1,849±2,190	684±372
Percentage change								
Mean±SD		+257±161		+85±95		−43±14		−51±31
$P <$		0.025		NS		0.01		0.025

Diabetic patients were studied before treatment and after 10–12 wk treatment of diabetes. The therapy was started in hospital in all subjects except S. S. and then continued as outpatients. Treatment was started with chlorpropamide in all patients. However, after 2 wk treatment J. S. was changed to insulin therapy.

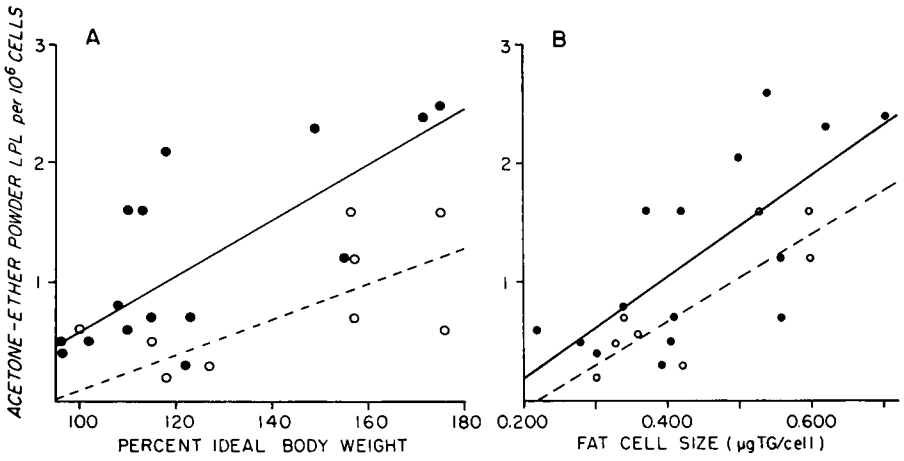

FIGURE 2 Relationship between acetone-ether powder LPL and percent of ideal body weight (A) or fat cell size (B) in control and untreated diabetic subjects. Controls (● and solid line); diabetics (○ and dashed line).

Group	y	x	Equation	r	P	Difference in Slopes	Difference in Elevation
Control	LPL	Ideal body wt	$y = 0.024x - 1.76$	0.76	<0.001	NS	<0.01
Diabetic	LPL	Ideal body wt	$y = 0.015x - 1.42$	0.67	<0.05		
Control	LPL	Cell size	$y = 4.19x - 0.63$	0.71	<0.01	NS	NS
Diabetic	LPL	Cell size	$y = 3.87x - 0.85$	0.85	<0.01		

The heparin-releasable LPL activity from control subjects, when expressed per 10^6 cells, was also related to percent of ideal body weight and fat cell size, although the correlations were weaker than with acetone-ether powder LPL (Fig. 3). Among untreated diabetic subjects, in contrast, heparin-releasable LPL was always low and not significantly related to these parameters of obesity. Both fat cell size ($r = 0.85$; $P < 0.001$) and fasting insulin levels ($r = 0.70$; $P < 0.01$) were correlated with percent of ideal body weight.

Effect of feeding on adipose tissue LPL. The feeding of a fat-containing formula diet to control subjects after

