Separata de Archivos Latinoamericanos de Nutrición, Vol. XXII, Nº 4, Dicl. 1972.

Intestinal malabsorption in malnourished children and during recovery. Duodenal contents of lipase, nitrogen, and micellar fat after fat stimulation[1]

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SUMMARY

Fifteen severely PCM children with edema were studied to determine the interactions between micellar fat, lipase activity and nitrogen content of duodenal aspirates when stimulated by a fatty emulsion, in order to gain knowledge on the basic mechanisms of fat malabsorption in PCM and during recovery. Results show that a limited number of children on admission may have decreased lipase production to be responsible for the malabsorption of fat, but that lipase activity recovers very early in treatment, even when children were receiving only 0.7 to 1 gram of protein/l.c.g. Micellarization of fat recovers later during protein repletion. Total nitrogen secretion into the duodenum is normal from admission throughout recovery. Consequently, lipase deficiency cannot explain the fat malabsorption and decreased micellarization of fat in PCM children and during recovery. These studies point out at the specific responsive capacity of the pancreas even when severe protein depletion exists, and suggest that alterations in others factors, such as in specific bile salts and other amphipaths, may be more directly involved in the process of fat malabsorption in PCM.

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1. This work was supported in part by the U. S. Public Health Service (National Institute of Arthritis and Metabolic Diseases), NIH Grant 2R22AM-12105-04.
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4. INCAP Publication I-657.
Recibido: 23-6-1972.

INTRODUCTION

Children with severe-protein calorie malnutrition (PCM) have frank malabsorption which affects a variety of nutrients (1). Prominent among these are dietary fats and purified ^{131}I triolein and oleic acid. The latter are malabsorbed in the same proportions and in a highly correlated fashion to the degree of protein depletion (1, 2). As a consequence, the absorption of these substances is corrected slowly and progressively in the course of protein repletion (1). The basic mechanisms involved in fat malabsorption in PCM are still largely unknown, although from recent experiments it appears that fat micellarization may be important in explaining the characteristics and the severity of the fat malabsorption in this syndrome and when diarrhea is present. Schneider *et al.* (3) have recently demonstrated that in severe PCM as well as in recovered children with diarrhea micellar fat is decreased below 4 mg of fat ml of duodenal aspirates when fat malabsorption is present. In these cases, unconjugated bile salts are elevated and conjugated bile salts are depressed in duodenal aspirates (4). Furthermore, micellar fat decreases logarithmically as the ratio of unconjugated to conjugated bile salts increases (4).

Other investigators (5, 6) have shown decreased lipase activity in PCM which could be responsible for the fat malabsorption and decreased micellarization. However, the previous studies in Guatemalan PCM children have provided indirect evidence against this possibility and suggested that even if lipase deficiency occurred in severe PCM, its activity rapidly returned to normal with dietary therapy, as has been shown by others (5, 7).

The purpose of this investigation was to validate the previously described indirect evidence of the adequacy of lipase activity in PCM children and during recovery, by direct determinations of the lipase activity simultaneous to fat micellarization in duodenal aspirates obtained during and after fat stimulation. Also, total, nitrogen in duodenal aspirates was measured since the degree of protein deficiency correlates with fat malabsorption and with micellar capacity at the duodenum (3), and total protein in duodenal aspirates has been highly correlated with pancreatic enzyme activity in the human and under a variety of circumstances (8-10).

MATERIALS AND METHODS

Fifteen edematous severely PCM children were the subjects of this study. They were rehabilitated through dietary means as outlined in other publications (1, 3, 11). Their pertinent clinical characteristics upon admission and at different stages of recovery, was described in Table 1. Also, two normal children of similar ages were studied. Upon admission, the children were rehydrated and were given a stabilization diet which provided 0.7 to 1 gram of protein and 70 to 100 calories per/l.c./day, 30% of which came from vegetable fat. The protein sources were either casein + 0.2% methionine or Incaparina*, and the carbohydrate sources were corn starch, sucrose, and dextrinomaltoses. Recovery was achieved by increasing the concentration of the diets progressively to provide 3 to 4 grams of protein and 120 to 180 calories/l.c./day. The proportion of fat calories remained constant at 30%. The children were studied the day after admission (ADM) after 6 to 9 days on the stabilization diets (STAB), when the children were recovering and their creatinine height index (CHI) (12) was between 0.60 and 0.80 (REC ±), and when they were fully recovered (REC) as judged by clinical appearance, weight for height above 0.95 and CHI greater than 0.85.

