Use of [15N]glycine in the measurement of apolipoprotein B synthesis in perfused rat liver

Julian B. Marsh[1] **and Margaret R. Diffenderfer**

Atherosclerosis Center and the Division of Nutrition, Department of Physiology and Biochemistry, Medical College of Pennsylvania, Philadelphia, PA 19129

Abstract Rat livers were perfused with [15N]glycine and unlabeled sodium benzoate by the single-pass technique via the portal vein or in retrograde fashion via the inferior vena cava. Perfusate [15N]hippurate enrichment was significantly greater than that of hepatic free glycine from 15 to 90 min, regardless of the direction of the perfusion. This result implies that differential labeling by periportal versus perivenous hepatocytes is not likely. When fasted animals were compared to those fed a chow diet or a sucrose-enriched diet, the labeling ratio of medium hippurate/hepatic free glycine decreased by only 9% in spite of a 5-fold decrease in the concentration of intrahepatic free glycine. Administration of nembutal to the intact animal significantly increased the enrichment of medium hippurate by 24% but did not affect the enrichment of the hepatic free glycine. We conclude that the difference between hippurate and free glycine enrichment is related to intracellular compartmentation of glycine transport. We suggest that measurement of the enrichment of hippurate after the administration of [15N]glycine with benzoate in intact animals or human subjects can therefore be used to estimate the enrichment of the intracellular precursor pool of glycine with a correction factor that does not vary appreciably under fed or fasted conditions. When uniformly labeled deuteroglycine was used as the tracer, enrichment of hepatic free glycine was decreased fivefold compared with [15N]glycine. Isotopic enrichments of $apoB_H$ and $apoB_L$ from the $d<1.063$ g/ml lipoprotein fraction isolated from the perfusion medium between 30 and 90 min averaged 3.7 and 4.1% excess, respectively. As measured by SDS-PAGE, twice as much $apoB_L$ as $apoB_H$ was secreted, indicating a larger intrahepatic pool of $apoB_L$. —**Marsh, J. B., and M. R. Diffenderfer.** Use of [15N]glycine in the measurement of apolipoprotein B in perfused rat liver. *J. Lipid Res.* 1991. **32:** 2019–2024.

Supplementary key words [15N]hippurate

Several stable isotopically labeled amino acids have been used for the measurement of apolipoprotein synthesis rates by the constant-infusion technique (1, 2) since the original study using [15N]glycine (1). In this approach, estimation of the isotopic enrichment of the intracellular amino acid precursor is required to calculate the fractional synthetic rate from the measured enrichment of the amino acid in the apolipoprotein. Since it is not feasible to measure intracellular glycine directly in humans, the plateau value for VLDL apoB has been used. This has two disadvantages: it requires a somewhat long infusion period for an accurate estimate of the plateau enrichment, and the level of enrichment is low when the plasma pool of VLDL is high. A more advantageous approach would be to estimate the glycine enrichment within the liver from that of the glycine conjugation product with benzoic acid, hippuric acid.

Infusion studies in humans (1) have shown that urinary hippurate was 20–30% more highly enriched than the plateau value for the enrichment in glycine of plasma VLDL apoB. This discrepancy was explored in a rat liver perfusion system where direct measurement of intrahepatic free glycine and glycyl tRNA could be carried out (3). In this study, the same 20–30% greater enrichment of hippurate compared to hepatic free glycine was found as in the human infusion study (1) and in earlier in vivo studies (4). However, it was not known whether the greater enrichment of hippurate would vary with the metabolic state, nor had the mechanism of the phenomenon itself been investigated. It was also not known whether deuteroglycine, with a very low natural abundance, would be preferable to [15N]glycine.