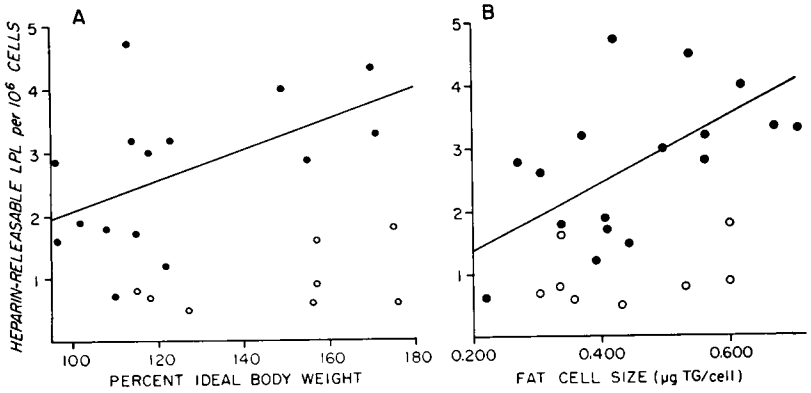

FIGURE 3 Relationship between heparin-releasable LPL and percent of ideal body weight (A) or fat cell size (B) in control and untreated diabetic subjects. Controls (●); diabetics (○). The slopes are for control subjects. Slope in A is $y = 0.025x - 0.36$ ($r = 0.53$; $P < 0.05$) and slope in B is $y = 5.30x - 0.35$ ($r = 0.61$; $P < 0.02$).

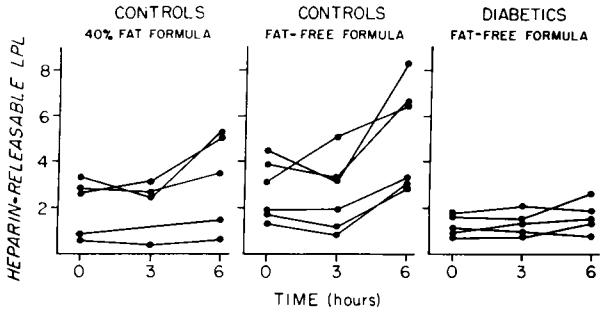

FIGURE 4 Effect of feeding of diets of different composition on adipose tissue heparin-releasable LPL activity in control and diabetic subjects. Mean fasting levels (fat-free diet): controls 2.8±1.3; diabetics 1.2±0.5 ($P < 0.02$). The enzyme activity is expressed as neq FFA/10^6 cells per min.

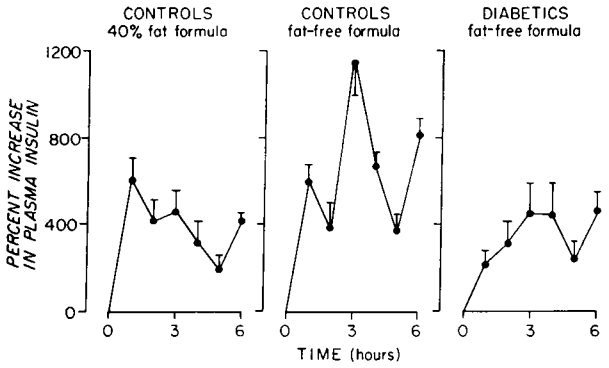

FIGURE 6 Effect of feeding of diets of different composition on plasma insulin in control and diabetic subjects. The results are plotted as mean (±SD) of the percent change above basal fasting value.

an overnight fast did not cause a significant increase in heparin-releasable LPL (47% increase; Fig. 4) or acetone-ether powder LPL (20% increase; Fig. 5). The mean plasma insulin level increased 408% (Fig. 6) and plasma glucose 22% above basal levels during feeding. In contrast, when control subjects were fed a fat-free carbohydrate-rich formula diet, there were marked increases in heparin-releasable LPL (89%; $P < 0.01$; Fig. 4) and acetone-ether powder LPL (80%; $P < 0.02$; Fig. 5). These changes occurred independently of the presence or absence of a familial form of hypertriglyceridemia. Plasma insulin increased 664% (Fig. 6) and plasma glucose 35% above basal levels during feeding with this diet. While the plasma glucose responses were about the same, the insulin responses were significantly higher on the fat-free carbohydrate-rich diet ($P < 0.01$). In contrast to the results in control subjects, heparin-releasable LPL and acetone-ether powder LPL did not increase significantly on the fat-free high carbohydrate diet in diabetic subjects (mean increases 32 and 31%, respectively; Figs. 4 and 5). The plasma insulin response was significantly lower in the diabetics as compared to control subjects (mean increase above basal level 346% in diabetics compared to 664% in controls; $P < 0.01$). Only one of the diabetics, an obese hyperglycemic patient (C. S.; Table III), was studied after treatment with chlorpropamide, which not only normalized the fasting plasma glucose, but also appeared to result in near normal LPL and insulin responses to feeding (Figs. 4 and 5). In control subjects the LPL responses to feeding were not affected by obesity. When the data from all subjects were pooled, there was a significant correlation between the increments in heparin-releasable LPL ($r = 0.66$; $P < 0.01$) (Fig. 7B), or acetone-ether powder LPL $r = 0.69$; $P < 0.01$ (Fig. 7A), and percent increase in plasma insulin during feeding. If the changes in insulin were expressed

