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Effects of Biotin Deficiency on Plasma and Tissue Fatty Acid Composition: Evidence for Abnormalities in Rats

DONALD M. MOCK, NELL I. MOCK, SUSAN B. JOHNSON, AND RALPH T. HOLMAN

Department of Pediatrics, Divisions of Gastroenterology and Nutrition, University of Iowa, Iowa City, Iowa 52242 [D.M.M., N.I.M.]; and The Hormel Institute, University of Minnesota, Austin, Minnesota [S.B.J., R.T.H.]

ABSTRACT. Abnormalities of fatty acid composition have been detected in the plasma of patients who developed frank biotin deficiency during parenteral nutrition. We sought to determine which abnormalities of fatty acid composition, if any, would be replicated in the biotin-deficient rat and to determine the relative temporal relationships of these abnormalities to biotin nutritional status. We measured fatty acid compositions of the phospholipids extracted from plasma, heart, and liver and assessed biotin nutritional status longitudinally in biotin-deficient and biotin-treated rats during progressive biotin deficiency. In the biotin-deficient group, significant increases relative to the biotin-treated group were detected in all three tissues in the odd-chain fatty acids 15:0 and 17:0. In the biotin-deficient rats, significant increases in 18:2ω6 in liver and 18:3ω6 in plasma and liver and significant decreases in 22:5ω6 were detected in plasma and liver. The constellation of fatty acid abnormalities observed in the biotin-deficient rats was not identical to that observed in biotin-deficient patients, but abnormalities in composition of odd-chain fatty acids were detected in both human and rat and therefore are attributable to biotin deficiency per se. The abnormalities in fatty acid composition were already present by wk 4 on the egg white diet; the cutaneous findings appeared between wk 3 and 6. These observations are consistent with the hypothesis that an abnormality in fatty acid metabolism may play a pathogenetic role in the cutaneous manifestations of biotin deficiency. (*Pediatr Res* 24: 396–402, 1988)

Abbreviations

BD, biotin deficient
BT, biotin treated
PL, phospholipid
ANOVA, analysis of variance
PCC, propionyl-CoA carboxylase
MCC, methylcrotonyl-CoA carboxylase
FAME, fatty acid methyl esters

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Correspondence and reprint requests Donald M. Mock, M.D., Ph.D., Department of Pediatrics, Divisions of Gastroenterology and Program in Human Nutrition, University of Iowa Hospitals and Clinics, Iowa City, IA 52242.
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Several lines of evidence (1–9), including preceding studies from our laboratories (4, 10), suggest that biotin deficiency causes abnormalities in fatty acid metabolism that are reflected in abnormalities in tissue fatty acid composition in man and in the rat. In the present study, we sought to determine which of the abnormalities observed in patients who developed biotin deficiency during parenteral nutrition would be replicated in an animal model of biotin deficiency and hence could be attributed to biotin deficiency per se. We also measured the time course of these abnormalities and of several biochemical parameters reflecting biotin nutritional status to determine whether these fatty acid abnormalities are markers for a lesser degree or an earlier stage of biotin deficiency.

METHODS

Male Sprague-Dawley rats of weanling age (approximately 28 days, 50–70 g) were purchased from Harlan Sprague-Dawley, Inc. (Madison, WI). Rats were housed in hanging wire, stainless steel cages, fed Purina Rodent Laboratory Chow *ad libitum* (Ralston Purina, St. Louis, MO), and given tap water *ad libitum* for 6 days until initiation of the experiment. At that time, 38 rats were randomly assigned to one of two treatment groups. The first group (BD) received Teklad diet no. 81079 *ad libitum*. The diet contained 30% spray-dried egg white, an amount that had reliably induced biotin deficiency previously as judged by urinary biotin excretion, by plasma concentrations of biotin, and by the appearance of the classical physical findings of biotin deficiency in the rat (11). The second group of rats (BT) were randomly paired to those in the BD group and pair-fed the same diet. The BT group received 250 μg of biotin dissolved in 1.25 ml of vehicle (0.01 M sodium bicarbonate) by interperitoneal injection twice weekly. The BD group received injections of vehicle only.

Ten pairs of rats (a BD rat and its pair-fed BT rat) were randomly chosen for 24-h urine collections at wk 2, 4, 6, 8, and 12. The rats were housed in metabolic cages designed to prevent fecal contamination of urine; in the rare instance when gross contamination was noted, the urine sample was discarded. During the 24-h collections, rats were allowed water *ad libitum*, but food was removed to prevent contamination of the urine with food (particularly with avidin). Studies from our laboratory demonstrate that contamination of urine by this egg white diet reduces the biotin detectable by our ^{125}I-avidin binding assay (Mock DM, unpublished data). After completing the urine collections at 4, 6, and 8 wk, three pairs of rats were randomly chosen for sacrifice by exsanguination via the abdominal aorta after anesthesia with pentobarbital. At 12 wk, all of the remaining rats were killed. After death, the livers and hearts were immediately removed, weighed, frozen in liquid nitrogen, and stored at −70° C. Plasma was separated from blood and stored at −20° C.