In the present experiments, the effects of fasting for 24 h and of sucrose feeding on the hippurate/free glycine 15N labeling ratio were measured to determine the extent to which metabolic conditions might affect the ratio and therefore the usefulness of glycine as a tracer in vivo. We also examined the effects of reversing the direction of perfusion flow in order to assess the role of perivenous versus periportal hepatocytes. In addition, evidence concerning the importance of intracellular glycine transport processes

Abbreviations: $apoB_H$ and $apoB_L$, high and low molecular weight forms of apolipoprotein B; DATD, N,N'-diallyltartardiamide; EDTA, ethylenediamine tetraacetic acid; MTBSTFA, N-methyl-N-*t*-butyldimethylsilyl trifluoroacetamide; PMSF, phenylmethyl sulfonyl fluoride; SDS-PAGE, sodium dodecylsulfate-polyacrylamide gel electrophoresis; TBDMS, *t*-butyldimethylsilyl; VLDL, very low density lipoproteins.

[1]To whom correspondence should be addressed at: Medical College of Pennsylvania, 3300 Henry Avenue, Philadelphia, PA 19129.

was sought by comparing the hippurate/free glycine labeling ratio in livers obtained with or without nembutal administration to the intact animal. We also compared the effectiveness of deuteroglycine and [^{15}N]glycine in labeling the hepatic free glycine pool.

MATERIALS AND METHODS

Liver perfusion

Male rats of the Sprague-Dawley strain were used. Liver perfusion in the single-pass mode was carried out at 37°C as previously described (3) after cervical fracture, except in those experiments where nembutal was administered. In the normal perfusion mode the liver remained in situ, but in the retrograde mode, after the inferior vena cava was cannulated, the superior vena cava was ligated and the liver was excised and placed on a wire screen with the outflow emerging from the severed portal vein. The perfusion flow rate was kept at approximately 3 ml/g liver per min by varying the inflow pressure (50–75 cm H_2O). The perfusion medium was Krebs-Ringer-bicarbonate buffer, gassed with 95% O_2–5% CO_2, pH 7.4, containing 0.3 mM glycine, enriched by a 1:1 dilution of 99% [^{15}N]glycine (or glycine-d_5) with glycine containing only the natural abundance of ^{15}N. The medium also contained 0.014 mM sodium benzoate, 0.1% glucose, and 0.2 mM glutamine.

Perfusion with labeled glycine was carried out for 15–90 min, after which the medium was switched to one devoid of glycine for 90 sec, and sections of the three largest lobes were quickly placed in 0.15 M NaCl at 0°C, rinsed briefly, and individually transferred to tubes containing 10% trichloroacetic acid at 0°C.

Apolipoprotein B isolation

Lipoproteins were isolated from a single collection of perfusion medium from 30 to 90 min. After removal of aliquots for measurement of medium glycine isotope enrichment, the medium was concentrated at 4°C in an Amicon TCF-10 apparatus using a PM10 membrane. The solution was then made 0.002 M with respect to EDTA and the protease inhibitor PMSF was added as described (5). The density was adjusted to 1.063 g/ml with NaBr and ultracentrifugation was carried out for 18 h at 5°C at 48,000 rpm in a Beckman Ti50 rotor. The top 10% of each tube was removed and dialyzed overnight at 4°C against 0.02 M phosphate buffer–0.002 M EDTA, pH 7.4. Partial delipidation with 10 volumes of ethyl ether was followed by addition of 2-mercaptoethanol, glycerol, and SDS (to 2% for each) and the solution was heated at 100°C for 2 min before SDS-PAGE analysis. The polyacrylamide gels were 3.5%, cross-linked with 27% DATD (6). After staining with Coomassie blue and destaining as described with 10% acetic acid (7), the gels were subjected to densitometric scanning at 605 nm. The bands corresponding to apoB_H and apoB_L were cut out and the gels were cleaved with 0.088 M periodic acid (6). The solution was adjusted to 10% trichloroacetic acid (TCA), and after centrifugation and washing of the precipitate by resuspension and recentrifugation in 10% TCA, the precipitated protein was hydrolyzed for 24 h at 110°C in 6 N HCl under nitrogen.

