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# L-Histidine-Induced Hypercholesterolemia: Characteristics of Cholesterol Biosynthesis in Rat Livers[1] (40280)

JIRAPA K. SOLOMON AND RONALD L. GEISON[2]

*Biomedical Research Unit, Waisman Center, and the Department of Nutritional Sciences, University of Wisconsin, Madison, Wisconsin 53706*

Dietary enrichment with high levels of single amino acids induced decreased food intake and growth suppression in young animals (1). The effects depend upon the kind and concentration of amino acid supplemented. Waisman and his colleagues have described in Rhesus monkeys a marked hyperlipemia associated with the dietary administration of L-histidine (2, 3). Histidine was the only amino acid of nine studied which induced this hyperlipemia. The hyperlipemia involved all circulating lipids. Serum phospholipids increased twofold, cholesterol two- to threefold and triglycerides three to eightfold. Geison and Waisman fed 5% and 8% excess L-histidine diets to rabbits for 4 weeks and found a 50% increase in the plasma cholesterol level (4). Phospholipid levels did not change. The effect in rabbits was less pronounced than that observed in monkeys.

Our report presents the effect of dietary L-histidine supplementation in rats. We observe alterations in the incorporation of [2-$^{14}$C] acetate or [1-$^{14}$C] octanoate into lipids, studied in liver slices taken from rats fed a diet supplemented 5% with L-histidine.

*Materials and methods.* The basic diet fed in all experiments was Purina Formulab Chow containing 23% protein, 6.5% fat, 0.58% histidine, carbohydrate, vitamins and minerals. L-histidine (free base) was purchased from Ajinomoto, Co., Tokyo. [2-$^{14}$C] acetate (specific activity 53.3 mCi/mmole), [1-$^{14}$C] octanoate (specific activity 3.5 mCi/mmole), *Aquasol* (scintillation solution) and *Protosol* were purchased from New England Nuclear Corp., Elmhurst, IL. DNA standard (from Salmon testes) was purchased from Sigma Chemical Co., St. Louis, MO. Diphenylalanine reagent was obtained from Allied Chemical, Palatine, IL. It was purified by recrystalizing from boiling hexane to obtain a white crystalline product. Bovine serum albumin (Nutritional Biochemicals, Cleveland, OH.) was used as protein standard. For homogenization, a motor-driven Potter-Elvehjem homogenizer or a *Polytron* (Brinkman Instruments) was used. Liver slices were made using a McIlwain tissue chopper (The Mickle Laboratory Engineering Co., England). Incubation of liver slices was performed in a Dubnoff Metabolic Shaking Incubator. All radioactivity measurements were obtained with a Nuclear Chicago scintillation counter, Isocap/300.

Male albino rats from Holtzman Rat Co., Madison, WI, were obtained at 21 days of age and weighed 55 ± 5 g. They were housed in individual wire-bottom cages with a light–dark cycle changing every three hours throughout all experiments. Rats in the control group were fed ground Purina Formulab Chow *ad lib.* In the histidine-treated group, L-histidine constituted 5% of the diet by weight. It was added to the ground chow diet and fed to the rats *ad lib.* Since histidine-treated rats eat less than untreated controls, a second control group (pair-fed controls) was used. This group was fed the same amount of food eaten by the histidine-treated group. After 4 days of feeding, the rats were killed by decapitation. Livers were isolated and either homogenized with 9 vol of distilled water for DNA and protein determinations or sliced for the *in vitro* experiments.

Liver protein was estimated by the method of Lowry *et al.* (5) using bovine serum albumin as standard. DNA estimation was done using the method of Schneider (6) with a DNA standard from Salmon testes.

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[2] Present Address: Sigma Chemical Co., P. O. Box 14508, St. Louis, MO 63178.



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Incubation of liver slices with [2-$^{14}$C] acetate or [1-$^{14}$C] octanoate was done under optimum conditions suggested by Dietschy and McGarry (7). $CO_2$ released was trapped by *Protosol* in a cup hanging above the incubation mixture and the radioactivity was counted by dropping the cup into a scintillation vial containing *Aquasol*. Liver slices were washed with 0.9% NaCl three times and homogenized in distilled water using the *Polytron*. The homogenate was extracted with chloroform–methanol in a 2:1 ratio. The extract was dried and dissolved in a small volume of chloroform, then spotted on a silica gel G plate (0.25 mm thick). The plate was developed in a solvent system which contained heptane–ether–acetic acid in a 75:25:5 ratio. Lipid fractions were visualized by spraying the plate with 0.1% 2′,7′-dichlorofluorescein in methanol. Each band was assayed for radioactivity and the rate of synthesis is expressed as nanomoles of the labeled precursor incorporated into the product per gram of liver per hour. The percent deviation from control in each fraction was calculated. All statistical analysis was done by using the twotailed Student's *t* test.