Portions of the plasma, liver, and heart were shipped on dry ice to the Hormel Institute, Austin, MN for fatty acid analysis. Three pairs of uncontaminated urine samples at each time point and three pairs of serum samples at wk 12 were randomly selected for measurement of biotin concentration with the ^{125}I-avidin assay of Mock and DuBois (12). Hepatic activities of the biotin-dependent enzymes propionyl-CoA carboxylase (EC 6.4.1.3) and methylcrotonyl-CoA carboxylase (EC 6.4.1.4) were assayed according to the method of Weyler *et al.* (13).

Fatty acid analysis. Lipids were extracted and the PL fraction was separated by thin-layer chromatography. The PL were converted to methyl esters, and the FAME were analyzed by capillary gas chromatography using methods described previously (10). All results are tabulated as the mean ± SD of the triplicate values.

STATISTICAL ANALYSIS

Fatty acid data were analyzed by two-way ANOVA using treatment (BD or BT) as one variable and time on the diet as the other. To confirm the expected increase in odd-chain fatty acids, one-tailed analysis was performed. Two-tailed analysis was used for all other comparisons. An n of 3 at each time point was used for the ANOVA; the data for the three pairs at 12 wk were chosen at random from the larger number of pairs sacrificed. A significant effect of treatment without an accompanying significant interaction between treatment and time was interpreted as evidence of an abnormality that was caused by biotin deficiency but was not becoming significantly more abnormal during the period of the study. A significant interaction of treatment with time was taken as evidence of an abnormality that was caused by biotin deficiency and that progressed with time on the egg white diet. A significant effect of time alone was taken as evidence of a phenomenon not related to biotin deficiency (*e.g.* maturational effect, dietary change from Chow to egg white diet, etc.).

To compare our findings to a previous publication of Kramer *et al.* (4), Student's two-tailed paired t tests were performed on fatty acid data of all pairs at wk 12, if suitable samples had been obtained at death. Student's two-tailed paired t tests were also used to test differences in plasma concentrations of biotin. A critical value of $p < 0.05$ was chosen as significant for both ANOVA and t testing.

RESULTS

Evidence of progressive biotin deficiency. The urinary excretion of biotin in the BD group decreased dramatically with time on the egg white diet (Fig. 1). In the BT group, urinary biotin increased with time (Table 1), suggesting that parenteral biotin supplement substantially exceeded that absorbed during the baseline period of Chow feeding. The mean plasma concentration of biotin at the termination of the study (wk 12) was significantly less ($p < 0.0016$) in the BD group (2.2 ± 1.5 pmol/ml, $n = 3$) than in the BT group (29 ± 7 pmol/ml, $n = 3$). After 4 wk on the egg white diet, the mean hepatic activities of two biotin-dependent carboxylases (PCC and MCC) of the BD group had declined to about 25% of the mean of the BT group and did not decline further in the subsequent 8 wk (Fig. 2); p values for treatment were $p < 0.005$ for both PCC and MCC. No significant effects of time or interaction were detected.By day 16, 42% of

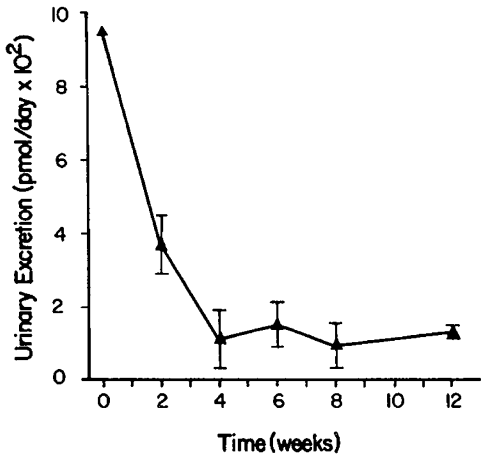

Fig. 1. Decline of urinary excretion of biotin as a function of time on the egg white diet. All points are depicted as mean ± 1 SD for a group of three rats except at 0 wk; the symbol depicts the mean of measurements of samples from two animals.

Table 1. *Biotin excreted in urine of treated rats**

	Urinary excretion	
Wk	pmol/day × 10^2	pmol/mg creatinine × 10^2
0	9.44	9.64
2	78.8 ± 69.6	72.7 ± 87.2
4	161 ± 161	37.8 ± 7.8
6	181 ± 47	56.7 ± 28.8
8	269 ± 234	43.8 ± 27.5
12	212 ± 121	28.8 ± 1.25

* Biotin excretion in urine as measured by ^{125}I-avidin assay in rats fed a diet containing raw egg white, but receiving twice weekly injections of biotin. All values are mean ± SD of $n = 3$ except wk 0 which is $n = 2$.

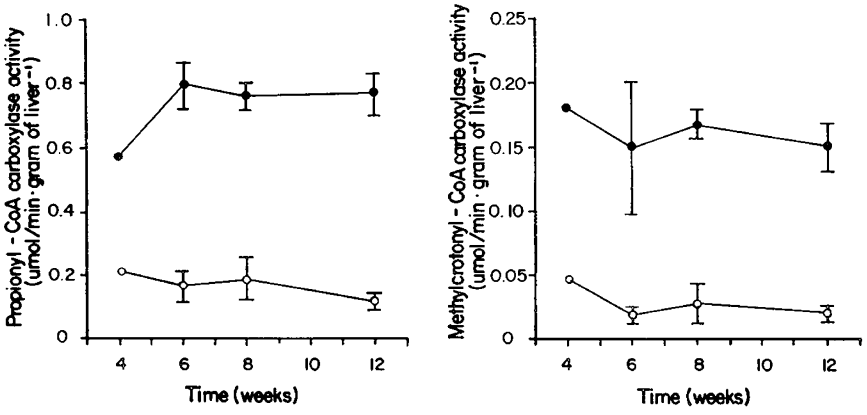

Fig. 2. Effect of biotin deficiency on hepatic propionyl-CoA carboxylase (*left*) and methylcrotonyl-CoA carboxylase (*right*) activities. Values for paired BT (●) and BD (○) rats are depicted as mean ± 1 SD for three pairs of rats at wk 6 and 8 and eight pairs at wk 12. At wk 4, data are available from only a single pair of rats.