Duodenal aspirates were obtained in the course of fat micellarization studies (3) where a lipid emulsion is instilled into the duodenum at a total dose of 2 ml per kilo divided in four equal doses delivered in 5 minutes every 15 minutes. This emulsion had 50% corn oil and 0.2 uc of trivalent ^{51}Cr as tracer for dilution. Constant suction was applied at the duodenum jejunum junction for 90 minutes. The analytical procedure has been described in detail (3). Any amount of aspirate was divided in two portions: one was used for determinations of micellar fat (3) and bile salts (4) and the other was frozen inmediately in dry ice and analyzed without delay for lipase activity by a modification of the method of Seligman and Nachlas (13). Lipase activity was calculated by subtracting that due to esterase from the total activity. Results are expressed as 10^3 lipase units per ml of duodenal aspirate (1 unit = change in OD equivalent to hydrolysis of 10 ug of β

* Incaparina: Vegetable mixture of protein of high biological value consisting of lime treated corn and cottonseed flour, supplemented with lysine.

TABLE Nº 1

CHARACTERISTICS OF THE PCM CHILDREN STUDIED ON ADMISSION AND DURING RECOVERY

Clinical Stage	No.	Age (months)	Days of Therapeutic Diet	Weight for Height (%)	Serum Proteins (g/100 ml)	Creatinine Height Index
Admission	15	40[a] (15-64)	1 (1-4)	76 (52-98)	4.2 (2.0-5.6)	0.47 (0.39-0.65)
Stabilization	12	40 (15-64)	7 (6-9)	81 (60-98)	4.1 (2.0-5.6)	0.47 (0.39-0.65)
During Recovery	14	41 (16-65)	35 (21-69)	95 (69-114)	7.0 (6.0-8.3)	0.72 (0.60-0.80)
Recovered	12	46 (20-67)	122 (71-176)	109 (100-126)	7.1 (6.0-7.6).	0.93 (0.85-1.03)

[a] Mean
(Range)

naphthyl laureate at 37.5°C in 5 hours). Since the volumes released into the duodenum vary from child to child even though the amount of emulsion per l.c./body weigth was identical —those with diarrhea secreting large amounts of fluid— lipase units are expressed both per ml of aspirate and per ml of aspirate corrected for dilution of the emulsion by means of a factor obtained from 51 Chromium counting. This factor is the ratio of dilution in the aspirates of the subject divided by the mean dilution obtained in fully recovered children without diarrhea. Total nitrogen and TCA precipitable nitrogen in duodenal aspirates were measured by the micro Kjeldahl technique (14). Over 95% of the total nitrogen was precipitated by TCA.

RESULTS

Figure 1 presents the individual lipase activities in children on admission, after stabilization, during recovery and when fully recovered. The activity values, uncorrected for dilution of the fat emulsion, show that on admission only one child had normal lipase activity. During the period of stabilization there was a tendency for a small rise in lipase activity. During recovery the activity was already similar to that obtained in fully recovered children and in the two control children. When activity values are calculated including the dilution of the fat emulsion, the values on admission are somewhat higher although 4 patients had less than 75×10^3 units/ml. During the period of stabilization there was a clear rise in the majority of children, reaching values similar or even higher to those of fully recovered children.

Table 2 shows the mean, standard error and range of lipase activity during the different clinical stages, and the significance of the differences between groups. The presence of diarrhea in the children studied was reflected in lower lipase activity per ml of aspirate but this difference disappeared when the amount of fluid secreted into the duodenum was taken into consideration. Schneider *et al.* (3) have shown that in malnourished and recovered children with diarrhea the volume of fluid secreted into the duodenum is significantly higher than that observed in children without diarrhea, regardless of nutritional status.

