Correlation between percentage of palmitic acid in adipose tissue and serum cholesterol level in patients with multiple sclerosis

WILLIAM INSULL, JR., HAROLD B. HOUSER, and ARTHUR S. LITTELL

Departments of Medicine, Preventive Medicine and Biometry, Case Western Reserve University, Cleveland, Ohio 44106; and Highland View Hospital, Cleveland, Ohio 44122

ABSTRACT To investigate mechanisms controlling the concentration of serum cholesterol we studied its relationship to the proportions of fatty acids in the lipids of adipose tissue of patients with multiple sclerosis. In 26 men the serum cholesterol concentration had a significant multiple linear regression on the proportion of palmitic, palmitoleic, and grouped longer-chain acids in adipose tissues. In 29 women the serum cholesterol had a significant regression on the proportion of palmitic acid and age. Whether these observations would hold for normal people must be determined in future studies.

SUPPLEMENTARY KEY WORDS palmitoleic acid · multiple regression

THE CONCENTRATION of plasma cholesterol is the most significant factor, after age, relating to the risk of arteriosclerotic coronary heart disease in men (1). Fatty acids in the diet are highly potent factors influencing the concentration of serum cholesterol. However, their exact mechanism of action remains unknown although the degree of unsaturation and the number of carbon atoms in the molecule appear to be important (2). Conceivably, the dietary acids may act locally upon the metabolism of the intestine or systemically after absorption and assimilation. Dietary fatty acids and endogenously synthesized fatty acids mix and are deposited for storage in the adipose tissue as triglycerides. They become available for metabolism throughout the body when mobilized from the adipose tissue as plasma free fatty acids (3). Study of the fatty acid mixture of adipose tissue and serum cholesterol concentration may provide clues about the systemic action of dietary fatty acids on serum cholesterol concentration.

METHODS

We studied Caucasian subjects, 26 men and 29 women, aged 29–62 yr, with multiple sclerosis. The age distribution by decade for men and women, respectively, was third decade 1 and 1, fourth decade 6 and 13, fifth decade 15 and 13, and sixth decade 4 and 2. The subjects had functional capacities on a modified Kurtzke scale varying from asymptomatic to bedridden (4), and none was hospitalized at the time of examination. The clinical status of the disease in each had been fairly stable for at least a year prior to study. They consumed uncontrolled diets whose composition, as determined by repeated determinations for a year prior to this study, approximated that of the usual American diet (5, 6). Their body weights averaged 101% (range 75–135%) of average values for sex and age (7).

The concentration of serum total cholesterol was measured by automated technique (AutoAnalyzer Method N-24, Technicon Instruments Corp., Chauncey, N. Y.). Adipose tissue obtained by needle biopsy of subcutaneous tissue over the buttocks was extracted and stored under nitrogen gas at −15°C until analysis (8). The relative proportions of 14 long-chain fatty acids in the extract were determined by gas–liquid chromatography of duplicate aliquots (9). Linoleic acid, arachidonic acid, and other fatty acids with 20 or more carbon atoms constituted together an average of 0.72% of the total fatty acids and were grouped in the calculations as "other acids." Approximately 50% of the arachidonic acid was recovered for analysis. The relationships of serum cholesterol concentration to 14 acids and age (15 variables) were evaluated separately for each sex by simple and multiple linear regression analysis. In the

latter, the smallest subset of significant variables for a regression was defined as the set of independent variables such that the omission of any variable from the set would significantly ($P < 0.05$) increase the sum of squared deviations from regression, whereas the addition of any other variable to the set would not significantly decrease the sum of squared deviations from the regression (10).

RESULTS

The concentrations of serum cholesterol for the females were 227 ± 54.2 (mean ± SD) and for the males 235 ± 54.8 mg/100 ml. The means were not significantly different. For each of the 14 fatty acids the average proportions and the standard deviations within each sex are presented in Table 1 along with the difference in means divided by its standard error. Only palmitic and 17:0 acids showed significant differences between the sexes in average proportion. By simple linear regression analysis the significant regressions were those of cholesterol on palmitic acid and "other acids" in men, and on palmitic acid, 15:0, and age in women (Table 2). In multiple linear regression analysis of cholesterol on 15 variables the smallest subset of significant variables in males consisted of palmitic, palmitoleic, and "other acids" (Table 3). In females the smallest set of significant variables consisted of palmitic and age. For neither sex was the regression of cholesterol on all variables significant.

