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Insulin resistance in uremia. Characterization of lipid metabolism in freshly isolated and primary cultures of hepatocytes from chronic uremic rats.

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Research Article

We have studied the mechanism(s) of hyperlipidemia and liver insulin sensitivity in a rat model of severe chronic uremia (U). Basal lipid synthesis was decreased in freshly isolated hepatocytes from U when compared with sham-operated ad lib.-fed controls (alfC). Basal lipid synthesis in pair-fed controls (pfC) was in between U and alfC. Similarly, the activity of liver acetyl CoA carboxylase, fatty acid synthetase, citrate cleavage enzyme, malate dehydrogenase, and glucose-6-phosphate dehydrogenase was diminished in U. Muscle and adipose tissue lipoprotein lipase was also decreased. Insulin stimulated lipid synthesis in freshly isolated hepatocytes from alfC. Hepatocytes from U and pfC were resistant to this effect of insulin. To ascertain if the insulin resistance in U was due to starvation (chow intake 50% of alfC) or to uremia itself, the U and pfC were intragastrically fed an isocaloric diet via a Holter pump the last week of the experimental period. Hepatocytes from orally fed U and pfC were also cultured for 24 h in serum-free medium. While freshly isolated and cultured U hepatocytes remained insulin resistant, those from pfC normalized, in vivo and in vitro, when they were provided with enough nutrients. Conclusions: (a) Hyperlipidemia in uremia is not due to increased synthesis, but to defect(s) in clearance. (b) Insulin does not stimulate lipid synthesis in uremia. This finding, along with our […]

Insulin Resistance in Uremia

CHARACTERIZATION OF LIPID METABOLISM IN FRESHLY ISOLATED AND PRIMARY CULTURES OF HEPATOCYTES FROM CHRONIC UREMIC RATS

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ABSTRACT We have studied the mechanism(s) of hyperlipidemia and liver insulin sensitivity in a rat model of severe chronic uremia (U).

Basal lipid synthesis was decreased in freshly isolated hepatocytes from U when compared with sham-operated ad lib.-fed controls (alfC). Basal lipid synthesis in pair-fed controls (pfC) was in between U and alfC. Similarly, the activity of liver acetyl CoA carboxylase, fatty acid synthetase, citrate cleavage enzyme, malate dehydrogenase, and glucose-6-phosphate dehydrogenase was diminished in U. Muscle and adipose tissue lipoprotein lipase was also decreased.

Insulin stimulated lipid synthesis in freshly isolated hepatocytes from alfC. Hepatocytes from U and pfC were resistant to this effect of insulin. To ascertain if the insulin resistance in U was due to starvation (chow intake 50% of alfC) or to uremia itself, the U and pfC were intragastrically fed an isocaloric diet via a Holter pump the last week of the experimental period. Hepatocytes from orally fed U and pfC were also cultured for 24 h in serum-free medium. While freshly isolated and cultured U hepatocytes remained insulin resistant, those from pfC normalized, in vivo and in vitro, when they were provided with enough nutrients.

Conclusions: (*a*) Hyperlipidemia in uremia is not due to increased synthesis, but to defect(s) in clearance. (*b*) Insulin does not stimulate lipid synthesis in uremia. This finding, along with our recent demonstration that insulin binding and internalization are not decreased in the uremic liver, suggests that a post-binding defect(s) in the liver plays an important role in the mechanism(s) of insulin resistance in uremia. (*c*) Cultured hepatocytes from uremic rats remain insulin resistant. This quality renders these cells useful in studying the postinsulin binding events responsible for the insulin-resistant state in the absence of complicating hormonal and substrate changes that occur in vivo.

INTRODUCTION

The precise mechanism(s) causing hypertriglyceridemia in renal failure remains unknown (1, 2). It could be due to either increased triglyceride production by liver and/or reduced peripheral removal. It seems well established that the clearance of triglycerides in uremia is decreased due to both qualitative and quantitative abnormalities in lipoprotein lipase (LPL)[1] (3–5). The evidence for altered lipid synthesis by liver in uremia is conflicting. It is important to define the role of the liver since a hyperinsulinemic insulin-resistant state is recognized in chronic renal failure (6). Although peripheral insulin insensitivity in uremia is well accepted, insulin action in the liver is unsettled. DeFronzo et al. (7) have demonstrated a normal suppression of hepatic glucose production by insulin in uremic patients. We have recently demonstrated in

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[1] *Abbreviations used in this paper:* AcCoAC, acetyl-coenzyme A carboxylase; CCE, citrate cleavage enzyme; FAS, fatty acid synthetase; G6PDH, glucose-6-phosphate dehydrogenase; LPL, lipoprotein lipase; MDH, malate dehydrogenase.

an uremic rat model that the liver is resistant to insulin with regard to amino acid transport (8). If the uremic liver were insulin sensitive to stimulation of lipid synthesis, it could be expected that the elevated basal immunoreactive insulin concentration would increase endogenous triglyceride production.

To define the role of the liver in the hyperlipidemia of uremia we studied lipid synthesis from tritiated water and [^{14}C]acetate in freshly isolated and primary cultures of rat hepatocytes. Hepatocytes were isolated from a rat model that leads to a predictable degree of severe uremia (8). In the experimental design, specific consideration was given to the nutritional status of the animals. Two sham-operated control groups, ad lib. fed and pair fed were studied with uremic rats. Also, uremic animals were fed intragastrically with an isocaloric diet. This allowed us to analyze the metabolic events due to either uremia or food restriction. Finally, our ability to isolate hepatocytes that respond (9) and process insulin (10) allowed us to characterize at the cellular level the insulin sensitivity of the uremic liver with regard to lipid synthesis.

METHODS

Chemicals. ^3H_2O (1 Ci/g) and [1-^{14}C]acetic acid, sodium salt (57 mCi/mmol) were obtained from New England Nuclear, Boston, MA. Crude collagenase (4177CLSII 41K22, 164 U/mg) was obtained from Worthington Biochemical Corp., Freehold, NJ; fraction V bovine albumin from Reheis Chemical Co., Kankakee, IL; and serum-free medium (Waymouth's MB752/1) from Gibco Laboratories, Grand Island, NY. Crystalline porcine insulin was kindly provided by Dr. Ronald Chance of Eli Lilly & Co., Indianapolis, IN. All other chemicals were reagent grade.

