Insulin Stimulation of Adipose Tissue Lipoprotein Lipase

USE OF THE EUGLYCEMIC CLAMP TECHNIQUE

CRAIG N. SADUR and ROBERT H. ECKEL, *Division of Endocrinology, Department of Medicine, University of Colorado Health Sciences Center, Denver, Colorado 80262*

ABSTRACT The role of insulin in the regulation of adipose tissue lipoprotein lipase activity in humans was investigated in 11 normal subjects and compared with the effects of 0.9% saline infusions in five control subjects. After a basal adipose tissue biopsy for lipoprotein lipase activity, insulin was rapidly infused to achieve and maintain serum levels of ~70 μU/ml while plasma glucose was kept at basal concentrations. Free fatty acids in serum fell to 27±3% of basal by 20 min ($t = 5.19$, $P < 0.001$) and triglycerides decreased to 77±3% of basal by 80 min ($t = 3.76$, $P < 0.01$). Adipose tissue lipoprotein lipase activity failed to increase significantly above that measured in controls by the first 3 h of the study. By 6 h of the infusion a stimulatory effect of insulin on adipose tissue lipoprotein lipase was found ($t = 3.94$, $P < 0.01$). There was no relationship between the amount of glucose infused and the insulin effect on the enzyme. The increase in adipose tissue lipoprotein lipase activity at 6 h, however, was inversely related to the basal lipase activity ($r = -0.690$, $P < 0.02$). Thus, insulin appears to stimulate adipose tissue lipoprotein lipase activity in humans. This effect of insulin is delayed when compared with antilipolysis and the fall in plasma triglyceride. The inverse relationship between insulin-stimulated adipose tissue lipoprotein lipase activity and basal enzyme activity suggests that adipose tissue itself is the main regulator of the lipase response to insulin.

INTRODUCTION

Adipose tissue lipoprotein lipase (ATLPL)[1] is an enzyme secreted from the fat cell and transported to an area on or near the capillary endothelium, where it hydrolyzes triglyceride (TG) into glycerol and free fatty acids (FFA) (1). These FFA are taken up, in turn, by the fat cell, where they are reesterified and stored as TG. This mechanism is responsible for nearly all the deposition of TG in adipose tissue stores (2, 3).

Thus far, the hormonal regulation of ATLPL has not been clarified. In both, rat adipose tissue (4) and cultured preadipocytes (5, 6), in vitro studies have demonstrated a stimulatory effect of insulin on ATLPL. Likewise, administration of glucose and/or insulin to experimental animals has resulted in an increase in the adipose tissue enzyme activity (7, 8). For instance, Borensztajn et al. (7) have shown that glucose administration increased ATLPL levels, an effect attributed to postprandial hyperinsulinemia. To test the role of insulin, they injected the hormone intraperitoneally into starved rats, producing a fivefold increase in enzyme activity by 3 h. The authors were, however, unable to distinguish a primary effect of insulin from other effects associated with insulin-mediated glucose metabolism.

In humans, there is more than a suggestive role of an insulin-stimulatory effect on the enzyme. Type I diabetic patients with poor glucose control have depressed levels of ATLPL and postheparin plasma lipoprotein lipase activity (9). With chronic administration of insulin and adequate glycemic control (9), enzyme activity in type I diabetics is normal (9, 10). Type II diabetics also have lower ATLPL during periods of suboptimal glucose control (11, 12). Pykälistö et al. (11) found that the heparin-releasable (presumably functionally available) ATLPL activity, which was significantly lower in untreated type II diabetics than in controls, did not significantly change in patients after 1 wk of either insulin or chlorpropamide therapy, despite an improvement in glucose tolerance. ATLPL significantly rose, however, after those same diabetics were treated for 10 to 12 wk. In contrast, in hypertriglyceridemic, obese diabetics, Taylor et al. (12) have shown that 1 wk of insulin therapy increased

---

Portions of this work were presented at the Annual Meeting of the American Federation for Clinical Research, San Francisco, CA, April 1981.

Received for publication 8 May 1981 and in revised form 12 January 1982.

[1] *Abbreviations used in this paper:* ATLPL, adipose tissue lipoprotein lipase; FFA, free fatty acids; HDL, high density lipoprotein; TG, triglyceride.

the extracted form (presumably total adipose tissue enzyme) of ATLPL, which reached levels comparable to those found in control subjects.

