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The role of TNF-alpha in chronic malnutrition in childhood

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Executive Summary

Malnutrition during childhood has profound effects not only on growth but more importantly on brain maturation and function. As such, malnutrition is one of the most important issues in international development. Children who fail to reach their intellectual potential represent a lost resource and often an added burden to their countries. Children in developing countries often have infections (particularly diarrheal diseases) associated with and exacerbating malnutrition. During diarrheal disease inflammatory mediators (cytokines) such as tumor necrosis factor alpha [TNFα], interleukin 1ß [IL-1ß], and IL-6 may be produced. When such cytokines are administered to animals, they cause clinical and laboratory abnormalities similar to those seen in malnourished children. It is not yet known whether the diarrhea-associated accelerated deterioration in children with poor nutrition is due to production of cytokines. The aim of this study was to define the role of cytokines in the clinical and metabolic abnormalities seen in severe malnutrition. We hypothesized that during diarrhea release of cytokines causes or contributes to the weight loss and metabolic derangements of severe malnutrition.

Our major findings have been in defining the metabolic changes in hormones (cortisol, insulin, thyroid stimulating hormone, thyroxine, glucagon, and growth hormone), and in serum lipids and lipoproteins (cholesterol, LDL, HDL, and triglycerides) and in demonstrating the frequency of elevations of proinflammatory cytokines and their modulators (TNFα, IL-1ß, IL-6, sTNF RI, sTNF RII, IL-1RA, interferon gamma) found in children on admission to hospital and during treatment. These findings lay the basis for further studies of pharmacologic agents in correcting these abnormalities. The significance of these findings for international development is that if they provide strong evidence for a role of inflammatory cytokines in malnutrition and as such they may lead to fundamentally new approaches to the problems of undernutrition. This project has developed skills in study design, protocol management, data management, and statistical evaluation in the Guatemalan team of physician who undertook it.

4. Research Objectives: The aim of this study was to define the role of cachectin/tumor necrosis factor alpha (TNFα) in the clinical and metabolic abnormalities seen in severely malnourished children. It has generally been thought that inadequate food intake explains the clinical and metabolic features of malnutrition. However, infection typically causes acute deterioration in underfed children, with death often the result. Infections trigger a variety of immunologic responses that, when well regulated, aid in the survival of the child. However, prolonged secretion of immune factors may have profound adverse effects. In particular, TNFα and the cytokines it induces (interleukin 1ß [IL-1ß] and IL-6) when present in excess, may carry a risk of adversely affecting survival. The metabolic (endocrine, electrolyte, serum lipid-carbohydrate-protein) and clinical (anorexia, edema, diarrhea, weight loss) features of chronic malnutrition in childhood can be mimicked by administration of TNFα to animals.

Specific research objectives: The original aim was to define the role of TNFα in malnutrition by achieving three objectives:

1. Determine the relationship between TNFα concentrations and clinical/metabolic abnormalities during recovery from severe malnutrition in a group of prospectively evaluated children at the Hospital General San Juan de Dios in Guatemala City.

2. Determine the relationship between serum TNFα and serum endotoxin (lipopolysaccharide) levels.

3. Determine the effect of agents that decrease TNFα on the clinical and metabolic abnormalities and recovery of children with severe malnutrition.

Based on data accumulated during the study of TNFα, we expanded the study to include measures of other inflammatory mediators to obtain additional evidence of activation of pro-inflammatory cytokines. We did this because we felt it was critically important to determine whether their was evidence for pro-inflammatory cytokine production in these children. We felt that if we could confirm this central feature of our hypothesis it would provide the basis for future interventions involving anti-inflammatory agents in severe malnutrition.

This study complements observations of other scientists on the role of infection in malnutrition. The interaction between infections and nutritional status is complex. Infections typically trigger progressive deterioration in marginally nourished children. For example, in rural India it has been shown that there is a correlation between weight for height (as an index of protein energy status) and risk of death from infectious agents. (7)

Diarrhea is often a precipitating event in malnourished children who deteriorate. It is estimated that each episode of diarrhea is responsible for a 0.083cm/day deficit in linear growth.(5) The adverse effects of diarrhea on growth have been documented in multiple studies. (35,48,34,47,9) Children living in developing countries may have as many as ten episodes of diarrhea per year.(45) In underdeveloped countries, two thirds of deaths occur in malnourished children. (8) These children often die after developing an episode of acute diarrhea that fails to resolve and is followed by progressive deterioration. Persistence of diarrhea with subsequent malnutrition occurs in 3-20% of children below 5 years of age in these settings. (45) Persistence of diarrhea is more likely to occur in those who already have some degree of malnutrition. (4) In Peru, Bangladesh, Brazil, India and Nepal 22-56% of deaths among young children are related to persistent diarrhea.(45) The case fatality rate in India was twenty fold higher in

those with persistent diarrhea than in those with acute diarrhea.(45) Morbidity due to diarrhea is greatest in children whose weight for height is <70% of standard. (55,7)

The role of diarrhea in protein calorie malnutrition has been emphasized in studies from Guatemala.(20,21,36) In this setting, chronic malnutrition is nearly always complicated by diarrhea. The risk is particularly high in the youngest children. An important hypothesis resulting from this work has been that malnutrition may in large part be the result of frequent infections. Fever, vomiting and anorexia contribute to decreased intake of nutrients. Anorexia (rather than maternal withholding of food) is the mechanism of the reduced intake of food in children suffering from diarrhea.(26) Malabsorption of fats also occurs during both acute and persistent diarrhea; steatorrhea may in turn contribute to loss of fat soluble vitamins. Carbohydrate intolerance with both decreased energy absorption and osmotic diarrhea further exacerbate the syndrome. Diarrhea thus worsens the underlying nutritional disease.

