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# Specific Changes of Bile Acid Metabolism in Spontaneously Diabetic Wistar Rats (40894)

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*Abstract.* Bile acid metabolism has been investigated in a newly described animal model depicting juvenile human diabetes (spontaneously diabetic Wistar (BB) rat) and compared to normoglycemic control from the Wistar strain. Diabetic animals used were on insulin treatment except for the last 24 hr. The plasma glucose levels (mg%) of diabetic rat (D) was significantly higher than control rats (C) (150 ± 35 in C vs 340 ± 32 in D). The total bile acid pool (mg/100 g) in D was significantly ($P < 0.05$) higher when compared to C (9.0 ± 0.8 in C vs 14.9 ± 1.7 in D). The pool of cholic acid was significantly ($P < 0.05$) increased while that of chenodeoxycholic acid was significantly ($P < 0.05$) decreased (cholic acid: 5.9 ± 0.45 in C vs 10.06 ± 1.2 in D; chenodeoxycholic 0.90 ± 0.1 in C vs 0.57 ± 0.06 in D). This increased the cholic/chenodeoxycholic acid ratios from 6.6 ± 0.4 in controls to 19.3 ± 2.4 in diabetic rats. These studies have shown diverse alteration in the concentration of the two primary bile acids in the diabetic state.

Epidemiological and clinical studies have noted an increase in the incidence of atherosclerosis in patients with diabetes mellitus (1, 2). It is believed that at least part of the effect of diabetes on atherosclerosis may be due to the noted increase in plasma cholesterol (3) in these patients. Unless the diabetes is severe, hypercholesterolemia is generally mild in controlled diabetes. Studies in animals, under experimentally induced diabetic conditions, have confirmed the occurrence of hypercholesterolemia (4, 5). The mechanism responsible for the mild hypercholesterolemia in diabetes has not been clearly explained. On the basis of cholesterol turnover studies Wong and Van Bruggen suggested (6) that there may be a depression of cholesterol degradation to bile acids in alloxan induced diabetic rats. However, studies by Nervi *et al.* (7, 8) noted an increase in bile acid pool in alloxan treated diabetic rats. These authors further noted an increase in cholic acid pool in these animals. In all of the animal studies discussed above, diabetes was induced experimentally with alloxan or streptozotocin. Therefore, the notion that some of the noted changes in cholesterol metabolism could have been due to the diabetes inducing agent itself, cannot be ruled out. Recently the availability of newly described animal model of spontaneous diabetes, the Wistar (BB) rat (9, 10) gave an ideal opportunity to investigate difference in cholesterol and bile acid metabolism during early stages of insulin-deficient diabetes without any drug manipulations. In this report we have compared some of the parameters of bile acid metabolism in spontaneously diabetic Wistar rats with that of age matched normoglycemic controls from the same colony.

*Materials and methods.* Spontaneously diabetic Wistar rats (6 months old) were obtained from Animal Resources Division, Health and Welfare, Canada. The diabetic animals were given protamine zinc insulin daily (dosage unit = body weight/50) from 70 to 75 days of age except on the day preceding the experiment. All animals were fed laboratory rat chow *ad libitum* during the course of the experiment. For determination of bile acid pool, the entire gastrointestinal tract and liver were dissected out. The tissues were homogenized with 10 ml of saline and weighed aliquots were taken for the determination of bile acids. Bile acids were extracted using a procedure described previously (11, 12). The bile acids extracted were deconjugated by basic



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hydrolysis, methylated, and quantitated as methyl ester trifluoroacetates by gas–liquid chromatography using hyocholic acid as an internal standard (11, 12). When needed bile acid methyl esters were purified from fatty acids by thin layer chromatography as described by Grundy *et al.* (13). A F&M Model 402 gas–liquid chromatograph with 1% QF-1 columns was used for the analysis. The operating conditions were: column 220°C and carrier gas, helium, 50 ml/min. For the thin layer chromatographic analysis of bile acid conjugation, aliquots of extracts from the small intestine were chromatographed on silica gel G using a solvent system (14) consisting of isopropyl acetate:propionic acid:propanol:water (4:3:2:1, v/v/v/v). Authentic standards of bile acids were spotted along with the sample. Plasma cholesterol was determined by the standard NIH method (15). Plasma glucose was estimated by the glucose oxidase technique with Beckman glucose analyzer.

