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Leptin Levels Are Associated with Immune Response in Malnourished Infants

ANA PALACIO, MARCELO LOPEZ, FRANCISCO PEREZ-BRAVO, FERNANDO MONKEBERG, AND LIANA SCHLESINGER

Institute of Nutrition and Food Technology, University of Chile, and University Iberoamericana, Santiago, Chile

Circulating leptin levels, proinflammatory and T helper cells type 1 (Th1), Th2 cytokine production, and lymphoproliferative response were measured in 15 infants with primary moderate protein calorie malnutrition on admission and after recovery of 10% of weight. Sixteen healthy, well nourished infants of comparable age served as controls. A significant deficit in the z-score of weight for age, weight for height, body mass index, and circulating leptin and growth factors were detected in malnourished infants on admission compared with controls ($P < 0.05$). These deficits were associated with a significant suppression of the lymphoproliferative response, Th1, and proinflammatory cytokine production ($P < 0.05$). After a 10% weight gain, a significant increase in circulating leptin levels was produced in parallel with a significant increase in Th1 activity, as revealed by an enhancement in interferon-γ and a suppression in IL-4 production. Concomitantly, the lymphoproliferative response became similar to that detected in control infants. Furthermore, a significant increase in IL-1 and TNFα production compared with that at admission was shown. These findings suggest an association between the increase in leptin and the immunological recovery observed following refeeding of malnourished infants. (*J Clin Endocrinol Metab* 87: 3040–3046, 2002)

ACUTE NUTRITIONAL deprivation affects immune function; in addition, it rapidly reduces circulating leptin in mice and humans (1–3). Furthermore, impaired cell-mediated immunity has been described in *ob/ob* and *db/db* mice, which are known to be defective in leptin or its receptor, respectively (4–6). Lord *et al.* (6) reported that the *in vitro* addition of leptin to mixed lymphocyte cultures increases the proliferative response of T lymphocytes and regulates the T helper type 1 (Th1)/Th2 balance. Moreover, Howard *et al.* (7) in a recent publication demonstrated that the regimen of recombinant leptin administration during acute starvation was able to completely reverse the effect of starvation on thymic involution and protected against the starvation-induced changes in both thymocyte number and subpopulations proportions. Also, these researchers showed that peripheral administration of recombinant leptin to *ob/ob* mice reduced thymocyte apoptosis and substantially increased both thymic cellularity and the $CD4^+ CD8^+/CD4^- CD8^-$ ratio (7).

Recently, decreased levels of leptin have been demonstrated in severe malnourished infants by Soliman and coworkers (8); however, no link between this decrease and *in vivo* immune responses has been established to date, despite the fact that a great body of evidence indicates that chronic nutritional deprivation suppresses cellular immune function (9–13).

The purpose of this study was to investigate whether the decrease in leptin levels is associated with alterations of cell-mediated immunity and with proinflammatory and Th1/Th2 balance in human protein calorie malnutrition (PCM) of moderate severity. Furthermore, to confirm these findings, cell-mediated immunity, proinflammatory cytokines, and the Th1 and Th2 profile were also determined when a significant increase in the circulating leptin level was observed in malnourished infants.

Subjects and Methods

Subjects

In 15 infants with primary moderately intense PCM (seven males and eight females) leptin, growth factor levels, proinflammatory, Th1 and Th2 cytokine production, and lymphoproliferative responses were determined on admission to a Close Nutritional Recovery Center of the Chilean Nutrition Foundation, and after a 10% weight gain. All infants had a birth weight over 2500 g and were free from infections at the time of study (C-reactive protein, <10 mg/liter). Sixteen healthy, well nourished infants (seven males and nine females) of comparable age served as the control group. Informed consent was obtained from the mothers after the aims of the project had been fully explained to them. This study was approved by the ethics in human research committee of the Institute of Nutrition and Food Technology, University of Chile (Santiago, Chile).

Nutritional status

Anthropometric measurements, weight, length, body mass index (BMI) (kilograms per square meter), and skinfold thickness were determined by a registered nurse on admission and after a 10% weight gain. Nude weight was obtained before the first morning feeding with an infant scale (Detecto, Brooklyn, NY) with a precision of 5 g. Body length to the nearest 0.1 cm was determined in the recumbent position using a portable infantometer. The means of three measurements for weight and length were evaluated comparing them to the National Center for Health Statistics standard. Weight for age (W/A) and weight for height (W/H) z scores were calculated with the PCTL9Z anthropometric subroutine (Center for Health Promotion and Education, National Center for Disease Control and Prevention, Atlanta, GA). The percentage of body fat was calculated by skinfold measurements using a Lange caliper (Cambridge, MD, and Washington DC) (14).