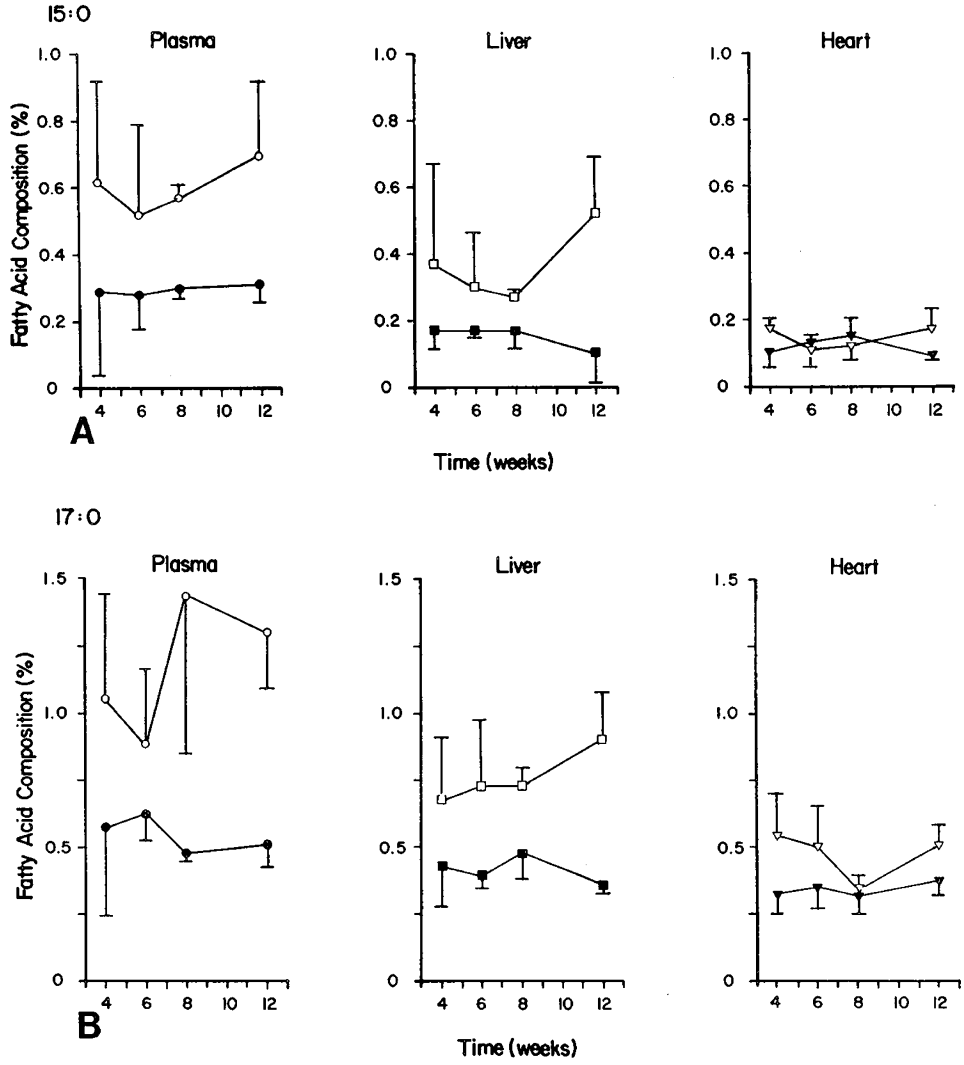

Fig. 3. Effect of biotin deficiency on content of odd-chain fatty acid in plasma, liver, and heart. For *A* (content of 15:0) and *B* (content of 17:0), data from BD rats are depicted by *open symbols* and BT rats by *closed symbols.* Means ± 1 SD from three pairs of rats are depicted. Results of analysis of variance are presented in Table 2.

the rats in the BD group were showing early periocular hair loss and mandibular lesions. By the 6th wk, all rats in the BD group were showing mandible lesions and loss of large amounts of hair. The ataxia and kangaroo gait were present in the majority of the BD animals by 12 wk. In the BT group, no signs of biotin deficiency were present at any time during the study.

Evidence for changes in fatty acid composition.[1] Odd-chain fatty acids (15:0, 17:0) in plasma and liver PL were highly significantly increased in the BD group relative to the BT group (Fig. 3; Table 2). The increase was evident at 4 wk and did not change significantly over the next 8 wk. In heart PL, 17:0 was significantly increased, and, for 15:0, a significant interaction of treatment with time was detected.

Linoleic acid (18:2ω6) was significantly increased in PL from liver but not plasma or heart when the BD group was compared to the BT group (Fig. 4*A*; Table 3). In all three tissues, significant increases with time, independent of treatment effects, were also detected.