*Results.* Table I shows the body weights, plasma glucose, and cholesterol levels in control and diabetic rats. The body weight of the diabetic rats was significantly ($P < 0.05$) lower than the controls. As expected the diabetic rats had significantly higher ($P < 0.01$) glucose levels in plasma. The plasma cholesterol levels of diabetic rats were significantly lower ($P < 0.01$) than that of control rats. Table II shows the bile acid pool in control and diabetic rats. The most significant differences between the two groups is the striking increase in the cholic acid pool ($P < 0.05$) and a decrease in the chenodeoxycholic acid pool ($P < 0.05$). The total bile acid pool in diabetic rats was significantly increased ($P < 0.05$). Analysis of the bile acid composition in control and diabetic rats (Table III) indicated a significant decrease in the percentage composition of chenodeoxycholic acid

TABLE I. BODY WEIGHT, PLASMA GLUCOSE, AND PLASMA CHOLESTEROL IN RATS

| Group | Body weight[a] (g) | Plasma glucose[a] (mg%) | Plasma cholesterol[a] (mg%) |
|---|---|---|---|
| Control (8) | 431 ± 11* | 150 ± 35** | 65.8 ± 1.1*** |
| Diabetic (8) | 360 ± 10* | 340 ± 32** | 52.3 ± 3.1*** |

[a] Mean ± SEM.
* $P < 0.05$ for difference between groups.
** $P < 0.01$ for difference between groups.
*** $P < 0.01$ for difference between groups.

TABLE II. BILE ACID POOL IN CONTROL AND DIABETIC RATS

| Bile acid[a] | Bile acid pool (μg/100 g body wt) Control (4)[b] | Diabetic (7)[b] | Statistical evaluation[c] |
|---|---|---|---|
| Lithocholic acid | 75.83 ± 52.74 | 49.33 ± 6.31 | N.S. |
| 3β-,12α-Dihydroxy-5β-cholanoic acid | 388.49 ± 93.34 | 558.61 ± 72.90 | N.S. |
| Deoxycholic acid | 543.38 ± 143.22 | 1,109.03 ± 293.83 | N.S. |
| 3α-,12β-Dihydroxy-5β-cholanoic acid | 17.35 ± 2.17 | 62.13 ± 20.35 | N.S. |
| Chenodeoxycholic acid | 903.70 ± 106.41 | 573.33 ± 63.24 | $P < 0.05$ |
| Hyodeoxycholic acid | 770.79 ± 257.85 | 503.88 ± 159.71 | N.S. |
| Cholic acid | 5,906.71 ± 450.94 | 10,628.22 ± 1,266.29 | $P < 0.05$ |
| β-Muricholic acid | 114.75 ± 45.44 | 96.58 ± 50.58 | N.S. |
| 3α-Hydroxy 7-keto-cholanoic acid | 54.08 ± 32.27 | 341.18 ± 198.83 | N.S. |
| 3α-Hydroxy 6-keto-cholanoic acid[d] | 52.23 ± 16.71 | 203.75 ± 162.29 | N.S. |
| 3-Keto-7α-hydroxy cholanoic acid | 207.52 ± 16.22 | 626.47 ± 263.16 | N.S. |
| 3α-,12α-Dihydroxy 7-keto-cholanoic acid | 0.98 ± 0.98 | 100.28 ± 76.60 | N.S. |
| 3α-,7α-Dihydroxy 12-keto-cholanoic acid | 13.88 ± 10.39 | 52.55 ± 19.28 | N.S. |
| Total bile acids | 9,049.69 ± 889.54 | 14,905.34 ± 1,741.03 | $P < 0.05$ |

[a] Bile acids were identified by GLC on the basis of comparison with the known standards as described in the text.
[b] Mean ± SEM.
[c] Student's *t* test of significance for unpaired samples.
[d] Tentative identification based on retention times in GLC.