Experimental model of chronic uremia. We have recently described the uremic rat model used in this study (8). Briefly, male Sprague Dawley rats weighing 200 g were anesthetized with ether. The flank of the rat was entered and the left kidney was separated from the adrenal gland and perirenal fat. The kidney was placed into a vinyl chamber measuring 0.9 cm^3, which was closed completely except for a 3-mm diam aperture for the renal pedicle. The enclosed kidney was replaced in the retroperitoneal space and the flank closed. 7 d after this operation, the right kidney was removed using a right flank approach leaving the right adrenal gland intact. The sham-operated controls were operated using the same technique to enter the retroperitoneal space. The kidneys were manipulated, but not removed, and a 1-cm^2 piece of vinyl was placed in the left retroperitoneal space.

Experimental protocol. The study animals included two experimental groups: group I which included animals orally fed a standard Purina rat chow (No. 5001, Ralston Purina Co., St. Louis, MO). These rats were subdivided into three groups: sham-operated rats ad lib. fed, uremic rats ad lib. fed, and sham-operated rats pair fed with the uremic rats. The studies were performed 4 wk after surgery. The animals were fasted for ~3 h before killing.

Group II included uremic and sham-operated pair-fed rats that were fed as in group I from the 1st to the 3rd wk of the experimental period and they were fed intragastrically during the 4th wk of the experimental period. A feeding catheter was inserted ~8 mm into the glandular portion of the stomach. This catheter was secured in place using a purse string suture and then tunneled through the fascia and under the skin to the midscapular region. With this model, there was no restriction of movement. The rats received, via a Holter pump, an isocaloric continuous infusion of 6.5% calories as protein, 27.8% calories as fat, and 65.6% calories as carbohydrates for 7 d as reported previously (11). The nitrogen and energy content of this diet met the requirements of the rat as listed by the National Research Council in Nutrient Requirement of Laboratory Animals (12).

Preparation of hepatocytes. Liver perfusion, hepatocyte isolation, and cultures were performed as previously described (13, 14).

Lipid synthesis. Freshly isolated hepatocytes (1–3 × 10^6 cells/ml) were incubated in the absence and presence of insulin at which time ^3H_2O (15 μCi/ml) or [^{14}C]acetate (5 mM 0.5 μCi/ml) were added to the cell suspension. Total lipids were extracted at different times as previously described (15). Lipid synthesis was linear during the experimental period. Any additions to the primary cultures were added at the time of the first medium change 4 h after plating (16). These cultures were then incubated in the absence and presence of different concentrations of insulin and [^{14}C]acetate or ^3H_2O for 20 h. Total lipids released into the medium, or present into the cells, were extracted (13).

Enzymes and serum chemistry determinations. Approximately 3 h after food deprivation, animals were killed by decapitation. The blood was collected and centrifugated to obtain the serum for measurements of urea nitrogen, creatinine, triglyceride, and cholesterol by standard laboratory techniques. Liver, soleus muscle, and epididymal fat pad were removed, weighed, and placed in ice-cold 0.25 M sucrose, 1 mM EDTA buffer at pH 7.4, and homogenized with the same buffer (1:5, wt/vol). The tissue homogenates were centrifuged at 15,000 *g* for 15 min at 4°C. The supernatant of the livers was recentrifuged at 100,000 *g* for 45 min at 4°C. The activity of fatty acid synthetase (FAS) (17), citrate cleavage enzyme (CCE) (18), glucose-6-phosphate dehydrogenase (G6PDH), and malate dehydrogenase (MDH) (19) were measured in the supernatant of liver. The activity of acetyl-coenzyme A carboxylase (AcCoAC) was measured in the liver fraction, after gel filtration and activation of crude enzyme, by $^{14}CO_2$ fixation as reported by Inoue and Lowenstein (20). Lipoprotein lipase activity was assayed in the 15,000-*g* supernatant of adipose tissue and soleus muscle using the method of Schotz et al. (21) with minor modifications (22). Finally, total fat (23), triglycerides (24), and cholesterol (25) were measured in the liver. All samples from each enzyme were analyzed within the same assay to minimize assay variability. All values were the mean of duplicate or triplicate determinations. For these assays, the intra- and interassay coefficients of variations were <10 and 20%, respectively.

RESULTS

Experimental model of chronic uremia. This experimental model of chronic uremia, using a vinyl chamber to prevent hypertrophy of the remnant kidney, leads to a predictable degree of severe uremia in 4 wk, with only 20% mortality (Table I). The uremic and pair-fed controls gained ~10% of their initial body weight whereas the ad lib.-fed controls gained ~90% during the 4-wk experimental period. This dif-

TABLE I
Morphometrics, Liver, and Serum Measurements from Uremic, Sham-operated, Pair-fed, and Ad Lib.-fed Controls

	Uremic	Pair-fed control	Ad lib.-fed control
Initial body weight (*g*)	210±3*	207±6*	175±16*
Final body weight (*g*)	236±9*	220±6*	333±13‡
Rat chow intake/28 d (*g*)	480±26*	476±27*	801±31‡
Mortality (%)	20*	3‡	4‡
Liver weight (*g*)	9.5±0.5*	7.9±2*	15.3±3.5‡
Total fat (*mg/g liver*)	40.3±1.8*	41.4±4.3*	36.5±0.9*
Triglyceride (*mg/g liver*)	9.0±0.5*	12.8±0.6‡	12.0±0.9‡
Total cholesterol (*mg/g liver*)	4.4±0.3*	4.0±0.1‡	4.5±0.1*
Free cholesterol (*mg/g liver*)	2.3±0.1*	2.0±0.1*	2.2±0.1*
Serum triglycerides (*mg/dl*)	119±31*	26±2‡	82±5*
Serum cholesterol (*mg/dl*)	75±7*	44±4*	52±2*
Serum creatinine (*mg/dl*)	2.1±0.2*	0.5±0.1‡	0.4±0.1‡
Blood urea nitrogen (*mg/dl*)	106±4*	21±1‡	25±2‡

Data are means±SE. Means in the same row that have different symbols are significantly different ($P < 0.05$).

ference in body weight gain was as a consequence of the difference in food intake (Table I). Blood urea nitrogen and serum creatinine were about four times greater in the uremic rats than in the pair-fed and ad lib.-fed controls. Serum triglyceride levels in the uremic animals were 50 and 350% above those of the ad lib.- and pair-fed controls, respectively. Serum cholesterol, however, was only mildly increased in the uremic animals.