The cytokines are host proteins secreted by a variety of cells that regulate the immune system. Their interactions are complex and incompletely understood. TNFα is produced primarily by macrophages in response to bacterial lipopolysaccharides and other immune and inflammatory stimuli.(27,56) It plays a key role in the initiation of inflammatory and immune responses by inducing production of other cytokines (e.g. IL-1, IL-6, etc) involved in recruiting and activating granulocytes, monocytes and lymphocytes.(41,2,53) The host benefit or injury resulting from TNFα depends on its concentration, duration of cell exposure, and presence of other mediators.(56) Although central to normal host immune responses, excess TNFα produced during infection may have deleterious effects.(22,13) The biologic effects of TNFα are dose and concentration related. Low concentrations regulate leukocytes and endothelial cells and are responsible for important physiologic host responses to infection (1). However, when TNFα release or dose is high, or continues for extended periods of time, it can in itself induce significant pathology. Excessive acute production of TNFα as seen in septic shock, can result in hemodynamic alterations, hypercoagulopathy, organ damage, and death (37). The continued release of TNFα is in part, responsible for the loss of skeletal protein and body fat, altered substrate utilization and increased energy demands associated with tissue wasting and organ dysfunction (37). Local production of TNFα at an inflammatory site may be beneficial for the host while the overproduction of systemic TNFα may result in fatal outcome. (50)

There are two lines of evidence suggesting a role for TNFα as a mediator of the nutritional consequences of infection. First, administration of TNFα to animals results in the catabolic changes like those that occur during infection. Although acute increases in TNFα may cause shock, low level chronic secretion is of greater significance in the long term consequences of TNFα. Low dose continuous administration of TNFα to animals is associated with anorexia, weight loss, edema, hypoalbuminemia, loss of body protein, lipid and cell mass and lethality.(11,25) The hypoalbuminemia that is responsible for the edema often seen in malnutrition, is likely to be due both to TNFα induced vascular permeability and to TNFα induced down regulation of the gene controlling synthesis of albumin. (46) In experimental animals receiving TNFα, anorexia, as manifest by reduction in food intake, has been reported (37,38). Loss of body weight that cannot be fully explained by the associated anorexia has also been observed (60). TNFα has anti-anabolic and catabolic effects (38). It can produce edema, loss of body protein, lipid and cell mass (39), endocrine abnormalities (40,12), and alterations in the distribution of zinc, iron and copper (28). The metabolic effects of TNFα include decreased lipogenic enzyme synthesis in adipocytes (causing cellular cachexia in vitro) and accelerated glycogenolysis in skeletal muscle cells, (56) decreased serum triiodothyronine, (40) The anemia so often seen in malnutrition, may be due in part to the decrease in red cell synthesis caused by TNFα.(38)

Second, production of TNFα has been related to cachexia in a variety of naturally occurring diseases. Persistent TNFα production occurs in chromic infection and malignancy. There is much evidence that TNFα is the central mediator of the wasting that accompanies chronic disease.(3) TNFα is increased in many syndromes characterized by wasting. Increased serum levels of TNFα have been reported in anorexia nervosa (58), acquired immune deficiency syndrome (30), parasitic infections (49), cancer-associated wasting (2), pulmonary tuberculosis (57), bacterial infections (59) and thermal injury (33). Serum TNFα measures are difficult to interpret because the half life is short (6 minutes),(3) although high levels in serum have been documented in some clinical conditions(43,18) and serum levels sometimes relate directly to severity of infection.(59,19) The failure to detect TNFα uniformly in a variety of inflammatory states may be due to kinetics of production, the paracrine and autocrine sites of action, sample timing, stage of infection, and number of samples obtained. For this reason measurements of other cytokines induced by TNFα are also of interest in that they may provide proof of production of such inflammatory events. TNFα IL-1, and/or IL-6 could explain the host non-specific responses resulting in cachexia.(15) However, there is no consensus regarding the relative importance of each of these mediators.

Solving the problem of the role of inflammation in malnutrition has profound social, economic and humanitarian implications for the developing world. The cost both in human misery and resources spent on dealing with the problem might well be decreased if therapy aimed at decreasing inflammation can be devised.

The unique aspect of this study is that it adds important data to our evolving understanding of the metabolic and clinical deterioration that precedes death in malnutrition syndromes. This project is scientifically innovative because it proposed a fundamentally new hypothesis to address the pathogenesis of the deterioration that occurs in children with severe malnutrition. The benefit of this research is that by defining a role for immune system dysfunction in the aberrations of malnutrition, we have laid the foundation for new diagnostic and therapeutic approaches.

