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Increased Ratio of Plasma Free Fatty Acids to Albumin During Normal Aging and in Patients with Coronary Heart Disease

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Summary

The ratio of free fatty acids (FFA) to albumin, its carrier protein, was determined in 118 healthy men of ages 20 to 69 years and in 83 patients with coronary heart disease (CHD) of ages 33 to 69 years. During aging in normal men, this ratio increased progressively from an average value of 0.755 ± 0.061 in the age 20–29 group to a value of 1.042 ± 0.105 in the 60–69-year-old group. In patients with CHD this ratio was approximately 18% higher in each 10-year cohort than the corresponding control value, rising from a value of 1.029 ± 0.081 in the 30–39-year-old group to a value of 1.212 ± 0.106 in the 60–69-year-old group. The compositional spectrum of FFA among representative groups was similar, although linoleic acid was slightly reduced as a function of aging and the development of CHD.

These results demonstrate that studies which measure only the absolute changes in FFA levels as a function of age or development of CHD tend to underestimate the magnitude of changes in FFA availability to tissues and for participation in biochemical reactions.

Key words: *Aging – Albumin – Coronary heart disease – Free fatty acids*

Introduction

Numerous studies have reported elevated levels of plasma free fatty acids (FFA) in patients with coronary heart disease (CHD), angina pectoris and systemic atherosclerosis [1–5]. While circulating FFA are a principal source of energy for

heart muscle, increase in the concentration of plasma FFA may have deleterious consequences on the maintenance of normal heart function and blood circulatory patterns. Free fatty acidemias are associated in experimental animals with the development of systemic hyperfibrinogenemia which can lead to correspondingly increased blood viscosities and with decreased endogenous blood fibrinolytic activity which may delay the clearance of deposited fibrin within the vascular system [6–10]. High plasma FFA values have also been proposed as inducers of myocardioarrhythmias [11]. However, the availability of plasma FFA to tissues and for participation in biochemical reactions is governed not by the absolute concentration of plasma FFA but rather by the ratio of FFA to albumin, its carrier protein [11–13]. Therefore, to determine more accurately the availability of plasma FFA in patients with coronary heart disease, both the FFA concentration and the albumin concentration were determined in 118 normal males aged from 20 to 69 years and in 83 patients with a history of CHD ranging in age from 33 to 69 years. From the values obtained the free fatty acid to albumin ratio for each individual was calculated in the two sample populations.

Methods

One hundred and eighteen normal Caucasian males aged from 20 to 69 years served as the controls in this study. All were in excellent physical condition and had no known clinical abnormalities. Subjects with a previous history of acute myocardial infarction ranged in age from 33 to 69 years and were accepted into the study provided they had electrocardiographic evidence of acute myocardial infarction between 6 months and 2 years previously. None of these patients studied had other complicating diseases such as diabetes mellitus or was currently receiving drugs such as corticosteroids or blocking agents which could alter plasma FFA levels. None of the control subjects or the patients smoked cigarettes or cigars.

Blood samples were obtained at 8:00 to 9:00 a.m. after an overnight fast from 8:00 p.m. of the previous evening. All of the volunteers in the study were specifically instructed to avoid intake of any type of beverage or food except water before the donation of the blood sample. After arrival in the laboratory in the morning, all of the blood donors were first allowed to sit quietly for 15 minutes to normalize any elevation of FFA induced by exertion or agitation.

Blood samples were obtained by venipuncture and the plasma FFA levels determined by microtitration, and albumin levels obtained by electrophoresis, by methods previously detailed [7]. The reproducibility of measurements of the FFA to albumin ratio was determined in 15 representative subjects (five 20–29-year-old normals, five 60–69-year-old normals, five 60–69-year-old patients with CHD) over a 3-month period at monthly intervals.

The composition of FFA was determined by extraction of serum (5 ml) with 24 volumes of chloroform–methanol (2 : 1) followed by washing of the extract with 0.5 volume of 0.04% $MgCl_2$. The chloroform phase was flash evaporated at 20°C, redissolved in chloroform (2 ml), then passed through a column containing 3 g silicic acid. FFA and neutral lipids were eluted with 25 ml chloroform, then flash

TABLE I

CHANGES IN PLASMA CONCENTRATION OF FFA, ALBUMIN AND FFA/ALBUMIN RATIO AS A FUNCTION OF AGING IN HEALTHY MALES AND IN PATIENTS WITH CORONARY HEART DISEASE

Values represent mean ± standard deviation. Individual assays were run in duplicate. Probability values were calculated using the two-tailed Student *t*-test.