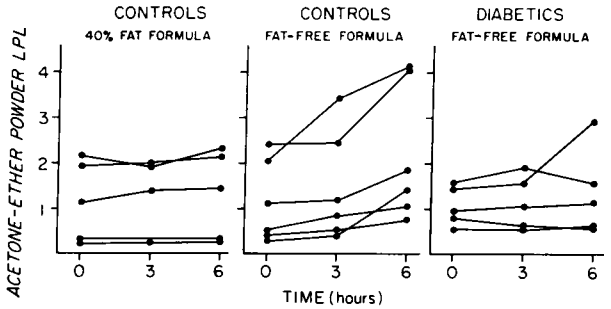

FIGURE 5 Effect of feeding of diets of different composition on adipose tissue acetone-ether powder LPL activity in control and diabetic subjects. The enzyme activity is expressed as neq FFA/10^6 cells per min.

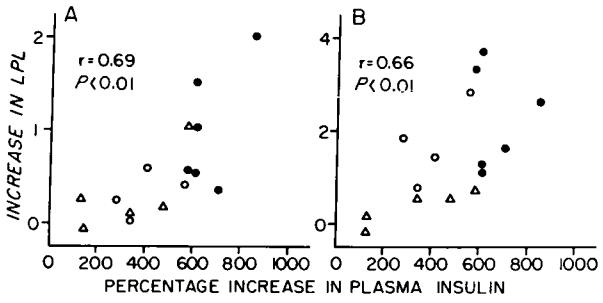

FIGURE 7 Relationship between increase in plasma insulin and adipose tissue LPLs during feeding. Controls fed fat-free diet (●); controls fed fat-containing diet (○); diabetics fed fat-free diet (△). Changes in plasma insulin during feeding has been calculated as follows: the mean value of 1, 2, 3, 4, 5, and 6 h plasma insulins after starting of feeding has been expressed as percent increase above basal fasting insulin for each subject. The LPL activity is expressed as neq FFA/10^6 cells per min.

in absolute instead of relative values, these correlations would be lost.

DISCUSSION

Results in the present study help to clarify the role of human adipose tissue LPL in hypertriglyceridemic states characterized by alterations in insulin secretion. On the basis of other studies with diabetic human subjects and animal models, it has been suggested that the mechanism of hypertriglyceridemia in diabetes is increased splanchnic TG production, decreased plasma TG removal, or a combination of both (40, 41). In animals in vivo the results are particularly dependent on the duration of insulin deficiency. After the acute induction of experimental diabetes, FFA esterification (42), the amount of very low density lipoprotein particles in the liver (43), and the rate of TG secretion (43) are all increased, even though at this stage impaired TG removal may also contribute to the hypertriglyceridemia (43). In animals with experimental diabetes for more than several hours, splanchnic TG secretion is decreased and impaired TG removal seems to be the sole mechanism of the hypertriglyceridemia (43). Thus, both duration and degree of insulin deficiency appear to be important variables in determining whether or not alterations in TG removal play the dominant role.