The subsequent intermediates in the ω6 pathway are the product of chain elongation of linoleic acid (20:2ω6), the product of desaturation of linoleic acid (18:3ω6, γ-linolenic acid), and the product of chain elongation of 18:3ω6 (20:3ω6). These are minor components of the total fatty acid profile. Data are provided in the appendix tables; results are summarized here. For 20:2ω6, a significant interaction of treatment with time was detected in plasma PL ($p < 0.05$). For 18:3ω6, both plasma and liver PL showed significant increases in BD compared to BT ($p < 0.01$, $p < 0.05$, respectively). No significant changes in 20:3ω6 were detected in any tissue. For arachidonic acid (20:4ω6), no significant treatment effects were detected. In all three tissues, 22:5ω6 (the elongation and desaturation product of 20:4ω6) tended to decrease in BD compared to BT; significance was reached in plasma and liver (Fig. 4*B*). For 22:5ω6 in plasma PL, a significant interaction of treatment with time was also detected (Table 3).

Table 2. *Significance of difference between BD and BT rats for odd-chain fatty acids*

Fatty acid		Plasma	Liver	Heart
15:0	Treatment	$p < 0.001$	$p < 0.0025$	NS
	Time	NS	NS	NS
	Interaction	NS	NS	$p < 0.05$
17:0	Treatment	$p < 0.0005$	$p < 0.0005$	$p < 0.005$
	Time	NS	NS	NS
	Interaction	NS	NS	NS

[1] Full tabular data for all determinations are in appendix following references.

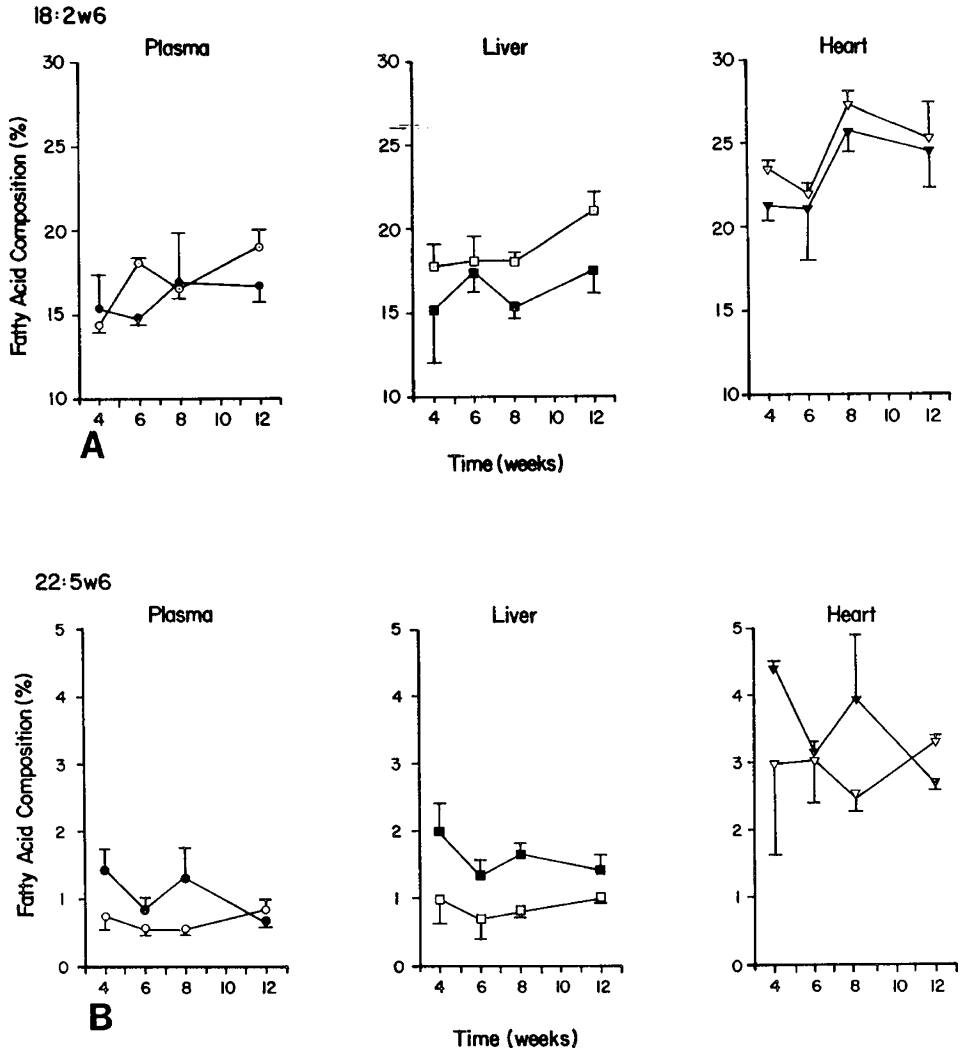

Fig. 4. Effect of biotin deficiency on content of 18:2ω6 and 22:5ω6 fatty acid in plasma, liver, and heart. *A*, 18:2ω6; B, 22:5ω6. *Symbols* as in Figure 3. Results of analysis of variance are presented in Table 3.