DISCUSSION

The interpretation of these observations must include consideration of the characteristics of the data and the methods of statistical analysis. The proportions of fatty acids, the pattern of differences between the sexes, and the correlation of fatty acids with age and body weight observed in these subjects are similar to those reported by us and other observers (9). The levels of cholesterol concentration, the absence of a significant regression of cholesterol on age among men, and the significant positive regression among women are in agreement with the known relationships between serum cholesterol and age (11, 12). With these measures, abnormality of systemic lipid metabolism with multiple sclerosis is not apparent here, nor has it been reported in the literature, although abnormalities of lipid metabolism have been observed in the brain (13–16).

In the simple linear regression analysis of cholesterol on fatty acids the similar standard errors of the slopes for males and females indicate that the slopes were estimated with equal precision in the two sexes. Palmitic acid was the only acid with slopes for both sexes significantly different from zero. In the multiple regression analysis the regression of serum cholesterol and the 15 variables together (14 acids plus age) was not significant. However, search for a subset of significant variables revealed in each sex a subset of variables giving significant reduction in the sum of squared deviations from the regression. Among the males this subset was palmitic, palmitoleic, and "other" acids, accounting for 66% of the total variance. Palmitoleic acid, although not significant individually, did significantly contribute to this subset. Among females the subset was palmitic acid and age, accounting for 31% of the total variance. 15:0 acid did not make a significant further contribution, even though its simple

TABLE 1 DISTRIBUTION OF MAJOR FATTY ACIDS IN ADIPOSE TISSUE OF 26 MALE AND 29 FEMALE CAUCASIANS WITH MULTIPLE SCLEROSIS

Fatty Acids				
Common Name	Symbols*	Males	Females	t†
		%		
Lauric	12:0	0.23 ± 0.20‡	0.26 ± 0.15‡	−0.8
Myristic	14:0	2.44 ± 0.63	2.54 ± 0.53	−0.6
Myristoleic	14:1	0.46 ± 0.21	0.54 ± 0.15	−1.6
—	15:0	0.36 ± 0.14	0.36 ± 0.14	0.0
—	15:?	0.11 ± 0.09	0.11 ± 0.08	0.0
Palmitic	16:0	21.12 ± 1.76	19.63 ± 1.82	3.1§
Palmitoleic	16:1	6.77 ± 1.43	7.16 ± 1.45	−1.0
—	16:2	0.51 ± 0.17	0.47 ± 0.13	0.8
—	17:0	0.36 ± 0.14	0.27 ± 0.14	2.3‖
—	17:?	0.44 ± 0.16	0.44 ± 0.15	0.0
Stearic	18:0	5.06 ± 1.24	4.83 ± 1.27	0.7
Oleic	18:1	51.72 ± 2.19	51.93 ± 2.59	−0.3
Linoleic	18:2	9.64 ± 3.06	10.61 ± 1.99	−1.4
"Others"	18:3, 20:+	0.69 ± 0.40	0.75 ± 0.43	−0.5

* Designated by number of carbon atoms:number of double bonds.
† Difference between means of sexes divided by SEM.
‡ Mean ± SD.
§ $P < 0.01$.
‖ $P < 0.05$.

TABLE 2 SIMPLE REGRESSIONS OF SERUM CHOLESTEROL ON ADIPOSE TISSUE FATTY ACIDS AND AGE

Independent Variables		Male				Female			
		Slope†	SE* of Slope	t‡	R^2§	Slope†	SE* of Slope	t‡	R^2§
Lauric	12:0	−63.7	55.4	−1.15	0.05	−87.9	68.7	−1.28	0.06
Myristic	14:0	2.1	17.9	0.12	0.00	34.2	18.5	1.84	0.11
Myristoleic	14:1	−1.0	52.1	−0.02	0.00	84.5	65.9	1.28	0.06
—	15:0	48.2	81.3	0.59	0.01	156.2	69.0	2.26‖	0.16
—	15:?	−11.0	119.7	−0.09	0.00	116.5	127.1	0.92	0.03
Palmitic	16:0	12.2	5.8	2.08‖	0.15	13.3	5.1	2.58‖	0.20
Palmitoleic	16:1	10.6	7.5	1.42	0.08	−1.1	7.2	−0.16	0.00
—	16:2	39.1	64.2	0.61	0.02	69.1	77.0	0.90	0.03
—	17:0	−44.3	78.4	−0.56	0.01	−61.7	71.9	−0.86	0.03
—	17:?	−14.8	70.9	−0.21	0.00	−83.4	67.4	−1.24	0.05
Stearic	18:0	−16.6	8.4	−1.97	0.14	7.1	8.1	0.88	0.03
Oleic	18:1	−4.0	5.0	−0.78	0.02	−5.0	3.9	−1.28	0.06
Linoleic	18:2	−0.3	3.7	−0.07	0.00	−7.2	5.0	−1.43	0.07
"Others"	18:3; 20:+	−73.5	23.8	−3.10¶	0.29	−20.5	24.1	−0.85	0.03
Age	—	−1.3	1.3	−1.01	0.04	2.3	1.1	2.12‖	0.14