Age	Number of patients		FFA (microequivalents/ml)		Albumin (microequivalents/ml)		Molar ratio FFA/albumin	
	Normal	CHD	Normal	CHD	Normal	CHD	Normal	CHD
20–29	26	–	0.560 [a] ± 0.086	–	0.742 [f] ± 0.076	–	0.755 [k] ± 0.061	–
30–39	20	11	0.572 [b] ± 0.078	0.637 [d] ± 0.083	0.684 [g] ± 0.075	0.620 [i] ± 0.074	0.831 [l] ± 0.077	1.029 [n] ± 0.081
40–49	18	20	0.578 ± 0.082	0.607 ± 0.048	0.652 ± 0.079	0.597 ± 0.055	0.893 ± 0.122	1.022 ± 0.095
50–59	27	22	0.594 ± 0.059	0.645 ± 0.080	0.607 ± 0.043	0.565 ± 0.058	0.991 ± 0.099	1.147 ± 0.159
60–69	27	30	0.611 [c] ± 0.061	0.665 [e] ± 0.069	0.587 [h] ± 0.064	0.556 [j] ± 0.053	1.042 [m] ± 0.105	1.212 [o] ± 0.106

P values of differences: $P < 0.001$ for [f] vs [h], [k] vs [m], [n] vs [o], [i] vs [n], [m] vs [o]; $P < 0.01$ for [a] vs [c], [b] vs [d], [c] vs [e]; $P < 0.025$ for [g] vs [i], [i] vs [j]; $P < 0.05$ for [h] vs [j]; $P < 0.25$ for [d] vs [e].

evaporated to dryness, and dissolved in 10 ml hexane. FFA were extracted from hexane into 3 ml of 50% ethanolic 0.1 N NaOH, the extract was then acidified with 0.1 N HCl, the fatty acids re-extracted into additional hexane, and the FFA methylated with 14% boron trifluoride [14]. Fatty acid methyl esters were quantitated on a column of 12% diethylene glycol succinate on Gas-Chrom Q (100–120 mesh) (Applied Science, State College, PA) programmed from 175°C to 225°C in a Beckman GC-65 gas chromatograph.

Results

The subjects were divided into 10-year cohorts for data analysis. In normal males the plasma FFA levels rose progressively from average value of 0.560 ($\pm$0.086) microequivalents per ml at ages 20–29 years to a value of 0.611 ($\pm$0.061) at 60–69 years, an average increase of 9% (see Table 1). In patients with CHD the values of FFA averaged about 8% higher than in normal controls in the corresponding 10-year age groups. The 60–69-year CHD group had an average FFA which was 19% higher than the 20–29-year normals. In contrast, plasma albumin concentrations diminished with age from an average value of 0.742 ($\pm$0.076) microequivalents per ml in the 20–29-year group to an average value of 0.587 ($\pm$0.064) in the 60–69-year-old normal subjects, an average decrease of 21%. In patients with CHD, the average values for albumin were approximately 8% below the corresponding values for the healthy subjects in each 10-year age group, with the 60–69-year-old CHD group averaging 25% less albumin than the 20–29-year-old normals.

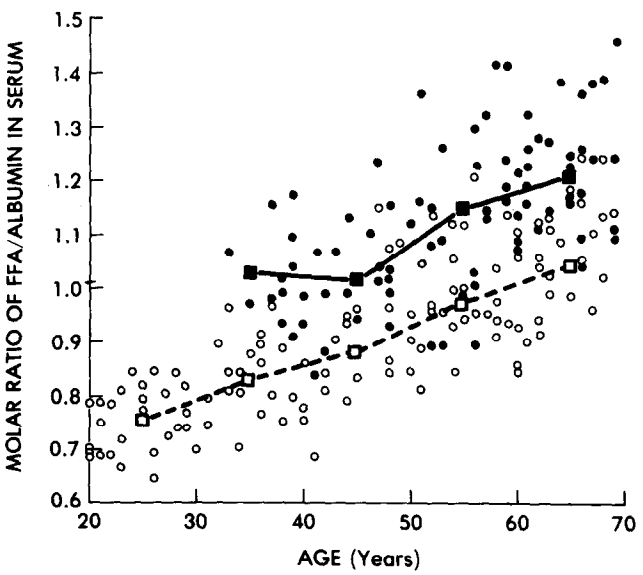

Fig. 1. Molar ratio of FFA/albumin in healthy males of ages 20–69 (open circles) and in patients of ages 33–69 with coronary heart disease (closed circles). The average values for the 10-year cohorts are given by open squares for the healthy group and by closed squares for the patient group.

