PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 168, 97–104 (1981)

# Acceleration of Low-Density Lipoprotein Catabolism in Man by Total Parenteral Nutrition (41240)

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*Abstract.* Since insulin enhances the catabolism of low-density lipoprotein (LDL) by cultured human cells, a potential role of insulin in the regulation of plasma LDL levels in man is suggested. To evaluate the possible effect of insulin on LDL catabolism *in vivo*, the disappearance rates of injected $^{125}$I-labeled autologous LDL were determined before and after endogenous hyperinsulinemia was evoked by total parenteral nutrition. Multicompartmental analysis of plasma decay curves showed a 26% increase in the fractional catabolic rate of LDL after total parenteral nutrition was started. An associated reduction of plasma cholesterol levels resulted from changes in both LDL and high-density lipoprotein. These results suggest that the reduction of LDL levels during total parenteral nutrition is at least partly accounted for by enhanced LDL catabolism. It is postulated that the enhanced LDL catabolism may result from the hyperinsulinemia that accompanies total parenteral nutrition, via insulin stimulation of receptor-mediated LDL catabolism.

Low-density lipoprotein (LDL) concentrations in plasma are determined in part by removal of LDL at extrahepatic tissue sites (1, 2). LDL is believed to be catabolized by cells. Approximately 30–50% of the LDL catabolized *in vivo* is believed to be degraded following its binding to specific cell surface receptors, the remainder of the catabolism being non-receptor-mediated (3, 4).

A primary factor that appears to regulate the LDL receptor *in vitro* is LDL concentration (4). However, studies using cultured human cells have suggested that certain hormones also play a role in the regulation of the LDL receptor. Insulin in physiological concentrations can increase LDL receptor number in cultured skin fibroblasts (5–7) as can thyroid hormone (7). On the other hand, glucocorticoids appear to inhibit LDL degradation (8). Although these effects were found using physiological concentrations of the hormone with cultured human cells, it is not clear whether these hormones play a regulatory role in LDL metabolism in humans *in vivo*. Therefore, to address the question of whether insulin may regulate the LDL receptor *in vivo*, we evaluated LDL catabolism before and during a perturbation that leads to dramatic changes in endogenous insulin levels. Intravenous hyperalimentation was chosen, since the onset of therapy previously has been shown to result in a rapid and marked sustained endogenous hyperinsulinemia (9). LDL catabolism was first evaluated prior to the institution of intravenous hyperalimentation; parenteral nutrition was then started, and LDL catabolism was reevaluated during the period of hyperinsulinemia that ensued. The effect of hyperinsulinemia evoked by a short-term infusion of exogenous insulin also was determined in one subject.

**Materials and Methods.** *Subjects.* Five patients (one male, four female, aged 52–63) who required longterm total parenteral nutrition (TPN) for a variety of gastrointestinal diseases (Table I) were admitted to the Clinical Research Center for evaluation. In three, the study was performed at the time TPN was initially started, while in the remaining two, TPN was temporarily stopped for the baseline evaluation, and then restarted. None was on drugs known to affect lipid metabolism.

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0037-9727/81/090097-08$01.00/0

TABLE I. PATIENT DATA

| Number | Age | Sex | Diagnosis | Percentage IBW |
|---|---|---|---|---|
| 1 | 52 | M | Crohn's disease | 88 |
| 2 | 63 | F | Scleroderma | 90 |
| 3" | 54 | F | Postsurgical short-bowel syndrome | 60 |
| 4 | 55 | F | Intestinal pseudo-obstruction | 82 |
| 5" | 56 | F | Postsurgical short-bowel syndrome | 98 |

" Previously receiving TPN.

One, non-TPN-requiring 35-year-old male with familial combined hyperlipidemia also was studied. Informed consent was obtained in all cases after the protocol had been approved by the University of Washington Human Subjects Review Committee.