Feeding in humans has resulted in higher ATLPL levels when compared with the fasting state (13, 14), a change that has been thought to be secondary to rises in insulin levels. Additional support for this was a greater increase in ATLPL after formula containing 85% carbohydrate than after formula containing 45% fat (11). These studies, however, have not been controlled for other variables, such as gut hormone responses to feeding and changes in glucose levels, which cloud the issue of the exact role of insulin regulation of ATLPL. In addition, there has been variability in the time of stimulatory response of ATLPL after feeding, from 60 min (13) to 6 h (14). This delayed effect might indicate, as was suggested by Chen et al. (15), that postprandial TG removal may occur through mechanisms other than ATLPL.

Because there has not yet been a satisfactory method to isolate the role of insulin from gut hormones that may be increased with eating, we therefore turned our attention to the euglycemic clamp technique (16), currently the best in vivo method, to show that insulin regulates ATLPL independently.

METHODS

Subjects. 11 subjects served as the study group and received intravenous insulin and glucose. Each was of average height and within 20% of ideal body weight according to the Metropolitan Life Insurance Company standards (17). The body mass index (weight divided by the square of the height) (18) was a mean±SEM of $2.06 \times 10^{-3} \pm 0.07$ kg/cm^2. The subjects ranged in age from 20 to 39 yr, 26.5±1.4 yr. Nine subjects were women, and two were men. Five subjects, three men and two women, comprised the control group, who received infusions of 0.9% (normal) saline. They too were of normal height and weight with a body mass index of $2.20 \times 10^{-3} \pm 0.05$ kg/cm^2 with an age range of 23 to 40 yr, 28.4±3.4 yr. All subjects were of stable weight for at least 3 mo before the study. Exercise histories were variable. No individual was taking drugs that affect glucose or lipid metabolism, such as oral contraceptives, other estrogenic compounds, diuretics, or other antihypertensive medications. All subjects were free of either acute or chronic illnesses. One study individual had a history of hypothyroidism but was euthyroid on full thyroid hormone replacement. All subjects underwent physical examinations and laboratory studies to confirm their state of health. Each ingested 40 g/m^2 of glucose and had a normal 3-h oral glucose tolerance curve, according to the guidelines of the National Diabetes Data Group (19). In addition, all subjects had a normal triiodothyronine resin uptake, total thyroxine, thyroid-stimulating hormone, electrolyte panel, liver panel, calcium, phosphorous, magnesium, complete blood count, and urinalysis. Fasting serum cholesterol and TG were normal for all subjects.

All studies were performed in the Clinical Research Center at the University of Colorado Health Sciences Center. Before the study, each subject received 2 d of isocaloric formula feeding containing 45% carbohydrate, 40% fat, and 15% protein. After an overnight fast, on the morning of the euglycemic clamp study, two intravenous lines were placed in opposite arms of the study subjects, one for infusion of fluids and one for sampling of blood. For the control group saline solution was substituted for the insulin and glucose, which was the only change from the protocol of the study group. A heating pad was placed over the arm where the samples were obtained from venous blood. Plasma glucose determinations were carried out, after plasma was immediately separated with a Beckman Microfuge (Beckman Instruments, Inc., Palo Alto, CA), by the glucose oxidase technique using the Beckman glucose analyzer (Beckman Instruments, Inc., Clinical Instruments Div., Fullerton, CA). Basal plasma glucose values were taken on the morning of the euglycemic clamp study and served as the standard, determining the varying glucose infusion needed to maintain euglycemia during the rest of the study.

A preinfusion, base-line adipose tissue biopsy was performed ~1 h after intravenous lines were inserted. After the buttock region was cleansed with an iodine solution, 3 ml of 1% lidocaine without epinephrine were infiltrated in the dermal region, forming a wheal. A number-11 scalpel blade was used for the dermal incision. A 15-gauge adipose tissue biopsy needle was inserted through the incision for retrieval of tissue by a suction technique. The same procedure was repeated on other biopsy sites of the buttocks, 20, 80, 180, and 360 min into the insulin infusion.

Insulin was infused in the study subjects in an exponentially decreasing manner over the first 10 min and then at 40 mU/m^2 per min to obtain a steady-state serum level of ~70 μU/ml. Glucose infusion was begun at 4 min into the insulin infusion. At that point and throughout the study a blood sample was determined every 5 min for plasma glucose concentration to determine the changes in glucose infusion rate needed to maintain euglycemia.

