Vol. 82, No. 2, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 30,1978 Pages 526-531

IMMEDIATE EFFECTS OF CARBON MONOXIDE ON THE METABOLISM OF CHYLOMICRON REMNANTS BY PERFUSED RAT LIVER

Ronald S. Gardner, David L. Topping* and Peter A. Mayes

Department of Physiology and Biochemistry, Royal Veterinary College, London NW1 OTU, U.K.

Received April 3,1978

SUMMARY:

Livers from fasted male rats were perfused with blood containing 30% carboxyhemoglobin. Chylomicron remnants (labelled with [^{3}H] cholesterol and [^{14}C] oleate), prepared in functionally hepatectomized rats, were added to the perfusate. Carboxyhemoglobin decreased hepatic uptake of remnant cholesterol and increased the amount of lipoprotein flushed out of the liver at the end of perfusion. Transfer of triacylglycerol fatty acids to phospholipid and formation of d>1.006 lipoproteins was diminished. Ketogenesis was increased and lipoprotein triacylglycerol secretion decreased. The data indicate an inhibition of hepatic remnant catabolism by carboxyhemoglobin and are discussed with reference to the possible role of smoking in atherosclerosis.

INTRODUCTION:

Pathological (1,2) and epidemiological studies (3,4) have linked cigarette smoking with an increased incidence of coronary heart disease. It has been proposed that of cigarette smoke constituents, CO is a major atherogenic agent accelerating the rate of cholesterol deposition in the intimal surface by limiting O_2 supply (5). After removal of chylomicron TGFA by lipoprotein lipase in extrahepatic tissues, remnant particles are produced, which are relatively richer in cholesterol (6) and which are subsequently metabolized by the liver (7). Type III hyperlipoproteinemia is associated with delayed remnant clearance and premature atherosclerosis. Remnants formed at the intimal surface of arteries may be potentially atherogenic (8).

In a study of the effects of chronic CO exposure on diet-induced atherogenesis in rabbits, it was found that d<1.006 lipoprotein cholesterol was

---

Abbreviations: COHb: carboxyhemoglobin; Hb: hemoglobin; TGFA: triacylglycerol fatty acids; VLDL: very low density lipoproteins (d<1.006).

*Present address: Division of Human Nutrition, CSIRO, Adelaide, Australia.

0006-291X/78/0822-0526$01.00/0
Copyright © 1978 by Academic Press, Inc.
All rights of reproduction in any form reserved.

increased (9). It was suggested that the rise was possibly a result of diminished hepatic metabolism of remnants. It was decided to examine the effects of CO (as COHb) on remnant metabolism by the perfused rat liver.

METHODS:

Livers from male Wistar albino rats (340-360g) were perfused in-situ for an experimental period of 90 min by a modification of the method of Mayes and Felts (10). To minimize hepatic lipoprotein secretion, 24h fasted animals were used for liver perfusions (7). The perfusate was 130ml of defibrinated whole rat blood, dialyzed against a Krebs-Henseleit buffer (11) containing glucose (5mM) and mixed amino acids (500mg/l). The rate of perfusion was 12ml/min and the pO_2 was maintained at 90-100mmHg.

Remnants, labelled with [1α,2α-^{3}H] cholesterol and [1-^{14}C] oleate TGFA, were prepared as described previously (7). At the start of the perfusion, remnants containing approx. 19μmol of TGFA, 0.6μCi of [^{3}H] and 2.0μCi of [^{14}C] were added to the perfusate. During the course of the perfusion the hepatic vein was occluded every 5 min for 3 sec in order to cause a greater exchange of lipoproteins in the extracellular spaces (7). In half of the perfusions, a concentration of COHb was maintained at 30% of total Hb (12), a concentration similar to that used in studies in experimental animals (5). Details of analytical methods have been given previously (7). The statistical significance of differences between the two experimental groups was determined by Analysis of Variance. Data are represented as the means ± SEM of three separate perfusions.

RESULTS AND DISCUSSION:

Concentration of metabolites in blood

Perfusate glucose concentrations did not differ between the two groups of livers and remained at 4.5-5.0μmol/ml throughout the experiment. In control perfusions, lactate concentrations, representing the resultant of erythrocyte production and hepatic utilization, were constant at 1.8-1.9μmol/ml of blood. In perfusions with COHb the lactate concentration decreased during the first 30 min reaching a significantly ($P<0.05$) decreased steady state concentration (0.6-0.7μmol/ml). COHb did not affect perfusate free fatty acid concentrations which remained steady at 0.3μmol/ml serum. However, secretion of VLDL by the perfused liver was significantly ($P<0.01$) decreased from 1.8 ± 0.3μmol of TGFA/g of liver/h in controls to 0.5 ± 0.2 in the presence of COHb. Ketogenesis was 0.7 ± 0.2μmol/g of liver/h in control perfusions and was significantly ($P<0.01$) increased to 3.3 ± 0.5 in COHb perfusions. The ratio of [3-hydroxybutyrate]/[acetoacetate] was 1.0 ± 0.2 during control perfusions but a significant ($P<0.01$) decrease to 0.6 ± 0.2 occurred in COHb perfusions, in-

dicating a decreased [NADH]/[NAD] ratio in mitochondria.