Four intermediates in the ω3 family of polyunsaturated fatty acids were measured; these were 18:3ω3 (linolenic acid), 20:5ω3, 22:5ω3, and 22:6ω3. Data are presented in the appendix tables. Of these, only 22:5ω3 was sigificantly increased in plasma PL of BD relative to BT at $p < 0.05$. Similarly, a significant interaction of treatment with time for increases of heart PL was found for 22:5ω3 ($p < 0.001$) and 22:6ω3 ($p < 0.02$). A trend toward increased content of 18:3ω3 in plasma was noted, but significance was not reached.

Table 3. *Significance of differences between BD and BT rats for ω6 fatty acids*

Fatty acid		Plasma	Liver	Heart
18:2w6	Treatment	NS	$p < 0.001$	NS
	Time	$p < 0.05$	$p < 0.01$	$p < 0.001$
	Interaction	NS	NS	NS
22:5w6	Treatment	$p < 0.002$	$p < 0.001$	NS
	Time	NS	$p < 0.05$	$p < 0.005$
	Interaction	$p < 0.02$	NS	NS

DISCUSSION

The data presented here provide evidence that biotin deficiency causes characteristic abnormalities in the fatty acid composition of PL in plasma, liver, and heart. The content of one or both of the two odd-chain fatty acids, 15:0, and 17:0, were significantly increased in all three tissues studied; plasma and liver had the most striking differences. Significant abnormalities were also detected in several of the ω6 intermediates in PL from plasma and liver. These gross abnormalities of composition could lead to abnormalities in prostaglandin composition or production, particularly if a given lipid subclass (*e.g.* phosphatidyl choline) is selectively affected. For the ω3 intermediates measured, biotin deficiency caused an increase in the percentage composition of 22:5ω3 in plasma and heart PL, and an increase in heart PL 22:6ω3 in the interaction of treatment with time.

In the documented cases of inadvertent biotin deficiency in man, the signs and symptoms of biotin deficiency typically required months to years to develop (14–21). In studies of biotin deficiency induced experimentally in man and in the rat, the characteristic findings tend to appear after about 6 wk (1, 3–8). In our study, the earliest cutaneous manifestations were noted by 4 wk, but were not uniformly and strikingly present until 6 wk. By the 6th wk, the hepatic activities of propionyl-CoA carboxylase and methylcrotonyl-CoA carboxylase of the BD group had decreased to about 25% of the values for the BT group, and biotin concentrations of plasma and excretion in urine had decreased to 40 and 10%, respectively. We selected 4, 6, 8, and 12 wk as the time points for analysis of fatty acid composition. Surprisingly, substantial differences in fatty acid composition between the BD and BT groups were already present by 4 wk. This finding emphasizes recent observations (7, 22–25)

that abnormalities in fatty acid composition can develop in a matter of a few weeks or less. Thus, the data from this study does not permit us to say when in the first 4 wk the abnormalities appeared. The timing relative to the onset of the cutaneous manifestations of biotin deficiency is consistent with the hypothesis than an abnormality (or abnormalities) in fatty acid metabolism may play a pathogenetic role in the cutaneous disease. Moreover, the early onset of abnormalities in 15:0 and 17:0 suggest that the composition of odd-chain fatty acids deserves further investigation as an early indicator of biotin status at the tissue level.

This study was begun before the publication of similar studies by Kramer *et al.* (4) and Suchy *et al.* (6). These two studies detected increases in the composition of odd-chain fatty acid, a finding confirmed in our study.

Our study also expands the information available. In the study of Suchy *et al.* (6) rats were not pair-fed and fatty acid compositions were determined at a single time point (12–14 wk). The method of fatty acid analysis used by Suchy *et al.* (6) did not allow evaluation of the polyunsaturated fatty acids but did selectively evaluate saturated acids up to 27:0 Suchy *et al.* (6) detected significant increases in all serum odd-chain fatty acids from 15:0 to 27:0. The study of Kramer *et al.* (4) used a pair-fed design and found some abnormalities in fatty acid composition (especially in 22:5ω6) that were due to the difference between pair and *ad libitum* feeding.

Our study also expands the study of Kramer *et al.* (4) in three important aspects. First, fatty acid composition was analyzed in three tissues (plasma, liver, and heart) rather than liver alone. Thus, comparison to clinical studies is facilitated. Second, serial sampling was done during progressive biotin deficiency rather than at a single time point. Thus, in the analysis of the data, we were able to evaluate differences due to treatment, trends in time not due to biotin deficiency, and interactions of treatment with time. Third, the temporal relationship of the fatty acid abnormalities to biotin nutritional status was assessed by measurement of hepatic carboxylase activities and urinary excretion of biotin.

The current study confirms the findings of Kramer *et al.* (4) that biotin deficiency causes an increase in the odd-chain fatty acids 15:0 and 17:0 in liver PL. Increases of these fatty acids were also demonstrated in plasma and heart PL in this study suggesting a generalized abnormality in PL composition. The current study also confirms the finding of Kramer *et al.* (4) that biotin deficiency causes an increase in 18:2ω6 in liver PL. A similar change was found in both plasma and heart PL, but only the abnormality in plasma PL reached statistical significance. A significant increase in 18:3ω6 of plasma and liver PL was detected in the current study; 18:3ω6 data were not reported by Kramer *et al.* (4). The data of the current study did not confirm the Kramer *et al.* (4) finding of a significant decrease in 20:3ω6 of liver PL, but a trend toward a decrease was apparently present. Our study detected a decrease in 22:5ω6 in both plasma and liver that was not found by Kramer et al. (4).