Measurement of isotopic enrichment of glycine and hippurate

Glycine was isolated from the perfusion medium, from the trichloroacetic acid extract of each of the lobes of the liver, and from the apoB hydrolysates by ion exchange chromatography as described (3). Hippurate was isolated from ethyl acetate extracts of perfusion medium and further purified on octadecyl silica columns (3). After derivatization with MTBSTFA (Regis, Cat. 270141) in acetonitrile, aliquots were injected onto a temperature-programmed fused silica capillary column (25 meter cross-linked 5% phenylmethyl silicone, Hewlett-Packard Ultra 2) coupled to a Hewlett-Packard model 5988A mass spectrometer operated in the selected ion monitoring mode. The glycine peak was monitored at *m/z* 246 and 247 (or 248 for deuteroglycine) and the hippurate was monitored at *m/z* 236 and 237 (or 238). Isotope enrichment was calculated from standard curves constructed from mixtures of enriched and unenriched glycine. To compensate for any differences in instrument performance, standard curves were run every day along with the unknown samples. A standard curve for hippurate labeled with ^{15}N by synthesis from labeled glycine and benzoyl chloride gave a slope that was 0.99 times that for [^{15}N]glycine, and not significantly different from 1.0. Therefore, the slope of the glycine standard curve was used to measure hippurate enrichment.

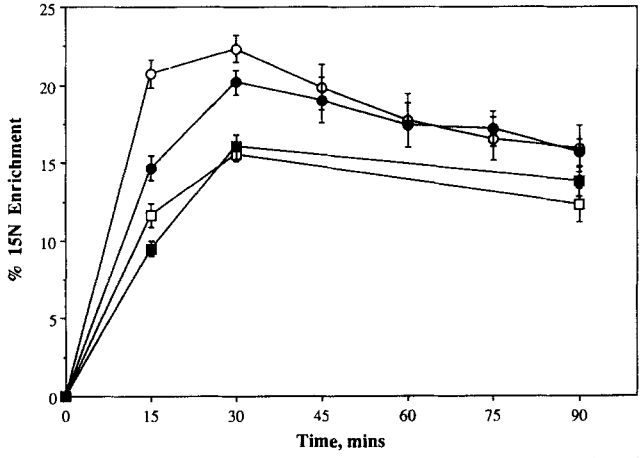

Fig. 1. ^{15}N enrichment of perfusate hippurate (top two curves) and hepatic free glycine (bottom two curves). Open symbols, normal perfusion; closed symbols, retrograde perfusion. The error bars represent the standard error of the mean. Each point represents the mean of 3–10 experiments.

TABLE 1. Body weight, liver weight, liver glycogen, liver free glycine content, and net glycine output by livers perfused under different conditions[a]

	(1) Normal Diet, Normal Perfusion	(2) Normal Diet, Retrograde Perfusion	(3) Normal Diet, Normal Perfusion, Nembutal	(4) Fasted, Normal Perfusion	(5) Normal Diet plus Sucrose Normal Perfusion
Body weight, g	247 ± 17.3 (10)	254 ± 22.5 (12)	255 ± 5.8 (4)	232 ± 10.4 (5)	254 ± 11.0 (6)
Liver weight, % body weight	5.08 ± 0.381 (10)	5.47 ± 0.465 (12)	5.26 ± 0.722 (4)	4.24 ± 0.173[b] (5)	5.29 ± 0.311 (6)
Liver glycogen, mg/g	27.1 ± 13.2 (4)	21.0 ± 4.84 (4)	24.7 ± 5.18 (4)	0.83 ± 1.00[c] (5)	16.8 ± 3.99 (6)
Liver free glycine, μmol/g	2.02 ± 0.836 (4)	2.72 ± 0.819 (4)	2.26 ± 0.473 (4)	0.53 ± 0.021[c] (5)	2.57 ± 0.338 (6)
Net glycine output, μmol/g/h[d]	4.3 ± 1.11 (3)	5.9 ± 0.56 (3)	6.6 ± 1.03[e] (4)	4.3 ± 0.43 (5)	4.8 ± 0.26 (6)