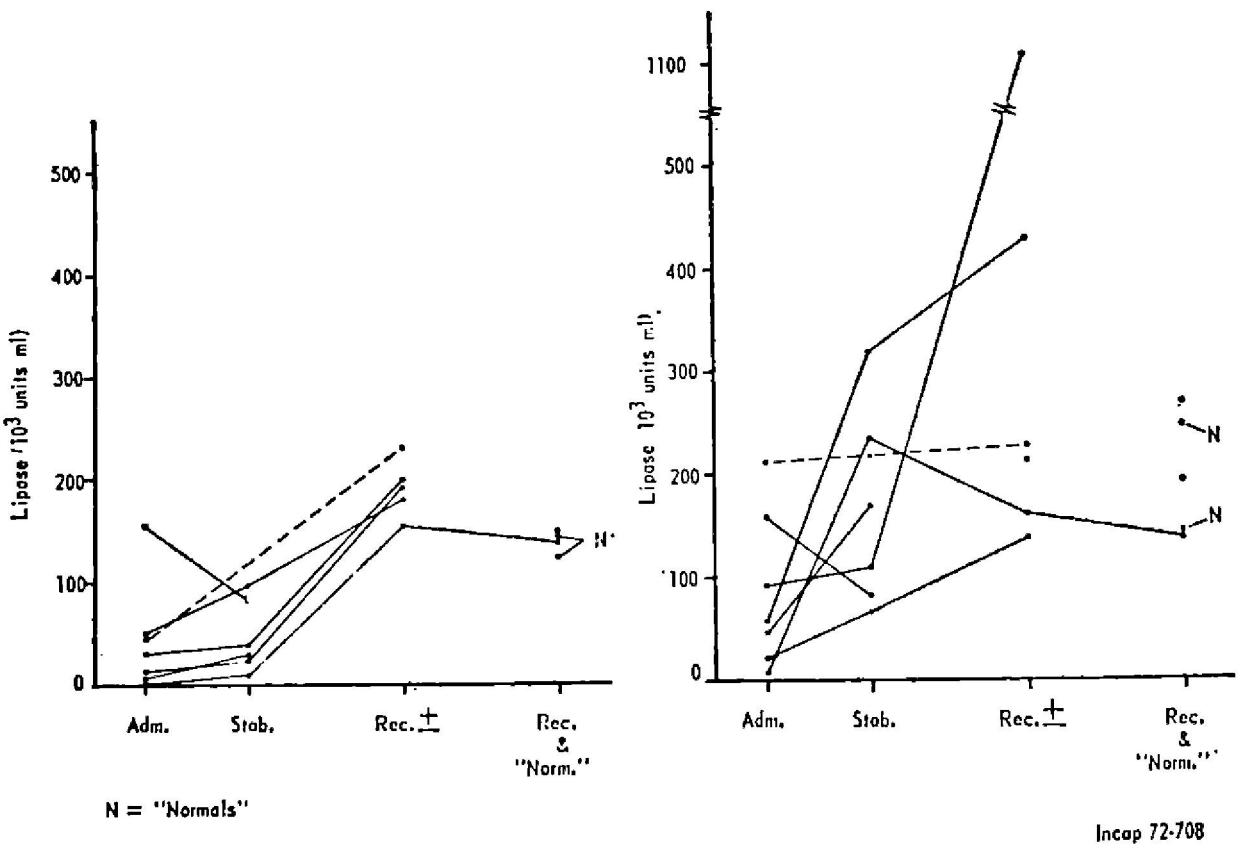

Figure 1: Lipase activity in duodenal aspirates of PCM children and during recovery. Stimulus consisted of fat instillation into the duodenum. On the left graph values are uncorrected for dilution of the fat emulsion. On the right, the values are corrected for dilution of the fat emulsion.

TABLE Nº 2

LIPASE ACTIVITY IN DUODENAL ASPIRATES OF PCM CHILDREN AND DURING RECOVERY. RESPONSE TO FAT INSTILLATION INTO THE DUODENUM

Clinical Stage		Admission	Stabilization	During Recovery	Recovered
Activity (10^3 units/ml)					
No. of Children		7	6	6	5
Mean		43.17	47.53	175.47	189.12
S. E.		20.06	14.00	13.65	53.27
Range	Diarrhea +	(3.0-9.8)	(-)	(101.2-228.0)	(-)
	Diarrhea -	(13.0-156.8)	(11.7-96.6)	(155.0-196.6)	(123.8-401.8)
Activity corrected for dilution (10^3 units/ml)					
No. of Children		7	6	6	5
Mean		83.04	162.90	367.02	197.32
S. E.		28.93	39.93	151.14	26.95
Range	Diarrhea +	(7.3-45.8)	(-)	(212.5-228.0)	(-)
	Diarrhea -	(22.2-212.6)	(66.2-318.2)	(138.0-1104.8)	(136.0-270.0)

Significance of differences: Uncorrected samples: Admission vs. during recovery, $p < 0.01$; vs. Recovered, $p < 0.05$. Stabilization vs. during recovery, $p < 0.01$, vs. Recovered, $p < 0.05$. Samples corrected for dilution: Adm vs. During recovery, $p < 0.02$.