For ω3 intermediates, Kramer *et al.* (4) detected significant increases in both 18:3ω3 and 22:6ω3. Similar trends were seen in the current study although significance was not reached for 18:3ω3 in liver, heart, or plasma. A significant increase in plasma and heart PL occurred in 22:5ω3 and 22:6ω3.

The study reported here and that of Kramer *et al.* (4) measured the effects of very similar treatments in the same species; yet some disagreements in results occurred. The study of Kramer *et al.* (4) evaluated fatty acid composition at a single time point (7 wk), whereas the current study measured trends in time from 4 to 12 wk. To provide a more direct comparison of the two studies, we used Student's paired *t* test to evaluate the differences in the content of the individual fatty acids from liver PL between pairs of BD and BT rats at a single time point (wk 12) in a fashion analogous to that of Kramer *et al.* (4). We detected significant statistical differences for the same fatty acids identified as significantly different by two-way AnOVA with an exception: 22:5ω6 showed no change by *t* test, but a decrease by ANOVA. In addition, *t* testing found significant differences in the comparison of 20:4ω6 which decreased and 22:4ω6 and 22:6ω3 which both increased in the BD group relative to the BT group. These results and the direction of change are almost identical with the findings of Kramer *et al.* (4). In summary, this method of comparison emphasizes the basic agreement between the findings of the two studies.

A previous paper from this laboratory described the abnormalities of fatty acid composition of serum PL, triglyceride, cholesteryl esters, and free fatty acid in three subjects who were profoundly biotin deficient. Abnormal increases in odd-chain fatty acids occurred during biotin deficiency; with biotin supplementation, those abnormalities normalized or decreased toward normal. Our study in rats provides additional evidence that increased content of odd-chain fatty acids is the result of biotin deficiency per se.

In serum PL of the three patients with biotin deficiency, linoleic acid (18:2ω6) decreased, 18:3ω6 and 20:3ω6 increased, arachidonic acid (20:4ω6) remained normal, and 22:4ω6 varied, with one patient having decreased amounts and two patients having increased amounts. Biotin treatment did not result in a consistent resolution in any of these abnormalities. In the current animal study, biotin deficiency significantly increased both linoleic acid (18:2ω6) and 18:3ω6 in plasma and liver, 20:3ω6 and 20:4ω6 did not change significantly in any tissue, and 22:5ω6, the chain elongation and desaturation product of arachidonic acid, decreased significantly in plasma and liver PL of BD rats. Thus the ω6 abnormalities of the patients were not generally found in this animal study; indeed, for some intermediates, opposite effects were seen. Based on a comparison of the human and the rat studies, we are not able to determine if the differences in abnormalities in the ω6 series are attributable to species differences or to other factors such as the differences in composition and/or route of administration of lipids or other nutrients.

The data presented here do not address the question of whether the fatty acid abnormalities have an etiologic role in any of the physical findings of biotin deficiency. The evidence is accumulating that essential fatty acid deficiency causes a scaly dermatitis and alopecia by affecting prostaglandin metabolism (26–29). One is tempted to speculate that the gross compositional abnormalities of ω6 fatty acids observed in this study might lead to abnormalities in prostaglandin composition and might in turn cause the cutaneous manifestations of biotin deficiency. Our ongoing studies of the effect of essential fatty acid on the cutaneous manifestations provide evidence for such a functional role (30).

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Appendix Table 1.
*Composition of ω6 fatty acids**