Potassium was supplemented in all subjects by 30 meq of carbohydrate-free KCl solution given the evening preceding the study and at ~1.5 and 3.5 h into the insulin infusion to maintain normokalemia.

TG determinations were performed by the enzymatic method of Dow Chemical Co. (20). The coefficient of variation of TG was 6%.

Total cholesterol was also measured enzymatically (21) with a coefficient of variation of 2%.

High density lipoprotein (HDL) cholesterol was measured in plasma from which very low density lipoproteins and low density lipoproteins were precipitated by 4% sodium phosphotungstate (22). The HDL cholesterol was measured in the supernatant after centrifugation in a Beckman TJ-6 centrifuge, 1,500 *g* for 30 min at 4°C. The coefficient of variation for HDL cholesterol including analytical and precipitation variation was 8%.

Serum insulin levels were measured by a double antibody radioimmunoassay with the technique of Desbuquois and Aurbach (23).

FFA were determined by use of ^{60}CO as a tracer, which formed a salt complex with available FFA (24).

Catecholamines were determined by radiometric assay, CAT-A-KIT (Upjohn Co., Kalamazoo, MI), a modification of the method described by Passon and Peuler (25).

The measurement of ATLPL in human adipose tissue was performed in tissue pieces of 40–50 mg and modified from the method previously described by Pykälistö et al. (11). For activity releasable with heparin, the tissue was incubated with heparin (13.3 μg/ml, Upjohn Co. beef lung) in Krebs Ringer phosphate buffer (pH 7.40 for 45 min at 37°C). 0.1 ml was taken off and incubated in a Dubnoff shaker with

0.1 ml substrate. The substrate was prepared with 5 mg of unlabeled triolein (Sigma Chemical Co., St. Louis, MO), 4 μCi of [1-^{14}C]triolein (Amersham Corp., Arlington Heights, IL), and 0.24 mg egg lecithin (Applied Science Labs, Inc., State College, PA). Emulsification of the triolein and lecithin was carried out with a mixture of 10% fatty acid-poor bovine serum albumin/normal human serum/2 M Tris HCl buffer/distilled water (4:1.5:5:9.5) for 100 s of sonification (10 s on, followed by 10 s off, for 10 cycles) using a sonicator (model W-220F, Heat Systems-Ultrasonics, Inc., Plainview, NY), at 4°C. After incubation of enzyme with substrate, the reaction was terminated with 3.25 ml of Belfrage and Vaughn extraction mixture (26). Fatty acids were extracted for 5 min on an Eberbach Corp. (Ann Arbor, MI) shaker, and after centrifugation by a Beckman model TJ-6 centrifuge at 600 *g* for 20 min, 0.5 ml of the upper phase of each sample was removed and counted in a Searle Mark III scintillation counter (Searle Radiographics Inc., Des Plaines, IL).

Student's *t* test for unpaired observations and Pearsons linear regression analysis were used for statistical analyses.

RESULTS

Base-line and euglycemic clamp data. The base-line data of both the 11 study and 5 control subjects are displayed in Table I. Basal plasma glucose values in all subjects ranged from 74 to 92 mg/dl. The glucose infusion in the study group was varied to maintain plasma glucose at the initial concentration. During the insulin infusion, plasma glucose varied little, ranging from 96.3 to 104.0% of basal value.

Mean±SEM for basal fasting TG, total cholesterol, and HDL cholesterol concentrations were similar between groups (Table I). Base-line serum insulin concentrations on the day of the study ranged from 1 to 18 μU/ml, with the exception of one subject who had a level of 37 μU/ml (Table I). A previously obtained insulin concentration (1 mo earlier) was < 2 μU/ml on this subject.

Catecholamines. There was no statistical difference between either the basal catecholamine levels (Table I) or the percentage change from basal between the two groups. Fig. 1 illustrates the changes in norepinephrine and epinephrine during the study. At all time points there was no statistically significant difference between the two populations. However, the insulin group showed a significant increase in norepinephrine over the study period ($r = 0.294$, $n = 86$ measurements, $P < 0.01$).