*Protocol.* During the first half of the study, patients were stabilized on their usual hypocaloric oral intakes (<600 cal per day in all cases). During this period, plastic central venous catheters were inserted (if not already present) and plasma was obtained for separation and iodination of LDL (density, 1.019–1.063 g/ml) for reinjection. Autologous LDL was iodinated with $^{125}I$ using the iodine monochloride method as modified for lipoproteins (1) as previously described (5, 6). At least 7 days separated the insertion of the catheter and the injection of the iodinated LDL. Supersaturated KI (250 mg q.i.d.) was administered for 3 days before the injection of $^{125}I$-LDL and continued for the duration of the study. Prior to the injection of $^{125}I$-LDL, a "day curve" (fasting plasma sample and hourly samples thereafter for 8 hr) of glucose and insulin was obtained. Approximately 50 $\mu$Ci $^{125}I$-labeled LDL was then injected intravenously. Blood samples were obtained 10 min after injection and daily thereafter until the end of the study for determination of plasma radioactivity. More than 97% of the radioactivity in plasma was trichloroacetic acid precipitable and previously has been shown to be entirely within LDL (1).

TPN was begun 9–13 days after the injection of $^{125}I$-labeled LDL. Plasma radioactivity determinations were continued for a further 7–10 days. Daily fasting plasma cholesterol, triglyceride, and insulin levels were determined, and lipoprotein lipids were quantified two to three times per week. A second plasma glucose and insulin "day curve" was performed after the TPN infusion rate exceeded 2 liters/day.

The effect of infusion of exogenous insulin was evaluated in the non-TPN-requiring subject who had been injected with $^{125}I$-LDL as part of another study in which LDL turnover rates were being determined. At the end of that study (14 days after the injection of the labeled LDL), blood samples were collected at six hourly intervals for 24 hr, and four 6-hr collections of urine were made. The radioactivity in these samples was measured and urine/plasma (U/P) ratios of radioactivity were calculated as a measure of LDL fractional catabolic rate (1). Insulin was then infused for 4 hr (2 mU/kg/min), with close monitoring of blood glucose levels on a glucose analyzer. Twenty percent dextrose was infused at a varying rate to maintain euglycemia; this rate averaged 306 mg glucose/min. The effect of the resulting hyperinsulinemia without hyperglycemia on the ratio of radioactivity in urine to plasma was determined.

*Total parenteral nutrition.* Therapy with 60% dextrose, 10% Travamin, plus the necessary vitamin, mineral, and electrolyte supplements (10) was administered continuously. Intralipid was not used in any case. After an initial infusion rate of 1 liter/day, the rate was increased to a maximum of 4 liters/day over a 2- to 3-day period. Daily monitoring of electrolytes and minerals were performed and replacements added to the TPN solution as necessary. In one instance insulin therapy was required for 1 day to control hyperglycemia. During the period of hyperalimentation, the patients continued to consume the same diet

as during the first phase of the study. Total calorie intake on TPN was markedly increased (mean, 3480 cal/day; range, 2800–4000).

*Assays.* Plasma cholesterol and triglyceride were determined by Standard Lipid Research Clinic methods using a Beckman Autoanalyzer II (11). HDL cholesterol was determined in plasma following heparin manganese precipitation (11). Plasma was separated by ultracentrifugation at $d = 1.019$ for the determination of very low and intermediate-density lipoproteins (VLDL and IDL; $d < 1.019$). LDL lipids were estimated by subtraction of HDL values from the values in the $d = 1.019$ infranate. Plasma insulin was determined by radioimmunoassay (12) and glucose using a glucose-oxidase method.

*Data analysis and statistics.* The plasma radioactivity data were analyzed in two stages, i.e., prior to the onset and during TPN. Before TPN was started, body weights were stable and a steady state was assumed. During this phase, each patient's plasma radioactivity curve displayed a biphasic decay. Thus the data were analyzed using the two-pool model for LDL (Fig. 1) described previously (1). The pool into which the $^{125}$I-labeled LDL was introduced is a rapidly turning over plasma-containing pool (hereafter referred to as the plasma pool). Since the exchange of this pool with the second has a time scale of days, and since LDL degradation occurs in a matter of a few hours, the sites of LDL degradation appear as part of the plasma pool. Consequently, catabolism occurs from this pool [L (0,1) in Fig. 1; this number also equals the fractional catabolic rate].

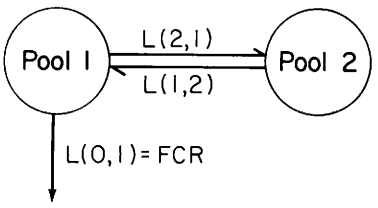


FIG. 1. Two-pool model for LDL catabolism. Pool 1, rapidly turning over plasma-containing pool; Pool 2, slowly turning over "extravascular" pool; L(2,1), fractional loss of LDL to pool 2 from pool 1; L(1,2), fractional loss of LDL to pool 1 from pool 2; L(0,1), fractional loss of LDL to outside the body from pool 1; FCR, fractional catabolic rate.