Support of this project by Hospital San Juan de Dios and the University of Texas Medical School at Houston: Patient care costs which were contributed by Hospital General San Juan de Dios for this study were approximately $1,000 per patient [approximately $80,000 total]; these costs include nursing and physician care during the time when the research personnel were not present, medications, and hospital overhead. The matching contribution at the University of Texas was that portion of ten percent of Dr. Cleary's salary not covered by this grant.

5. Methods and Results: Extensive demographic information was collected on each child admitted to the study and entered into the database by Dr. Monica Soto de González. 77 children [38 male/39 female] were enrolled. There were 14 children less than six months of age, 21 between six and twelve months, 21 between twelve and eighteen months, 14 between eighteen and twenty four months and 7 over twenty four months. Many of these children had been low birth weight (21% <2.5kg). They typically had very prolonged diarrhea prior to admission (mean 282 hours). Vomiting and fever were common (51% and 30%, respectively). Many (51%) were dehydrated on admission. Multiple infections other than gastroenteritis were present in these very ill infants; 40% had pneumonia and 12% had urinary tract infection on admission. They had often failed outpatient antibiotic management (29% had received antibiotics prior to admission). The children were quite ill as reflected in the fact that there were three deaths and that the survivors were hospitalized for an average of 20 days.

We defined the frequency of inflammatory diarrhea [as indicated by positive fecal leukocytes and

fecal blood] in this population. Surprisingly, we have found that 85% of children admitted to the hospital with diarrhea associated with severe malnutrition have evidence of inflammatory diarrhea. This finding has not previously been reported in the literature on pediatric malnutrition. It suggests either that an important element in the pathophysiology has not been previously appreciated or that an invasive pathogen not detected by routine stool analyses is present.

We defined the common metabolic events occurring during the various types of malnutrition with diarrhea. The following table summarizes mean data on hormone measurements at admission and during the course of treatment for severely malnourished children. Blood samples for these analyses were collected weekly from each child. The children were classified as having marasmus, kwashiorkor, or marasmus-kwashiorkor based on clinical presentation using the criteria of McLaren. The data below for measurements of hormones and serum lipids are expressed as group arithmetic means and standard deviations.

Table 1: Relationship between clinical category of malnutrition and hormones

Hormone	day after admission	MARASMUS	MARASMUS-KWASHIORKOR	KWASHIORKOR
TSH				
	0	3.0+/-2.5	4.4+/-2.5	2.9+/-0.8
	7	3.7+/-3.0	5.0+/-3.0	3.2+/-1.5
	14	6.8+/-9.2	4.9+/-2.9	4.1+/-0.5
[normal range 0.38 - 4.8 uU/mL]				
Cortisol				
	0	24.2+/-11.4	21.5+/-12.5	32.5+/-20.4
	7	15.9+/-4.4	16.7+/-6.5	24.2+/-15.3
	14	17.9+/-8.2	16.1+/-4.4	15.0+/-4.2
[normal range 7 - 25 ug/dL]				
Thyroxine				
	0	5.5+/-3.0	6.9+/-4.6	5.1+/-4.0
	7	6.8+/-2.5	8.2+/-3.6	7.9+/-2.2
	14	8.1 +/-3.6	8.2+/-2.9	6.0+/-2.0
[normal range 6.8 - 14.4 ug/dL]				
Insulin				
	0	3.8+/-4.0	2.6+/-2.5	5.6+/-7.8
	7	12.5+/-17.4	5.3+/-10.1	1.4+/-1.3
	14	2.8+/-1.7	3.5+/-1.2	3.4+/-1.7
[normal range 0 - 30 uU/mL]				

Table 1 (continued): Relationship between clinical category of malnutrition and hormones

Hormone day after admission	MARASMUS	MARASMUS-KWASHIORKOR	KWASHIORKOR
Glucagon			
0	127+/-68	129+/-74	42+/-20
7	124+/-108	110+/-54	70+/-18
14	143+/-97	110+/-34	56+/-11
[normal range 40 - 130 pg/mL]			
Growth hormone			
0	11.2+/-10.3	13.6+/-9.2	8.3+/-2.6
7	11.8+/-10.5	13.6+/-9.7	13.5+/-7.8
14	9.8+/-8.9	7.8+/-8.4	13.0+/-4.2
[normal range 0 - 7 ng/mL]			

These studies showed that there are metabolic abnormalities in Guatemalan children with diarrhea and malnutrition that varied with the type of malnutrition and with the stage of recovery. Elevation in TSH was common in marasmus and marasmus-kwashiorkor but not in kwashiorkor. Others have reported that TSH is low in marasmus. Thyroxine was often very low in all three groups as expected. It is usually said that capture of iodine is diminished in marasmus and that its metabolism is more rapid than normal in kwashiorkor. Cortisol levels were frequently low even late in recovery in all groups; the literature has contradictory findings on this question. Insulin levels were within the normal range and not different between the groups. Unexpectedly, the growth hormone concentration was routinely elevated in all groups. Others have reported that growth hormone is depressed in marasmus and higher than normal in Kwashiorkor. Glucagon was elevated only in the marasmus and marasmus-kwashiorkor groups so that the mean values for each group were at the upper range of normal. As reported by others, glucagon levels are not elevated in kwashiorkor.

Serum lipid studies showed that cholesterol levels were quite low on admission and gradually rose during therapy and that triglycerides were high (greater than 95 percentile for age based on US standards) in all groups through the first two weeks of treatment.