Lipid metabolism in uremia. Fig. 1 shows the time course of [^{14}C]acetate and $^{3}H_2O$ incorporation into total lipids in freshly isolated hepatocytes from uremic, pair-fed and ad lib.-fed controls. Basal lipid synthesis was markedly decreased in both the uremics and pair-fed controls when compared with ad lib.-fed control animals. Also, whereas maximal insulin concentration (1×10^{-7} M) stimulated lipid synthesis above the basal level in hepatocytes from the ad lib.-fed rats, those from food-restricted animals (uremics and pair-fed controls) were refractory to insulin. As potential methodological problems exist with the use of [^{14}C]acetate to access lipid synthesis (26), we studied both [^{14}C]acetate and $^{3}H_2O$ incorporated into lipids. It should be recognized that since the pool of endogenous acetyl-CoA was not determined, the [^{14}C]acetate data represented only apparent incorporation of acetate into lipids. However, since the results of [^{14}C]acetate and $^{3}H_2O$ incorporation into lipids were qualitatively similar, acetate serves as an adequate substrate to evaluate lipid synthesis in hepatocytes from uremic and nonuremic animals. A similar conclusion was reached by Beynen et al. (27) using hepatocytes from normal rats in the absence and presence of insulin and glucagon, and by Caro and Amatruda (15) using hepatocytes from normal and fasted rats in the absence and presence of insulin in assessing suitability of acetate for lipid synthesis.

Fig. 2 shows the activity of various key liver lipogenic enzymes from the uremic, pair-fed, and ad lib.-fed animals. AcCoAC catalyzes the carboxylation of acetyl-CoA to malonyl-CoA. This irreversible reaction is the committed step in fatty acid synthesis. Fatty acid synthetase is the enzyme system that catalyzes the synthesis of saturated long-chain fatty acids from acetyl-CoA, malonyl-CoA, and NADPH. CCE provides a mechanism for transfer of intramitochondrial acetyl-CoA to the cytosol, where fatty acid synthesis occurs. MDH and G6PDH catalyzes the reaction that generates much of the NADPH needed for fatty acid synthesis. The activity of all the lipogenic enzymes studied was lower in the uremic animals when compared with that in the ad lib.-fed controls. The activity of AcCoAC and G6PDH in the uremic animals was also lower than in the pair-fed animals. In the pair-fed animals, the activity of FAS, CCE, and MDH was intermediate to the previous two groups.

We next studied LPL activity in adipose and muscle tissue to ascertain a possible defect in triglyceride clearance that might explain the observed hyperlipidemia in uremia. Fig. 3 shows that LPL activity was decreased in the uremic animals when compared with that in the ad lib.-fed controls. In the pair-fed animals, LPL activity in the adipose tissue was normal but decreased in the muscle tissue.

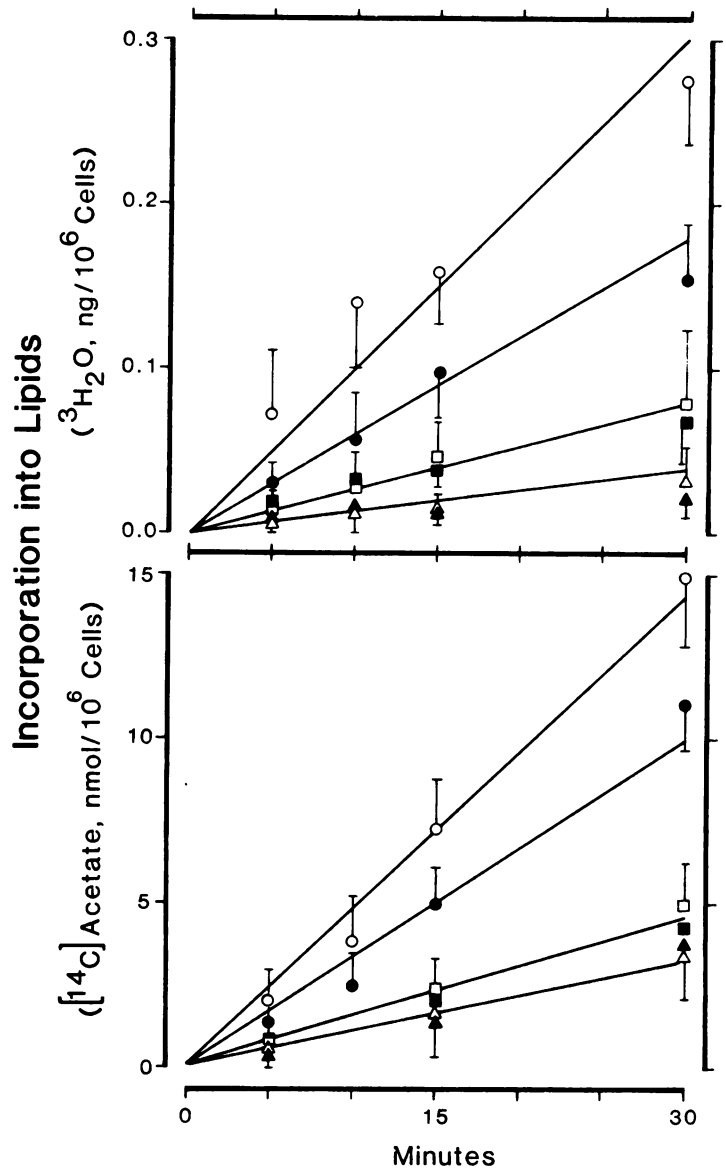

FIGURE 1 Time course of lipid synthesis. Freshly isolated hepatocytes from ad lib.-fed controls (● ○), pair-fed controls (■ □), and uremic rats (▲ △) were incubated in the absence (filled) and the presence (open) of insulin 1×10^{-7} M. ^3H_2O (15 μCi/ml) (*upper* panel) and [1-^{14}C]acetic acid (sodium salt, 5 mM, 0.01 mCi/mmol) (*lower* panel) incorporation into lipids was studied. At different times, total lipids were extracted into a mixture of chloroform, water, and methanol. Each point is the mean (±SE) of triplicate samples from six different experiments.

Insulin action in uremia. As demonstrated in Fig. 1, maximal insulin concentration (1×10^{-7} M) failed to stimulate lipid synthesis above basal in the uremic animals. However, since the hepatocytes from the pair-fed animals were also refractory to insulin, it was important to determine whether insulin resistance in uremia was due to the food restriction or uremia per se. In an attempt to answer this question, we used both an in vivo and an in vitro approach.