[a]The numbers represent the means ± the SEM. The numbers in parentheses represent the number of experiments.
[b]Significantly different from the mean for all fed animals ($P < 0.01$).
[c]Significantly different from the mean for all fed animals ($P < 0.001$).
[d]Calculated from the difference between the isotopic enrichment of perfusion medium free glycine before (49.5%) and after (43–47%) 30 min of perfusion, the average flow rate of 2280 ml/h, the average liver weight, and the initial concentration of glycine (0.3 mM).
[e]Significantly different from the mean of all other normal perfusions (the average of columns 1, 4, and 5, which was 4.5 ± 0.29); $P < 0.03$.

Other methods

Hepatic free glycine was measured in an aliquot of the trichloroacetic acid extract by the isotope dilution method. Protein concentrations were measured by the bicinchoninic acid method of Smith et al. (8). Trichloroacetic acid-soluble glycogen was measured using the phenol-sulfuric acid method (9) after precipitation of glycogen with 2 volumes of ethanol, using glucose as a weight standard. Statistical comparisons were carried out using Student's *t* or Mann-Whitney non-parametric methods.

RESULTS

Effects of retrograde perfusion

Fig. 1 shows the time course of the ^{15}N enrichment of medium hippurate and liver free glycine in normal and retrograde perfusion modes. Except for the 15-min time point, no statistically significant differences between the two modes of perfusion were observed. We ascribe the difference at 15 min to the swelling of the liver which results from retrograde perfusion at the relatively high flow rate of 3 ml/g per min. However, the livers appeared to adjust to this circumstance since measurement of both total protein and d<1.063 g/ml protein output from 30 to 60 min was not significantly altered (data not shown) and liver glycogen and free glycine content were not significantly different from normally perfused liver (**Table 1**).

Effects of nembutal administration

Administration of the standard 60 mg/kg dose of nembutal intraperitoneally prior to surgery resulted in a highly significant increase in medium hippurate enrichment at 15 and 30 min with no significant change in liver free glycine enrichment at 30 min (**Fig. 2**).

Effects of fasting and dietary sucrose

The fasted rats had extremely low levels of liver glycogen and a fivefold reduction in hepatic free glycine content (Table 1). As shown in **Table 2,** there was no difference in the average enrichment of hepatic free glycine and a slight though significant (9%) decrease in the hippurate/hepatic free glycine ratio. Dietary sucrose enrichment had no significant effect on any parameter measured.

^{15}N enrichment of apoB

The ^{15}N enrichment of $apoB_H$ and $apoB_L$ is shown in **Table 3,** along with estimates of their secretion rates in

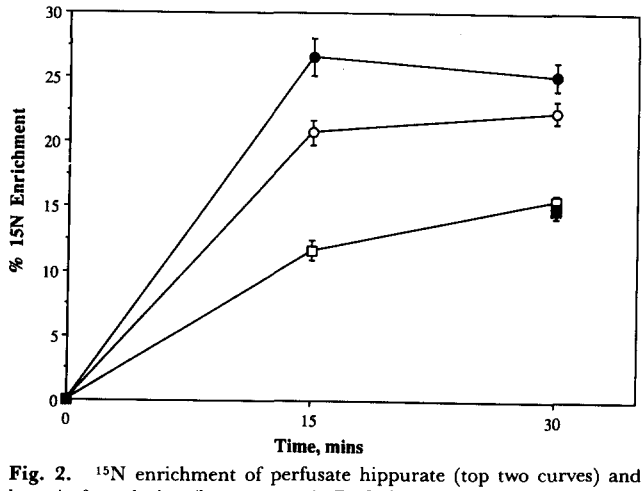

Fig. 2. ^{15}N enrichment of perfusate hippurate (top two curves) and hepatic free glycine (botton curve). Perfusions were carried out in the normal manner. Open symbols, livers perfused after cervical fracture of the rat; closed symbols, after nembutalization. The error bars represent the standard error of the mean. Each point represents the mean of 3–10 experiments.

