APPENDIX V-c

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Plasma and Red Blood Cell Fatty Acid Composition in Children With Protein-Calorie Malnutrition

TO:

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Summary

Plasma and red cell fatty acids were measured in 44 children with third degree malnutrition; 22 children with marasmus, 11 children with kwashiorkor, and 11 with marasmic kwashiorkor(MK). The presence of edema, dermatosis, hair changes and hypoalbuminemia were positively correlated with the red cell percentage linoleic acid($r=0.54, p<0.001$) and negatively correlated with red cell percentage arachidonic acid($r=-0.45, p<0.005$). Red cell percentage linoleic acid in kwashiorkor was decreased from the control value of 15.3 percent to 10.9 percent. In marasmus and MK, there was significantly greater decreases, to 7.7 percent and 8.0 percent, respectively. Red cell arachidonic acid in kwashiorkor was significantly($p<0.001$) decreased to 9.8 percent(control value of 13.1 percent), while in marasmus there was no significant change. Only one sample, plasma from an infant with marasmus, had any detectable eicosatrienoic acid(20:3,w9). Similar changes in fatty acid composition have been reported in infants with acrodermatitis enteropathica, in an infant with biotin deficiency, and in biotin deficient rats.

Speculation

In essential fatty acid deficiency, a dermatosis results from altered epidermal prostaglandins secondary to decreaed arachidonic acid concentration. The significantly decreased percentage arachidonic acid in kwashiorkor might also alter epidermal prostaglandins, contributing to the dermatosis that is characteristically seen in many of these children.

INTRODUCTION

Protein-calorie malnutrition (PCM) encompasses three clinical syndromes: kwashiorkor marasmus and a mixed form, marasmic-kwashiorkor(MK). Kwashiorkor is characterized by edema, dermatosis, enlarged fatty liver, changes in hair pigment and texture. and decreased serum albumin in the presence of normal sub-cutaneous fat. Marasmic infants are generally younger and lack any specific physical or laboratory findings other than wasting of muscles and decreased subcutaneous fat. Many infants with marasmus exhibit some. but not all, of the signs of kwashiorkor and are designated marasmic-kwashiorkor(MK). The McLaren score has proven useful in differentiating among the three syndromes.(26)

The etiology and pathogenesis of the forms of PCM remain unclear. Animals fed a high carbohydrate,low protein diet often develope kwashiorkor. Those on a low carbohydrate,low protein diet exhibit marasmus. However, studies comparing the dietary histories of infants with kwashiorkor and marasmus frequently have failed to show any such dietary correlation.(28) Even so, protein deficiency could explain some of the findings in kwashiorkor. The edema of kwashiorkor results in part from the low serum albumin concentration, and the fatty liver is felt to be the result of decreased lipoprotein synthesis.(42)

The dermatosis of kwashiorkor may be the result of tryptophan deficiency, since it resembles the dermatosis in pellagra. Human essential fatty acid(EFA) deficiency also causes a desquamative dry dermatosis, as well as retarded growth. In EFA-deficient animals a scaly dermatosis with increased skin permeability, depigmentation,fatty lung, and fatty liver have been documented.(3) Naismith suggested that essential fatty acid deficiency occurs in kwashiorkor. (28) He showed that the weaning foods of children with kwashiorkor in Nigeria contained less than the minimum recommended intake of linoleic acid (less than one percent of total energy intake). Plasma linoleic and arachidonic acid levels were decreased, while the concentration of eicosatrienoic acid, an abnormal fatty acid, was increased, thus satisfying Holman`s criterion for EFA deficiency (an

eicoatrienoic to arachidonic acid ratio of greater than 0.4).(18) Other studies of children with kwashiorkor also have reported a decrease in the ratio of unsaturated to saturated fats in plasma (23,35) and in red cell lipids. (33)

Previous studies evaluated EFA's in kwashiorkor only. In the present study, a broader spectrum of malnutrition was studied in order to understand the peculiar features of kwashiorkor. Plasma lipids reflect changes in diet over a few days. Red cell lipids were measured because they reflect changes in lipid concentration and diet over a few weeks.(12) Red cell lipids should not be sensitive to the acute crisis that resulted in hospital admission or to effects of treatment during the first week of hospitalization. The present study correlated the presence of edema, dermatosis, hair changes, and hypoalbuminemia with the fatty acid composition of plasma and red cell lipids. Children with kwashiorkor exhibited changes in fatty acid composition similar to those seen in children with acrodermatitis enteropathica and biotin deficiency.