Uremic and pair-fed animals received intragastrically an isocaloric diet during the final week of the experimental period (group II of the experimental protocol). Their daily body weight increased 5.7±1.4 and 3.7±0.9 g, respectively. Fig. 4 demonstrates the

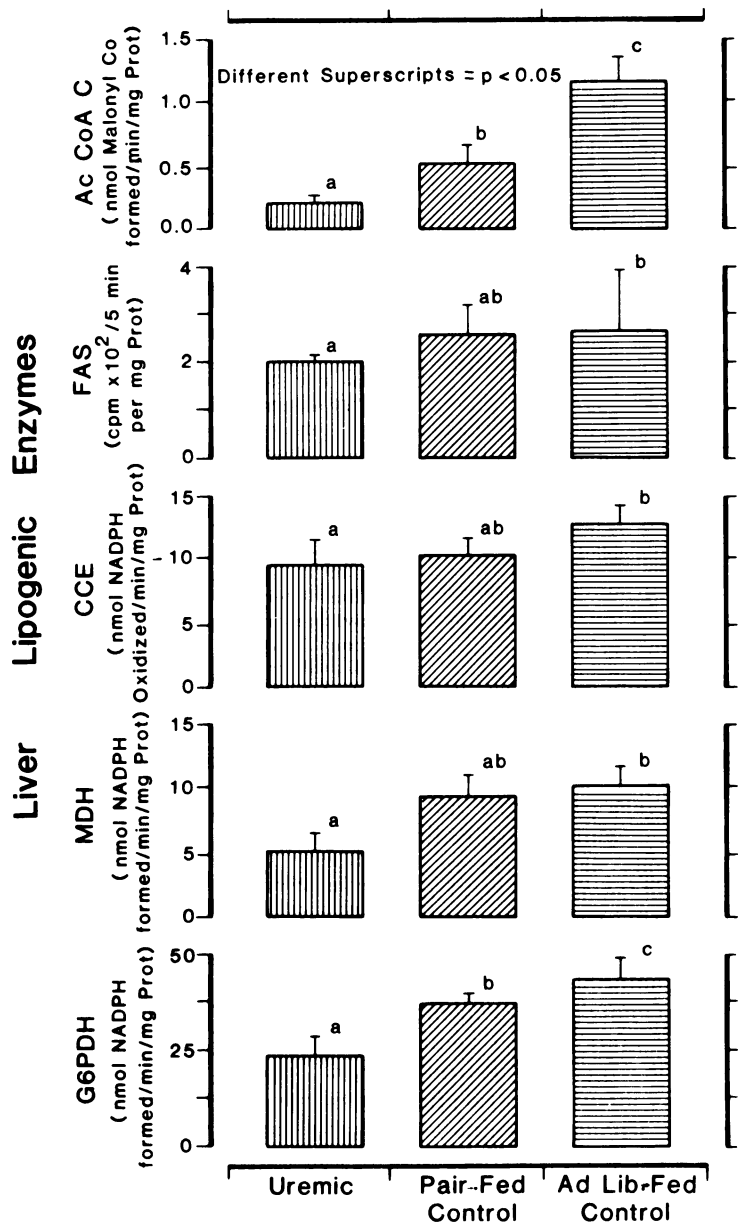

FIGURE 2 Activity of liver lipogenic enzymes. Liver homogenates were prepared from ad lib.-fed controls (horizontal lines), pair-fed controls (oblique lines), and uremic animals (vertical lines). The activity of AcCoAC, FAS, CCE, MDH, and G6PDH was measured as described in the Methods section. Each bar represents the mean (±SE) from six different animals. Different superscriptions on each bar indicates $P < 0.05$ by analysis of variance.

time course of lipid synthesis in the absence and presence of insulin (1×10^{-7} M) in hepatocytes from these intragastrically fed animals. Hepatocytes from the uremic animals remained refractory to insulin whereas the hepatocytes from the pair-fed controls became responsive to insulin.

It was interesting to note that in these intragastrically fed uremic and pair-fed controls, the basal lipid synthesis was ~20 times higher than in respective orally ad lib -fed uremics and their pair-fed controls (Fig. 1). The enhanced lipid synthesis during the intragastric feeding period was partially due to a four-

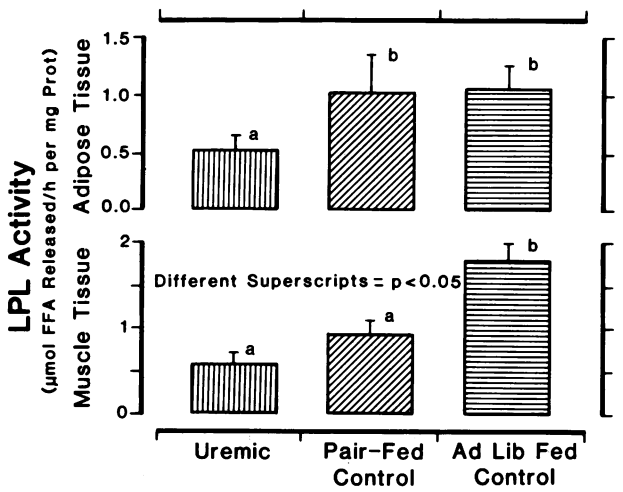

FIGURE 3 LPL activity. Adipose tissue (*upper* panel) and muscle tissue (*lower* panel) homogenates were prepared from ad lib.-fed controls (horizontal lines), pair-fed controls (oblique lines), and uremic animals (vertical lines). The activity of LPL was measured as described in the Methods section. Each bar represents the mean (±SE) from six different animals. Different superscripts on each bar indicates $P < 0.05$ by analysis of variance.

fold increase in the activity of the AcCoAC, the rate-limiting step in liver lipogenesis (Fig. 5). The relationship between basal lipid synthesis and AcCoAC activity was maintained in the two groups of animals during the two experimental feeding regimens. There were no significant changes in the activities of the other lipogenic enzymes studied in the liver (Fig. 5) during the intragastric feeding. The increased lipogenesis observed in these animals, who were food restricted for 3 wk and fed with an isocaloric diet for 1 wk, is consistent with the observation of others (28–29) who found that feeding a high-carbohydrate, low-fat diet to fasted rats greatly enhanced the capacity of the liver to synthetize lipids. Also, the difference in diet and feeding schedule between the orally fed and intragastrically fed animals might have played a role in the differences in basal lipogenesis observed. Fig. 6 also demonstrates that in these intragastrically fed animals, a defect in the triglyceride removal system persisted in the uremic animals as evaluated by LPL activity.