TABLE 2. Effect of fasting and sucrose feeding on ^{15}N enrichment of liver free glycine and perfusate hippurate[a]

	Hippurate (% ^{15}N)		Liver Free Glycine (% ^{15}N)	Ratio, Hippurate/Glycine
	15 min	30 min	30 min	30 min
Normal diet	21.0 ± 1.08 (10)	22.3 ± 0.866 (7)	15.5 ± 0.442 (4)	1.41 ± 0.0674 (4)
Normal diet plus sucrose	18.9 ± 0.607 (6)	21.4 ± 0.714 (6)	14.7 ± 0.291 (6)	1.46 ± 0.0254 (6)
Fasted	18.5 ± 1.31 (5)	19.3 ± 1.39 (5)	14.5 ± 0.903 (5)	1.33 ± 0.0156[b] (5)

[a]The numbers represent the means ± the SEM. The numbers in parentheses represent the number of experiments.

[b]Significantly different from the mean in fed rats (1.44 ± 0.0299, n = 10); $P < 0.03$. The means were derived from the ratios in each individual experiment.

the d<1.063 g/ml lipoprotein fraction. The total apoB secretion averaged 24 μg/g per h, of which two-thirds was $apoB_L$. However, there was no significant difference in isotopic enrichment between the two forms of apoB.

Hepatic free glycine and medium hippurate isotopic enrichment from deuteroglycine

As shown in **Table 4,** deuteroglycine did not preserve its label in the pool of hepatic free glycine in perfusions carried out after administration of nembutal. Hippurate enrichment in the medium averaged 1.4%, only 6% of that seen with [^{15}N]glycine. In the absence of nembutal, at 30 min, an enrichment of only 2.9% for hepatic free glycine and of 4.4% for medium hippurate was measured. These values are 19% of the corresponding values for [^{15}N]glycine shown in Table 2.

DISCUSSION

The use of stable isotopically labeled amino acids, such as [^{15}N]glycine, in studies of lipoprotein metabolism by the primed constant infusion technique (1) requires an estimate of the isotopic enrichment of the glycine precursor pool. This estimate has been achieved by measuring either the enrichment of the glycine of VLDL-apoB at its plateau (1, 2) or that of the conjugation product with benzoic acid, hippuric acid (1). A disadvantage in estimating by means of the conjugation product is that hippurate is more highly labeled than hepatic free glycine (3). If a correction factor is to be used, it is important to know how variable the factor might be under different metabolic conditions. It is also relevant to understand the underlying mechanism for the difference between free glycine and hippurate enrichment.

One possible explanation for the greater enrichment of hippurate is a difference in the conjugation capacity of periportal and perivenous hepatocytes. Since a diffusion gradient and a continuous dilution of the labeled glycine occur as the perfusion inflow continues, periportal cells would conjugate benzoate with glycine having an ^{15}N enrichment closer to that of the plasma (or perfusion medium) itself. If these cells were enzymatically more active than the perivenous cells, and if secreted hippurate does not re-enter the downstream hepatocytes, one would expect a greater enrichment of hippurate than that of the average free glycine of the whole liver. Reversal of the glycine gradient by retrograde perfusion should therefore diminish the greater enrichment of hippurate. The results of our experiments do not support this hypothesis. Early in the retrograde perfusion, at 15 min, there was a decreased hippurate/free glycine labeling ratio. We attribute this to the effects of the swelling of the liver when perfused at a high rate in the retrograde direction. From 15 min onward, there was no significant effect of reversal of the direction of flow on the relative enrichment of hippurate. It is more likely, therefore, that the phenomenon reflects an intracellular compartmentation process. An effect of nembutal administration—a significant increase in the enrichment of hippurate with little change in the enrichment of hepatic free glycine—would support this sugges-