The peripheral removal of plasma TG seems to be related to the activity of LPL, located in tissues near or at capillary endothelium (2, 30). Measurements of tissue LPL activity in diabetic rats have shown a decrease in association with insulin deficiency, that was restored to normal by treatment with insulin (12, 13). Moreover, in vitro LPL synthesis in rat adipose tissue was promoted by insulin (9, 11 44), suggesting that this hormone is essential for the maintenance of normal adipose tissue LPL activity, and supports previous conclusions concerning LPL in diabetes based on PHLA (25, 45). In the present study, adipose tissue LPL was found to be low in untreated diabetic patients. In particular, the heparin-releasable LPL activity, which correlates best with tissue uptake of TGs (4, 7, 46), was decreased. This suggests that the entry of circulating TG into adipose tissue was impaired in these patients. A decrease in plasma TG and a concomitant increase in adipose tissue LPL during therapy further supports the concept that the hypertriglyceridemia among these diabetic patients was caused at least in part by defective TG removal by this tissue. Since LPL is present in many other tissues, e.g., heart, diaphragm, lung, and mammary gland (1-3), adipose tissue may not be the only peripheral organ responsible for TG removal. Thus, on the basis of the present study, apart from stressing adipose tissue participation, one cannot evaluate quantitatively the degree of defective TG removal in diabetic humans. The present results do not exclude the contribution of increased splanchnic TG production to the development of hypertriglyceridemia in diabetes, especially in those who were obese or had a familial form of hypertriglyceridemia (47-49). Thus, patient selection would be a major factor influencing the interpretation of the mechanism of hypertriglyceridemia in diabetes.

Since there is evidence for the existence of several forms of LPL in rat adipose tissue, LPL activity in ammonium extracts of acetone-ether powder was measured. This activity appears to more closely represent the total LPL activity in adiopse tissue (2). It behaved differently from heparin-releasable LPL in diabetes: acetone-ether LPL was not significantly decreased in the untreated state and treatment of diabetes did not increase activity significantly. There are at least three possible explanations for this difference: (*a*) since diabetic patients in this study were, as a group, more obese than controls, and since this study also shows that acetone-ether powder LPL is related to obesity, increased fat cell size may counteract the effect of diabetes on acetone-ether powder LPL; (*b*) the release of LPL from tissue by heparin is an energy-requiring process (50), which may partially explain the particular dependence of heparin-releasable LPL on glucose and insulin metabolism, and (*c*) and the enzyme composition of these two preparations may be different. In animal adipose tissue two forms of LPL exist: one is apparently related to newly synthesized enzyme, and the second can be readily displaced by heparin and may be the product of the former (29, 51). The acetone-ether powder LPL in our study may measure mainly the first enzyme form and the heparin-releasable predominantly the second form.

Adipose tissue LPL per 10^6 fat cells was directly correlated with obesity and with the increased insulin levels associated with obesity. Thus, obese subjects with basal hyperinsulinism had significantly higher LPL activity in their fat cells. This would indicate that the larger fat cells in these obese subjects were not "resistant" to the effects of circulating insulin as far as LPL is concerned.

Since the number of fat cells appears to be normal in adult onset obesity (52, 53), the expression of LPL activity per cell may provide a quantitative evaluation of the plasma TG removal system of adipose tissue in adults with varying degrees of obesity (36). If so, the plasma TG removal capacity, as related to adipose tissue, would appear to be increased in obesity. There is evidence from animal studies indicating that obesity is accompanied by increased splanchnic TG production (47), which when coupled with increased removal, may explain the mild elevation of plasma TG associated with obesity.

The changes in adipose tissue LPL after food intake also appeared to be related to changes in insulin. Food intake caused variable responses in adipose tissue LPL among individual subjects, as shown recently in another human study (54). The magnitude of insulin response to feeding may be a main determinant of the increase in adipose tissue LPL activity. Thus, in control subjects, the rise of both plasma insulin and adipose tissue LPL activity was dependent upon the amount of carbohydrate in the diet. In untreated diabetic patients, in the absence of adequate insulin response, even high carbohydrate feeding was not associated with an increase in adipose LPL activity. Furthermore, when all the results from dietary studies were pooled, the percentage increment in plasma insulin and the rise in adipose tissue LPL were significantly correlated. Since the control and diabetic subjects represented varying degrees of obesity, it is not surprising that there was no correlation between the induction of LPL and the absolute changes in insulin. It has been previously shown that the effect of obesity on insulin levels can be eliminated as a variable, if the changes in plasma insulin are expressed as percent from basal level instead of absolute changes (55).