MATERIALS & METHODS

SUBJECTS--Forty-four children with third degree malnutrition (weight less than sixty percent of ideal weight for age) were studied in three hospitals in Lima,Peru. Their average weight, height. and head circumference were respectively,52%, 84% and 94% of the ideal for age, based on the Boston standard. They ranged in age from one to twenty-seven months (average 12 months) and were hospitalized an average of four days before blood collection.

All children were examined by a single observer (J.Wolff). The physical findings of dermatosis, hair changes, and edema were scored on a scale of 0 to 3: 0 for no abnormality, 1 for slight, 2 for moderate and 3 for marked change. Hepatomegaly was considered present when the liver span (measured by percussion and palpation) was greater than 9 cm.

The majority of infants (92 percent) had gastroenteritis with dehydration. A minority (18 percent) also had pneumonia or sepsis . One marasmic child had keratomalacia but there was no evidence of specific nutritional deficiencies in other children. Seventy percent of the subjects were receiving intravenous fluids. In addition, 54 percent of the subjects had been offered a formula containing evaporated milk, ricewater and sugar during the days of hospitalization prior to blood collection. Control subjects were eleven children who were totally recovered from third degree malnutrition. Their blood was obtained after they had been hospitalized in the Instituto de Investigacion Nutricional for over two months.

METHODS--Blood was collected at the time of physical examination by venipuncture into heparinized tubes containing one drop of a 0.1% butylhydroxy toluene (BHT)solution. The whole blood was centrifuged at 2000g. for fifteen minutes. The packed cells were washed three times in five volumes of 0.89 percent saline. Lipids of the packed cells were extracted with isopropanol and methanol by the method of Rose and Oklander. (32)

Plasma lipids were extracted by a modification of a method by Sperry and Brand.(24,38) Fatty acid methyl esters were prepared as described by Christie by transesterification of the extracted lipids using a methanolic HCl reagent.(6) Partial purification of fatty acid methyl esters was accomplished by column chromatography over 6% hydrated Al_2O_3. Reference fatty acid methyl esters were purchased from Supelco,Inc..

Gas chromatographic analysis was done using a model 5700A Hewlett-Packard Gas Chromatograph coupled to a Hewlett-Packard model 3385A Reporting Integrator. Two 6 ft stainless steel columns, 1/8 in. OD, packed with 15% Ethylene Glycol Succinate coated on 100/120 mesh Gas-Chrom Q pretreated with H_3PO_4 were used. The carrier gas was He at a flow rate of 30 ml/min. Standard mixes and samples were auto-injected. Initial oven temperature of 140°C was held for 16 min. after injection, then heated at a rate of 1°C/min. to a final temperature of 200°C. Upper temperature was maintained for 32 min. before recycling. Detection was by dual differential FID with a H_2 flow of 30ml/min. and air at 240ml/min. Detector and injector heated zones were at 250°C.

The plasma fatty acids measured are from the phospholipid, triglyceride, cholesterol ester, and free fatty acid pool. The red cell fatty acids are from the same pool in red cell membranes. Each fatty acid value was expressed as its percentage of the subject's total fatty acids.

Serum total protein and albumin concentrations were determined by the Biuret method and acetate gel electrophoresis. Total and free cholesterol plasma concentration were measured with a BMC(Boehring-Mannheim Corp.) kit, which utilizes an enzymatic colormetric assay.(2)

RESULTS

CLINICAL FINDINGS- On the basis of the McLaren score, twenty-two children had marasmus, eleven had kwashiorkor and eleven had marasmic-kwashiorkor . The presence of hypoalbuminemia, dermatosis, edema, and hair changes correlated significantly in degree with each other and with the McLaren score(Table 1).

CHOLESTEROL MEASUREMENTS- Compared to controls,plasma free cholesterol concentration was normal in marasmus,but twice normal in kwashiorkor (Table 2). There was a negative correlation between plasma free cholesterol concentration and serum albumin.

