

Fatty Acid Composition of Serum Lipids in 5-Year-Old White Carneau and Show Racer Pigeons*† (33703)

FRANKLIN YOUNG (Introduced by John R. Beaton)

Department of Food and Nutritional Sciences, University of Hawaii, Honolulu, Hawaii 96822

Swell *et al.* (1) hypothesized from a study of several species of animals that the level of arachidonic acid in serum cholesterol esters may be related to susceptibility to atherosclerosis. Young and Middleton (2) showed that the levels of arachidonic acid in cholesterol esters of both serum and liver from 8-month-old atherosclerosis-susceptible White Carneau pigeons (WC) are significantly lower than those of the atherosclerosis-resistant Show Racer pigeons (SR) of the same age. The aortas from neither breed exhibited any visible gross lesion at necropsy, as would be expected of pigeons of this age (3). The incidence of aortic atherosclerosis, as evidenced by the presence of lesions, in the White Carneau breed is much greater than in the Show Racer breed between the ages of 4 and 8 years (4). The present study was undertaken to determine whether or not the greater difference in incidence between these two breeds is reflected in greater difference in the arachidonic acid levels of the serum sterol esters. In addition, there might be other differences in the fatty acid composition of serum lipids accompanying the greater difference in the incidence of the disease between these two breeds. Also included in this study were pigeons from the F₁ generation of Show Racer and White Carneau matings since it has been reported that offsprings from such matings result in pigeons which are intermediate in their susceptibility to aortic atherosclerosis (5).

Materials and Methods. Fifteen 5-year-old

male pigeons of the Show Racer (SR), of the White Carneau (WC) breeds and of the F₁ generation of SR and WC matings were used. The diet consisted of a mixture of corn, wheat, milo, and peas, fed *ad libitum*. Individual blood samples were obtained weekly from the alar veins until 5 ml of serum had been accumulated. This required 3–4 weekly blood samples. Lipids were extracted from the serum (6) and fractionated into sterol esters, glycerides, and phospholipids by thin-layer chromatography (2). The component fatty acid methyl esters of the various lipid fractions were prepared (7) and analyzed in duplicate by gas-liquid chromatography (2). Fatty acids were quantified using the disc integrator of the recorder. Comparisons of the fatty acid values were made between SR and WC, between SR and F₁, and between WC and F₁.

To determine whether or not any changes occur in the fatty acid composition of serum sterol esters of pigeons when they grow old and/or when they develop aortic atherosclerosis, the data from this study were compared with those of an earlier study (2). Comparisons were made between the two age groups within each breed.

Student's *t*-test (8) was used to evaluate the significance of differences between mean values. In the tables, brackets connect the values which differ significantly from each other, and the probability values are shown in the footnote of each table.

Results and Discussion. Fatty acid compositions of serum glycerides of SR, WC, and the F₁ cross are shown in Table I with 18:1 fatty acid being the most abundant for all breeds and cross, followed by 18:2 and 16:0 fatty acids. No significant difference was found between the proportions of fatty acids in serum glycerides of SR and WC. The lack of such difference also has been reported for humans (9). Serum glycerides of SR had a

* Part of this study was made while author was associated with the Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, N. C. This study was supported by grants from USPHS (HE-05277 and HE-11484), Hawaii Heart Assn., and Biomedical Sciences Support, University of Hawaii.

† Journal Series No. 1010 of the Hawaii Agr. Expt. Sta.

TABLE I. Mean Relative Percentages of Fatty Acids of Serum Glycerides.

Breed ^a	No. of birds	(No. of carbons:double bonds)					
		16:0	16:1	18:0	18:1	18:2	20:4
SR	14	20.0	4.6	8.3	41.7] ^b	22.6] ^c	0.9
F ₁	15	20.6	4.7	8.7	38.3]	24.8]	0.8
WC	15	21.0	4.9	9.1	39.7	22.9	0.7

^a SR, Show Racer; F₁, offspring of SR × WC; WC, White Carneau.

^b $p < .005$.

^c $p < .025$.

significantly higher proportion of 18:1 fatty acid and significantly lower proportion of 18:2 fatty acid than did those of F₁. The significance of these findings, if any, is not known. Other workers have shown that the blood glyceride fatty acid composition may be influenced by the fatty acid composition of dietary fat (10, 11). Since in this study all birds were fed the same diet, any differences observed would not be attributable to dietary factors.