The interpretation of these in vivo experiments is difficult because of other associated changes that might occur in the hormonal and substrate milieu. Therefore,

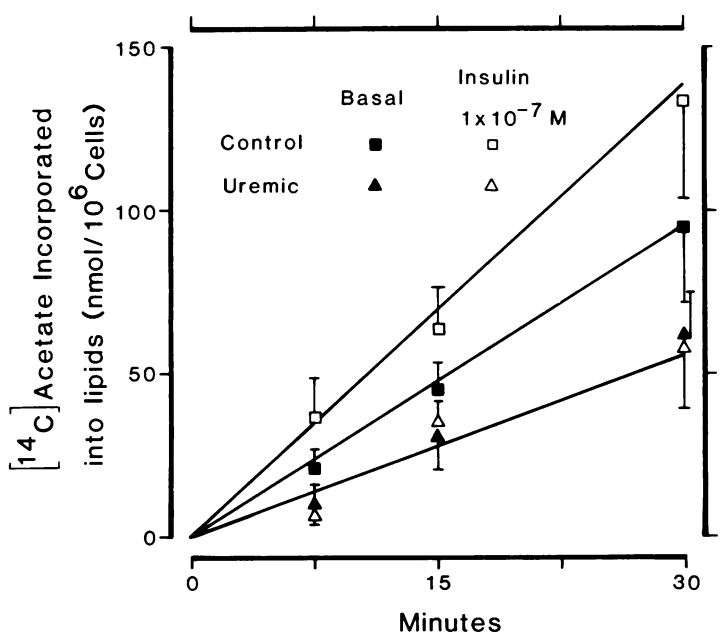

FIGURE 4 Time course of lipid synthesis. Hepatocytes were isolated from pair-fed controls (■ □) and uremic animals (▲ △) intragastrically refed an isocaloric diet during the last week of the experimental period. The cells were incubated in the absence (filled) and presence (open) of insulin 1×10^{-7} M. [1-^{14}C]Acetic acid (sodium salt, 5 mM, 0.01 mCi/mmol) incorporation into lipids was studied and, at different times, total lipids were extracted into a mixture of chloroform, water, and methanol. Each point is the mean (±SE) of triplicate samples from three different experiments.

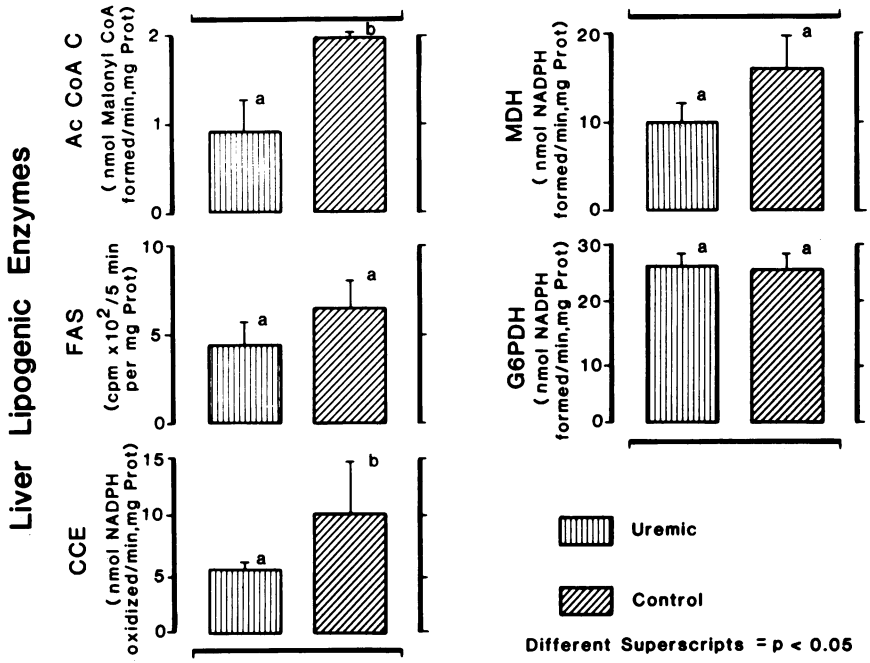

FIGURE 5 Activity of liver lipogenic enzymes. Liver homogenates were prepared from pair-fed controls (oblique lines) and uremic animals (vertical lines) intragastrically refed an isocaloric diet during the last week of the experimental period. The activity of AcCoAC, FAS, CCE, MDH, and G6PDH was measured as described in the Methods section. Each bar represents the mean (±SE) from six different animals. Different superscripts on each bar indicates $P < 0.05$ by t test.

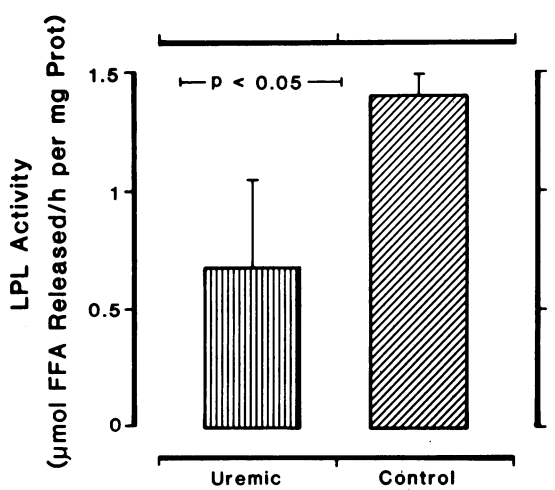

FIGURE 6 LPL activity. Adipose tissue homogenates were prepared from pair-fed controls (oblique lines) and uremic animals (vertical lines) intragastrically refed an isocaloric diet during the last week of the experimental period. The activity of LPL was measured as described in the Methods section. Each bar represents the mean (±SE) from three different animals.

to clarify the role of uremia itself in insulin resistance, we cultured hepatocytes from uremic, pair-fed control, and ad lib.-fed control animals in a chemically defined medium free of serum. Fig. 7 demonstrates the insulin dose-response curves of [^{14}C]acetate incorporation into lipids in the cells and medium (*upper* panel), cells alone (*middle* panel), and the medium alone (*lower* panel). Although the basal lipid synthesis in the uremic cultured hepatocytes appears lower than in the other two groups, it was not significantly different. The distribution of lipids between the cells and the medium under basal conditions was equal. No differences were observed in this distribution pattern among all three groups. Despite 24-h culture in synthetic medium, hepatocytes from the uremic animals remained refractory to insulin whereas the cultured cells from the pair-fed animals, which were initially refractory to insulin when freshly isolated, responded to insulin at every concentration used (paired t test, $P < 0.05$–<0.01). Inability of insulin to stimulate lipid synthesis in cultured hepatocytes from uremic animals could not be due to differences in the acetate pool, since the results were qualitatively similar using $^{3}H_2O$.