TPN was commenced midway during the turnover study. At that time the plasma radioactivity decay curve was in its second phase. Our aim was to see whether TPN altered this curve, and if so, to determine why. During TPN the subjects probably were not in the steady state; to overcome this difficulty, the following assumptions were made. We assumed that the onset of any change in LDL metabolism induced by TPN occurs "immediately." Thus, the major effect TPN has on the parameters of the model are immediate, and additional effects due to the non-steady state are assumed to be minor. Since it is hypothesized that the effect of TPN on the model parameters is mediated by insulin, and since (a) insulin levels change very rapidly after starting TPN, and (b) the effects of insulin on LDL receptors occurs within 4 hr *in vitro* (5), this assumption appears to be reasonable. Therefore, linear kinetic analysis also was used during the second phase of the study. Plasma radioactivity data were fitted by nonlinear least-squares using the SAAM computer program (13) and a PDP-10 computer.

Comparison of pre- and post-treatment values was performed using the paired *t* test. Values are shown as mean ± standard deviation.

**Results.** The plasma radioactivity decay curves up to the time that TPN was started were consistent with the two-pool model in all cases. However, the onset of TPN was associated with an immediate break in the plasma radioactivity decay curves (Fig. 2). On the basis of the biexponential decay observed during the first phase of the experiment (i.e., before starting TPN), a "predicted" plasma radioactivity curve was extrapolated for each patient to indicate how the plasma radioactivity would have decayed if TPN had not been started. In each case, the observed decay of plasma radioactivity after starting TPN was more rapid than would be predicted if TPN had no effect on LDL metabolism (Fig. 2). Further, the changes in each case were observed as

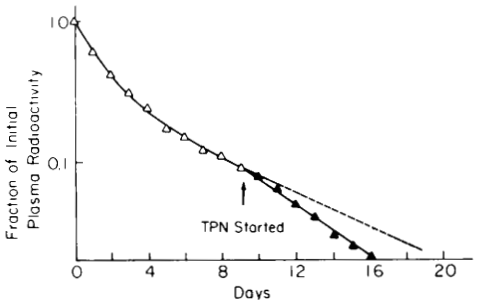


FIG. 2. Effect of total parenteral nutrition (TPN) on the plasma radioactivity curve in patient 4. All patients showed the same pattern. – – –, predicted plasma decay based on data obtained before starting TPN; △——, observed data before starting TPN; ▲——, observed data after starting TPN; ↑, TPN started.

early as the first datum obtained after commencement of therapy.

To determine which rate constants of the model were affected by therapy, initially all the rate constants were allowed to change following the onset of TPN. When this was done, the estimates of the standard deviations of the rate constants were very large. This suggests that not all of them change. Each rate constant [L(0,1); L(2,1); L(1,2)] was then held constant in turn, while the other two were allowed to change. Changes in fractional catabolic rate [L(0,1)] and L(1,2) while holding L(2,1) constant were found to be both necessary and sufficient to explain the observed downward deviation in the plasma radioactivity decay curves. In all subjects the fractional catabolic rate increased (mean change, 26 ± 17%), while L(1,2) increased by 17, 26, and 26% in three patients, but did not change in the other two (Table II).

TPN was associated with a reduction in plasma cholesterol levels in all five subjects, reaching a nadir between 2 and 6 days following the institution of therapy (Table III, Fig. 3). The reduction in plasma cholesterol was accounted for by a reduction in both LDL and HDL cholesterol. LDL cholesterol levels fell in all but one patient (Table IV). In that patient, plasma for lipoprotein determination was not obtained at the time of her nadir in plasma cholesterol. HDL cholesterol levels also fell in all five patients (Table IV). Despite a greater per-