FFA. Insulin had a dramatic effect on lipolysis (Fig. 2A). FFA fell significantly within 20 min of the insulin infusion, from 323±50 to 81±14 nmol/ml, or 27±3.3% of basal. This change was statistically dif-

TABLE I
Base-line Data Before Insulin or Saline Infusion

Subject	Age	Sex	Wt	Wt/Ht² × 10⁻³	Glucose	Insulin	Norepinephrine	Epinephrine	FFA	TG	Total cholesterol	HDL cholesterol	ATLPL
	yr		*kg*	*kg/cm²*	*mg/dl*	*μU/ml*	*pg/ml*		*nmol/ml*	*mg/dl*	*mg/dl*	*mg/dl*	*neq/g/min*
Insulin													
E.J.	20	F	51.6	1.90	85	37	200	53	198	78	174	58	19.1
K.K.	24	F	52.3	1.92	90	6	270	48	314	56	155	57	39.1
J.O.	27	M	72.8	2.17	90	4	325	18	130	68	138	39	43.6
C.B.	25	M	77.3	2.52	89	5	261	41	322	87	142	46	17.9
R.M.	39	F	48.0	1.97	80	3	255	103	131	74	180	64	23.0
D.M.	28	F	61.4	2.40	89	18	129	42	257	131	143	28	30.7
L.M.	26	F	59.0	1.86	79	7	306	89	284	132	151	41	33.0
T.K.	29	F	55.7	1.86	86	7	153	55	478	50	190	70	22.2
M.B.	24	F	46.7	1.85	74	7	189	76	623	43	176	58	10.8
P.W.	25	F	61.4	2.17	79	11	386	132	573	60	150	66	7.4
D.W.	25	F	51.6	2.09	83	13	147	33	243	65	150	64	25.1
Mean	26.5			2.06	84.0	10.7	238.3	62.7	323.0	76.7	159.0	53.7	24.7
±SEM	±1.4			±0.07	±1.6	±2.9	±24.7	±10.2	±50.3	±9.0	±5.3	±4.0	±3.4
Saline													
J.H.	23	F	69.3	2.26	92	8	224	22	149	113	207	70	19.0
M.B.	27	F	64.5	2.37	78	6	120	22	249	79	173	64	7.7
D.S.	25	M	72.3	2.19	85	5	242	46	314	90	167	46	1.0
R.M.	40	F	50.5	2.10	85	11	—	—	230	53	182	59	17.9
K.F.	27	M	68.0	2.10	85	1	154	52	387	129	192	34	13.0
Mean	28.4			2.20	85.0	6.2	185.0	35.5	265.8	92.8	184.2	54.6	11.7
±SEM	±3.4			±0.05	±2.2	±1.7	±28.8	±7.9	±41.8	±13.2	±7.1	±6.5	±3.3

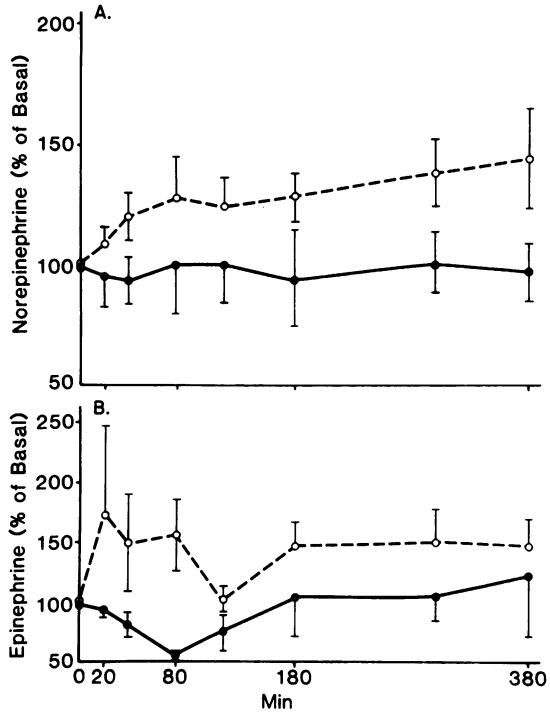

FIGURE 1 Norepinephrine and epinephrine. Norepinephrine (A) and epinephrine (B), as a percentage of basal levels, are both plotted against time during the insulin (40 mU/m^2 per min) infusion (open circles, broken line) and saline infusion (closed circles, solid line). Data are presented as mean±SEM.

ferent from that of the control group ($t = 5.19$, $P < 0.001$). Further suppression of lipolysis was soon after obtained, and FFA levels were maintained at the consistently low range for the remainder of the infusion. The control group, however, demonstrated a rise in FFA during the saline infusion.