Fatty acid	Time	Plasma BD	Plasma BT	Liver BD	Liver BT	Heart BD	Heart BT
18:2ω6 (linoleic acid)	4 wk	14.50 ± 0.21	15.35 ± 1.89	17.38 ± 1.51	15.32 ± 2.27	23.27 ± 0.34	21.45 ± 0.77
	6 wk	17.96 ± 0.01	14.76 ± 0.35	17.91 ± 1.02	17.50 ± 0.95	22.11 ± 0.34	21.39 ± 3.07
	8 wk	16.42 ± 0.41	16.59 ± 2.90	17.92 ± 0.29	15.55 ± 0.61	27.14 ± 0.86	25.84 ± 1.13
	12 wk	18.89 ± 1.02	16.33 ± 0.52	21.12 ± 0.89	17.42 ± 0.75	25.13 ± 2.40	24.39 ± 1.53
		Two-way ANOVA results					
	Treatment	$0.1 < p > 0.05$		$p < 0.001$		NS	
	Time	$p < 0.05$		$p < 0.01$		$p < 0.001$	
	Interaction	NS		NS		NS	
20:2ω6	4 wk	0.37 ± 0.16	0.48 ± 0.42	0.47 ± 0.16	0.45 ± 0.05	0.30 ± 0.04	0.33 ± 0.02
	6 wk	0.29 ± 0.03	0.31 ± 0.03	0.35 ± 0.07	0.36 ± 0.08	0.39 ± 0.11	0.38 ± 0.16
	8 wk	0.37 ± 0.01	0.32 ± 0.09	0.32 ± 0.05	0.33 ± 0.01	0.17 ± 0.02	0.21 ± 0.04
	12 wk	0.47 ± 0.11	0.15 ± 0.01	0.39 ± 0.04	0.49 ± 0.34	0.27 ± 0.12	0.17 ± 0.02
		Two-way ANOVA results					
	Treatment	NS		NS		NS	
	Time	NS		NS		$p < 0.01$	
	Interaction	$p < 0.05$		NS		NS	
18:3ω6	4 wk	0.26 ± 0.03	0.16 ± 0.05	0.29 ± 0.07	0.23 ± 0.02	0.18 ± 0.03	0.13 ± 0.01
	6 wk	0.17 ± 0.01	0.12 ± 0.11	0.31 ± 0.04	0.27 ± 0.09	0.17 ± 0.02	0.13 ± 0.02
	8 wk	0.16 ± 0.03	0.12 ± 0.10	0.39 ± 0.09	0.17 ± 0.03	0.10 ± 0.02	0.16 ± 0.05
	12 wk	0.24 ± 0.05	0.12 ± 0.11	0.53 ± 0.18	0.33 ± 0.13	0.12 ± 0.11	0.10 ± 0.02
		Two-way ANOVA results					
	Treatment	$p < 0.01$		$p < 0.05$		NS	
	Time	NS		NS		NS	
	Interaction	NS		NS		NS	
20:3ω6	4 wk	0.51 ± 0.24	0.60 ± 0.28	0.28 ± 0.06	0.39 ± 0.14	0.29 ± 0.07	0.34 ± 0.08
	6 wk	0.41 ± 0.18	0.39 ± 0.04	0.29 ± 0.05	0.33 ± 0.10	0.33 ± 0.02	0.32 ± 0.02
	8 wk	0.38 ± 0.03	0.41 ± 0.17	0.31 ± 0.02	0.31 ± 0.03	0.28 ± 0.01	0.30 ± 0.06
	12 wk	0.32 ± 0.03	0.40 ± 0.12	0.28 ± 0.11	0.31 ± 0.02	0.25 ± 0.05	0.27 ± 0.02
		Two-way ANOVA results					
	Treatment	NS		NS		NS	
	Time	NS		NS		NS	
	Interaction	NS		NS		NS	
20:4ω6 (arachidonic acid)	4 wk	22.03 ± 1.25	27.53 ± 7.18	35.43 ± 4.14	31.57 ± 1.66	26.36 ± 4.11	26.27 ± 1.37
	6 wk	22.97 ± 0.51	21.38 ± 1.14	32.38 ± 2.81	33.63 ± 4.18	26.94 ± 3.13	26.94 ± 4.18
	8 wk	19.47 ± 1.01	21.77 ± 3.76	30.12 ± 0.13	30.71 ± 3.03	27.94 ± 2.29	29.11 ± 2.13
	12 wk	15.39 ± 0.90	17.22 ± 4.53	29.94 ± 3.20	33.33 ± 1.98	24.90 ± 3.20	26.92 ± 2.04
		Two-way ANOVA results					
	Treatment	NS		NS		NS	
	Time	$p < 0.01$		NS		NS	
	Interaction	NS		NS		NS	
22:4ω6	4 wk	0.72 ± 0.10	0.94 ± 0.22	0.59 ± 0.10	0.85 ± 0.01	1.72 ± 0.42	2.11 ± 0.07
	6 wk	0.55 ± 0.01	0.84 ± 0.14	0.75 ± 0.35	0.72 ± 0.17	1.78 ± 0.41	2.23 ± 0.18
	8 wk	0.60 ± 0.01	0.78 ± 0.35	0.62 ± 0.03	0.82 ± 0.09	1.46 ± 0.02	1.60 ± 0.18
	12 wk	0.74 ± 0.07	0.60 ± 0.20	0.74 ± 0.10	0.73 ± 0.10	1.56 ± 0.13	1.31 ± 0.07
		Two-way ANOVA results					
	Treatment	NS		NS		NS	
	Time	NS		NS		$p < 0.005$	
	Interaction	NS		NS		NS	
22:5ω6	4 wk	0.73 ± 0.18	1.45 ± 0.28	0.99 ± 0.31	2.00 ± 0.39	2.99 ± 1.32	4.46 ± 0.10
	6 wk	0.55 ± 0.09	0.84 ± 0.14	0.69 ± 0.27	1.32 ± 0.24	3.03 ± 0.63	3.11 ± 0.14
	8 wk	0.56 ± 0.01	1.31 ± 0.46	0.79 ± 0.04	1.65 ± 0.14	2.50 ± 0.21	3.96 ± 0.93
	12 wk	0.86 ± 0.06	0.67 ± 0.26	1.01 ± 0.04	1.41 ± 0.22	3.32 ± 0.09	2.70 ± 0.07
		Two-way ANOVA Results					
	Treatment	$p < 0.002$		$p < 0.001$		NS	
	Time	NS		$p < 0.05$		$p < 0.005$	
	Interaction	$p < 0.02$		NS		NS	

* Data for the composition of ω6 fatty acids are tabulated as mean ± SD. Significance from two-way ANOVA is expressed as p values for treatment, time, and interaction of treatment with time.