TABLE II. EFFECT OF TPN ON RATE CONSTANTS OF LDL CATABOLIC PARAMETERS

| Subject | $L(2,1)^{a}$ ($days^{-1}$) | $L(1,2)^{bef[a]}$ ($days^{-1}$) | $L(1,2)^{dur[a]}$ ($days^{-1}$) | dur/bef | $L(0,1)^{bef[a]}$ ($days^{-1}$) | $L(0,1)^{dur[a]}$ ($days^{-1}$) | dur/bef |
|---|---|---|---|---|---|---|---|
| 1 | 0.379 (0.076) | 0.552 (0.125) | 0.533 (0.122) | 0.97 | 0.411 (0.013) | 0.452 (0.051) | 1.10 |
| 2 | 0.459 (0.040) | 0.332 (0.041) | 0.314 (0.031) | 0.95 | 0.600 (0.017) | 0.710 (0.085) | 1.18 |
| 3 | 0.224 (0.014) | 0.250 (0.047) | 0.292 (0.033) | 1.17 | 0.364 (0.013) | 0.498 (0.067) | 1.37 |
| 4 | 0.201 (0.025) | 0.302 (0.089) | 0.378 (0.074) | 1.26 | 0.303 (0.014) | 0.454 (0.071) | 1.50 |
| 5 | 0.360 (0.035) | 0.296 (0.028) | 0.372 (0.052) | 1.26 | 0.542 (0.011) | 0.623 (0.057) | 1.15 |

[a] See Fig. 1 for a diagram of the model of LDL metabolism used.

*Note.* Pool 1, Rapidly turning over plasma-containing pool; pool 2, "extravascular" pool; L(2,1), the fraction of pool 1 entering pool 2 per day [Note: In deriving the parameters, L(2,1) was held constant (see text)]; L(1,2), the fraction of pool 2 entering pool 1 per day; L(0,1), the fraction of pool 1 lost from the body per day, fractional catabolic rate; bef, before commencement of TPN; dur, during TPN. Numbers in parentheses are standard deviations.

TABLE III. PLASMA LIPID RESPONSE TO PARENTERAL NUTRITION

| Patient No. | Cholesterol (mg/dl) | | | Triglyceride (mg/dl) | | |
|---|---|---|---|---|---|---|
| | | TPN | | | TPN | |
| | Basal[a] | Nadir | Rebound | Basal | Peak | Rebound |
| 1 | 88 | 46 (4) | 128 | 83 | 113 (4) | 60 |
| 2 | 109 | 72 (6) | 138 | 118 | 205 (12) | 78 |
| 3 | 223 | 203 (5) | 222 | 196 | 635 (6) | 210 |
| 4 | 185 | 118 (5) | 180 | 106 | 140 (10) | — |
| 5 | 123 | 112 (2) | 164 | 114 | 250 (4) | 145 |
| $\bar{x} \pm$ SD | 146 ± 56 | 110 ± 60 | 166 ± 37 | 123 ± 43 | 269 ± 212 | 123 ± 68 |

*Note.* Numbers in parentheses refer to the day after institution of TPN on which the nadir or peak occurred.
[a] Basal values are the means of the daily values prior to commencing TPN.

centage fall in HDL cholesterol, this lipoprotein fraction accounted only for 38% of the reduction in total plasma colesterol.

Plasma triglyceride levels rose in all cases following the institution of TPN (Table III). There was no correlation between the reduction in HDL cholesterol and the increase in $d < 1.019$ triglycerides. Although all patients consumed less than 600 cal/day, all were relatively weight stable during the period prior to intravenous therapy. Likewise, following the institution of TPN, despite a marked increase in maximal calorie intake (mean, 3480 cal/day), negligible weight change occurred in four patients while the fifth gained 1.5 kg. Thus, weight change is unlikely to account for the observed changes in lipoprotein metabolism.

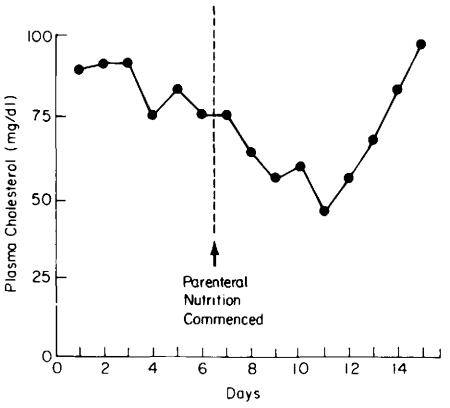


FIG. 3. Time course of changes in plasma cholesterol level in relation to total parenteral nutrition (TPN) in patient 1. The arrow designates the commencement of TPN.