TG. Similarly, insulin decreased TG concentrations (Fig. 2B). TG levels did not change significantly for the first 60 min of the insulin infusion; however, by 80 min, levels fell to 77±3.1% of base line ($t = 3.76$, $P < 0.01$) when compared with the saline infusion group. This trend continued during the remainder of the infusion. This effect appeared to follow the fall in FFA and was weakly but not significantly inversely related to the insulin effect on ATLPL at 6 h.

Total cholesterol and HDL cholesterol. Total cholesterol and HDL cholesterol were compared before, during, and at the end of the insulin infusion. By the end of the infusion, the total cholesterol decreased from 159±5.3 to 138±4.3 mg/dl, whereas the HDL cholesterol fell less, from 54±4.0 to 50±4.4 mg/dl. Neither basal ATLPL nor the ATLPL response to insulin correlated with basal HDL levels.

ATLPL activity. The effect of insulin on ATLPL can be seen in Fig. 3. Basal ATLPL ranged from 7.4 to 43.6 neq FFA/g per min, mean = 24.7±3.4 neq FFA/g per min. There was no correlation between basal ATLPL and catecholamines or any apparent relationship of the enzyme activity to exercise history. However, basal ATLPL was inversely correlated with the basal FFA ($r = -0.663$, $P < 0.05$). Initially, adipose tissue biopsies were performed at 80 min with no demonstrable stimulatory effect of insulin on ATLPL. In subsequent studies, biopsies were performed at an earlier timepoint (20 min) and at a later timepoint (180 min) to examine the possibility of a rapid or delayed response which might have been missed at 80 min. At 20 min, no effect was found, but at 180 min into the insulin infusion, insulin increased ATLPL above basal values in eight of the 11 subjects. The infusion was continued for another 3 h, at which time all subjects increased above basal. When compared with saline controls, a significant stimulatory response was found ($t = 3.94$, $P < 0.01$).

Neither the basal ATLPL nor insulin-mediated increases in ATLPL was related to basal serum insulin concentrations. There was also no relationship between the amount of gluocse infused and the increase in ATLPL ($r = 0.131$, P = NS).

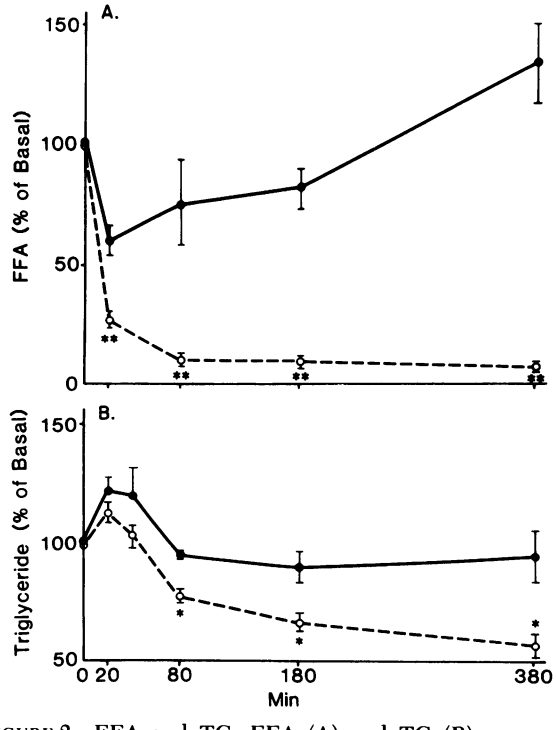

FIGURE 2 FFA and TG. FFA (A) and TG (B), as a percentage of basal levels, are plotted against time during the insulin (40 mU/m^2 per min) infusion (open circles, broken line) and saline infusion (closed circles, solid line). *, $P < 0.01$; **, $P < 0.001$.

Although there was an insignificant trend present between the insulin response of ATLPL and the basal activity at 3 h ($r = -0.423$), the 6-h insulin-mediated response was significantly dependent on the basal enzyme activity ($r = -0.690$, $P < 0.02$) (Fig. 4).