Table 2: Lipid changes in children with malnutrition on admission and during the course of therapy

lipid	day	MARASMUS	MARASMUS-KWASHIORKOR	KWASHIORKOR
cholesterol				
	0	114+/-40	120+/-40	95+/-39
	7	136+/-37	129+/-25	101+/-18
	14	146+/-49	156+/-50	112+/-30

[cholesterol for age 0-4 years less than 117 is less than the fifth percentile; 156 is 50th percentile]

LDL	0	34+/-33	32+/-19	39+/-37
	7	46+/-33	31+/-16	16+/-10
	14	41+/-52	43+/-33	18+/-15

[These values are well below the 5th percentile for age value of 65mg/dL]

HDL	0	56+/-19	58+/-30	29+/-20
	7	66+/-19	68+/-30	72+/-21
	14	75+/-20	91+/-23	80+/-26

[5%ile=39; 50%ile=56; 95%ile=76]

triglycerides				
	0	125+/-70	150+/-97	136+/-66
	7	145+/-68	158+/-79	141+/-12
	14	154+/-119	136+/-45	111+/-11

[The 95%ile is greater than 102]

The study treatment groups (ibuprofen vs placebo) were similar on admission (children who died are excluded from the following tables).

Table 3: Comparability of ibuprofen and placebo groups

	Ibuprofen	placebo
AGE in months	15.8 ± 8.6	14.1 ± 7.1
Birthweight (kg)	2.91 ± 0.67	2.73 ± 0.51
Weight/Age	53.5 ± 8.4	54.9 ± 11.7
Height/Age	85.91 ± 5.4	86.03 ± 6.01
Weight/Height	73.1 ± 11.4	72.7 ± 10.1

[data expressed as mean ± sd]

The most striking effect of ibuprofen therapy on the course of illness was that its use was associated with significantly lower ($P < 0.007$) maximal daily temperature. Other outcome variables showed little effect as shown in the summary tables below. In each of the following tables week 1 represents the sample evaluated on admission, week 2 the sample collected between 6-8 days later, week 3 the sample at day 13-15, and week 4 the sample at day 20-22. There are less patients sampled (n in the tables) in the later weeks reflecting discharge of children from the hospital.

There was no difference in leukocyte counts between the groups before or during therapy. This data suggests that inflammation as reflected by leukocyte counts was not affected by ibuprofen therapy.

Table 4: Total white blood cell count each week during therapy

week	n	ibuprofen	n	placebo
1	37	14613 ± 7545	35	14357 ± 8059
2	35	11531 ± 6213	35	12078 ± 3890
3	26	12875 ± 9450	25	13272 ± 7072
4	11	9254 ± 2987	7	11328 ± 2588

Metabolic changes during treatment with ibuprofen: It is known that TNFα reduces hepatic albumin synthesis. We evaluated the ability of ibuprofen to block this effect. Serum Albumin levels were higher by two weeks of ibuprofen therapy but not one week after completion of therapy. This data suggests that although ibuprofen has a modest effect on speeding recovery of serum albumin levels, this particular regimen was not associated with major persistent differences in albumin.

Table 5: Serum albumin during each week of therapy

week	n	ibuprofen	n	placebo
1	37	3.4 ± 1.2	34	3.4 ± 1.0
2	34	3.9 ± 1.2	33	3.9 ± 1.1
3	25	4.5 ± 1.2	23	4.0 ± 1.1
4	10	4.7 ± 0.8	6	5.0 ± 2.0

Serum lipid changes are summarized in the following four tables. Malnourished children have a paradoxical hypertriglyceridemia with very low density lipoproteins but diminished total cholesterol, high density and low density lipoproteins.(6) It is hypothesized that these effects may relate to cytokine modulation. TNFα and IL-1 stimulate lipolysis and decrease lipoprotein lipase activity in cultured fat cells.(23) However, as shown below our regimen to block cytokine lipolytic effects was not successful.

Table 6: Serum triglyceride changes during each week of therapy

week	n	ibuprofen	n	placebo
1	37	187 ± 109	35	172 ± 151
2	34	168 ± 75	31	194 ± 120
3	23	198 ± 117	23	215 ± 115
4	10	153 ± 94	4	272 ± 149

Table 7: Serum cholesterol changes during each week of therapy

week	n	ibuprofen	n	placebo
1	37	103 ± 38	35	113 ± 61
2	34	116 ± 34	31	132 ± 41
3	23	140 ± 46	23	135 ± 42
4	10	139 ± 64	10	139 ± 65

Table 8: Serum HDL cholesterol changes during each week of therapy

week	n	ibuprofen	n	placebo
1	37	40 ± 29	35	40 ± 26
2	34	48 ± 29	31	52 ± 24
3	23	58 ± 34	23	59 ± 30
4	9	73 ± 23	5	67 ± 23

Table 9: Serum LDL cholesterol changes during each week of therapy

week	n	ibuprofen	n	placebo
1	37	26 ± 21	35	41 ± 39
2	34	37 ± 23	31	40 ± 34
3	23	44 ± 40	23	33 ± 27
4	9	48 ± 48	5	47 ± 26

Hormonal changes during therapy were characterized because it is hypothesized that cytokine mediated inflammatory changes cause the metabolic abnormalities seen in malnutrition in part through hormones. Our data suggest that although there is evidence of upregulation of proinflammatory cytokines in these children (see cytokine data below), our treatment regimen did not block these

effects.