Commencement of TPN was in all cases associated with a prompt and marked increase in basal plasma insulin levels. Mean integrated levels of plasma insulin during the "day curve" increased from 15 ± 10 $\mu$U/ml before TPN to 174 ± 52 $\mu$U/ml ($P < 0.005$) during TPN. Fasting plasma glucose levels increased from 94 ± 14 to 131 ± 23 mg/dl ($P$ = NS) and integrated levels during the "day curve" increased from 110 ± 31 to 142 ± 16 mg/dl ($P$ = NS).

To test the role of insulin per se on LDL catabolism, a single non-TPN-requiring subject was infused with exogenous insulin and sufficient glucose to maintain euglycemia in the presence of hyperinsulinemia. Plasma insulin levels increased from a basal value of 46 $\mu$U/ml to a new steady-state level of 542 $\mu$U/ml during the infusion. The hyperinsulinemia was accompanied by a doubling in LDL fractional catabolic rate as determined by the urine to plasma ratio from 0.28 ± 0.03 to 0.55 day$^{-1}$.

**Discussion.** This study was designed to evaluate whether TPN, with its accompanying metabolic effects including hyperinsulinemia, is associated with a change in the degradation of LDL *in vivo*. Kinetic parameters based on computer-modeling of data obtained after the injection of radiolabeled LDL were used to evaluate LDL catabolism before and after TPN was started. Ideally the study order should have been reversed in some cases. However, therapeutic considerations in these

TABLE IV. PLASMA LIPOPROTEIN RESPONSE TO TOTAL PARENTERAL NUTRITION

| Patient No. | LDL Cholesterol (mg/dl) | | | HDL Cholesterol (mg/dl) | | |
|---|---|---|---|---|---|---|
| | Pre[a] | Post | % reduction | Pre[a] | Post | % reduction |
| 1 | 64 | 32 | 50 | 23 | 12 | 48 |
| 2 | 56 | 58 | −4 | 36 | 19 | 47 |
| 3 | 109 | 72 | 34 | 51 | 26 | 49 |
| 4 | 104 | 52 | 50 | 47 | 23 | 51 |
| 5 | 35 | 29 | 17 | 35 | 26 | 26 |
| $\bar{x} \pm$ SD | 74 ± 32 | 49 ± 18 | 29 ± 23 | 38 ± 11 | 21 ± 6 | 44 ± 10 |

[a] The pretreatment value was the mean of two to four determinations made before commencing TPN. The post value represents the lowest value obtained after TPN was started.

patients precluded this possibility. The results demonstrate that there was an immediate increase in the fractional catabolic rate of LDL in all cases. Associated with the institution of TPN, plasma cholesterol levels fell rapidly, as previously noted by others (14, 15). Our data demonstrate that the reduction of plasma cholesterol levels is due to a reduction of both LDL and HDL.

LDL levels could fall as a result of enhanced LDL catabolism, reduced LDL production or both. Since LDL–cholesterol, and hence presumably apo-LDL, mass was changing during phase 2 of the study, we cannot accurately calculate LDL synthesis rates during TPN and cannot compare LDL synthesis in the two phases of the study. Therefore, we cannot exclude the possibility that a reduced input of LDL is accompanying the observed changes in LDL removal during TPN. In fact, it is likely that LDL synthesis also is reduced by TPN, at least in some cases, e.g., in case 1, LDL cholesterol fell by 50%, while LDL FCR increased by only 10%. Further, two reports have shown that LDL levels fell during TPN in subjects with homozygous familial hypercholesterolemia, i.e., who have no LDL receptors (16, 17). In those subjects, the rate of change of plasma cholesterol was slower (nadir in 2 to 6 weeks) than in our patients (nadir in 2 to 6 days). This is consistent with the idea that the fall in plasma cholesterol in homozygous familial hypercholesterolemia occurs by a mechanism other than the LDL receptor-mediated pathway (e.g., via reduced LDL production. Hence, it is probable that the more rapid cholesterol lowering in our patients was determined by rapid changes in LDL catabolism that were measured directly, but a simultaneous reduced input of LDL also could have played a role.

The data obtained do not permit definite resolution of (i) whether the increase in FCR is due to increase in LDL receptor-mediated degradation, and, if so, (ii) whether the increase in LDL receptor activity is mediated by insulin as has been shown to occur *in vitro* (5, 6). However, there is some rationale for suggesting that insulin might be involved.