*Results.* [2-$^{14}$C] Acetate and [1-$^{14}$C] octanoate were found to incorporate into every fraction of liver lipids and the released $CO_2$ at various rates. L-histidine primarily affected the incorporation of the labeled substrates into cholesterol and triglycerides. When [2-$^{14}$C] acetate was used as substrate, the increase in its incorporation compared to controls was found to be 107% for unesterified cholesterol and 100% for cholesterol esters (Fig. 1-A). Both increases were statistically significant, $P < 0.02$ and $P < 0.01$ respectively. The opposite effect was observed in the case of triglycerides, which showed a significant ($P < 0.05$) decrease of 36%.

When [2-$^{14}$C] octanoate was used as substrate, there were significant ($P < 0.02$) increases in the incorporation of the labeled substrate of 90% and 71% for unesterified cholesterol and cholesterol esters, respectively, compared to controls (Fig. 1-B). The incorporation into triglycerides was significantly ($P < 0.01$) decreased by 39%.

Histidine did not significantly alter the incorporation of the labeled substrates into other liver lipids such as phospholipids, free fatty acids, monoglycerides and diglycerides. Histidine did not alter the activity of the tricarboxylic acid cycle, as indicated by the insignificant change of the incorporation of the labeled substrates into released $CO_2$.

The effects of excess dietary L-histidine on cell size as estimated by liver DNA and protein contents are shown in Table I. The DNA to protein ratio in histidine-treated rat livers was significantly lower than the ratio observed in both *ad lib.* ($P < 0.05$) and pair-fed ($P < 0.01$) controls. The decrease in the ratio was 19.4% compared to pair-fed controls. Liver DNA and protein contents in histidine-treated rats were significantly ($P < 0.01$)

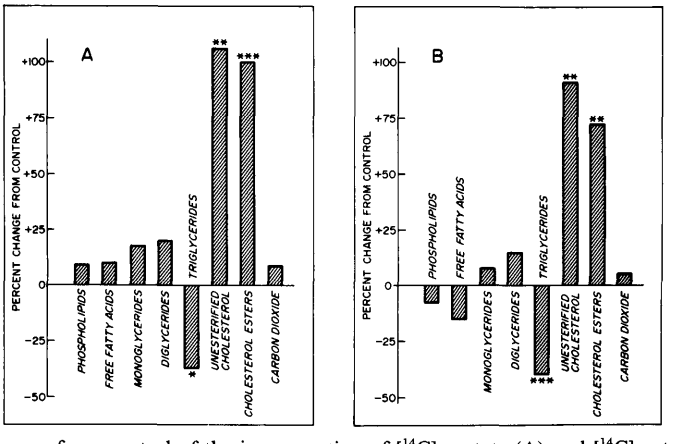


FIG. 1. Percent change from control of the incorporation of [$^{14}$C] acetate (A) and [$^{14}$C] octanoate (B) into liver lipid fractions and $CO_2$ by liver due to L-histidine supplementation. The conditions of the incubation and separation of lipid fractions are described in the methods. Asterisks indicate *P* values for comparison with *ad lib.* controls: $*P < 0.05$, $**P < 0.02$ and $***P < 0.01$.

TABLE I. EFFECT OF 5% DIETARY L-HISTIDINE SUPPLEMENTATION ON DNA AND PROTEIN CONTENTS IN RAT LIVER.[a]

| Diet | DNA (mg/100 g liver) | Protein (g/100 g liver) | $\frac{\text{DNA}}{\text{protein}} \times 10^3$ |
|---|---|---|---|
| *AD LIB*: 95% Chow + 5% L-Histidine (5) | 147.7 ± 11.9[b] | 18.3 ± 0.5[b] | 8.0 ± 0.5[b, d] |
| *AD LIB*: Chow (5) | 166.0 ± 3.3 | 17.1 ± 0.6 | 9.8 ± 0.4 |
| Pair-Fed: Chow (5) | 224.5 ± 3.0[c] | 22.6 ± 0.5[c] | 10.0 ± 0.3 |

[a] Results are expressed as mean ± S.E.M.
[b] Significantly different from pair-fed controls, $P < 0.01$.
[c] Significantly different from *ad lib.* controls, $P < 0.001$.
[d] Significantly different from *ad lib.* controls, $P < 0.05$.
[e] (N) = number of rats per group.

lower than levels in the pair-fed controls.