These changes also were manifest in the altered ratio of cholesterol ester to free cholesterol . The ratio in both marasmus and marasmic-kwashiorkor was reduced from the control ratio of 2.52 to approximately 1.8. The ratio in kwashiorkor was reduced even more to 0.87. A significant positive correlation existed betwen serum albumin and the ratio of cholesterol ester to free cholesterol (Table 1).

Total plasma cholesterol concentration was normal in marasmus and marasmic-kwashiorkor and elevated in kwashiorkor, but not significantly. There was no correlation between total plasma cholesterol concentration and serum albumin.

PLASMA FATTY ACIDS- Only one sample,plasma from an infant with marasmus, had any detectable eicosatrienoic acid(20:3 w9), but the ratio of 20:3 w9 to 20:4 w6 (arachidonic acid) was less than 0.4. Thus, Holman's criterion for the diagnosis of EFA deficiency was not met.

Percentage linoleic acid(18:2 w6) was one-third that of controls in all PCM children (Table 3). Percentage arachidonic acid(20:4 w6) was decreased only in kwashiorkor. The ratio of 18:3/20:3(w6 series) in the kwashiorkor group was twice as large as that in the control group or in the marasmus group. The ratio of 18:2/20:4(w6 series) in the kwashior-

kor group was also twice as large as in the marasmus group. There was a significant negative correlation between serum albumin and 20:3 and 20:4 of the w6 series.

RED CELL FATTY ACIDS- The red cell fatty acid results were similar to the plasma results. Eicosatrienoic acid(20:3 w9) was not detected in any of the red cell samples. Serum albumin was correlated with most of the red cell percentage fatty acids (Table 4). The linoleic series' data were analyzed further because of the strong correlations of serum albumin with these four fatty acids (Table 5). Linoleic acid(18:2) in kwashiorkor was decreased from the control value of 15.3 percent to 10.9 percent. In marasmus and marasmic-kwashiorkor, there were significantly greater decreases to 7.7 percent and 8.0 percent, respectively. Arachidonic acid (20:4) in kwashiorkor was significantly($p<.001$) decreased to 9.8 percent (control value of 13.1 percent), while in marasmus there was no significant change. There was also a significant difference between percentage arachidonic acid values in marasmus and kwashiorkor($p<.001$). The percentage 20:3 intermediate was significantly decreased in all PCM children, but significantly more so in kwashiorkor than in marasmus or marasmic-kwashiorkor($p<.005$). There was no change in the percentage 18:3 values. Serum albumin was negatively correlated with percentage linoleic acid($p<.001$) and positively correlated with percentage 20:3 and arachidonic acid($p<.005$).

The ratio of linoleic to arachidonic acid(18:2/20:4) was normal in kwashiorkor but was significantly decreased in marasmic-kwashiorkor and marasmus. The ratio of 18:2/18:3 was decreased in all PCM children while the ratio of 20:3/20:4 was decreased significantly only in kwashiorkor and marasmic-kwashiorkor. The ratio of 18:3/20:3 was doubled in kwashiorkor and similar to controls in marasmus. Serum albumin was significantly negatively correlated with 18:2/20:4, 18:2/18:3, and 18:3/20:3 and positively with 20:3/20:4.

PLASMA AND RBC FATTY ACID CORRELATIONS- Percentage plasma oleic, linoleic, and arachidonic acid values were significantly correlated($r=0.8, p<.001$)with their respective red cell values. The ratio of red cell to plasma values for linoleic and arachidonic acid was

significantly correlated with serum albumin.

DISCUSSION

Children with both kwashiorkor and marasmus had a significant decrease in red cell and plasma linoleic acid concentrations. This presumably is due to decreased dietary intake of linoleic acid compounded by fat malabsorption . (29,40) However, despite the higher concentration of linoleic acid in the kwashiorkor group, that of arachidonic acid was significantly lower than the levels in the marasmus group or the control group. This resulted in a linoleic/arachidonic acid ratio (in red cells) of 0.67 in marasmus and 1.29 in kwashiorkor. Percentage arachidonic acid was significantly negatively correlated with the McLaren score, its associative findings, and percentage linoleic acid. Percentage linoleic acid was positively correlated with the McLaren score. Despite decreased linoleic acid in our children with kwashiorkor and marasmus, the abnormal eicosatrienoic acid was not present, contrary to Naismith's finding in Africa.