TABLE 3. Secretion rate and ^{15}N enrichment of $apoB_H$ and $apoB_L$ in perfused liver[a]

	$ApoB_H$	$ApoB_L$
Secretion Rate,[b] μg/g/h	8.4 ± 1.24 (6)	16.0 ± 2.14 (6)[c]
^{15}N enrichment, % excess	3.7 ± 0.694 (6)	4.1 ± 0.939 (6)

[a]The numbers represent the means ± the SEM. The numbers in parentheses represent the number of experiments.

[b]These values are based on measurements of the total d<1.063 g/ml protein output of 73 ± 12.1 μg/g/h in eight experiments and the percent of that total represented by $apoB_H$ and $apoB_L$ as judged by dye binding after SDS-PAGE. For $apoB_H$ and $apoB_L$, a dye binding ratio was estimated from the measured dye binding ratio of 2.11 in a pooled sample of rat plasma d<1.063 g/ml apoproteins compared with previous measurements of this ratio, 1.44, in a pooled sample of rat plasma lipoproteins separated by SDS-column chromatography and measured by the Lowry method. The corrected dye binding ratio was thus estimated at 2.11/1.44 or 1.47. It should be noted that the B-100/B-48 dye binding ratio in human plasma has been reported to be 4–5 times higher than this (14).

[c]Significantly different from the mean for $apoB_H$ ($P = 0.01$).

TABLE 4. Enrichment of deuterium in liver free glycine and perfusate hippurate from deuteroglycine[a]

Experiment No.	Time[b]	Liver Free Glycine	Perfusate Hippurate
	min	*% enrichment*	
1	30		2.2
	60	<0.05	0.8
2	30		1.5
	60	<0.05	1.3
3	10		4.0
	20		4.8
	30	2.9	4.4

[a]Livers were perfused as described in the text except for the substitution of deuteroglycine (labeled 99% with deuterium) for [^{15}N]glycine. In experiments 1 and 2, the rats were nembutalized. Analysis of deuterium enrichment of the TBDMS derivatives of glycine and hippurate was carried out as described, monitoring for *m/z* plus 2 fragments, representing the non-exchangeable atoms attached to the α carbon.

[b]Hippurate was analyzed in samples obtained from the 0–30 min and 30–60 min perfusate collection periods in experiments 1 and 2 and from 9–10, 19–20, and 29–30 min in experiment 3. Glycine was obtained from livers at the end of each perfusion.

tion. Glycine conjugation is an intramitochondrial process (10) and barbiturates are known to affect mitochondrial permeability (11). It would appear that the transport rate of free glycine from the extracellular fluid to the intramitochondrial space exceeds its rate of mixing with the cytosolic pool used to charge its tRNA.

In these experiments, the net output of glycine when the perfusion fluid contained 0.3 mM glycine was calculated by the isotope dilution of the effluent (Table 1). Net glycine output averaged 4.5 μmol/g per h, and it was not affected by fasting or sucrose feeding. It was significantly increased to 6.6 μmol/g per h in the nembutalized rats, in spite of the fact that the hepatic free glycine concentration was not altered. This points again to the effects of barbiturates on permeability.

As for the variability of hippurate production under different metabolic conditions, a fivefold decrease in the size of the free glycine pool was observed in the livers from fasted rats, but there was only a 9% decrease in the hippurate/free glycine labeling ratio in these animals. A sucrose-enriched diet had no significant effect on the ratio. Therefore, dietary changes would not be expected to produce marked changes in the ability to estimate the glycine precursor pool from measurements of plasma (12) or urinary hippurate (1).