The replacement of Hb by COHb in the perfusate, lowers the O_2 consumption of the perfused liver (12) which can account for many of the hepatic effects of COHb. In the present experiments, the decreased O_2 consumption would lead to a decreased ATP turnover and probably to a lower [ATP]/[ADP] ratio (13) which would, in turn, activate pyruvate dehydrogenase. Therefore more pyruvate and lactate would be oxidized to acetyl-CoA and account for the decreased lactate concentrations found in COHb perfusions. The increased production of acetyl-CoA from pyruvate might then switch fatty acid oxidation via the citric acid cycle to ketogenesis, as indicated by the increased production of ketone bodies.

Metabolism of chylomicron remnants

Of the original remnant [^{3}H] cholesterol added to the perfusate 15.1 ± 1.1% was taken up into the liver at the end of control perfusions whereas only 8.4 ± 1.8% was found in the livers of COHb perfusions ($P<0.05$). This finding, taken together with the corresponding data for the uptake of remnant ^{14}C-TGFA (11.4 ± 1.5 and 7.2 ± 1.9 respectively) supports the proposal that COHb inhibits the uptake of remnants by the liver.

In both control and COHb-treated perfusions there was a substantial and quantitatively similar hydrolysis of cholesteryl esters, amounting to 22.9% and 25.4% of the added cholesteryl ester, respectively. However a significantly ($P<0.05$) greater proportion of the hydrolyzed cholesteryl ester was present in the perfusate lipoproteins ($d<1.006$) and a significantly ($P<0.05$) smaller proportion was present in the liver at the end of COHb perfusions (Fig. 1).

Fatty acid was transferred from triacylglycerol to phospholipid in the liver and perfusate lipoproteins ($d<1.006$) of both groups of perfusions. There was no effect of COHb on the magnitude of the transfer within the liver, but the accumulation of phospholipid enriched lipoprotein ($d<1.006$) in the perfusate was significantly decreased in COHb perfusions ($P<0.05$). Thus, total

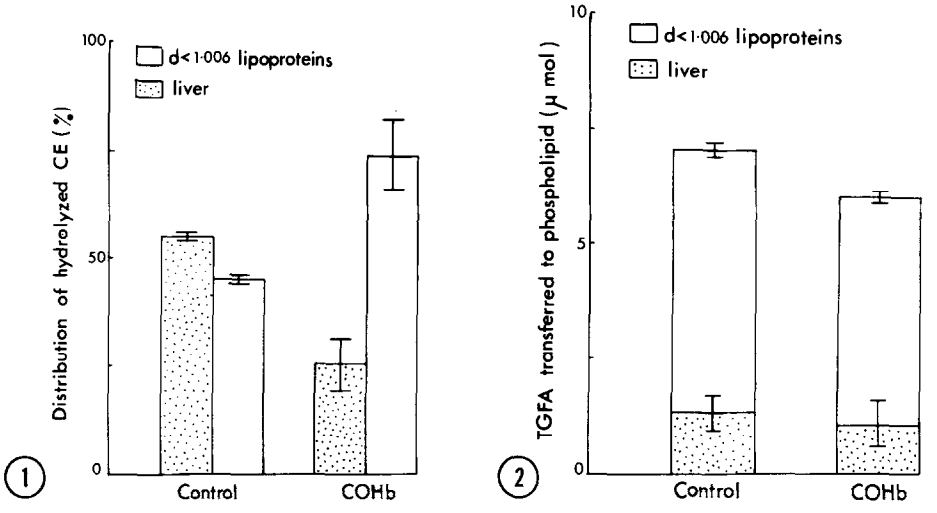

Figure 1: Effect of COHb on the distribution of remnant cholesteryl ester (CE) hydrolyzed by the perfused rat liver.

Figure 2: Effect of COHb on the transfer of ^{14}C-labelled remnant TGFA to phospholipids by the perfused rat liver.

net transfer of [^{14}C] TGFA to phospholipid was significantly ($P<0.01$) less in COHb than in control experiments (Fig. 2). The mean value of $0.8 \pm 0.4\%$ for the oxidation of [^{14}C] TGFA in COHb perfusions was lower than $1.6 \pm 0.3\%$ found in controls but the difference, although indicating decreased oxidation of remnants in the presence of COHb, was not statistically significant at the 5% level.