TABLE 2

COMPOSITION (%) OF SERUM FREE FATTY ACIDS IN NORMAL PERSONS AND IN PATIENTS WITH CARDIOVASCULAR HEART DISEASE

Ten subjects in each grouping. P values on C 18:2 differences: [a] vs [b], $P < 0.025$; [a] vs [c], $P < 0.005$; [b] vs [c], $P < 0.01$.

	Normal persons (20–29 years)	Normal persons (60–69 years)	Patients with CHD (60–69 years)
C 14:0	2.4 ± 0.3	2.1 ± 0.4	2.3 ± 0.3
C 16:0	27.4 ± 0.9	28.2 ± 0.6	28.3 ± 0.8
C 16:1	6.7 ± 0.7	7.4 ± 0.7	7.6 ± 0.8
C 18:0	12.1 ± 0.8	14.1 ± 0.5	14.4 ± 0.7
C 18:1	30.1 ± 1.2	29.7 ± 0.9	29.1 ± 1.1
C 18:2	14.1 ± 1.7 [a]	12.1 ± 1.5 [b]	10.2 ± 1.5 [c]
> C 18:2 *	7.2 ± 0.5	6.5 ± 0.6	6.2 ± 0.5

* > C 18:2 = C 18:3 + C 20:3 + C 20:4 + C 20:5 + C 22:6.

These relatively modest changes in the FFA and albumin concentrations among the various groups are considerably amplified when one considers the physiologically meaningful parameter, the ratio of the FFA to albumin, its carrier protein. This ratio rises progressively with age from a value of 0.755 (±0.061) in the 20–29-year-old normals to a value of 1.042 (±0.105) in the 60–69-year-old normal subjects, an average increase of 38%. In the patients with CHD, each 10-year age group averages approximately 17% higher in this ratio than corresponding normal subjects, with the value for the 60–69-year-old CHD group of 1.212 (±0.106) being 60% higher than the 20–29-year-old normal value. A scattergram of the individual FFA/albumin ratios for the two populations is given in Fig. 1.

The variability of individual FFA/albumin measurements as determined in the 15 subjects over the 3-month period had an average standard deviation of 9.1%. There was little difference in variance among the three groups. Individual standard deviations ranged from 4.9% to 14.1% for 3 measurements.

The compositional spectrum of plasma FFA among representative groups of subjects was similar with a slight tendency toward a decrease in the relative linoleic content in the 60–69-year-old normal group (12.2 ± 1.4% vs 14.1 ± 1.6% in 20–29-year-old normals) and a further enhancement of this decrease in the 60–69-year-old patients with CHD (10.2 ± 1.3%) (Table 2). The linoleic content of FFA in patients with CHD and systemic atherosclerosis has been previously reported to be either decreased [1,15] or unchanged [16].

Discussion

The results suggest that studies which measure changes in the absolute concentration of FFA as a function of age or the development of CHD and other forms of blood flow impairments may considerably underestimate the change in FFA availa-

bility that actually occurs. Many studies have demonstrated that the availability of FFA to tissues or isolated cells and the ability of FFA to participate in biochemical reactions is controlled not by the absolute FFA concentrations but rather by the ratio of the FFA to albumin, its carrier protein [11–13]. Increased FFA/albumin ratios may be caused by either an excessive fat ingestion, or an increased mobilization of fatty acids from the adipose stores or by a decreased utilization of the fatty acids by bodily tissues [6].