TABLE Nº 3

MICELLAR FAT IN DUODENAL ASPIRATES OF PCM CHILDREN AND DURING RECOVERY

Clinical Stage		Admission	Stabilization	During Recovery	Recovered
Micellar Fat (mg/ml)					
No. of Children		10	6	10	10
Mean		1.86	1.95	6.28	5.79
S. E.		0.51	0.55	0.92	0.91
Range	Diarrhea +	(0.3-3.4)	(0.5-3.8)	(3.2)	(1.5-4.5)
	Diarrhea -	(1.4-5.3)	(0.7-1.8)	(2.9-12.5)	(5.5-11.6)

Significance of differences: Admission and Stabilization vs. During recovery and Recovered, $p < 0.01$.

Micellar fat was low on admission, remained low during stabilization and rose during recovery (Table 3). This contrasts with lipase values which rose earlier (stabilization period). The influence of diarrhea on fat micellarization is particularly evident among the recovered children, who suffered a decrease in their micellar capacity to levels wich overlapped those observed while the children were still malnourished. The values for micellar fat are all corrected for dilution.

Table 4 shows the results of total nitrogen in duodenal aspirates. There is no difference between the nitrogen concentration from admission to full nutritional recovery. The nitrogen concentrations presented in this Table represent values uncorrected and corrected for dilution. Diarrhea affects the uncorrected values but do not affect the corrected ones.

Since simultaneous micellar lipid, total nitrogen and lipase activity were not available in all the children studied, the results of these determinations in the children where the three determinations were done at different clinical stages, are presented in Table 5. These results indicate the variability observed between different children within each category.

Total nitrogen and lipase activity correlate with a coefficient of 0.594 when all the samples with these two determinations are considered. This correlation is relatively poor and is due to the following facts: malnourished children upon admission occasionally have low lipase activity in spite of adequate total nitrogen concentrations. The opposite is true during recovery, where a rebound in lipase activity seems to occur and which is not necessarily manifested in a rise in nitrogen.

With regards to micellar fat, there is a poor negative correlation with nitrogen concentration ($r = -0.440$). However, micellar fat was below the lower limit of normality (4 mg of fat/ml of aspirate) in all the cases who had nitrogen concentrations below 75 mg/100 ml. The contrary is not true; total nitrogen concentrations above 75 mg/100 ml were observed in 13 children who had less than 4 mg of micellar fat/ml of aspirate. Eleven of these cases were severely malnourished (Adm. or Stab.), 9 of which presented diarrhea at the time of study. The two other children were recovering and one had diarrhea at the time of study. Micellar fat and lipase activity

TABLE Nº 4

TOTAL NITROGEN IN DUODENAL ASPIRATES OF PCM CHILDREN AND DURING RECOVERY

Clinical Stage	Admission and Stabilization		During Recovery and Recovered	
Diarrhea	+	-	+	-
Total N (mg/ml)				
No. of Children	11	5	5	18
Mean	59.5	127.7	79.26	102.23
S. E.	8.5	35.6	9.21	7.51
Range	(18.0-125.0)	(55.7-215.7)	(57.0-107.3)	(55.8-179.8)
Total N corrected for dilution (mg/ml)				
No. of Children	11	5	5	16
Mean	113.30	184.20	115.72	138.02
S. E.	27.11	62.32	13.34	16.88
Range	(40.6-356.2)	(75.0-379.9)	(91.3-165.0)	(74.7-330.8)

TABLE Nº 5

INDIVIDUAL RESULTS OF MICELLAR LIPID, TOTAL NITROGEN AND LIPASE ACTIVITY IN THE CHILDREN WHO HAD THE THREE DETERMINATIONS DONE SIMULTANEOUSLY

Admission			Stabilization			During Recovery			Recovered		
ML[a]	N	Lipase	ML	N	Lipase	ML	N	Lipase	ML	N	Lipase
0.7	45	7	0.7	380	234	4.3	77	161	5.6	120	193
0.3	67	12	0.9	128	66	12.5	75	138	3.6	91	142
2.7	128	91	0.7	54	108	3.5	135	228	5.5	148	136
2.9	57	157	8.9	79	84	4.4	128	212	7.4	317	246
0.3	69	54				4.4	407	1105	7.1	116	147
						12.8	199	428			

ä ML= Micellar lipid (mg/ml)
N = Nitrogen (mg/100 ml)
Lipase= (10^3 u/ml)

also correlate poorly ($r = 0.233$) but the comments made from the micellar fat total nitrogen also apply, as no children with lipase activity below 75×10^3 units/ml had micellar fat above 4 mg/ml of duodenal aspirate. Children upon admission or with diarrhea may have adequate lipase activity and yet their micellar fat is abnormally low.