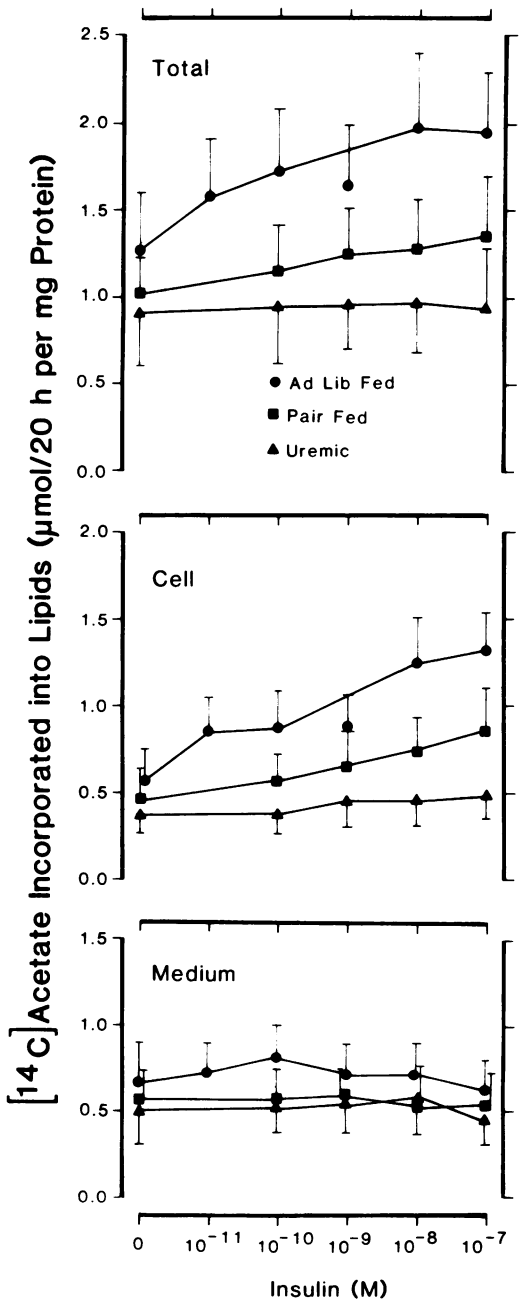

FIGURE 7 Dose-response curve for insulin-stimulated lipid synthesis. Primary cultures of hepatocytes were prepared from ad lib.-fed controls (●), pair-fed controls (■), and uremic animals (▲). Cultures were incubated with [1-^{14}C]acetic acid (sodium salt, 5 mM, 0.01 mCi/mmol) in the absence and presence of different concentrations of insulin. After 20 h of incubation, lipids from the cells and medium (*upper* panel), from the cells only (*middle* panel), and the medium only (*lower* panel), were extracted into a mixture of chloroform, water, and methanol. Each point is the mean (±SE) of triplicate samples from six different experiments.

In cultured hepatocytes from ad lib.-fed controls, the insulin-stimulated lipid synthesis expressed as the absolute value was higher than that in the uremic rats at insulin concentrations from 1×10^{-9} M to 1×10^{-7} M. Insulin response in cultured hepatocytes from pair-fed animals was intermediate to the other two groups. However, if the data were expressed as the absolute increment above basal, there was no statistical difference between the ad lib.-fed and pair-fed controls. The insulin response in the two control groups was higher than in the uremic group at every insulin concentration except at insulin 1×10^{-9} M, at which the insulin response in the uremic animals was not different from that in the pair-fed animals. It is important to note that insulin favored the accumulation of lipids within the cells without affecting the amount of lipids secreted into the medium. From Fig. 7 it can be calculated that the ratio of lipids in medium to cells decreases with increasing concentrations of insulin in the insulin-sensitive hepatocytes (controls) but is unchanged in the insulin-resistant cells (uremic).

DISCUSSION

The present studies were undertaken to evaluate the mechanism of hyperlipidemia and the role of the liver in the insulin-resistant state of uremia. We have developed a model in the rat that leads to a predictable degree of severe uremia. The uremic animals have elevated serum triglyceride and normal serum cholesterol when compared with their pair-fed, age-, and weight-matched controls. Also, these uremic rats are hyperinsulinemic and normoglycemic (8). These characteristics of experimental uremic rats are similar to those found in uremic humans (30, 31), thus making it a suitable animal model to investigate these problems.

Increased lipid production, reduced peripheral tissue removal, or a combination of both could contribute to hyperlipidemia in uremia (1, 2). It is presently well established that abnormalities of lipoprotein lipase, the tissue enzyme system that mediates triglyceride removal, exist in uremia (3–5). We have confirmed in this study that adipose and muscle tissue activities of LPL are decreased in this uremic rat model. However, it is unclear whether liver lipid synthesis is also altered in uremia.

Hepatic triglyceride synthesis from [^{3}H]glycerol in uremic patients (32), from [1-^{14}C]acetate in an in vivo uremic rat model (33), and from [2-^{14}C]acetate in liver slices from uremic rats (34), has been reported to be increased. In contrast to these studies, Cattram et al. (35) concluded that hypertriglyceridemia in uremic patients could not be explained by increased endog-

enous triglyceride production. Also, Bagdade et al. (36) and Gregg et al. (37) studied triglyceride secretion rate in a uremic rat model by in vivo administration of Triton W-R-1339 to inhibit peripheral triglyceride removal, and found it to be normal. Several methodological differences could explain these apparent conflicting data. First, the two clinical studies (32, 33) compared their results in uremic patients with those of Kekki and Nikkila (38) in normal men using a radioisotopic technique that has only been validated for nonuremic subjects (39). Second, in the experiments using [^{14}C] acetate (33, 34), the acetate pool was not measured and the data were not validated by measuring lipid synthesis from tritiated water (40). Third, it is not known if Triton W-R-1339, a nonionic detergent, has any effect on the liver or other important target organs other than inhibition of triglyceride removal from the circulation. In addition, the effect(s) that inhibition of triglyceride removal might have on lipid synthesis in different metabolic states is not known.