Ambient plasma insulin levels rose approximately 10- to 15-fold following the institution of intravenous hyperalimentation. We have previously demonstrated that physiological concentration of insulin *in vitro* enhances the degradation of LDL by cultured human skin fibroblasts by increasing the number of LDL receptors, the effect being observed as early as 4 hr after exposure of the cells to insulin (5). Since LDL degradation *in vivo* is believed to be mediated to a considerable extent via the LDL receptor pathway (3, 4), it is of interest that the extent of stimulation of LDL fractional catabolic rate in the present study (26%) is quantitatively similar to the extent of stimulation of LDL degradation by insulin *in vitro*, reported previously (6). Within the context of the model, an increased number of LDL receptors *in vivo* would be expected to result in an increased fractional catabolic rate, since the sites of catabolism of LDL are assumed to be part of the

rapidly turning over plasma-containing pool (pool 1) and the rate of degradation from this pool [L(0,1)] equals the fractional catabolic rate. The change in L(1,2) in three of the five subjects could reflect more sites in pool 1 to which LDL from pool 2 could return, bind, and be metabolized. It is not clear why only three of the five subjects showed this particular change. Therefore, the present findings support the hypothesis that insulin can modulate the LDL receptor *in vivo*, thereby influencing the catabolism of LDL. This raises the question of why plasma cholesterol levels fell during TPN in subjects who lacked LDL receptors on a genetic basis (16, 17). Possibly, TPN (or insulin) could have influenced non-receptor-mediated LDL removal in these and our subjects. No direct measurements of the kinetics of LDL removal are provided in the two studies of homozygous familial hypercholesterolemics and preliminary data from our laboratory suggests that insulin does not stimulate the catabolism by cultured fibroblasts and smooth muscle cells of LDL that has been modified so that it is not recognized by the LDL receptor (Chait, unpublished observations). Thus the TPN-induced reduction of plasma cholesterol and LDL levels in homozygous familial hypercholesterolemia presumably is accounted for by reduced LDL production.

Because of the many other changes that occur as a result of TPN, it is possible that the kinetic changes we observed were due to some factor other than insulin. However, the study in the single subject infused with insulin suggests that this hormone is indeed playing a regulatory role in LDL catabolism *in vivo*. Increased LDL degradation would be expected to accelerate both the loss of radioactivity from plasma and its appearance in urine as free radioiodide, manifested as an increased ratio of radioactivity in urine-to-plasma (U/P) ratio. The marked increase in this ratio, observed by as early as 4 hr of insulin infusion in the subject not receiving TPN, thus further supports the contention that insulin can indeed enhance LDL catabolism *in vivo*.

It is of interest that HDL cholesterol levels also fell following the commencement of TPN. Changes in HDL cholesterol are frequently mirrored by a reciprocal change in VLDL triglyceride (18); this was not the case in the present study and in patients with homozygous familial hypercholesterolemia (17), suggesting that the changes in HDL levels observed were unrelated to changes in plasma triglyceride metabolism. The reason for the reduction in HDL following TPN is not clear and speculation concerning the mechanism of this change is difficult in the light of the present uncertainty of the metabolic fate of HDL.

Whether or not insulin modulates circulating levels of cholesterol and LDL on a more long-term basis remains speculative. Supportive evidence is provided by the positive correlation between plasma glucose and LDL cholesterol levels observed in treated insulin-dependent diabetes [(19), Eckel and Bierman, unpublished observations], suggesting that the better insulinized the patient, the lower the level of LDL cholesterol. Also, non-insulin-dependent diabetics, who frequently are insulin resistant, also appear to have increased levels of LDL (20–22) which are reduced by insulin therapy (20, 21). In addition, sucrose feeding, which is associated with increased insulin secretion, has been shown to be associated with increased clearance of LDL and lower circulating cholesterol and LDL levels (23). Thus, circulating levels of insulin might interact *in vivo* with other factors known to influence LDL metabolism (4) to regulate the level of circulating cholesterol.

The expert technical assistance of Martha Jensen and the skillful typing of Sharon Kemp are gratefully acknowledged. Supported in part by NIH Grant AM 02456, Juvenile Diabetes Foundation Grant 78 R 159, a grant from Reynolds Industries, and the Graduate Student Research Fund, University of Washington. This work was performed at the University Hospital Clinical Research Center (RR-37). Alan Chait is a recipient of Special Emphasis Career Award: Diabetes/Cardiovascular K01 AM 00592. David Foster is a recipient of a Research Career Development Award (HL 00576).

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Received February 10, 1981. P.S.E.B.M. 1981, Vol. 168.