Although the results of this study indicate that insulin has an effect on adipose tissue LPL activity, it must be emphasized that the final determinant of adipose tissue LPL may merely be the balance between insulin and various lipolytic hormones, particularly those related to the diabetic state, such as glucagon (56) and catecholamines (57). It is known from human studies that this hormonal balance also varies with the nutritional state (58). Previous animal studies have shown that the amount of insulin and catecholamines in vitro, e.g. in the incubation medium, determines the rate of the synthesis of adipose tissue LPL (9, 11, 44, 59, 60).

Although the main purpose of the present study was to assess adipose tissue LPL in diabetes and obesity, some hypertriglyceridemic nondiabetic control subjects were included for two reasons: (*a*) some of the diabetic patients had one of the familial forms of hypertriglyceridemia, and (*b*) the elevations of plasma TG, per se, may lead to decreased adipose tissue LPL as a result of elution of endothelial enzyme into the circulation (61). Since (*a*) treatment of diabetes was always accompanied by an increase in adipose tissue LPL regardless of the presence or absence of familial hypertriglyceridemia, and (*b*) there was no relationship between LPL activity and plasma TG among control and diabetic subjects, the untreated diabetes, as such, seemed to be the primary reason for low LPL activities found in untreated diabetic patients.

In conclusion, results in the present study demonstrate an association among human adipose tissue LPL activity, fat cell size, and insulin. Insulin appears to be an important hormone for the maintenance of normal adipose tissue LPL activity and may be responsible for the increase in enzyme activity in enlarged fat cells. Changes in plasma insulin after food intake seem to result in a rapid adaptation of adipose tissue LPL. Consequently, the peripheral TG removal system becomes sensitive to fluctuations in caloric balance. Insulin deficiency, as seen in untreated diabetic subjects, appears to result in low adipose tissue LPL activity both in the fasting state and after eating. This seems to be an important factor in the development of hypertriglyceridemia of diabetes in man.

ACKNOWLEDGMENTS

The authors would like to express their gratitude to Ms. Martha Kimura, Martha Pleasant, Shirley Corey, Mary Stewart, and Dr. Jean Leik for their assistance in this study.

This work was partially supported by NIH project grant AM 06670, training grant AM 05498, and fellowship AM 03433 (U. S. Public Health Service). The travel of Dr. Pykälistö to the United States was partially supported by the Duodecim Foundation, Helsinki, Finland.

REFERENCES

1. Korn, E. D. 1955. Clearing factor, a heparin-activated lipoprotein lipase. I. Isolation and characterization of the enzyme from normal rat heart. *J. Biol. Chem.* **215**: 1–14.
2. Robinson, D. S. 1963. The clearing factor lipase and its action in the transport of fatty acids between the blood and the tissues. *Adv. Lipid Res.* **1**: 133–182.
3. Pykälistö, O. J., W. C., Vogel, and E. L. Bierman. 1974. The tissue distribution of triacylglycerol lipase, monoacylglycerol lipase and phospholipase A in fed and fasted rats. *Biochim. Biophys. Acta.* **369**: 254–263.
4. Bezman, A., J. M. Felts, and R. J. Havel. 1962. Relation between incorporation of triglyceride fatty acids and heparin-released lipoprotein lipase from adipose tissue slices. *J. Lipid Res.* **3**: 427–431.
5. Garfinkel, A. S., N. Barker, and M. C. Schotz. 1967. Relationship of lipoprotein lipase activity to triglyceride uptake in adipose tissue. *J. Lipid Res.* **8**: 274–280.
6. Nestel, P. J., W. Austin, and C. Foxman. 1969. Lipoprotein lipase content and triglyceride fatty acid uptake in adipose tissue of rats of differing body weights. *J. Lipid Res.* **10**: 383–387.
7. Borensztajn, J., and D. S. Robinson. 1970. The effect of fasting on the utilization of chylomicron triglyceride fatty acids in relation to clearing factor lipase (lipoprotein lipase) releasable by heparin in the perfused rat heart. *J. Lipid Res.* **11**: 111–117.
8. Robinson, D. S. 1960. The effect of changes in nutritional state on the lipolytic activity of rat adipose tissue. *J. Lipid Res.* **1**: 332–338.
9. Wing, D. R., M. R. Salaman, and D. S. Robinson. 1966. Clearing-factor lipase in adipose tissue. Factors influencing the increase in enzyme activity produced on incubation of tissue from starved rats *in vitro*. *Biochem. J.* **99**: 648–656.