Appendix Table 2.
*Composition of ω3 fatty acids**

Fatty acid	Time	Plasma BD	Plasma BT	Liver BD	Liver BT	Heart BD	Heart BT
18:3ω3 (linolenic) acid)	4 wk	0.04 ± 0.06	0 ± 0	0.05 ± 0.03	0.02 ± 0.02	0.08 ± 0.02	0.18 ± 0.17
	6 wk	0 ± 0	0 ± 0	0.05 ± 0	0.05 ± 0.05	0.04 ± 0.05	0.03 ± 0.05
	8 wk	0 ± 0	0 ± 0	0.03 ± 0.03	0.02 ± 0.02	0.09 ± 0.03	0.07 ± 0.02
	12 wk	0.11 ± 0.13	0.02 ± 0.04	0.05 ± 0.06	0.03 ± 0.03	0.05 ± 0.08	0.05 ± 0.05
		Two-way ANOVA results					
	Treatment	NS		NS		NS	
	Time	NS		NS		NS	
	Interaction	NS		NS		NS	
20:5ω3	4 wk	0.02 ± 0.00	0.43 ± 0.46	0.05 ± 0.02	0.04 ± 0.01	0.10 ± 0.05	0.02 ± 0.02
	6 wk	0.02 ± 0.00	0.02 ± 0.00	0.04 ± 0.04	0.12 ± 0.11	0.11 ± 0.18	0.24 ± 0.30
	8 wk	0.03 ± 0.01	0.04 ± 0.02	0.49 ± 0.49	0.07 ± 0.02	0.07 ± 0.01	0.04 ± 0.04
	12 wk	0.02 ± 0.00	0.64 ± 1.09	0.18 ± 0.25	0.27 ± 0.37	0.03 ± 0.05	0.02 ± 0.02
		Two-way ANOVA results					
	Treatment	NS		NS		NS	
	Time	NS		NS		NS	
	Interaction	NS		NS		NS	
22:5ω3	4 wk	0.37 ± 0.11	0.35 ± 0.30	0.75 ± 0.44	0.51 ± 0.04	1.48 ± 1.01	0.87 ± 0.06
	6 wk	0.38 ± 0.04	0.24 ± 0.19	0.54 ± 0.09	0.50 ± 0.06	1.02 ± 0.21	0.96 ± 0.11
	8 wk	0.34 ± 0.08	0.29 ± 0.05	0.55 ± 0.11	0.48 ± 0.01	1.10 ± 0.04	4.21 ± 0.82
	12 wk	0.60 ± 0.15	0.27 ± 0.23	0.69 ± 0.06	0.56 ± 0.05	1.29 ± 0.02	0.85 ± 0.20
		Two-way ANOVA results					
	Treatment	$p < 0.05$		NS		NS	
	Time	NS		NS		$p < 0.001$	
	Interaction	NS		NS		$p < 0.001$	
22:6ω3	4 wk	2.95 ± 0.32	2.71 ± 0.05	5.48 ± 0.53	5.50 ± 1.65	5.11 ± 0.92	7.15 ± 2.51
	6 wk	2.67 ± 0.12	2.27 ± 0.32	5.02 ± 0.21	6.13 ± 0.62	5.87 ± 1.5	5.83 ± 2.34
	8 wk	2.39 ± 0.26	2.58 ± 0.43	4.79 ± 0.19	4.52 ± 0.58	6.32 ± 0.83	5.24 ± 1.31
	12 wk	3.80 ± 1.04	1.96 ± 1.38	6.46 ± 0.85	5.15 ± 0.21	8.84 ± 0.79	3.80 ± 0.69
		Two-way ANOVA results					
	Treatment	$0.1 < p > 0.05$		NS		NS	
	Time	NS		NS		NS	
	Interaction	NS		NS		$p < 0.02$	

* Data for the composition of the ω3 fatty acids are tabulated at mean ± SD. Significance from two-way ANOVA is expressed as p values for treatment, time, and interaction of treatment with time. Zero values indicate fatty acid was less than the limit of detectability at 0.1%.

Appendix Table 3.
*Composition of ω9 fatty acids**

Fatty acid	Time	Plasma BD	Plasma BT	Liver BD	Liver BT	Heart BD	Heart BT
18:1ω9	4 wk	4.29 ± 0.27	4.06 ± 0.76	3.10 ± 0.43	2.85 ± 0.26	3.01 ± 0.25	3.10 ± 0.25
	6 wk	4.30 ± 0.13	3.74 ± 0.18	3.59 ± 0.11	2.79 ± 0.30	3.73 ± 0.43	3.36 ± 0.39
	8 wk	4.67 ± 0.37	3.75 ± 0.10	3.73 ± 0.37	13.11 ± 17.32	3.36 ± 0.39	2.99 ± 0.33
	12 wk	5.07 ± 0.11	4.93 ± 0.67	3.46 ± 0.14	3.05 ± 0.21	3.01 ± 0.33	3.22 ± 0.37
		Two-way ANOVA results					
	Treatment	NS		NS		NS	
	Time	NS		NS		NS	
	Interaction	NS		NS		NS	
20:3ω9	4 wk	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	6 wk	0.00 ± 0.00	0.06 ± 0.10	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.13 ± 0.23
	8 wk	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	12 wk	0.00 ± 0.00	0.05 ± 0.10	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
		Two-way ANOVA results					
	Treatment	NS		NS		NS	
	Time	NS		NS		NS	
	Interaction	NS		NS		NS	

* Data for the composition of the ω9 fatty acids are tabulated as mean ± SD. Significance from two-way ANOVA is expressed as p values for treatment, time, and interaction of treatment with time. Zero values indicate fatty acid was less than the limit of detectability at 0.01%.