DISCUSSION

The results from this investigation point at three facts: 1) children withouth diarrea appeared to have less lipase activity and nitrogen concentration than children without diarrhea. Although this finding may be explained by different secretion rates into the duodenum, the number of cases with and without diarrhea here reported is not sufficiently large to allow definitive conclusions 2) as suspected by the indirect evidence obtained by us, and through a physiologic stimulation by means of fat (15, 16), lipase activity even when low or essentially absent upon admission recovers rapidly and cannot account for the slow recovery in fat absorption seen in children during recovery from PCM (1). Calculations based in the lipolytic activity of duodenal lipase suggest that only a concentration of less than 75×10^3 units of lipase per ml, corrected for dilution, can be of significance in producing fat malabsorption. This seems to be corroborated by the findings in this study in that all the children with lipase activity below this level were unable to micellarize lipid properly. It is only upon admission that this case may be present in PCM, and 3) lipase activity in severe PCM is low in spite of near normal protein content of duodenal aspirates; this activity increases even when total serum proteins remain unchanged during the period of stabilization. The diet in this period contained 30% of the calories in the form of fat, which amounts to a fat intake of 2.3 g/l.c.k./day. This finding suggests that the physiologic reserve of the pancreas, at least in terms of lipase production, is present and is able to respond to proper stimulation even when protein repletion is not taking place; furthermore, it suggests an adaptative mechanism of pancreatic lipase secretion as has been shown for other enzymes (17, 18). This is not in agreement with the interpretation of the data on PCM that Barbezat and Hansen

give based on serum albumin-lipase correlations (5); their data also indicate elevated lipase secretion early during nutritional recovery.

Alterations in fat micellarization and absorption in PCM and diarrhea cannot be adscribed to lipase deficiency alone. Probably abnormalities in bile salts and in other amphipaths play an important role as has been suggested by studies of bile salt concentration in the duodenum (4) and of ileal function in PCM (1, 19).

ACKNOWLEDGMENTS

The authors wish to thank Drs. M. Béhar and L. J. Mata for fruitful discussions and encouragement, and to Mrs. Cristina de Campos and Mr. Rubén D. Mendoza for their technical help. Gratitude is expressed to Mrs. Sara de Castañeda for her secretarial help.

These studies were possible thanks to the donation of the necessary X-Ray equipment by the Research Corporation, Inc.

RESUMEN

Malabsorción intestinal en niños desnutridos y durante su recuperación. Contenido duodenal de lipasa, nitrógeno y grasa micelar después de estimulación con grasa

Quince niños con desnutrición proteínico-calórica (DPC) severa, del tipo edematoso, fueron estudiados con el propósito de determinar las interacciones entre grasa micelar, actividad de lipasa y nitrógeno total en el contenido duodenal, consecutivas a la estimulación con una emulsión de grasa. El objetivo de este estudio era conocer mejor los mecanismos básicos causantes de la mala absorción de grasa en la DPC y durante la recuperación. Los resultados muestran que la producción disminuída de lipasa puede ser responsable de la mala absorción de grasa en un número limitado de niños con DPC al ingreso al hospital. Sin embargo, la actividad de lipasa se recuperó muy tempranamente en el curso de la rehabilitación nutricional, aun cuando los niños recibían únicamente de 0.7 a 1.0 g de proteína/kilo/día. La capacidad de micelarizar grasa se recupera más tarde durante la repleción proteínica. La secreción de N total al duodeno es normal desde el ingreso y a lo largo de la recuperación nutricional. Por lo tanto, la deficiencia de lipasa no puede explicar ni la mala absorción de grasa ni el defecto en micelarización de las grasas en niños con DPC y durante la recuperación. Estos estudios señalan la capacidad de respuesta específica del páncreas aún en la depauperación proteínica severa y sugieren que alteraciones en otros factores, tales como sales biliares específicas y otras substancias amfipáticas pueden estar más directamente involucradas en el proceso de mala absorción de grasas en la DPC.

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