DISCUSSION

The euglycemic clamp provides a valuable tool for the study of the roles of insulin and glucose not only in ATLPL regulation but in lipid metabolism in general. The dramatic and early fall in FFA illustrates that insulin, as expected, has a profound and probable pharmacologic effect upon the dynamic processes of fuel metabolism. Most likely, insulin inhibits lipolysis; however, a stimulatory effect on the reesterification of FFA to form intracellular TG cannot be ruled out. The rise in FFA during saline infusion is an expected response to continued fasting. The effect of insulin upon TG also shows a highly significant downward trend throughout the infusion. This drop occurs ~1 h after the fall in FFA. The exact cause of the decrease in TG remains unknown, but probably involves a combination of activation of the removal mechanism (27) and suppression of very low density lipoprotein production rate, which is mediated by the decrease in FFA presented to the liver for very low density lipoprotein synthesis (28). Further evidence that several factors may control this insulin-mediated fall in TG is the lack of a significant correlation between the fall in TG and the increase in ATLPL in the present study.

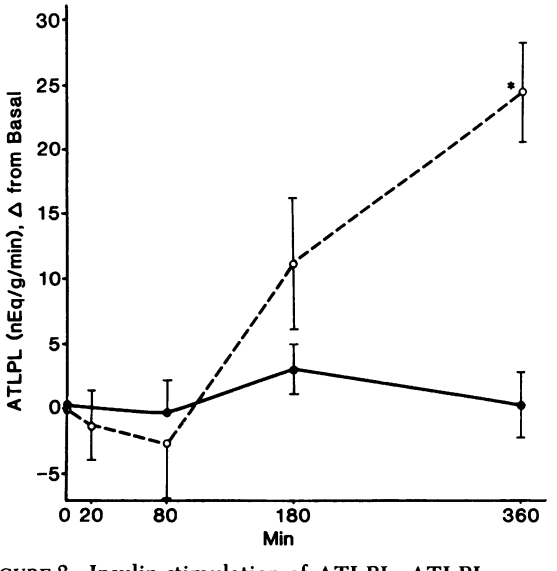

FIGURE 3 Insulin stimulation of ATLPL. ATLPL as a percentage of basal ATLPL is plotted over time during the insulin (40 mU/m² per min) infusion (open circles, broken line) and saline infusion (closed circles, solid line). *, $P < 0.01$.

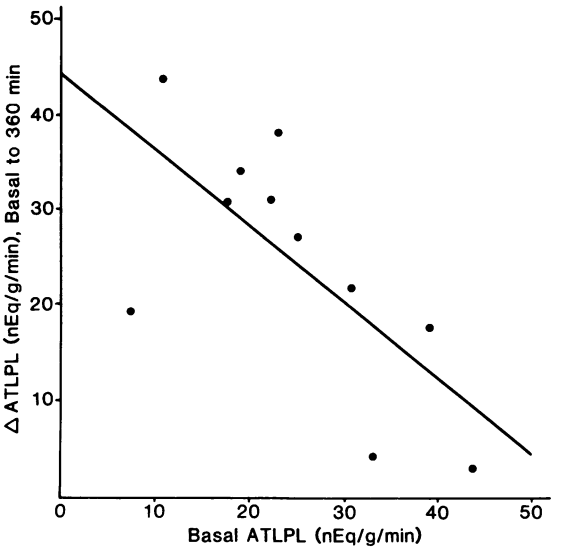

FIGURE 4 Insulin-induced stimulation of ATLPL. Insulin-stimulated ATLPL at 360 min is plotted as an increase above basal vs. basal ATLPL. $r = -0.690$, $P < 0.02$.

The effects of stress from the adipose tissue biopsies were monitored by determinations of catecholamines before and throughout the infusions. Although variations in basal levels were wide among individuals, no significant differences were found between the control and study groups. There was also no relationship between basal or changes in catecholamines and the ATLPL response to insulin. There was, however, an upward trend in norepinephrine levels during insulin administration, a stimulatory response described previously at higher insulin concentrations over shorter time intervals by others (29).

Insulin has a definite, but delayed, effect on ATLPL, with a significant rise in enzymatic activity occurring by 6 h when compared with controls infused with saline. Although the insulin stimulatory effect demonstrated in rat ATLPL occurred during variations in plasma glucose concentrations (7), the present investigation removes alterations in plasma glucose as a possible variable.

Although there was no relationship between the amount of glucose infused and the ATLPL response to insulin, it is impossible to conclude that insulin-mediated effects on glucose metabolism have no relationship to the ATLPL response. Glucose utilization can only be approximated by the amount of glucose infused because hepatic glucose output was not measured. However, studies by Kolterman et al.[2] indicate that total suppression of hepatic glucose output occurs by 45 min at similar insulin concentrations. Over a 6-

[2] Kolterman et al. Personal communication.

h insulin infusion the amount of glucose infused is a reasonable estimate of glucose utilization. It would therefore appear that the effect of insulin on ATLPL is largely independent of insulin-mediated glucose metabolism.