10. Pokrajac, N., W. J. Lossow, and I. L. Chaikoff. 1967. The effect of nutritional state on lipoprotein lipase activity in isolated rat adipose tissue cells. *Biochim. Biophys. Acta.* **139**: 123–132.
11. Pykälistö, O. 1970. Regulation of adipose tissue lipoprotein lipase by free fatty acids. Doctorate Thesis. University of Helsinki, Helsinki, Finland.
12. Schnatz, J. D., and R. H. Williams. 1963. The effect of acute insulin deficiency in the rat on adipose tissue lipolytic activity and plasma lipids. *Diabetes.* **12**: 174–178.
13. Kessler, J. I. 1963. Effect of diabetes and insulin on the activity of myocardial and adipose tissue lipoprotein lipase of rats. *J. Clin. Invest.* **42**: 362–367.
14. Persson, B. 1973. Lipoprotein lipase activity of human adipose tissue in health and in some diseases with hyperlipidemia as a common feature. *Acta Med. Scand.* **193**: 457–462.
15. Frederickson, D. S., K. Ono, and L. L. Davis. 1963. Lipolytic activity of post-heparin plasma in hyperglyceridemia. *J. Lipid Res.* **4**: 24–33.
16. Porte, D., Jr., D. D. O'Hara, and R. H. Williams. 1966. The relation between postheparin lipolytic activity and plasma triglyceride in myxedema. *Metab.* (*Clin. Exp.*). **15**: 107–113.
17. Bagdade, J. D., D. Porte, Jr., and E. L. Bierman. 1968. Hypertriglyceridemia. A metabolic consequence of chronic renal failure. *N. Engl. J. Med.* **279**: 181–185.
18. Glueck, C. J., A. P. Kaplan, R. I. Levy, H. Greten, H. Gralnick, and D. S. Fredrickson. 1969. A new mechanism of exogenous hyperglyceridemia. *Ann. Intern. Med.* **71**: 1051–1062.
19. Man, E. B., and J. P. Peters. 1935. Serum lipoids in diabetes. *J. Clin. Invest.* **14**: 579–594.
20. Albrink, M. J., and E. B. Man. 1958. Serum triglycerides in health and diabetes. *Diabetes.* **7**: 194–201.
21. New, M. I., T. N. Roberts, E. L. Bierman, and G. G. Reader. 1963. The significance of blood lipid alterations in diabetes mellitus. *Diabetes.* **12**: 208–212.
22. Nikkilä, E. A. 1973. Triglyceride metabolism in diabetes mellitus. *Prog. Biochem. Pharmacol.* **8**: 271–299.
23. Bagdade, J. D., D. Porte, Jr., and E. L. Bierman. 1967. Diabetic lipemia. A form of acquired fat-induced lipemia. *N. Engl. J. Med.* **276**: 427–433.
24. Wilson, D. E., P. H. Schreibman, and R. A. Arky. 1969. Post-heparin lipolytic activity in diabetic patients with a history of mixed hyperlipemia. Relative rates against artificial substrates and human chylomicrons. *Diabetes.* **18**: 562–566.
25. Brunzell, J. D., D. Porte, Jr., and E. L. Bierman. 1975. Reversible abnormalities in post-heparin lipolytic activity during the late phase of release in diabetes mellitus. *Metab.* (*Clin. Exp.*). **24**: 1123–1138.
26. Ehnholm, C., W. Shaw, W. Harlan, and V. Brown. 1973. Separation of two types of triglyceride lipase from human postheparin plasma. *Circulation.* **18**(Suppl. IV): 443. (Abstr.)
27. Krauss, R. M., R. I. Levy, and D. S. Fredrickson. 1974. Selective measurement of two lipase activities in postheparin plasma from normal subjects and patients with hyperlipoproteinemia. *J. Clin. Invest.* **54**: 1107–1124.
28. Robinson, D. S. 1970. Assimilation, distribution, and storage. The function of plasma triglycerides in fatty acid transport. *Compr. Biochem.* **18**: 51–116.
29. Garfinkel, A. S., and M. C. Schotz. 1972. Separation of molecular species of lipoprotein lipase from adipose tissue. *J. Lipid Res.* **13**: 63–68.