To establish if increased lipid synthesis could partially explain the hyperlipidemia of uremia, we have examined both *de novo* lipid synthesis from tritiated water and [^{14}C]acetate and the activities of important key lipogenic enzymes in the liver. The results of our studies clearly indicate that in uremia lipid synthesis is decreased (Figs. 1 and 2); therefore, hyperlipidemia in uremia is solely due to defect(s) in clearance as previously suggested (1–5, 33–35).

The primary role of nutrition in the regulation of lipid synthesis is well recognized (15, 28, 29). Several lines of evidence demonstrate that decreased food intake cannot solely explain the decreased lipid synthesis in the uremic animals. First, when compared with their orally pair-fed controls, the uremic animals demonstrate a decreased incorporation of $^{3}H_2O$ and [^{14}C]acetate into lipids. Also, the activities of liver AcCoAC and G6PDH are significantly decreased (Figs. 1 and 2). Secondly, uremic animals intragastrically fed an isocaloric diet still demonstrate a decrease in *de novo* lipid synthesis and activity of AcCoAC when compared with their controls (Figs. 4 and 5).

The mechanism by which lipid synthesis is decreased in uremia is presently unknown. The animals studied had a severe degree of uremia and they could have had multiple defects not evaluated, which could partially be responsible for the altered lipid metabolism. It is apparent, however, that extracellular factor(s) may be responsible for the decreased basal lipid synthesis. When the freshly isolated hepatocytes from uremic animals are cultured in a well-defined chemical medium free of serum for 24 h, they partially recover the ability to synthesize lipids in the absence of insulin, comparable to the ad lib.-fed animals (Fig. 7). One of the extracellular factor(s) involved may be glucagon, which is increased in the uremic rats (231±22 pg/ml) when compared with their pair-fed (75±11 pg/ml) and ad lib.-fed control animals (155±17 pg/ml; unpublished observation). Glucagon is known to inhibit AcCoAC in the liver (41).

Peripheral insulin resistance in uremia is well recognized (6); however, the role of the liver is unsettled. We have recently demonstrated that the liver in uremia is insulin resistant with regard to amino acid transport, which is not due to defects either in insulin binding or its internalization (8). DeFronzo et al. (7) have demonstrated a normal suppression of hepatic glucose production by insulin in uremic patients. It is important to recognize that in a given metabolic state, one tissue, but not another, may be resistant to insulin (42, 43). Furthermore, a given cell may be resistant to one but not another hormone action (44). Therefore, the designated "hormone-resistant state" should be qualified for each specific tissue and individual hormone action.

The present study clearly demonstrates that livers of uremic rats are also insulin resistant with regard to lipid synthesis. However, freshly isolated hepatocytes from both uremic and pair-fed animals were refractory to insulin, suggesting that nutritional deprivation might have rendered hepatocytes insulin resistant with regard to lipid synthesis. To determine whether the insulin-resistant state was due to nutritional deprivation or uremia itself, we used two approaches. First, we intragastrically fed an isocaloric diet to the uremics and to the pair-fed controls (Fig. 4) Secondly, we cultured hepatocytes from the orally ad lib.-fed uremic animals and their pair-fed controls for 24 h (Fig. 7). We have demonstrated that hepatocytes from the uremic animals remained insulin resistant, while those from pair-fed controls responded normally to insulin when they were provided with enough nutrients in vivo or in vitro.

Primary cultures of isolated rat hepatocytes from uremic rats are insulin resistant similar to the freshly isolated hepatocytes from uremic rats. This quality renders these cultures useful for studying the postinsulin binding events responsible for the insulin-resistant state in the absence of the complicating hormonal and substrate changes that occur in vivo.