Table 10: Serum TSH changes during each week of therapy

week	n	ibuprofen	n	placebo
1	36	3.4 ± 2.7	35	3.2 ± 3.1
2	32	3.8 ± 3.8	30	3.7 ± 2.4
3	24	4.7 ± 3.2	23	5.6 ± 7.0
4	10	3.6 ± 2.5	5	3.4 ± 2.8

[normal range 0.38 - 4.8 uU/mL]

Administration of TNFα in animals and humans causes increased plasma corticosterone. IL-1 and IL-6 stimulate the release of corticotrophin-releasing hormone in vitro, and that this response is antagonized by the cyclooxygenase inhibitors, indomethacin and ibuprofen.(61) Likewise, the effects of IL-6 on the hypothalamic pituitary axis are probably mediated by cyclooxygenase products.(42) However, ibuprofen pretreatment failed to prevent IL-1 induced elevations of plasma corticosterone and activation of the hypothalamic pituitary axis in mice.(14) The stimulatory effects of PLA2 and arachidonic acid on ACTH secretion are not effected by products generated by the cyclooxygenase or lipoxygenase pathways.(10)

Table 11: Serum cortisol changes during each week of therapy

week	n	ibuprofen	n	placebo
1	36	27 ± 19	35	26 ± 17
2	31	18 ± 6	30	18 ± 8
3	24	16 ± 6	23	16 ± 7
4	10	18 ± 14	5	14 ± 7

[normal range 7 - 25 ug/dL]

Thus, the stress related increase in cortisol was not corrected by ibuprofen treatment.

Table 12: Serum T4 changes during each week of therapy

week	n	ibuprofen	n	placebo
1	36	6.0 ± 3.2	34	6.4 ± 3.0
2	32	7.6 ± 3.2	30	7.6 ± 2.8
3	24	8.7 ± 2.4	23	7.3 ± 2.9
4	10	8.3 ± 3.8	5	6.6 ± 1.5

[normal range 6.8 - 14.4 ug/dL]

It is thought that increased production and release of prostaglandins under the control of TNFα exerts direct effects on carbohydrate metabolism in muscle cells including a net release of amino acids from peripheral tissues. Glucagon and TNFα act synergistically to increase the uptake of circulating amino acids by hepatocytes. IL-1 may suppress in vivo insulin release at least in part through the mediation of prostaglandin synthesis and that this effect can by blocked by ibuprofen.(51) However, our data do not confirm this observation at the doses used in this clinical study.

Table 13: Serum Insulin changes during each week of therapy

week	n	ibuprofen	n	placebo
1	36	3.6 ± 3.9	35	4.0 ± 3.9
2	32	5.6 ± 9.3	30	5.2 ± 8.9
3	24	7.8 ± 14.4	23	1.9 ± 1.9
4	10	4.1 ± 5.3	5	4.0 ± 3.6

[normal range 0 - 30 uU/mL]

Table 14: Serum Glucagon changes during each week of therapy

week	n	ibuprofen	n	placebo
1	35	97 ± 69	34	102 ± 82
2	31	116 ± 93	29	93 ± 56
3	20	114 ± 68	23	110 ± 83
4	10	102 ± 52	5	114 ± 95

[normal range 40 - 130 pg/mL]

Table 15: Serum Growth hormone changes during each week of therapy

week	n	ibuprofen	n	placebo
1	36	11.5 ± 7.6	35	13.6 ± 9.7
2	32	14.4 ± 9.7	30	11.1 ± 8.5
3	23	7.4 ± 6.4	23	10.7 ± 8.6
4	10	7.9 ± 13.5	5	5.6 ± 3.9

[normal range 0 - 7 ng/mL]

Cytokine Studies: Some data suggest that ibuprofen significantly inhibits eicosanoid synthesis induced by TNFα and it also diminished TNFα-induced mortality.(16) TNFα stimulates arachidonic acid metabolism in vivo. Other data suggest that ibuprofen reverses the deleterious hemodynamic and metabolic effects seen in live E. coli septic shock without depressing the endogenous production of TNFα or IL-6.(10) Such data have suggested that ibuprofen might be helpful in treating sepsis and other situations in which TNFα is elevated and contributes to the adverse outcome. Ibuprofen has not lowered TNFα in several studies.(44,32,24) However, this is controversial because other data have shown suppression of both IL-1 and TNFα production suppression by ibuprofen.(17) We initially had planned on studying only TNFα. However, the finding of values of zero in a number of patients who had clear evidence of severe infections coupled with the fact that the half life of TNFα in serum is only 6 minutes prompted us to evaluate other indicators of inflammation. Data on TNFα is as follows.

Table 16: Serum TNFα [pg/mL] during each week of therapy

week	n	ibuprofen	n	placebo
1	13	16 ± 20	12	21 ± 21
2	12	12 ± 10	13	15 ± 14
3	8	26 ± 33	9	14 ± 14

[Serum TNFα is normally < 15pg/mL]

TNFα is produced in response to a number of stimuli, including bacterial lipopolysaccharide (endotoxin), bacterial exotoxins, fungi, and viruses.
TNFα production by blood monocytes was not significantly different between ibuprofen and placebo groups. Summary statistics for unstimulated and LPS stimulated monocyte TNFα are shown in table 17.