30. Blanchette-Mackie, E. J., and R. O. Scow. 1971. Sites of lipoprotein lipase activity in adipose tissue perfused with chylomicrons. Electron microscopic cytochemical study. *J. Cell Biol.* **51**: 1–25.
31. Bierman, E. L., J. D. Bagdade, and D. Porte, Jr. 1968. Obesity and diabetes: The odd couple. *Am. J. Clin. Nutr.* **21**: 1434–1437.
32. Sims, E. A. H., R. F. Goldman, C. M. Gluck, E. S. Horton, P. C. Kelleher, and D. W. Rowe. 1968. Experimental obesity in man. *Trans. Assoc. Am. Physicians Phila.* **81**: 153–170.
33. Hirsch, J., and R. B. Goldrick. 1964. Serial studies on the metabolism of human adipose tissue. I. Lipogenesis and free fatty acid uptake and release in small aspirated samples of subcutaneous fat. *J. Clin. Invest.* **43**: 1776–1792.
34. Sjöström, L., P. Björntorp, and J. Vrána. 1971. Microscopic fat cell size measurements of frozen-cut adipose tissue in comparison with automatic determinations of osmium-fixed fat cells. *J. Lipid Res.* **12**: 521–530.
35. Goldrick, R. B. 1967. Morphological changes in the adipocyte during fat deposition and mobilization. *Am. J. Physiol.* **212**: 777–782.
36. Pykälistö, O. J., P. H. Smith, and J. D. Brunzell. 1975. Human adipose tissue lipoprotein lipase: comparison of assay methods and expressions of activity. *Proc. Soc. Exp. Biol. Med.* **148**: 297–300.
37. Bierman, E. L., D. Porte, Jr., D. D. O'Hara, M. Schwartz, and F. C. Wood, Jr. 1965. Characterization of fat particles in plasma of hyperlipidemic subjects maintained on fat-free high-carbohydrate diets. *J. Clin. Invest.* **44**: 261–270.
38. Samols, E., and D. Bilkus. 1964. A comparison of insulin immunoassays. *Proc. Soc. Exp. Biol. Med.* **115**: 79–84.
39. Snedecor, G. W., and W. G. Cochran. 1967. Statistical methods. The Iowa State University Press, Ames, Iowa. 6th edition. 432–436.
40. Nikkila, E. A., and M. Kekki. 1973. Plasma triglyceride transport kinetics in diabetes mellitus. *Metab.* (*Clin. Exp.*). **22**: 1–22.
41. Kissebah, A. H., P. W. Adams, and V. Wynn. 1974. Inter-relationship between insulin secretion and plasma free fatty acid and triglyceride transport kinetics in maturity onset diabetes and the effect of phenethylbiguanide (Phenformin). *Diabetologia.* **10**: 119–130.
42. Balasse, E. O., D. M. Bier, and R. J. Havel. 1972. Early effects of anti-insulin serum on hepatic metabolism of plasma free fatty acids in dogs. *Diabetes.* **21**: 280–288.
43. Reaven, E. P., and G. M. Reaven. 1974. Mechanisms for development of diabetic hypertriglyceridemia in streptozotocin-treated rats. Effect of diet and duration of insulin deficiency. *J. Clin. Invest.* **54**: 1167–1178.
44. Patten, R. L. 1970. The reciprocal regulation of lipoprotein lipase activity and hormone-sensitive lipase activity in rat adipocytes. *J. Biol. Chem.* **245**: 5577–5584.
45. Bagdade, J. D., D. Porte, Jr., and E. L. Bierman. 1968. Acute insulin withdrawal and the regulation of plasma triglyceride removal in diabetic subjects. *Diabetes.* **17**: 127–132.
46. Rogers, M. P., and D. S. Robinson. 1974. Effects of cold exposure on heart clearing factor lipase and triglyceride utilization in the rat. *J. Lipid Res.* **15**: 263–272.
47. Robertson, R. P., D. J. Gavareski, J. D. Henderson, D. Porte, Jr., and E. L. Bierman. 1973. Accelerated tri-