It is remarkable that the isotopic enrichment of hepatic free glycine after 30 min of perfusion was the same in all of the circumstances reported in these experiments. In the fasted rats, where the free glycine pool was greatly diminished, greater isotope enrichment would be expected. Since this did not occur, dilution of labeled glycine by unlabeled glycine coming from increased endogenous proteolysis probably compensated almost exactly for the effect of the decreased pool size. Since the net output of glycine was not increased by fasting, we must assume that the decreased pool size was accounted for by the increased catabolism of glycine through gluconeogenic and other metabolic pathways.

One metabolic pathway known to be important in glycine metabolism is the serine hydroxymethylase reaction, which labilizes the α hydrogen atoms (13). The speed of this reaction in rat liver probably accounts for the very low enrichment of hepatic free glycine and hippurate from deuteroglycine. Deuteroglycine, therefore, is not a useful stable isotopic tracer in spite of the fact that it has a negligible natural abundance.

In considering the problem of which stable isotope-labeled amino acid to choose for the study of lipoprotein metabolism in human subjects, Lichtenstein et al. (2) have shown the equivalence of deuterated leucine, valine, and lysine using the primed constant infusion method. These labeled amino acids have a negligible natural abundance which is an important consideration when the plasma pool size of the apolipoprotein in question is relatively large with a low fractional synthetic rate. The constant infusion method requires an estimate of the isotopic enrichment of the precursor amino acid. This was obtained from the plateau value for VLDL apoB, which is not easy to estimate. Furthermore, apoB-48 may reach a different plateau value than apoB-100 and has been reported to be present at 17% of the total in VLDL from fasted humans (14). The present experiments suggest that measuring hippurate enrichment from [^{15}N]glycine, as originally reported (1), can circumvent the difficulty of relying on VLDL apoB production, when a simple correction factor that does not vary appreciably under fasted or fed conditions is used. Its main drawback is the 1% natural abundance of ^{15}N. Recently, Parhofer et al. (15) have shown the equivalence of [1-^{13}C]leucine and [^{15}N]glycine in the measurement of apoB kinetic parameters. They also found that the constant infusion and the bolus dose techniques gave equivalent results that were closely correlated with one another. The bolus dose method yields more information but requires multicompartmental modeling. It would appear at present that the choice of labeled amino acid and of its method of administration will depend on the particular apolipoprotein being investigated.

The present study extends our previous observations (3) on total apoB synthesis and secretion using [^{15}N]glycine in perfused rat liver. The value of 24 μg/g per h for the total secretion of apoB in the d<1.063 g/ml fraction agrees closely with the value of 22 μg/g per h in our earlier study (3) in which apoB was measured after isopropanol precipitation. We have now found that twice as much $apoB_L$ as $apoB_H$ was secreted, yet the isotopic enrichment was about the same. This indicates a larger intrahepatic pool of $apoB_L$. An increased intrahepatic pool

size of apoB_L compared to apoB_H has also been inferred from differential labeling patterns of the two forms of apoB by Swift, Padley, and Getz (16) and by Sparks et al. (17). Mathematical analysis of the kinetics of apoB production in the perfused rat liver may yield additional information relating to the secretory process.

This work was supported in part by the Atherosclerosis Center of the Medical College of Pennsylvania, NIH grant HL-22633, and the Howard Heinz Endowment. We wish to thank Marie Ragni and Dr. Michael Bennett for their helpful advice, and Dr. Edward Fisher for his critical reading of the manuscript.

Manuscript received 1 May 1991 and in revised form 17 September 1991.