The observed increase in the serum FFA level as a function of aging may be due to the decreased metabolic rate of tissues associated with aging [17] and may represent an inability of bodily tissues to metabolize the quantity of fatty acid presented to the tissues. It is possible that the reduction in albumin is related to the same general phenomenon as the accumulation of fatty acids. Decreases in serum albumin are often associated with starvation and inadequate nourishment [18]. The decreased metabolism of energy-producing substrates associated with aging may mimic the decrease in albumin observed with inadequate caloric intake of starvation. This line of reasoning would suggest that the increased FFA and decreased albumin found in the CHD group may suggest that these individuals are aging at a somewhat more rapid rate than the normal subjects.

It is possible that an increased plasma FFA/albumin ratio may also represent one of the lipid abnormalities which predisposes to the development of CHD. A variety of conditions which are associated with insufficient blood flow and the development of CHD are also characterized by an increase in FFA/albumin ratio. This ratio rises during normal aging of humans and a wide variety of conditions or stresses such as psychogenic stress, tissue trauma and infections induce the mobilization of FFA from the tissues and the development of free fatty acidemias [6–8]. Excessive saturated fatty intake has been reported to increase plasma FFA in humans [6], while excessive cholesterol intake has been reported to do the same in rats [19]. In addition, in metabolic diseases such as diabetes mellitus, which are characterized by inadequate tissue perfusion, patients are often found to have elevated FFA levels [6].

If an increased FFA/albumin ratio is one of the causative factors leading to CHD and circulatory impairments, what is a possible mechanism by which this could occur? In previous studies I have emphasized the close relationship between alterations in the serum FFA/albumin ratio and consequent effects on the fibrinogen/fibrinolytic system [7,8,20]. Plasma fibrinogen concentrations and antifibrinolytic activities are closely associated with concomitant changes in FFA metabolism. Animals placed on diets rich in saturated fats develop high fibrinogen levels and lowered blood fibrinolytic activity [8]. Conditions or factors (drugs, hormones, psychogenic stress, tissue damage, toxins) which increase the serum FFA concentration or the FFA/albumin ratio, are associated with increases in blood fibrinogen concentrations and decreases in endogenous fibrinolytic activity [8]. On the other hand, a wide variety of FFA-lowering drugs induce a lowering of the blood fibrinogen concentration and an increase in endogenous blood fibrinolytic activity which is mediated by a reduction in the synthesis of fibrinogen and antifibrinolysins [8,20]. Conditions which increase blood fibrinogen concentrations and decrease

endogenous fibrinolytic activity have been proposed as directly influencing certain causative events that reduce blood flow [21]. Fibrinogen is a major determinant of blood and plasma viscosity [21], is a major contributor to the heparin-neutralizing activity of plasma [22] and high fibrinogen concentrations accelerate platelet [23,24] and erythrocyte aggregation [24,25]. The combination of rheological changes in blood induced by hyperfibrinogenemia combined with a decrease in the rate of clearance of fibrin by the fibrinolytic system may facilitate the development of atheromatous lesions [20–22].

Thus, diverse conditions such as acute psychogenic stress, infections and tissue trauma, excessive administration of fat-mobilizing hormones (e.g. amphetamine abuse) as well as the normal patterns of human aging may, by increasing the FFA/albumin ratio and subsequently perturbing the fibrinogen/fibrinolytic system, produce impairments in the patterns of blood flow similar to that associated with the excessive dietary intake of nutritional lipids. The results described in this paper, and considerations thereof, suggest that procedures that lower the FFA/albumin ratio in older humans and in patients with CHD may be of value in the maintenance of vascular patency on aging humans.