* Standard error.
† Concentration of serum cholesterol (mg/100 ml) ÷ per cent of fatty acid.
‡ Ratio of the slope to its standard error.
§ Coefficient of determination.
‖ $P < 0.05$.
¶ $P < 0.01$.

TABLE 3 MULTIPLE REGRESSION ANALYSIS OF CHOLESTEROL ON 14 ADIPOSE TISSUE FATTY ACIDS AND AGE

Independent Variables	Males				Females			
	Deviation from Regression				Deviation from Regression			
	Degrees of Freedom	Sum of Squares	Mean Square	R^2*	Degrees of Freedom	Sum of Squares	Mean Square	R^2*
None (total)	25	75244	—	—	28	82073	—	—
15 variables	10	20738	2074†	0.72	13	23546	1811‖	0.71
Palmitic, palmitoleic, and "others"	22	25575	1162‡	0.66	25	63497	2540¶	0.23
Palmitic and age	23	63686	2769§	0.15	26	56384	2169**	0.31

* R^2 is coefficient of determination.
† $F = (54506 \div 15)/(20738 \div 10) = 1.75$ $P > 0.05$.
‡ $F = (49669 \div 3)/(25575 \div 22) = 14.25$ $P < 0.001$.
§ $F = (11558 \div 2)/(63686 \div 23) = 2.09$ $P > 0.05$.
‖ $F = (58527 \div 15)/(23546 \div 13) = 2.15$ $P > 0.05$.
¶ $F = (18576 \div 3)/(63497 \div 25) = 2.44$ $P > 0.05$.
** $F = (25688 \div 2)/(56385 \div 26) = 5.92$ $P < 0.01$.

regression coefficient was significantly different from zero. Although the regression of the complete set of 15 variables was not statistically significant, the presence of palmitic acid as a significant variable in the simple linear regression and in both subsets of significant variables suggests that the relationship between this acid and cholesterol may be biologically significant.

The close association between the concentration of serum cholesterol and the proportions of palmitic acid in adipose tissue suggests that the metabolism of these two lipids may be linked. Up to the present no direct metabolic connection has been demonstrated. Palmitic acid has unique characteristics compared to other fatty acids: as the pivotal acid in fatty acid metabolism, it is the major terminal product of the de novo biosynthesis of fatty acids, the precursor for the biosynthesis of other long-chain saturated and monoenoic acids (17), and the major saturated fatty acid in human depot fat, plasma cholesteryl esters, and the diet fats (9, 2, 18). In adipose tissue of Americans, palmitic acid is exceptional as its proportion has no correlations with those of the other acids, indicating independence from them (9).

Several processes appear to influence the concentration of serum cholesterol, but their mechanisms are poorly defined at present (2). Saturated fatty acids in the diet have been observed to elevate serum cholesterol while unsaturated fatty acids lower it. After mathematical analysis of diets and the response of serum cholesterol concentration, Keys, Anderson, and Grande have suggested (19) that the hypercholesterolemic action of

dietary saturated fatty acids may be due primarily to palmitic acid. Similar studies by Hegsted, McGandy, Myers, and Stare (20) indicated that myristic acid was the most hypercholesterolemic dietary acid, but that palmitic acid also had significant effects, especially when evaluated in combination with myristic acid (20). The mechanism of action of these fatty acids remains unknown. Balance studies have demonstrated that lowering plasma cholesterol levels by substitution in the diet of unsaturated fatty acids for saturated fatty acids is associated with an augmented excretion of fecal steroids. These observations are, however, inconstant among investigators and are not regarded as conclusive (21). Our observation that the relative mass of the adipose tissue depot of palmitic acid, as estimated from the skinfold thickness and fatty acid analysis of lipids in subcutaneous fat, is significantly associated with serum cholesterol level suggests a close relationship of the metabolism of these two substances within the body (4). Palmitic acid of adipose tissue can arise from two sources, the diet and endogenous synthesis. The relative importance of these two sources in relation to cholesterol concentration is not known, although the observations of Keys et al. (19) and Hegsted et al. (20) indicate the importance of dietary palmitic acid.