glyceride secretion. A metabolic consequence of obesity. *J. Clin. Invest.* **52**: 1620–1626.
48. Reaven, G. M., D. B. Hill, R. C. Gross, and J. W. Farquhar. 1965. Kinetics of triglyceride turnover of very low density lipoproteins of human plasma. *J. Clin. Invest.* **44**: 1826–1833.
49. Brunzell, J. D., W. R. Hazzard, A. G. Motulsky, and E. L. Bierman. 1975. Evidence for diabetes mellitus and genetic forms of hypertriglyceridemia as independent entities. *Metab. (Clin. Exp.).* **24**: 1115–1122.
50. Stewart, J. E., and M. C. Schotz. 1971. Studies on release of lipoprotein lipase activity from fat cells. *J. Biol. Chem.* **246**: 5749–5753.
51. Garfinkel, A. S., and M. C. Schotz. 1973. Sequential induction of two species of lipoprotein lipase. *Biochim. Biophys. Acta.* **306**: 128–133.
52. Salans, L. B., S. W. Cushman, and R. E. Weismann. 1973. Studies of human adipose tissue. Adipose cell size and number in nonobese and obese patients. *J. Clin. Invest.* **52**: 929–941.
53. Sjöström, L., and P. Björntorp. 1974. Body composition and adipose tissue cellularity in human obesity. *Acta Med. Scand.* **195**: 201–211.
54. Nilsson-Ehle, P. 1974. Human lipoprotein lipase activity: comparison of assay methods. *Clin. Chim. Acta.* **54**: 283–291.
55. Bagdade, J. D., E. L. Bierman, and D. Porte, Jr. 1967. The significance of basal insulin levels in the evaluation of the insulin response to glucose in diabetic and nondiabetic subjects. *J. Clin. Invest.* **46**: 1549–1557.
56. Unger, R. H. 1972. Glucagon and diabetes mellitus. *Adv. Metab. Disord.* **6**: 73–98.
57. Christensen, N. J. 1974. Plasma norepinephrine and epinephrine in untreated diabetes, during fasting and after insulin administration. *Diabetes.* **23**: 1–8.
58. Goodner, C. J., and D. Porte, Jr. 1972. Determinants of basal islet secretion in man. *Handb. Physiol.* **7**: 597–609.
59. Nikkila, E. A., and O. Pykälistö. 1968. Regulation of adipose tissue lipoprotein lipase synthesis by intracellular free fatty acid. *Life Sci.* **7**(Pt. II): 1303–1309.
60. Nestel, P. J., and W. Austin. 1969. Relationship between adipose lipoprotein lipase activity and compounds which affect intracellular lipolysis. *Life Sci.* **8**(Pt. II): 157–164.
61. Shafrir, E., and Y. Bialɛ. 1970. Effect of experimental hypertriglyceridaemia on tissue and serum lipoprotein lipase activity. *Eur. J. Clin. Invest.* **1**: 19–24.