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References

1 Schrade, W., Biegler, R and Bohle, E., Fatty acid distribution in the lipid fractions of healthy persons of different ages, patients with atherosclerosis, and patients with idiopathic hyperlipidemia, J. Atheroscler. Res., 1 (1961) 47.
2 Jenkins, D.J.A., Welborn, T.A. and Goff, D.V., Free fatty acids, α-hydroxybutyrate, and ischaemic heart disease, Lancet, i (1970) 865.
3 Inoue, S., Ohta, M., Iizuka, T. and Murao, S., Glucose tolerance, serum insulin and lipid abnormalities in patients with coronary heart disease, Jpn. Heart J., 16 (1975) 670.
4 Urbaszek, V.W., Heiland, G., Pankau, H. and Modersohn, D., Der Einfluss koronarwirksamer Substanzen auf die Konzentration der unveresterten Fettsäuren im Serum bei Patienten mit Angina pectoris, Z. Ges. Inn. Med., 30 (1975) 15.
5 Ceremuzynski, L., Lada, W., Matwiszyn, B. and Lawecki, J., Patients with intractable angina — Free thyroxine index, immunoreactive insulin and free fatty acids in blood, free adrenaline and noradrenaline in urine, Europ. J. Cardiol., 7 (1978) 353.
6 Pickart, L. and Thaler, M., Fatty acids, fibrinogen and blood flow — a general mechanism for hyperfibrinogenemia and its pathologic consequence, Med. Hypothesis, 6 (1980) 545.
7 Pickart, L., Free fatty acidemia as an inducer of systemic hyperfibrinogenemia and fibrinolytic inhibition, Inflammation, 5 (1981) 61.
8 Pickart, L., Fat metabolism, the fibrinogen/fibrinolytic system and blood flow — New potentials for the pharmacological treatment of coronary heart disease, Pharmacology, 23 (1981) 271.
9 Saldeen, T., The microembolism syndrome, Microvasc. Res., 11 (1976) 227.
10 Wegener, T., Bagge, L. and Saldeen, T., Effect of nicotinic acid on the post-traumic increase in free fatty acids and fibrinolysis inhibition in the rat, Uppsala J. Med. Sci., 84 (1979) 195.
11 Miller, N.E., Mjøs, O.D. and Oliver, M.F., Relationship of epicardial ST segment elevation to the plasma free fatty acid/albumin ratio during coronary occlusion in dogs, Clin. Sci. Mol. Med., 51 (1976) 209.

12 Nestel, P.J. and Steinberg, D., Fate of palmitate and of linoleate perfused through the isolated rat liver at high concentrations, J. Lipid Res., 4 (1963) 461.
13 MacKenzie, C.G., MacKenzie, J.B., Reiss, O.K. and Wisneski, J.A., Identification of albumin-bound fatty acids as the major factor in serum-induced lipid accumulation in cultured cells, J. Lipid Res., 11 (1970) 536.
14 Morrison, W.R. and Smith, L.H. Preparation of fatty acid methyl esters and dimethylacetals from lipids and boron fluoride–methanol, J. Lipid Res., 5 (1964) 600.
15 Waremboorg, H., Biserte, G., Bertrand, M., Jaillard, J. and Sezille, G., Les acids gras du sérum dans diverses affections pathologiques, Presse Méd., 75 (1967) 523.
16 Antonini, F.M., D'Allessandro, A., Bucalossi, A., Contini, P. and Serio, M., Analyse en chromatographie gazeuse des acides gras estérifiés du sérum chez des patients normaux, diabétiques et atherosclèreux, avant, pendant, et après lipolyse noradrénalinique, Rev. Athérosclèr. (Paris), 9 (1967) 14.
17 Shock, N.W., Systems integration. In: C.E. Finch and L. Hayflick (Eds.), Handbook of the Biology of Aging, Van Nostrand Reinhold, New York, 1977, pp. 717–719.
18 Bhanthumnavin, K. and Schuster, M.M., Aging and gastrointestinal function, In: C.E. Finch and L. Hayflick (Eds.), Handbook of the Biology of Aging, Van Nostrand Reinhold, New York, 1977, pp. 717-719.
19 Tsai, A.C., Romsos, D.R. and Leveille, G.A. Effect of dietary cholesterol on hepatic lipogenesis and plasma insulin and free fatty acid levels in rats, J. Nutr., 105 (1975) 939.
20 Pickart, L., Suppression of acute-phase synthesis of fibrinogen by a hypolipidemic drug (clofibrate), Int. J. Tissue Reac., 3 (1981) 65.
21 Editorial, Haemostatic factors and coronary heart disease, Lancet, i (1981) 22.
22 O'Brien, J.R. and Etherington, M.D., Coronary heart disease, fibrinogen, and plasma heparin neutralizing activity, Lancet, i (1981) 495.
23 Copley, A.L., Fibrinogen, platelets and a new theory of atherogenesis, Thromb. Res., 14 (1979) 249.
24 Dormandy, J. and Ried, H.L. Controlled defibrinogenation in the treatment of peripheral vascular disease, Angiology, 29 (1978) 80.
25 Dintenfass, L. Rheology of Blood, Butterworths, London, 1976.