Flushing out the liver at the end of the perfusion resulted in 3.2% of [^{3}H] and 8.8% of [^{14}C] being removed in lipoproteins from the total amount of radioactivity present in the livers of control perfusions. In COHb perfusions both of these values were significantly increased, to 20.8% ($P<0.001$) and 14.7% ($P<0.05$) respectively (Fig. 3). Of the lipoproteins flushed out of the liver at the end of the perfusion approximately 34% was in the $d>1.006$ fraction in controls. This was decreased to 22% in the presence of COHb ($P<0.01$).

Whereas transfer of fatty acids from TGFA to phospholipids was significantly decreased in COHb experiments, total hydrolysis of cholesteryl esters was not. This may indicate that an energy dependent step, sensitive to lowered

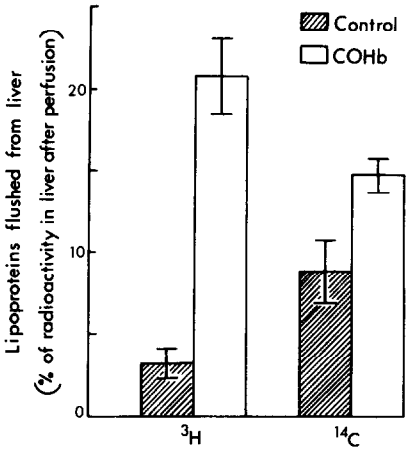

Figure 3: Effect of COHb on the recovery of lipoproteins from the liver after perfusion for 90 minutes. The liver was flushed with 4x20ml of Krebs-Henseleit bicarbonate buffer.

O_2 consumption, is involved in the transacylation to phospholipids but not in simple hydrolysis of cholesteryl esters. However, with COHb, a significantly greater proportion of the cholesterol resulting from hydrolysis, was released into the perfusate within lipoproteins ($d<1.006$). Thus the present investigation has shown that in the presence of COHb, the liver not only reacts with less and retains less remnant lipid, but also it does not trap the products of their metabolism so effectively. Taken together, these two tendencies would lead in-vivo to the elevation of both triacylglycerol and cholesterol in the blood. Such a delay in clearance may be significant in the deposition of lipids, especially lipoprotein cholesterol, in arteries.

A link between triacylglycerol - rich lipoproteins, including remnants, and atherosclerosis has been proposed (8). Recently the similarity between Type III hyperlipoproteinemic patients and smokers in having a high incidence of peripheral vascular disease has been attributed to the accumulation of cholesterol-rich remnants (14). The present investigation supports the hypothesis that CO from cigarettes (as COHb) restricts hepatic metabolism, via reduced O_2 consumption, which leads to a delay in clearance of remnants. The production

of an abnormal remnant degradation product, such as a particle containing more unesterified cholesterol, must also be considered as potentially atherogenic.

ACKNOWLEDGEMENTS:

The skilled assistance of G. Coates and P. Dave and the support of the Medical Research Council are gratefully acknowledged.

REFERENCES:

1. Jenkins, C.D., Rosenman, R.H., and Zyzanski, S.J. (1968) Circulation 38, 1140-1155.
2. U.S. Department of Health, Education and Welfare, Washington, D.C. (1973).
3. Auerbach, O., Hammond, E.C., and Garfinkel, L. (1965) New England J. Med. 273, 775-779.
4. Sackett, D.L., Gibson, R.W., Bross, I.D.J., and Pickren, J.W. (1968) New England J. Med. 279, 1413-1420.
5. Astrup, P. (1972) Brit. Med. J. 2, 447-452.
6. Redgrave, T.G. (1973) Biochem. J. 136, 109-113.
7. Gardner, R.S., and Mayes, P.A. (1978) Biochem. J. 170, 47-55.
8. Zilversmit, D.B. (1973) Circ. Res. 23, 633-638.
9. Davies, R.F., Topping, D.L., and Turner, D.M. (1976) Atherosclerosis 24, 527-536.
10. Mayes, P.A., and Felts, J.M. (1966) Proc. Eur. Soc. Study Drug Toxicity 7, 16-29.
11. Krebs, H.A., and Henseleit, K. (1932) Hoppe-Seyler's Z. Physiol. Chem. 210, 33-66.
12. Topping, D.L. (1975) Biochem. J. 152, 425-427.
13. Mayes, P.A., and Felts, J.M. (1976) Biochem. J. 156, 685-689.
14. Topping, D.L., Dwyer, T., and Weller, R.A. (1977) Lancet 2, 1327-1328.