In feeding studies, Goldberg et al. (14) showed that the basal level of ATLPL played a major role in subsequent elevations in enzyme activity. Similarly, the magnitude of the stimulatory effect of insulin on ATLPL in the present euglycemic clamp study proves to be a function of the basal enzyme activity. Interestingly, the control group had a significantly lower basal ATLPL than the study group ($t = 2.34$, $P < 0.05$). If the insulin effect on ATLPL were simply secondary to the intravenous fluid administration, the inverse relationship of basal ATLPL to the subsequent change in ATLPL would predict that the control group would have the greater response. In fact, as shown in Fig. 3, an essentially flat response occurred, which provides further evidence that insulin was responsible for the stimulatory response.

Because feeding is not part of the protocol, the present study cannot address the question of the role of insulin-stimulated ATLPL in chylomicron removal. It may be that an insulin infusion in the presence of fat feeding may lead to an earlier and more pronounced increase in ATLPL.

The phenomenon of basal ATLPL determining the absolute change in subsequent stimulated enzyme activity is intriguing. To speculate, the primary regulatory mechanism of TG removal may be the adipose tissue or adipocyte itself. With high basal activity, adipose tissue is little affected by various stimuli, e.g., insulin administration or oral feedings. With a lower basal level, however, ATLPL responds more to extraadipose regulatory mechanisms, which may eventually prove to be nothing more than secondary factors in ATLPL control.

The euglycemic clamp technique is a tool that can be adapted to the study of lipid metabolism. A stimulatory role of insulin on ATLPL in humans, independent of enteric factors and possibly glucose metabolism, has now been provided. The interaction of other variables which may determine or augment the ATLPL response to insulin, e.g., oral fat, can now be systematically approached.

ACKNOWLEDGMENTS

We sincerely thank Dr. Orville G. Kolterman for insulin radioimmunoassays, Deborah J. Krinitzsky and Debbie Gwinner for technical assistance, and Sally Frye and Joni Foti for secretarial skills.

Support for this work was provided by National Institutes of Health grant AM 26356 and grant RR-00051 from the General Clinical Research Center at the University of Colorado Health Sciences Center.

REFERENCES

1. Scow, R. O., E. J. Blanchette-Mackie, and L. G. Smith, 1980. Transport of lipid across capillary endothelium. *Fed. Proc.* **39:** 2610–2617.
2. Hollenberg, C. H. 1966. The origin and glyceride distribution of fatty acids in rat adipose tissue. *J. Clin. Invest.* **45:** 205–216.
3. Taskinen, M., and E. A. Nikkilä. 1977. Lipoprotein lipase activity in adipose tissue and in postheparin plasma in human obesity. *Acta Med. Scand.* **202:** 399–408.
4. Ashby, P., and D. S. Robinson. 1980. Effects of insulin, glucocorticoids, and adrenaline on the activity of rat adipose-tissue lipoprotein lipase. *Biochem. J.* **188:** 185–192.
5. Eckel, R. H., W. Y. Fujimoto, and J. D. Brunzell. 1978. Insulin regulation of lipoprotein lipase in cultured 3T3-L1 cells. *Biochem. Biophys. Res. Commun.* **84:** 1069–1075.
6. Spooner, P. M., S. S. Chernick, M. M. Garrison, and R. O. Scow. 1979. Insulin regulation of lipoprotein lipase activity and release in 3T3-L1 adipocytes. *J. Biol. Chem.* **254:** 10021–10029.
7. Borensztajn, J., D. R. Samols, and A. H. Rubenstein. 1972. Effects of insulin on lipoprotein lipase activity in the rat heart and adipose tissue. *Am. J. Physiol.* **223:** 1271–1275.
8. Garfinkel, A. S., P. Nilsson-Ehle, and M. C. Schotz, 1976. Regulation of lipoprotein lipase induction by insulin. *Biochim. Biophys. Acta.* **424:** 264–273.
9. Taskinen, M., and E. A. Nikkilä. 1979. Lipoprotein lipase activity of adipose tissue and skeletal muscle in insulin-deficient human diabetes. *Diabetologia.* **17:** 351–356.
10. Nikkilä, E. A., J. K. Huttenen, and C. Ehnholm. 1977. Postheparin plasma lipoprotein lipase and hepatic lipase in diabetes mellitus. *Diabetes.* **26:** 11–21.
11. Pykälistö, O. J., P. H. Smith, and J. D. Brunzell. 1975. Determinants of human adipose tissue lipoprotein lipase. *J. Clin. Invest.* **56:** 1108–1117.
12. Taylor, K. G., D. J. Galton, and G. Holdsworth. 1979. Insulin-independent diabetes: a defect in the activity of lipoprotein lipase in adipose tissue. *Diabetologia.* **16:** 313–317.
13. Nilsson-Ehle, P., S. Carlstrom, and P. Belfrage. 1975. Rapid effects on lipoprotein lipase activity in adipose tissue of humans after carbohydrate and lipid intake. *Scand. J. Clin. Lab. Invest.* **35:** 373–378.
14. Goldberg, A. P., A. Chait, and J. D. Brunzell. 1980. Postprandial adipose tissue lipoprotein lipase activity in primary hypertriglyceridemia. *Metab. Clin. Exp.* **29:** 223–229.
15. Chen, Y. I., J. Howard, V. Huang, F. B. Kraemer, and G. M. Reaven. 1980. Dissociation between plasma triglyceride concentration and tissue lipoprotein lipase deficiency in insulin-deficient rats. *Diabetes.* **29:** 643–647.
16. Insel, P. A., J. E. Liljenquist, J. D. Tobin, R. S. Sherwin, P. Watkins, R. Andres, and M. Berman, 1975. Insulin control of glucose metabolism in man. *J. Clin. Invest.* **55:** 1057–1066.
17. Metropolitan Life Insurance Company. 1959. New