The relative percentages of the major fatty acids of serum phospholipids of SR, WC, and F₁ cross between SR and WC are presented in Table II. As in the case of glyceride fatty acid composition, there was no significant difference between SR and WC. In patients with atherosclerosis however, it has been reported that the serum phospholipids contain significantly higher level of 16:0 fatty acid and lower levels of 18:2 and 20:4 fatty acids compared to controls of comparable age (9). The proportion of 18:1 fatty acid of serum phospholipids in F₁ of this study was significantly lower than that of SR. In addition, the proportion of 18:2 fatty

acid of the former was significantly higher than that of either SR or WC. It would be speculative to attach any significance to these differences at the present time with respect to susceptibility to aortic atherosclerosis.

The fatty acid compositions of serum sterol esters are presented in Table III. No difference was observed in the proportions of fatty acids between SR and WC. This is in contrast with the data from an earlier study using 8-month-old pigeons (2). In that study, the Show Racer males had a significantly higher proportion of arachidonic acid in serum sterol esters than did White Carneau males. The data of the present study seem to indicate that the difference in the proportions of arachidonic acid in the serum sterol esters between young SR and WC disappears with age. In the present study, it was observed also that the proportion of 18:1 fatty acid of the serum sterol esters of the F₁ is significantly lower and the proportion of 18:2 fatty acid significantly higher than that of either the SR or the WC. These differences are most probably not related to resistance to aortic atherosclerosis since it has been shown

TABLE II. Mean Relative Percentages of Fatty Acids of Serum Phospholipids.

Breed ^a	No. of birds	(No. of carbons:double bonds)					
		16:0	16:1	18:0	18:1	18:2	20:4
SR	15	16.3	1.0	31.3	15.2] ^d	28.8] ^b	5.6
F ₁	15	16.1	1.0	30.1	14.1]	31.2] ^c	5.9
WC	15	15.5	1.3	31.2	14.7	29.1]	6.3

^a See footnote a of Table I.

^b $p < .001$.

^c $p < .025$.

^d $p < .05$.

TABLE III. Mean Relative Percentages of Fatty Acids of Serum Sterol Esters.

Breed ^a	No. of birds	(No. of carbons:double bonds)					
		16:0	16:1	18:0	18:1	18:2	20:4
SR	15	9.1	3.6	3.1	24.7] ^b	55.1] ^c	1.9
F ₁	15	8.9	3.6	3.3	21.0] ^c	58.2] ^c	2.1
WC	14	8.8	4.1	3.4	23.7]	54.8]	2.1

^a See footnote *a* of Table I.^b *p* < .001.^c *p* < .005.

that pigeons from a cross of a resistant and susceptible breed exhibit a degree of resistance intermediate between the two parent breeds (5).

The comparison of relative percentage of fatty acids of serum sterol esters of 8-month-old and 5-year-old pigeons is presented in Table IV. The serum sterol esters of 5-year-old WC had significantly higher proportion of 18:0 fatty acid than those of 8-month-old birds. In both the SR and WC, it was found that the 5-year-old birds had significantly lower 18:1 fatty acid than did their 8-month-old counterparts. Arachidonic acid was present at significantly lower level in the serum sterol esters of the 5-year-old SR than of the 8-month-old SR. Although the values of arachidonic acid between the young and old WC did not differ significantly, the trend was in the same direction as in the SR.

The differences in fatty acid composition

of serum sterol esters observed between young and old pigeons suggest changes as the birds age and/or as they develop aortic atherosclerosis. Further studies are necessary to determine whether the changes are due to old age or the development of atherosclerosis, or both. Data of the present experiment do not provide support for, nor eliminate, the possibility of a cause-effect relationship between differences of fatty acid composition at 8 months of age and the later appearance of atherosclerotic lesions. Long-term experiments with periodic autopsy of animals will be necessary to observe possible correlation of changes in the fatty acid composition with the development of the disease.

Summary. A comparative study was made of the fatty acid compositions of serum glycerides, phospholipids, and sterol esters of White Carneau pigeons (WC) which are susceptible to naturally-occurring aortic atherosclerosis and Show Racer pigeons (SR) which are resistant. The significantly lower proportion of arachidonic acid in the serum sterol esters of 8-month-old WC compared to SR, was not observed in 5-year-old pigeons. No significant difference was observed in the fatty acid compositions of the three lipid fractions between 5-year-old WC and SR. Significant differences in the fatty acid compositions of all three serum lipid fractions were found between F₁ generation of SR × WC and either one of their parent breeds. Significant changes in the fatty acid composition of serum sterol esters were noted between 8-month-old and 5-year-old pigeons of both breeds.

The author gratefully acknowledges the technical assistance of Colin Huddleston, the Hawaii Heart

TABLE IV. Comparison of Mean Relative Percentages of Fatty Acids of Serum Sterol Esters of Young and Old Pigeons.