Table 17: Serum TNFα [pg/mL] during each week of therapy
[Monocyte stimulation in vitro with LPS]

week	n	unstimulated	lps stimulated
1	13	16 ± 56	337 ± 562
2	13	16 ± 36	342 ± 518
3	9	28 ± 84	190 ± 480
4	4	6 ± 12	290 ± 437

Since these studies failed to show an effect of therapy on TNFα or even clear evidence of inflammation in many patients as indicated by TNFα, we evaluated other cytokines in these children. Increased peripheral protein catabolism occurs with tumor necrosis factor, interleukin-1, and interleukin-6 administration in animals and humans. Individual cytokines often influence the synthesis, release, and activity of other cytokines. The paracrine release and rapid inactivation of tumor

necrosis factor may explain low levels in illnesses in which there is reason to believe that it is produced.(54) Serum concentrations of soluble tumor necrosis factor receptors may be better indicators of TNFα synthesis.

Table 18: Serum soluble TNFα receptor I [pg/mL] during each week of therapy

week	n	ibuprofen	n	placebo
1	6	4068 ± 1561	7	3243 ± 1676
2	5	3488 ± 1648	7	3118 ± 1666
3	4	3352 ± 1063	4	2840 ± 1814

[The normal range for sTNF RI is 749-1966, mean 1198 pg/mL]

Table 19: Serum soluble TNFα receptor II [pg/mL] during each week of therapy

week	n	ibuprofen	n	placebo
1	7	4749 ± 769	8	4912 ± 684
2	6	4811 ± 849	8	4616 ± 983
3	5	5024 ± 833	6	4618 ± 953

[The normal range for sTNF RII is 1003-3170, mean 1725 pg/mL]

Serial weekly IL-1ß levels were measured in thirteen patients and found to be zero throughout illness. No further studies of IL-1ß were done. However, IL-1 receptor antagonist, a major regulator of the effects of IL-1, was present in serum.

Table 20: Serum IL-1 receptor antagonist [pg/mL] during each week of therapy

week	n	ibuprofen	n	placebo
1	8	4003 ± 3545	6	4443 ± 4894
2	6	1808 ± 227	6	2166 ± 510
3	5	1910 ± 234	6	2265 ± 288

[Serum IL-1RA is normally <300 pg/mL; values >1050 occur in <2% of normal sera]

Ibuprofen inhibits IL-6 production by adherent cells stimulated with lipopolysaccharide. (29) We therefore anticipated that IL-6 might be lowered in the treated group. However, as shown below, ibuprofen did not appear to inhibit IL-6 production in vivo.

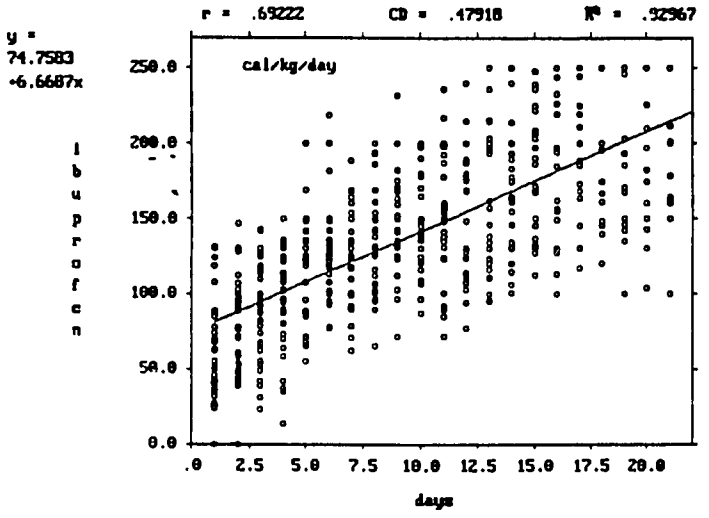
Table 21: Serum IL-6 [pg/mL] during each week of therapy

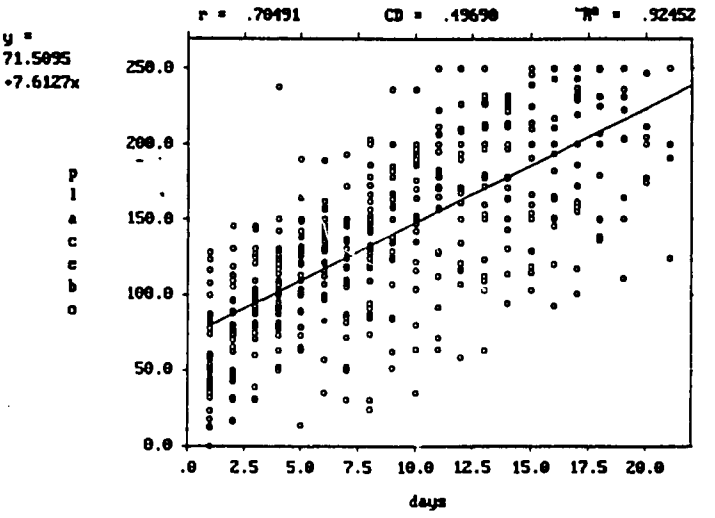
week	n	ibuprofen	n	placebo
1	10	38.7 ± 15.6	15	35.9 ± 17.8
2	10	30.8 ± 17.5	15	47.0 ± 49.3
3	9	42.3 ± 29.5	10	44.3 ± 47.2

[Serum IL-6 is normally < 12.5 pg/mL]

Interferon gamma levels were not significantly affected by ibuprofen treatment and did not change significantly over time [data not shown].