These findings of decreased arachidonic acid without eicosatrienoic acid have been reported in children with acrodermatitis enteropathica, in biotin deficient rats, and in a 12 month old child with documented biotin deficiency secondary to parenteral alimentation and short-gut syndrome.(5,24,25,27) Both the child with biotin deficiency and children with acrodermatitis enteropathica had a scaly dermatosis and alopecia--all common findings in kwashiorkor. Zinc deficiency has not been found in children with kwashiorkor.(37) The basis of the similar abnormal pattern of plasma fatty acid composition in kwashiorkor and acrodermatitis enteropathica remains unclear. An hypothesis for how biotin deficiency can cause abnormal fatty acid composition is explained below.

The differences in linoleic acid and arachidonic acid between kwashiorkor and marasmus could be explained by alterations in the synthesis or breakdown of these fatty acids. It is not known whether the catabolism of certain unsaturated fats are specifically and differentially affected by dietary or hormonal changes. (11,41) The influence of dietary and hormonal changes on the synthesis of unsaturated fatty acids has been studied

in animals. The major pathway of conversion of linoleic acid to arachidonic acid occurs in three steps: desaturation to 18:3, elongation to 20:3, and desaturation to 20:4. (figure 1) (15) Desaturation of fatty acids is decreased by increased blood sugar, glucagon, epinephrine, lack of insulin, cyclic AMP and fasting. It is increased by protein intake.(4,9,10)

Assuming no significant differences in the catabolism or utilization of these fatty acids, the pattern in kwashiorkor of decreased 18:2/18:3 and 20:3/ 20:4 and increased 18:3/20:3 ratio is consistent with either an increased rate of desaturation or a decreased rate of elongation, or both. Since desaturation is decreased, not increased, in the fasting state, decreased elongation seems likely.(10,14) Elongation occurs mainly in microsomes, where it requires malonyl CoA; a minor pathway occurs in mitochondria and utilizes acetyl CoA. Biotin is required for the production of malonyl CoA and is thus required for the major pathway of elongation.(22,39,41) Perhaps children with kwashiorkor differ from those with marasmus by having some form of biotin deficiency. This hypothesis of impaired elongation could be proved by in vivo measurement of elongation using labelled compounds or by tissue studies in vitro.

A pure deficiency in linoleic acid is accompanied by an increase in eicosatrienoic acid (20:3 w9). It is thought that in the non-deficient state linoleic acid competitively inhibits conversion of oleic to eicosatrienoic acid because of linoleic acid's greater affinity for the desaturating and elongating enzymes it shares with oleic acid. With decreased linoleic acid, the naturally produced oleic acid is converted to the abnormal eicosatrienoic acid(Figure 1).(15) Children with EFA deficiency have decreased linoleic and arachidonic acid, and increased eicosatrienoic acid. Children with kwashiorkor, acrodermatitis enteropathica, or biotin deficiency have decreased linoleic and arachidonic acid, and no eicosatrienoic acid. Children with marasmus have decreased linoleic acid, normal arachidonic acid, and no eicosatrienoic acid. Decreased elongation would explain

the absence of eicosatrienoic acid in kwashioror ,but not in marasmus. Most likely, different enzyme systems of desaturation and elongation exist for the three different EFA series and are affected differently by metabolic disturbances. (39)

The decrease in arachidonic acid and 20:3 w6 in kwashiorkor might explain some of its signs. Both are precursors of prostaglandins. (8) In particular, the dermatosis in EFA deficiency is the result of altered epidermal prostaglandins due to a decrease in its precursors:20:3(w6) and arachidonic acid.(21,30,31) Also, eicosatrienoic acid(w9) inhibits prostaglandin synthesis and the interconversion of prostaglandins. (43,45,46) The significantly decreased 20:3(w6) and arachidonic acid levels in kwashiorkor might also alter epidermal prostaglandins, causing a dermatosis. The dermatosis in kwashiorkor could be different from that in EFA-deficiency because of the absence of eicosatrienoic acid in kwashiorkor. Altered prostaglandins also affect melanocyte function and the formation of edema and could contribute to the changes in pigmentation and edema in kwashiorkor. (34,44) The role that prostaglandins play in the pathogenesis of kwashiorkor, acrodermatitis enteropathica, and biotin deficiency remains to be determined.