*Discussion.* L-histidine induces in young rats a hypercholesterolemia which occurs after a brief period of feeding (4 days). Histidine-treated rats are smaller than controls, have larger livers and 30–40% higher levels of plasma cholesterol (8). In the present study, the incorporation of [2-$^{14}$C]acetate into cholesterol by liver was found to increase by 100% with the feeding of an L-histidine enriched diet. However, Dietschy and McGarry (7) have shown that the acetyl-CoA available for cholesterol synthesis in the cytosol is not in isotopic equilibrium with the intramitochondrial pool. In order to verify the result, [1-$^{14}$C] octanoate was used as substrate under the same conditions. Octanoate is incorporated into cholesterol by the cytosolic biosynthesis pathway only after its intramitochondrial oxidation to acetyl-CoA. In this way the $C_2$ units entering the cholesterol biosynthetic pathway were in isotopic equilibrium with the intramitochondrial $C_2$ pool. Increases in the incorporation of the [1-$^{14}$C] octanoate into unesterified cholesterol and cholesterol esters by 90% and 71%, respectively, were observed.

The second significant effect of histidine on liver in this study was the 36–39% decrease in the incorporation of the labeled substrates into triglycerides. Kerr *et al.* (3) showed that histidine-induced hyperlipemia in monkeys was easily detected by the appearance of a "creamy" serum reflecting the predominant presence of triglyceride-laden chylomicrons. The decrease in triglyceride synthesis observed in our experiments was in accord with the absence of "creamy" serum in the rat.

Dietschy and McGarry have shown the concentrations of the labeled substrates used in these experiments (4 m*M* for acetate and 1.1 m*M* for octanoate) to be saturating for the metabolic process under study (7). They also have reported that octanoate was a more efficient precursor than acetate for sterol synthesis. This contrasts with the data obtained in the present study where we observed that in liver the rate of cholesterol synthesis from [2-$^{14}$C] acetate was not significantly different from that obtained with [1-$^{14}$C] octanoate.

Changes in the liver DNA to protein ratio support an increase in hepatic cell size which correlates well with previous results showing a 100% increase in liver glycogen content (8). In summary, the present study demonstrates that dietary enrichment with L-histidine induces specific effects in cholesterol and triglyceride synthesis in weanling rats. These effects might represent the regulation of specific enzymes in cholesterol biosynthesis and lipogenesis such as β-hydroxy-β-methylglutaryl coenzyme A reductase and fatty acid synthetase.

*Summary.* A diet supplemented 5% with L-histidine caused a 100% increase in the incorporation of [2-$^{14}$C] acetate or [1-$^{14}$C] octanoate into cholesterol in liver slices of weanling rats after four days of feeding. The incorporation of the labeled substrates into triglycerides decreased 38%. The hepatic DNA-to-protein ratio decreased 19% with histidine feeding, suggesting an increase in hepatocyte size.

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1. Daniel, R. G., and Waisman, H. A., Growth **32**, 255 (1968).
2. Kerr, G. R., Wolf, R. C., and Waisman, H. A., Proc. Soc. Exp. Biol. Med. **119**, 561 (1965).
3. Kerr, G. R., Wolf, R. C., and Waisman, H. A., *in*

"Symposia of the Zoological Society of London" (R. N. T-W Fiennes, ed.), No. 17, p. 371, Academic Press, London/New York (1966).
4. Geison, R. L., and Waisman, H. A., Proc. Soc. Exp. Biol. Med. **133,** 234 (1970).
5. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193,** 265 (1951).
6. Schneider, W. C., *in* "Methods in Enzymology" (Colowick, S. P. and Kaplan, N. O., eds.), Vol. III, p. 680, Academic Press, New York (1957).
7. Dietschy, J. M., and McGarry, J. D., J. Biol. Chem. **249,** 52 (1974).
8. Solomon, J. K., and Geison, R. L., Fed. Proc. **36,** 1157 (Abstr.) (1977).

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