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REFERENCES

1. Bagdade, J. D. 1975. Disorders of carbohydrate and lipid metabolism in uremia. *Nephron.* 14:153–162.
2. Meuck, C. C., and E. Ritz. 1980. Hyperlipoproteinemia in renal insufficiency. *Nephron.* 25:1–7.
3. 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.
4. Felts, J. M., B. Zacherle, and I. Staprons. 1976. Mechanism of hyperlipidemia in chronic failure. Proceedings of the 9th Annual Contr. Conference of Artificial Kidney Program, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, Department of Health, Education and Welfare, Publication No. NIH 77-467m. 14.
5. Murase, T., D. C. Cattron, B. Rubenstein, and G. Steiner. 1975. Inhibition of lipoprotein lipase by uremic plasma, a possible cause of hypertriglyceridemia. *Metab. Clin. Exp.* 24:1279–1286.
6. DeFronzo, R. A., R. Andres, P. Edgar, and W. G. Walker. 1973. Carbohydrate metabolism in uremia: A review. *Medicine (Baltimore).* 52:469–481.
7. DeFronzo, R. A., A. Alvestrand, D. Smith, and R. Hendler. 1981. Insulin resistance in uremia. *J. Clin. Invest.* 67:563–568.
8. Kauffman, J. M., and J. F. Caro. 1983. Insulin resistance in uremia. Characterization of insulin action binding and processing in isolated hepatocytes from chronic uremic rat. *J. Clin. Invest.* 71:698–708.
9. Caro, J. F., and J. M. Amatruda. 1982. Glucocorticoid-induced insulin resistance. The importance of postbinding events in the regulation of insulin binding, action, and degradation in freshly isolated and primary cultures of rat hepatocytes. *J. Clin. Invest.* 69:866–875.
10. Caro, J. F., G. Muller, and J. A. Glennon. 1982. Insulin processing by the liver. *J. Biol. Chem.* 257:8459–8466.
11. Lanza-Jacoby, S., H. S. Sitren, N. R. Stevenson, and F. E. Rosato. 1982. Changes in circadian rhythmicity of liver and serum parameters in rats fed a TPN solution by continuous and discontinued intravenous or intragastric infusion. *J. Parenter. Int.* 6:496–502.
12. National Research Council. 1978. National Academy of Sciences. Nutrition Requirements of Laboratory Animals. No. 10, Washington, DC.
13. Cech, J. M., R. B. Freeman, Jr., J. F. Caro, and J. M. Amatruda. 1980. Insulin action and binding in isolated hepatocytes from fasted streptozotocin-diabetic and older spontaneously obese rats. *Biochem. J.* 188:839–845.
14. Caro, J. F., and J. M. Amatruda. 1980. Functional relationships between insulin binding, action, and degradation. *J. Biol. Chem.* 255:10052–10055.
15. Caro, J. F., and J. M. Amatruda. 1982. The regulation of lipid synthesis in freshly isolated and primary cultures of hepatocytes from fasted rats: the primary role of insulin. *Metab. Clin. Exp.* 31:14–18.
16. Caro, J. F., and J. M. Amatruda. 1980. Insulin receptors in hepatocytes: postreceptor events mediated down-regulation. *Science (Wash. DC).* 210:1029–1131.
17. Hsu, R. Y., R. H. W. Butterworth, and S. W. Porter. 1969. Pigeon liver fatty acid synthetase. *Methods Enzymol.* 14:33–34.
18. Takeda, R. F., Suzuki, and H. Inoue. 1969. ATP citrate lyase. *Methods Enzymol.* 13:153–160.
19. Kaplan, M. D., and G. H. Fried. 1973. Adaptive enzyme responses in adipose tissue of obese hyperglycemic mice. *Arch. Biochem. Biophys.* 158:711–719.
20. Inoue, H., and M. Lowenstein. 1975. Acetyl coenzyme A carboxylase from rat liver. *Methods Enzymol.* 25:3–11.
21. Schotz, M., A. Garfinkel, R. J. Huebolter, and J. L. Stewart. 1970. A rapid assay for lipoprotein lipase. *J. Lipid Res.* 11:68–69.
22. Hietenan, E., and M. R. C. Greenwood 1977. A comparison of lipoprotein lipase activity and adipocyte differentiation in growing male rats. *J. Lipid Res.* 18:480–490.
23. Bligh, E. G., and N. J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* 37:911–917.
24. Levy, A. L., and C. Keylown. An automated colorimetric (non-fluorometric) assay for triglyceride. *In* Advances in Automated Analysis. Futura Publishing Co., Inc., Mount Kisco, NY. 1:497–502.
25. DeHoff, J. K., L. M. Davidson, and D. Kritchevsky. 1978. An enzymatic assay for determining free and total cholesterol in tissue. *Clin. Chem.* 24:433–435.
26. Dietschy, J. M., and J. D. McGarry. 1974. Limitations of acetate as a substrate for measuring cholesterol synthesis in liver. *J. Biol. Chem.* 249:52–58.
27. Beynen, A. C., W. J. Vaartges, and M. J. H. Geelen. 1979. Opposite effects of insulin and glucagon in acute hormonal control of hepatic lipogenesis. *Diabetes.* 28:828–835.
28. Tepperman, J., and H. M. Tepperman. 1961. Metabolism of glucose 1-C^{14} and glucose 6-C^{14} by liver slides of refed rats. *Am. J. Physiol.* 200:1069–1073.
29. Allmann, D. W., D. D. Hubbard, and D. M. Gibson. 1965. Fatty acid synthesis during fat-free refeeding of starved rats. *J. Lipid Res.* 6:63–74.
30. Reaven, G. M., R. S. Swenson, and M. L. Sanfelippo. 1980. An inquiry into the mechanism of hypertriglyceridemia in patients with chronic renal failure. *Am. J. Clin. Nutr.* 33:1476–1484.
31. Ponticelli, C., G. Barbi, A. Cantaluppi, C. Donati, G. Annoni, and D. Brancaccio. 1978. Lipid abnormalities in maintenance dialysis patients and renal transplant recipients. *Kidney Int.* 8 (Suppl.):872–878.
32. Cramp, D. G., T. R. Tickner, D. J. Beale, J. F. Moorhead, and M. R. Willis. 1977. Plasma triglyceride secretion and metabolism in chronic renal failure. *Clin. Chim. Acta.* 76:237–241.
33. Morin, R. J., M. V. Srikantaiah, and W. D. Davidson. 1980. Effect of uremia on incorporation of acetate into rat plasma and tissue lipids. *Metab. Clin. Exp.* 311–316.
34. Nitzan, M. 1971. Hepatic lipogenesis in acute uremic syndrome. In vitro studies with rat liver slices. *Nutr. Metab.* 13:292–297.
35. Cattran, D. C., S. A. Fenton, D. R. Wilson, and G. Steiner. 1976. Defective triglyceride removal in lipemia associated with peritoneal and hemodialysis. *Ann. Intern. Med.* 85:29–33.
36. Bagdade, J. D., E. Yee, D. E. Wilson, and E. Shafrid. 1978. Hyperlipidemia in renal failure: studies of plasma lipoproteins, hepatic triglyceride production, and tissue

lipoprotein lipase in a chronically uremic rat model. *J. Lab. Clin. Med.* 91:176–186.
37. Gregg, R. C., A. Diamond, C. E. Mondon, and G. M. Reaven. 1977. The effect of chronic uremia and dexamethasone in triglyceride kenetics in the rat. *Metab. Clin. Exp.* 26:875–882.
38. Kekki, M., and E. A. Nikkila. 1971. Plasma triglyceride metabolism in the adult nephrotic syndrome. *Eur. J. Clin. Invest.* 1:345–351.
39. Farquhar, J. W., R. C. Gross, R. M. Wagner, and G. M. Reaven. 1965. Validation of an incompletely coupled two-compartment nonrecycling catenary model for turnover of liver and plasma triglyceride in man. *J. Lipid Res.* 6:119–34.
40. Lowenstein, J. M. 1972. Is insulin involved in regulating the rate of fatty acid synthesis. *Handb. Physiol.* 1 (Endocrinology):415–424.
41. Geelen, M. J. H., A. C. Beynen, R. Z. Christiansen, M. J. Lepreau-Jose, and D. M. Gibson. 1978. Short-term effects of insulin and glucagon on lipid synthesis in isolated rat hepatocytes. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 95:326–330.
42. Livingston, J. N., P. Cuatrecasas, and D. H. Lockwood. 1972. Insulin insensitivity of large fat cells. *Science (Wash. DC).* 177:626–628.
43. Livingston, J. N., and D. H. Lockwood. 1974. Direct measurements of sugar uptake in small and large adipocytes from young and adult rat. *Biochem. Biophys. Res. Commun.* 61:989–996.
44. Amatruda, J. M., H. W. Newmeyer, and C. L. Chang. 1982. Insulin-induced alteration in insulin binding and insulin action in primary culture of rat hepatocytes. *Diabetes.* 31:145–148.