weight standards for men and women. *Statistical Bulletin.* **40:** 1–4.
18. Keys, A., F. Fidanza, M. J. Karvonen, N. Kimura, and H. L. Taylor. 1972. Indices of relative weight and obesity. *J. Chronic Dis.* **25:** 329–343.
19. National Diabetes Data Group. 1979. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. *Diabetes.* **28:** 1039–1057.
20. The Dow Chemical Company. 1978. Triglycerides Determination. Midland, MI.
21. Richmond, W. 1973. Preparation and properties of a cholesterol oxidase from *Nocardia Sp.* and its application to the enzymatic assay of total cholesterol in serum. *Clin. Chem.* **19:** 1350–1356.
22. Lopes-Virella, M. F., P. Stone, S. Ellis, and J. A. Colwell. 1977. Cholesterol determination in high-density lipoproteins separated by three different methods. *Clin. Chem.* **23:** 882–884.
23. Desbuquois, B., and G. D. Aurbach. 1971. Use of polyethylene glycol to separate free and antibody-bound peptide hormones in radioimmunoassays. *J. Clin. Endocrinol. Metab.* **33:** 732–738.
24. Chlouverakis, C., and D. Hojnicki. 1974. A modified radiochemical assay for serum free fatty acid determination. *Clin. Chim. Acta.* **54:** 91–93.
25. Passon, P. G., and J. D. Peuler. 1973. A simplified radiometric assay for plasma norepinephrine and epinephrine. *Anal. Biochem.* **51:** 618–631.
26. Belfrage, P., and M. Vaughn. 1969. Simple liquid-liquid partition system for isolation of labeled oleic acid from mixtures with glycerides. *J. Lipid Res.* **10:** 341–344.
27. Brunzell, J. D., D. Porte, Jr., and E. L. Bierman, 1979. Abnormal lipoprotein-lipase mediated plasma triglyceride removal in untreated diabetes mellitus associated with hypertriglyceridemia. *Metab. Clin. Exp.* **28:** 901–907.
28. Havel, R. H., J. P. Kane, E. O. Balasse, N. Segel, and L. V. Basso. 1970. Splanchnic metabolism of free fatty acids and production of triglycerides of very low density lipoproteins in normotriglyceridemic and hypertriglyceridemic humans. *J. Clin. Invest.* **49:** 2017–2035.
29. Rowe, J. W., J. B. Young, K. L. Minaker, A. L. Stevens, J. Pallotta, and L. Landsberg. 1981. Effect of insulin and glucose infusions on sympathetic nervous system activity in normal man. *Diabetes.* **30:** 219–225.