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Table 1. Correlation among physical and laboratory findings[1] for all PCM children.

	MS	HC	HE	ED	DE	AB	FC	TC	E/F	LA	AA
MS	-	.36 II	.15 NS	.89 I	.84 I	-.80 II	-.65 I	.22 NS	-.48 IV	.54 IV	-.45 III
HC	.36 II	-	-.04 NS	.30 I	.36 I	-.40 II	.34 I	.14 NS	-.25 NS	.43 III	-.31 I
HE	.15 NS	.04 NS	-	.13 NS	.26 NS	.08 NS	.09 NS	.19 NS	-.04 NS	-.06 NS	-.08 NS
ED	.89 IV	.31 I	.13 NS	-	.74 IV	-.77 IV	.56 IV	.20 NS	.45 III	.50 IV	-.36 I
DE	.84 IV	.36 I	.26 NS	.74 IV	-	.63 IV	.53 IV	.16 NS	-.38 II	.48 III	-.44 III
AB	-.80 IV	-.40 I	.08 NS	-.77 IV	-.63 IV	-	-.53 IV	-.01 NS	.56 IV	-.63 IV	.50 IV
FC	-.65 IV	.34 I	.09 NS	.56 IV	.53 IV	-.53 IV	-	.63 IV	-.59 IV	.63 IV	-.29 NS
TC	.22 NS	.14 NS	.19 NS	.20 NS	.16 NS	-.01 NS	.63 IV	-	.16 NS	.26 NS	-.34 I
E/F	-.48 IV	-.25 NS	-.04 NS	-.45 III	-.38 II	.56 IV	-.59 IV	.16 NS	-	.06 NS	.48 IV
LA	.54 IV	.43 III	-.06 NS	.50 IV	.48 III	-.63 IV	.63 IV	.26 NS	.06 NS	-	-.60 IV
AA	-.45 III	-.31 I	-.08 NS	-.36 I	-.44 III	.50 IV	-.29 NS	-.34 I	.48 IV	-.60 IV	-

1 McLaren score=MS, hair changes=HC, hepatomegaly=HE, edema=ED, dermatosis=DE, albumin=AB, free plasma cholesterol concentration=FC, total plasma cholesterol concentration=TC, cholesterol ester/free cholesterol ratio=E/F, percentage red cell linoleic acid=LA, percentage red cell arachidonic acid=AA.
2 NS=not significant, I=$p<0.05$, II=$p<0.01$, III=$p<0.005$, IV=$p<0.001$.

Table 2. Plasma cholesterol concentrations(mg/dl).

	controls n=11	kwashiorkor n=11	marasmus n=22	marasmic kwashiorkor n=11
free cholesterol	30±10	67±16	40±8	39 ±15
total cholesterol	105±32	128±52	109±25	97 ±22
CE/FC ratio[1]	2.52±0.55	0.87±0.51	1.81±0.74	1.77±0.97

1 The means of the ratios of cholesterol ester to free cholesterol(CE/FC/) were obtained by averaging the ratios for individual subjects.

Table 3. Percentage plasma fatty acids of the linoleic series(w6).

Fatty acid	Control n=11	Kwashiorkor n=30	Marasmus n=17	MK n=10
18:2	43.7±6.6	16.6±4.3	14.4±4.5	14.5±5.9
18:3	1.4±0.6	1.0±0.3	1.1±0.5	1.0±0.5
20:3	1.3±1.0	0.6±0.3	1.1±0.8	0.7±0.3
20:4	3.5±1.6	2.7±0.7	4.3±1.7	3.7±1.4
18:2/20:4[1]	12.5	6.1	3.3	3.9

1 The ratio was calculated by dividing the means obtained for each group.

Table 4. Correlations of red cell percentage fatty acids with serum albumin.