Both food and water intake are suppressed by peripheral administration of IL-1 and that such suppression can be blocked by ibuprofen.(52) We therefore evaluated caloric intake per kg per day. As shown in the following two figures, we were unable to demonstrate an effect of ibuprofen on anorexia in the group of children using this dose schedule.





Our observations are like those of McCarthy et al who found that although IL-1 and TNFα are potent induces of prostaglandin synthesis and injection of PGE, IL-1, or TNFα decreases food intake in healthy animals, ibuprofen had no effect on food intake or body weight.(31)

6. Impact, Relevance and Technology Transfer:

These findings are of use in Guatemala and elsewhere in that they demonstrate the frequency with which inflammation is present during severe malnutrition and the complex metabolic abnormalities related to malnutrition. The study has not yet defined how best to decrease the effects of these inflammatory mediators.

The impact of this project on the individual collaborators in Guatemala and the US has been the development of research skills and expertise for the design, management, performance, and analysis of a complex biomedical problem. The laboratory of San Juan de Dios has gained experience in analysis of hormones and lipids. This is the first large multidisciplinary study that Dr. Prado has organized. The managerial skills that he has developed will continue to be useful to him as he continues to do research and train future generations of Guatemalan pediatricians. Drs. Ernesto Almendares and Mónica Soto de González, both of whom have recently completed pediatrics residencies, have had their first experience in conducting a major research study. It is likely that this research training and experience will influence one of these two physicians (Dr. Soto de González) to pursue a career in research that will likely have a positive impact on the future of Guatemalan children. The Microlab machine which was purchased to allow complete biochemical profiles to be run on small samples of blood has been made available to the hospital for use in children not enrolled in the study. This has improved patient care by allowing laboratory monitoring of infants who previously could not be monitored using laboratory equipment that required large volumes of blood sample.

An unexpected impact of the study has been that we have defined a major nosocomial salmonella infection problem that was not previously fully appreciated. The frequency of stool cultures positive for this organism was significantly greater ($p < 0.001$) in stool cultures after admission than on admission to the hospital. Because many of these children did not have diarrhea, the problem would not ordinarily have been detected. This nosocomial outbreak situation has been addressed by the Infection Control Committee. It is unclear whether the detection of this problem accounts for the unexpectedly low death rate that occurred in the study population. These studies have raised interest locally in further defining the role of inflammation in children with severe malnutrition.

The hormonal data have suggested that there is a block in production of thyroxine in the face of elevated TSH levels. These data, coupled with other recent work on dietary iodine deficiency in Guatemala, have been interpreted as suggesting a nutritional defect in dietary iodine; this finding may lead to an important new nutritional supplement to the therapy given this population of children.

7. Project Activities/Outputs:

The multidisciplinary team that Dr. Prado has assembled to do this research has been very productive. Three abstracts were presented recently at the XXXVII Congreso Nacional de Pediatría held in Guatemala City, February 27 - March 3, 1995. One of these presentations received an award as the outstanding research project at the meeting. The abstracts have previously been forwarded to

USAID.

Dr. Heinz Chávez, the Chief of Pediatric Endocrinology has taken a leading role in the analysis of the hormone studies. His abstract, "Hormona hipofisiaria en desnutrido severo hospitalizado y en recuperación nutricional," earned him The Nestle's Award for Original Research given by Asociacion Pediatrica de Guatemala on March 9, 1995.

Dr. Prado has presented data from this study at the XXXVII Congreso Nacional de Pediatría in an abstract "Lípidos y lipoprcteinas en niños severamente desnutridos".

Dr. Susana de Forna, the nutritionist recruited to the study by Dr. Prado, presented "Informe preliminar de hallazgos bacteriológicos en coprocultivos y urocultivos de niños severamente desnutridos durante el tratamiento hospitalario".

Additional abstracts are currently being prepared for presentation to pediatric research meetings in the US.

Meetings during the project: The investigators have regularly visited each others' laboratories during this study. The initial three months of the grant were spent setting up the study, hiring personnel, purchasing supplies and working out logistical issues. In the proposal as originally submitted, we had planned to start the enrollment of patients as soon as approval was granted. However, after the grant was revised in January 1993, it appeared that it would not be funded and we were required to put our activities on hold. In mid-August 1993, we were notified that the grant might be funded and we sent the requested additional information. In early October 1993, we were notified that the study was funded to begin on Sept 30, 1993. When we received this letter in early October, we immediately set up a number of meetings. The first of these meetings took place within two weeks of notification that the study was to be funded. We began work even before the appropriate subcontracts could be executed between the University of Texas and Patronato Pro-Departamento de Pediatría on behalf of Hospital General San Juan de Dios (signed Oct 26, 1993 by The University of Texas).