Several hypotheses may be proposed to explain the relationship between palmitic acid and serum cholesterol concentration: (*a*) palmitic acid may directly, or indirectly, control the concentration of serum cholesterol, elevating it by influencing the biosynthesis, catabolism, or esterification of cholesterol or the synthesis of plasma lipoprotein; (*b*) the metabolism of palmitic acid and the mechanism governing the serum concentration of cholesterol may be controlled by a common factor or factors in the body, at present unknown; (*c*) some factor from outside the body associated with palmitic acid may also influence serum cholesterol concentration.

Further study will be needed to establish the validity of these preliminary observations for normal people and to evaluate the hypotheses we have proposed.

The technical assistance of Mrs. Elizabeth Kuhn and Mrs. Judith Jones is gratefully acknowledged. Dr. W. Insull, Jr. is an Established Investigator of the American Heart Association.

This work was supported by U.S. Public Health Service Grants HE 5284, HE 06304, AM 07474, GM 12302, and HD 00669.

Manuscript received 30 November 1968; accepted 2 June 1969.

References

1. Truett, J., J. Cornfield, and W. Kannel. 1967. *J. Chron. Dis.* **20:** 511.
2. Goodman, D. S. 1965. *Physiol. Rev.* **45:** 747.
3. Renold, A. E., and G. F. Cahill. 1965. *In* Adipose Tissue. American Physiological Society, Washington, D. C. 625.
4. Fink, S. L., and H. B. Houser. 1966. *Arch. Phys. Med. Rehabil.* **47:** 56.
5. Insull, W., Jr., H. B. Houser, and A. S. Littell. 1966. *J. Clin. Invest.* **45:** 1026.
6. Houser, H. B., W. Insull, Jr., J. C. Vandervort, A. S. Littell, and A. J. Sorenson. 1966. *Circulation.* **34**(III): 19.
7. 1959. *Statist. Bull. Metrop. Life Ins. Co.* **40:** 2.
8. Hirsch, J., J. W. Farquhar, E. H. Ahrens, Jr., M. L. Peterson, and W. Stoffel. 1960. *Amer. J. Clin. Nutr.* **8:** 499.
9. Insull, W., Jr., and G. E. Bartsch. 1967. *Amer. J. Clin. Nutr.* **20:** 13.
10. Snedecor, G. W., and W. G. Cochran. 1967. *In* Statistical Methods. Iowa State University Press, Ames. 385.
11. Adlersberg, D., L. E. Schaefer, A. G. Steinberg, and C. Wang. 1956. *J. Amer. Med. Ass.* **162:** 619.
12. Lewis, L. A., F. Olmsted, I. H. Page, E. Y. Lawrey, G. V. Mann, F. J. Stare, M. Hanig, M. A. Lauffer, T. Gordon, and F. E. Moore. 1957. *Circulation.* **16:** 227.
13. Baker, R. W. R., R. H. S. Thompson, and K. J. Zilkha. 1964. *J. Neurol. Neurosurg. Psychiat.* **27:** 408.
14. Cumings, J. N., R. C. Shortman, and T. Skrbic. 1965. *J. Clin. Pathol.* (*London*). **18:** 641.
15. Baker, R. W. R., R. H. S. Thompson, and K. J. Zilkha. 1963. *Lancet.* **i:** 26.
16. Cumings, J. N., and H. Goodwin. 1968. *Lancet.* **ii:** 664.
17. Olson, J. A. 1966. *Annu. Rev. Biochem.* **35**(II): 559.
18. Dayton, S., M. L. Pearce, S. Hashimoto, L. J. Fakler, E. Hiscock, and W. J. Dixon. 1962. *N. Engl. J. Med.* **266:** 1017.
19. Keys, A., J. T. Anderson, and F. Grande. 1965. *Metabolism.* **14:** 776.
20. Hegsted, D. M., R. B. McGandy, M. L. Myers, and F. J. Stare. 1965. *Amer. J. Clin. Nutr.* **17:** 281.
21. Moore, R. B., J. T. Anderson, H. L. Taylor, A. Keys, and I. D. Frantz, Jr. 1968. *J. Clin. Invest.* **47:** 1517.