TABLE III. BILE ACID COMPOSITION IN CONTROL AND DIABETIC RATS

| Bile acid | Bile acid composition % | | |
|---|---|---|---|
| | Control (4)[a] | Diabetic (7)[a] | Statistical evaluation[b] |
| Lithocholic acid | 0.77 ± 0.52 | 0.38 ± 0.08 | N.S. |
| 3β-,12α-Dihydroxy-5β-cholanoic acid | 4.25 ± 0.77 | 3.88 ± 0.40 | N.S. |
| Deoxycholic acid | 5.81 ± 1.38 | 7.39 ± 1.76 | N.S. |
| 3α-,12β-Dihydroxy-5β-cholanoic acid | 0.19 ± 0.01 | 0.41 ± 0.12 | N.S. |
| Chenodeoxycholic acid | 10.04 ± 0.75 | 4.04 ± 0.46 | $P < 0.01$ |
| Hyodeoxycholic acid | 8.14 ± 2.25 | 3.55 ± 0.98 | N.S. |
| Cholic acid | 65.78 ± 1.85 | 71.53 ± 1.78 | N.S. |
| β-Muricholic acid | 1.26 ± 0.49 | 0.64 ± 0.31 | N.S. |
| 3α-Hydroxy 7-keto-cholanoic acid | 0.64 ± 0.37 | 1.95 ± 0.93 | N.S. |
| 3α-Hydroxy 6-keto-cholanoic acid[b] | 0.57 ± 0.17 | 1.37 ± 1.02 | N.S. |
| 3-Keto-7α-hydroxy cholanoic acid | 2.40 ± 0.39 | 3.92 ± 1.17 | N.S. |
| 3α,-12α-Dihydroxy 7-keto-cholanoic acid | 0.02 ± 0.02 | 0.63 ± 0.47 | N.S. |
| 3α-,7α-Dihydroxy 12-keto-cholanoic acid | 0.15 ± 0.10 | 0.33 ± 0.12 | N.S. |

[a] Mean ± SEM.
[b] Tentative identification based on retention time in GLC.
[c] Student's $t$ test of significance for unpaired samples.

($P < 0.01$). In view of the (a) increase in the pool of cholic acid and (b) decrease in the pool of chenodeoxycholic acid, the ratios of the primary bile acids were computed on the basis of their concentration and also on the basis of the sum of their concentration and their respective metabolites (Table IV). Cholic acid/chenodeoxycholic acid ratios in diabetic rats were significantly higher ($P < 0.05$) when compared to normal rats. Thin layer chromatographic evaluation of the bile acid conjugates showed no qualitative differences in conjugation between the two groups of animals.

*Discussion.* The results of our study clearly demonstrate that during spontaneous diabetes there is an increase in bile acid pool. The data are in agreement with reports of increased bile acid pool in alloxan induced diabetic rats (7, 8). Thus, it appears that in diabetes there is a genuine increase in bile acid synthesis. Our studies however, do not rule out the possibility that decreased bile acid excretion could have partly contributed to the increase in pool size. The question whether the diabetes itself or some accompanying biochemical changes eg: increase in ketone bodies, are responsible for the noted changes in bile acid pool, has not been completely resolved in this study. The diabetic rats were on insulin treatment since 2 months of age. Under these conditions, the concentration of ketone bodies in the plasma never exceeds 0.5 m*M* (9). Hence it is unlikely that the ketone bodies are responsible for the noted changes. It is also unlikely that nutrient deprivation due to diabetes is responsi-

TABLE IV. CHOLIC/CHENODEOXYCHOLIC RATIO IN BILE ACIDS

| Group | Cholic acid / Chenodeoxycholic Acid | Cholic acid + metabolites / Chenodeoxycholic acid + metabolites |
|---|---|---|
| Control (4) | 6.66 ± 0.49* | 7.32 ± 0.86* |
| Diabetic (7) | 19.32 ± 2.49* | 20.30 ± 2.32* |

* $P < 0.05$ for differences between control and diabetic rats.

ble for the noted increase in bile acid pool in these animals, because dietary restriction has been shown to decrease the bile acid synthesis and excretion (16).