red cell fatty acids[1]	correlations with serum albumin
16:0	-0.16(NS)[2]
16:1 w7[3]	-0.55(IV)
18:0	0.38(I)
18:1 w9	0.09(NS)
18:2 w6	-0.59(IV)
18:3 w6	0.15(NS)
20:0	0.72(III)
20:3 w6	0.52(III)
20:4 w6	0.46(III)
22:1 w9	0.05(NS)
22:6 w7	0.11(NS)
24:0	0.57(IV)
24:1 w9	0.10(NS)
Ratios for the linoleic(w6) series[4]	
18:2/20:4	-0.49(III)
18:2/18:3	-0.49(I)
18:3/20:3	-0.41(I)
20:3/20:4	0.41(I)

1 These were all the red cell fatty acids measured(besides 20:3 w9).
2 NS=not significant, I=p<0.05, II=p<0.01, III=p<0.005, IV=p<0.001
3 w notation indicates the position of the first double bond numbered from the methyl terminus.
4 These ratios were calculated by averaging the ratios for individual subjects.

Table 5. Percentage red cell fatty acids of the linoleic(w6) series.

fatty acids	controls	kwashiorkor	marasmus	MK
18:2	15.3±1.9 (11)[1]	10.9±2.0 (13)	7.7±2.2 (19)	8.0±2.8 (10)
18:3	0.7±0.1 (8)	0.7±0.1 (11)	0.7±0.1 (10)	0.7±0.2 (5)
20:3	1.7±0.6 (11)	0.8±0.4 (11)	1.3±0.3 (14)	1.0±0.4 (8)
20:4	13.1±1.7 (11)	9.8±2.6 (13)	12.2±1.9 (19)	11.3±2.4 (10)
Ratios[2]				
18:2/20.4	1.19±0.24 (11)	1.26±0.79 (13)	0.67±0.31 (19)	0.78±0.41 (10)
18:2/18:3	22.9±3.7 (8)	16.4±5.5 (11)	10.7±5.5 (10)	10.8±6.0 (5)
18:3/20:3	0.52.±0.31 (8)	1.10±0.61 (10)	0.68±0.18 (10)	1.00±0.40 (5)
20:3/20:4	0.13±0.05 (11)	0.08±0.03 (11)	0.11±0.03 (14)	0.09±0.04 (8)

1 Number of subjects in sample.
2 Ratios were obtained by averaging the ratios calculated for individual subjects.

ω-3 Series α-Linoleate Sequence	ω-6 Series Linoleate Sequence	ω-9 Series Oleate Sequence
Δ-9,12,15-octadecatrienoic acid (c18:3) (α-linoleic acid)	Δ-9,12-octadecadienoic acid (18:2) (linoleic acid)	Δ-9-octadecaenoic acid (c18:1) (oleic acid)
↓ desaturation	↓ desaturation	↓ desaturation
Δ-6,9,12,15-octadecatetraenoic acid (c18:4)	Δ-6,9,12-octadecatrienoic acid (c18:3)	Δ-6,9-octadecadienoic acid (c18:2)
↓ elongation	↓ elongation	↓ elongation
Δ-8,11,14,17-eicosatetraenoic acid (c20:4)	Δ-8,11,14-eicosatrienoic acid (c20:3) (dihomo-γ-linolenic acid)	Δ-8,11-eicosadienoic acid (c20:2)
↓ desaturation	↓ desaturation	↓ desaturation
Δ-5,8,11,14,17-eicosapentaenoic acid (c20:5)	Δ-5,8,11,14-eicosatetraenoic acid (c20:4) (arachidonic acid)	Δ-5,8,11-eicosatrienoic acid (c20:3)
↓ elongation	↓ elongation	
Δ-7,10,13,16,19-docosapentaenoic acid (c22:5)	Δ-7,10,13,16-docosatetraenoic acid (c22:4)	
↓ desaturation	↓ desaturation	
Δ-4,7,10,13,16,19-docosahexaenoic acid (c22:6)	Δ-4,7,10,13,16-docosapentaenoic acid (c22:5)	

Fig. 1. Metabolic pathways for the biosynthesis of polyunsaturated fatty acids from linoleic, α-linolenic, and oleic acids. Δ Notation signifies positions of double bonds numbered from carboxyl terminal of acid. ω Notation signifies the position of the first double bond numbered from the methyl terminus of acid.