REFERENCES

1. Cryer, D. R., T. Matsushima, J. B. Marsh, M. Yudkoff, P. M. Coates, and J. A. Cortner. 1986. Direct measurement of apolipoprotein B synthesis in human very low density lipoprotein using stable isotopes and mass spectrometry. *J. Lipid Res.* **27:** 508–516.
2. Lichtenstein, A. H., J. S. Cohn, D. L. Hatchey, J. B. Millar, J. M. Ordovas, and E. J. Schaefer. 1990. Comparison of deuterated leucine, valine, and lysine in the measurement of human apolipoprotein A-I and B-100 kinetics. *J. Lipid Res.* **31:** 1693–1701.
3. Matsushima, T., D. R. Cryer, K. E. Winkler, J. B. Marsh, and J. A. Cortner. 1989. Measurement of apolipoprotein B synthesis in perfused rat liver using stable isotopes: [^{15}N]hippurate as a measure of the intracellular [^{15}N]glycine precursor enrichment. *J. Lipid Res.* **30:** 841–846.
4. Garfinkel, D., and A. Lajtha. 1963. A metabolic inhomogeneity of glycine in vivo. I. Experimental determination. *J. Biol. Chem.* **238:** 2435–2439.
5. Winkler, K. E., and J. B. Marsh. 1989. Characterization of nascent high density lipoprotein subfractions from perfusates of rat liver. *J. Lipid Res.* **30:** 979–987.
6. Späth, P. J., and H. Koblet. 1979. Properties of SDS-polyacrylamide gels highly cross-linked with N,N'-diallyltartaramide and the rapid isolation of macromolecules from the gel matrix. *Anal. Biochem.* **93:** 275–285.
7. Marsh, J. B. 1976. Apoproteins of the lipoproteins in a non-recirculating perfusate of rat liver. *J. Lipid Res.* **17:** 85–90.
8. Smith, P. K., R. I. Krohn, G. T. Hermanson, A. K. Mallia, F. H. Gartner, M. D. Provenzano, E. K. Fujimoto, N. M. Goeke, and D. C. Klink. 1985. Measurement of protein using bicinchoninic acid. *Anal. Biochem.* **150:** 76–85.
9. Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **28:** 350–356.
10. Gatley, S. J., and H. S. A. Sherratt. 1977. The synthesis of hippurate from benzoate and glycine by rat liver mitochondria. Submitochondrial localization and kinetics. *Biochem. J.* **166:** 39–47.
11. Swiercynski, J., and Z. Aleksandrowicz. 1974. Inhibition of ion transport across the mitochondrial membrane by amytal. *Biochim. Biophys. Acta.* **373:** 66–75.
12. Arends, J., F. Chiu, and D. M. Bier. 1990. Analysis of plasma hippurate in humans using gas chromatography-mass spectrometry: concentration and incorporation of infused [^{15}N]glycine. *Anal. Biochem.* **191:** 401–410.
13. Stover, P., and V. Schirch. 1990. Serine hydroxymethyltransferase catalyzes the hydrolysis of 5,10-methenyl-tetrahydrofolate to 5-formyltetrahydrofolate. *J. Biol. Chem.* **265:** 14227–14233.
14. Hidaka, H., H. Kojima, Y. Nakajima, T. Aoki, T. Nakamura, T. Kawabata, T. Nakano, Y. Harano, and Y. Shigeta. 1990. Apolipoprotein B-48 analysis by high-performance liquid chromatography in VLDL: a sensitive and rapid method. *Clin. Chim. Acta.* **189:** 287–296.
15. Parhofer, K. G., P. H. R. Barrett, D. M. Bier, and G. Schonfeld. 1991. Determination of kinetic parameters of apolipoprotein B metabolism using amino acids labeled with stable isotopes. *J. Lipid Res.* **32:** 1311–1323.
16. Swift, L. L., R. J. Padley, and G. S. Getz. 1987. Differential labeling of rat hepatic Golgi and serum very low density lipoprotein apoprotein B variants. *J. Lipid Res.* **28:** 207–215.
17. Sparks, J. D., C. E. Sparks, M. Bolognino, A. M. Roncone, T. K. Jackson, and J. M. Amatruda. 1988. Effects of non-ketotic streptozotocin diabetes on apolipoprotien B synthesis and secretion by primary cultures of rat hepatocytes. *J. Clin. Invest.* **85:** 804–811.