Our study also demonstrates that there are specific changes in the pool size of the two primary bile acids (chenodeoxycholic and cholic acid). While there is an increase in the pool size of cholic acid, there is a definite reduction in chenodeoxycholic acid pool size. The reduction in chenodeoxycholic acid pool during diabetes has not been previously reported. Chenodeoxycholic acid can be synthesized via two pathways starting from the cholesterol molecule (17). The first pathway in which the ring hydroxylation preceedings side chain oxidation, involves 3$\alpha$, 7$\alpha$-dihydroxy-5$\beta$-cholestane as an intermediate (18) while the other pathway (side chain degradation prior to ring hydroxylation) involves 3$\beta$-hydroxy-$\Delta^5$-cholenoic acid as an intermediate. It is possible that in diabetes one of the pathways of chenodeoxycholic acid synthesis is decreased. On the other hand, increase in cholic acid suggests a specific increase in the 12$\alpha$-hydroxylation of 7$\alpha$-hydroxy-cholesten-3-one (20). Studies on the activity of these key enzymes need to be done to demonstrate specific sites of bile acid biogenesis that are affected in diabetes.

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1. Palumbo, P. J., Elveback, L. R., and Chu, C. P., *et al.*, Diabetes **25**, 566 (1976).
2. Ostarder, L. D., Jr., Francis, I., Jr., and Hayne, N. S., *et al.*, Ann. Inter. Med. **62**, 1199 (1965).
3. Bennion, L. J., and Grundy, S. M., New Eng. J. Med. **296**, 1356 (1977).
4. Wong, R. K. L., and Van Bruggen, J. T., J. Biol. Chem. **235**, 26 (1960).
5. Maruhama, T., Metabolism **14**, 78 (1965).
6. Wong, R. K. L., and Van Bruggen, J. T., J. Biol. Chem. **235**, 30 (1960).
7. Nervi, F. O., Ganzalez, A., and Valdivieso, V. D., Metabolsim **23**, 495 (1976).
8. Nervi, F. O., Severin, C. H., and Valdivieso, V. D., Biochim. Biophys. Acta **529**, 212 (1978).
9. Nakhooda, A. F., Like, A. A., and Chappel, C. I., *et al.*, Diabetes **26**, 100 (1976).
10. Nakhooda, A. F., Like, A. A., and Chappel, C. I., *et al.*, Diabetologia **14**, 199 (1978).
11. Subbiah, M. T. R., Tyler, N. E., Buscaglia, M. D., and Marai, L., J. Lipid Res. **17**, 78 (1976).
12. Li, J. R., Dinh, D. M., Ellefson, R. D., and Subbiah, M. T. R., Metabolism **28**, 151 (1979).
13. Grundy, S. M., Ahrens, E. H., and Miettinen, T. A., J. Lipid Res. **6**, 397 (1965).
14. Hofmann, A. F., J. Lipid Res. **3**, 127 (1962).
15. LRC Manual of Lab Operation Vol 1, Lipids and Lipoprotein Analysis," NHLI-DHEW Publication No. 75-628. U.S. Government Printing Office, Washington D.C. (1974).
16. Subbiah, M. T. R. and Siekert, R. B., Jr., Brit. J. Nutr. **41**, 1 (1979).
17. Danielsson, H., *in* "Bile Acids, Chemistry, Physiology and Metabolism" (P. P. Nair and D. Kritchevsky, eds.), Vol. 2, p. 1. Plenum Press, New York (1973).
18. Bjorkhem, I., Danielsson, H., and Einarsson, K., Eur. J. Biochem. **2**, 244 (1967).
19. Mitropoulos, K. A., and Myant, N. B., Biochem. J. **103**, 472 (1967).
20. Einarsson, K., Eur. J. Biochem. **5**, 101 (1968).

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