Dr. Prado visited Houston **October 14-22, 1993** to work on logistical issues related to modifying case report forms, setting up data bases, hiring and training personnel specifically for this study, and ordering laboratory supplies. After returning to Guatemala, Dr. Prado assembled a multidisciplinary team that included the following personnel:

-Dr. Susana de Forno, a pediatric nutrition specialist who runs the malnutrition ward at Hospital General "San Juan de Dios", was recruited to aid in patient enrollment and evaluation.

-Dr. Mónica Soto de González, a past pediatric chief resident, was recruited to become the study coordinator, responsible for day to day management of each of the patients.

-Dr. Heinz Chávez Meyer, Chief of Pediatric Endocrinology at Hospital "San Juan de Dios", who had previously trained in endocrinology in Germany, was recruited to aid in the assessment of hormonal changes.

-Dr. Ernesto Almendares, a recently trained pediatrician, was recruited to assist in care of the patients.

-Remei Gordillo, a microbiologist, was recruited to analyze the patients stool, blood, and urine cultures, and to perform the macrophage assays for TNFα as well as the routine serum chemistries.

-Fabiola de Ponce, a system's engineer with extensive experience in computer programming, was recruited to assist in data management; she designed the management system.

-Cecilia Sánchez, head of the laboratory of nuclear medicine at Hospital General "San Juan de Dios", was recruited to assist in hormone measurements.

Dr. Cleary visited the study site in Guatemala **November 19-22, 1993** to finalize preparations for beginning patient enrollment. Remei Gordillo was trained to do the LPS stimulation of monocyte studies during this visit.

Dr. Cleary visited Guatemala **January 6-10, 1994** to review initial patient enrollment, computerized data entry, laboratory problems, and financial management.

Dr. Cleary visited Guatemala **August 19-22, 1994** to review progress on the study.

Dr. Prado visited Houston **October 10-14, 1994** to review progress on the study.

Dr. Cleary visited Guatemala **March 9-14, 1995** to review progress.

Dr. Cleary visited Guatemala **Sept 25-28, 1995** to review progress with Dr. Monica Soto and Dr. David Prado and to coordinate data analysis. Some patients enrolled were still in the hospital at that time so it was decided to continue collection data until those patients were discharged although the date for completion of the study was technically passed at the end of the month.

Dr. Cleary visited Guatemala **Nov 28-Dec 1, 1995** to work on data analysis and begin preparation of reports, abstracts and publications. Drs. Soto and Prado were trained in the use of EPISTAT, a data analysis program brought to Guatemala and installed by Dr. Cleary.

Project publications:

1. Chávez Meyer H, Prado D, de Forno S, de González MS, Sánchez C, Gordillo R, Almendares E, Cleary T. Hormonas hipofisiarias en desnutridos severos hospitalizados y en recuperación nutricional. XXXVII Congreso Nacional de Pediatría, Guatemala City, Guatemala. Feb 27-Mar 3, 1995.

2. de Forno S, de González MS, Prado D, Gordillo R, Almendares E, Cleary TG. Informe preliminar de hallazgos bacteriológicos en coprocultivos y urocultivos de niños severamente desnutridos durante el tratamiento hospitalario. XXXVII Congreso Nacional de Pediatría, Guatemala City, Guatemala. Feb 27-Mar 3, 1995.

3. Prado D, de González MS, Gordillo R, de Forno SM, Almendares E, Cleary TG. Lípidos y lipoproteinas en niños severamente desnutridos. XXXVII Congreso Nacional de Pediatría, Guatemala City, Guatemala. Feb 27-Mar 3, 1995.

Additional abstracts and manuscripts are currently in preparation to report the findings of this study.

8. Project Productivity:

The major goal of this project was accomplished although not precisely in the way we had originally envisioned. We showed that inflammatory mediators are present in the serum of children with severe malnutrition. Our findings from the studies of TNFα caused us to change our strategy for evaluation of inflammation. We focused on soluble TNFα receptors, IL-1ß, IL-1 receptor antagonist, and IL-6 because elevation in these cytokines represents clear evidence for upregulation of the inflammatory mediator cascade. This achievement is extremely important and represents a set of findings not previously reported. The shift in our direction based on early measures of TNFα made us re-allocate resources and use of clinical specimens so that additional cytokines could be measured. Because small volumes of blood were available from these infants we chose to not perform limulus assays as originally planned. We felt that the finding of many patients with levels of zero TNFα made it extremely unlikely that LPS would be present in their sera. This interpretation made it reasonable to use these blood samples for other cytokine measurements instead.

The second major goal of this study was not accomplished although the studies aimed at achieving this goal were successfully completed. We had anticipated that ibuprofen might block the inflammatory mediator induced metabolic changes of malnutrition. Although the dose of ibuprofen was adequate to have an antipyretic effect, we could not convincingly demonstrate an effect on other clinical or metabolic parameters.

9. Future work:

These studies have convincingly demonstrated that proinflammatory cytokines are present early in the course of children admitted to the hospital with life threatening malnutrition. These studies lay the basis for further attempts to modulate inflammation and thereby correct more rapidly the metabolic and clinical abnormalities. We are currently planning to develop a protocol to assess the impact of other antiinflammatory agents on outcome of malnutrition.

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