Breed	(No. of carbons:double bonds)				
	16:0	18:0	18:1	18:2	20:4
SR ^a	8.8	3.2	27.0] ^a	54.5	3.1] ^a
SR ^b	9.1	3.1	24.7]	55.1	1.9]
WC ^a	9.0	2.9] ^a	26.9] ^a	55.8	2.4
WC ^b	8.8	3.4]	23.7] ^a	54.8	2.1
F ₁ ^b	8.9	3.3	21.0	58.2	2.1

^a Eight-month-old, mean value for 60 birds.^b Five-year-old, mean value for 15 birds.^a *p* < .001.^a *p* < .025.^a *p* < .05.

Association Junior Research Fellow, Grayce M. Greene, and Judy K. Kirk.

1. Swell, L., Field, H., Jr., and Treadwell, C. R., *Proc. Soc. Exptl. Biol. Med.* **104**, 325 (1960).
2. Young, F. and Middleton, C. C., *Proc. Soc. Exptl. Biol. Med.* **123**, 816 (1966).
3. Lofland, H. B., Clarkson, T. B., Prichard, R. W., and Netsky, M. G., *Circulation* **20**, 973 (1959).
4. Lofland, H. B., Jr. and Clarkson, T. B., *Circulation Res.* **7**, 234 (1959).
5. Clarkson, T. B. and Lofland, H. B., *Circulation Res.* **9**, 106 (1961).
6. Young, F., Middleton, C. C., and Lofland, H.

- B., Jr., *Proc. Soc. Exptl. Biol. Med.* **117**, 613 (1964).
7. Stoffel, W., Chu, F., and Ahrens, E. H., Jr., *Anal. Chem.* **31**, 307 (1959).
8. Snedecor, G. W., "Statistical Methods" Iowa State Univ. Press, Ames, Iowa (1956).
9. Schrade, W., Biegler, R., and Bohle, E., *J. Atherosclerosis Res.* **1**, 47 (1961).
10. Ahrens, E. H., Jr., Hirsch, J., Peterson, M. L., Insull, W., Jr., Stoffel, W., Farquhar, J. W., Miller, T., and Thomasson, H. J., *Lancet* **1**, 115 (1959).
11. Swell, L., Law, M. D., and Treadwell, C. R., *J. Nutr.* **76**, 429 (1962).

Received Sept. 19, 1968. P.S.E.B.M., 1969, Vol. 130.

Glucagon and Epinephrine Responsive Adenyl Cyclase Activity of Regenerating Rat Liver* (33704)

F. F. BECKER¹ AND M. W. BITENSKY¹
(Introduced by C. A. Stetson)

*Departments of Pathology and Medicine, New York University School of Medicine,
New York, New York 10016*

The effect of the liver on the regulation of blood glucose depends in large part upon the interaction between glucagon or epinephrine with hepatic adenyl cyclase (1). The activation of membrane-associated adenyl cyclase by either hormone is followed by a rapid rise in the intracellular concentration of 3'5'-cyclic adenosine monophosphate (cyclic A). This cyclic nucleotide is known to cause the enzymic activation of glycogen phosphorylase and the concomittant inactivation of glycogen synthetase. As a result of this change in the activities of the glycogen regulatory enzymes, hepatic glycogen undergoes stepwise phosphorolysis and glucose is made available to the hepatocyte and remote tissues.

Following 70% hepatectomy of the rat, a series of profound structural and biochemical alterations are observed to occur in a large number of the residual hepatocytes (2). Throughout the process of regeneration the hepatocyte glycogen stores are significantly

depleted (3). This depression of glycogen appears to result from a greatly increased utilization of glucose which reflects the cells' heightened metabolic activity. During this time some areas of the hepatocyte membranes undergo degenerative change, subsequent to which a significant quantity of new membrane is elaborated (4, 5). Although it would appear advantageous to the cells' metabolic economy that they maintain their adenyl cyclase levels, the activity of this enzyme during the regenerative process has not been examined.

The present paper describes the behavior of the hepatic adenyl cyclase system at varying intervals following 70% hepatectomy.

Materials and Methods. Sprague-Dawley male rats weighing approximately 200 g were used throughout the studies except where specifically noted. They were fed and watered *ad libitum*. Seventy percent hepatectomy was performed as described previously. Sham operation was identical in all manipulations save for the handling and removal of the liver (6). Following sacrifice, the same liver lobes were utilized in each experimental

* Supported by Grants from the American Cancer Society E-355 D and P-432.

¹ Career Scientists of the Health Research Council of the City of New York.