Abbreviations: BMI, Body mass index; Con A, concanavalin A; IFNγ, interferon-γ; IGFBP, IGF-binding protein; LPS, lipopolysaccharide; PBMC, peripheral blood mononuclear cells; PCM, protein calorie malnutrition; Th1 and Th2, T helper cells type 1 and type 2; W/A, weight for age; W/H, weight for height.

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Endocrinological measurements

A venous blood sample was obtained after an overnight, 6-h fast, and serum was separated and kept frozen at −20 C until analysis of leptin and growth factors was performed. Serum leptin was measured by RIA (Linco Research, Inc., St. Charles, MO) as previously described and validated (15). The intra- and interassay coefficients of variation were 3.4 and 3.6 ng/ml, respectively.

IGF-I and IGF-binding protein-3 (IGFBP-3) were determined by ELISA. The IGF-I intra- and interassay coefficients of variation were 4.5% and 7% respectively. The IGFBP-3 intra- and interassay coefficients of variation were 4.2% and 6.8%.

Cytokine measurements

Induction of IL-1 TNFα and IL-6. Peripheral blood mononuclear cells (PBMC) were isolated by Hypaque gradient (Sigma, St. Louis, MO) and washed twice in PBS, and 2.5×10^6 cells/ml were resuspended in RPMI 1640 culture medium supplemented with gentamicin, glutamine, 0.5% heat-inactivated AB plasma, and 1 μg/ml indomethacin. Two hundred microliters of cell suspension were dispensed in 96-well flat-bottom microtiter plates in the absence (spontaneous cytokine release) or the presence of endotoxin [lipopolysaccharide (LPS), 1 μg/ml]. Cultures were incubated for 18 h at 37 C in 5% CO_2 and 95% air. Supernatants were collected and kept at −70 C until assays for released cytokines were performed.

Induction of Th1 and Th2 cytokines. The same methodology for cell isolation and culture conditions was applied for induction of these types of cytokines; their production per well was stimulated with 5 μg PHA, 5 ng phorbol acetate myristate, and 0.2 μg ionomycin, and the incubation period was 48 h.

Cytokine determinations

All cytokine determinations were performed using specific, commercial, ELISAs (R&D Systems, Inc., Minneapolis, MN). The assay sensitivity was 3.6 pg/ml.

Lymphoproliferative assay

Isolated PBMC were cultured in the presence of PHA and concanavalin A (Con A) for 3 and 5 d, respectively. Unstimulated cells served as controls. Eighteen hours before harvesting, the cells were incubated with 18.5 GBq tritiated thymidine (specific activity, 247.9 GBq/mol) and harvested in an automatic cell harvester (16). Results were expressed as counts per minute.

Statistical analysis

Parametric results were analyzed using Student's paired and unpaired tests. Nonparametric data were logarithmically transformed to yield normal distributions before parametric analyses were performed. *P* value less than 0.05 was accepted as statistically significant.

Results

Anthropometric determinations

Table 1 shows a significant deficit in W/A, W/H, BMI, and percentage of sc fat in the malnourished infants on admission compared with well nourished controls ($P < 0.05$). A significant increase in the W/A z-score and BMI was induced after 30 d of refeeding, the time interval necessary for 10% weight gain.

Endocrinological determinations

Serum leptin levels and IGF-I were significantly lower in the PCM children on admission (Fig. 1). After a 10% weight gain, PCM infants experienced significant increases in serum leptin and IGF-I levels, reaching the values observed in the control group. IGFBP-3 had a similar behavior, with mean ± SD values on admission and after 10% weight gain of 2703 ± 333.3 and 2959 ± 343.6 ng/ml ($P < 0.05$, respectively; mean ± SD of control group, 3213 ± 146.7 ng/ml; $P < 0.05$ *vs.* PCM admission values).

No correlation between log leptin levels and body fat percentage was found in PCM infants on admission; however, after the 10% weight gain, this correlation became as significant as in the control group (Fig. 2). A similar situation was observed when the correlation between leptin and IGF-I levels was evaluated in PCM infants (Fig. 3); a significant correlation became apparent after 10% weight recovery in PCM children (Fig. 3).

TABLE 1. Anthropometric characteristics of PCM infants

	PCM admission (n = 15)	PCM 10% wt gain (n = 15)	Control (n = 16)
Age (months)	14.6 ± 6.1	15.7 ± 6.5	13.7 ± 6.7
W/A (z-score)	−2.7 ± 0.4[a,b]	−2 ± 0.4[a]	0.2 ± 0.5
W/H (z-score)	−1.3 ± 0.6[a]	−1 ± 0.6[a]	0.3 ± 0.2
Body fat (%)	11.8 ± 2.6[a]	13.6 ± 2.7[a]	16.4 ± 3.9
BMI (kg/m²)	14.5 ± 1.02[a,b]	15.5 ± 1.01[a]	17.4 ± 0.8

Results are expressed as the mean ± SD.
[a] $P < 0.05$ *vs.* control.
[b] $P < 0.05$ *vs.* PCM after 10% weight gain.

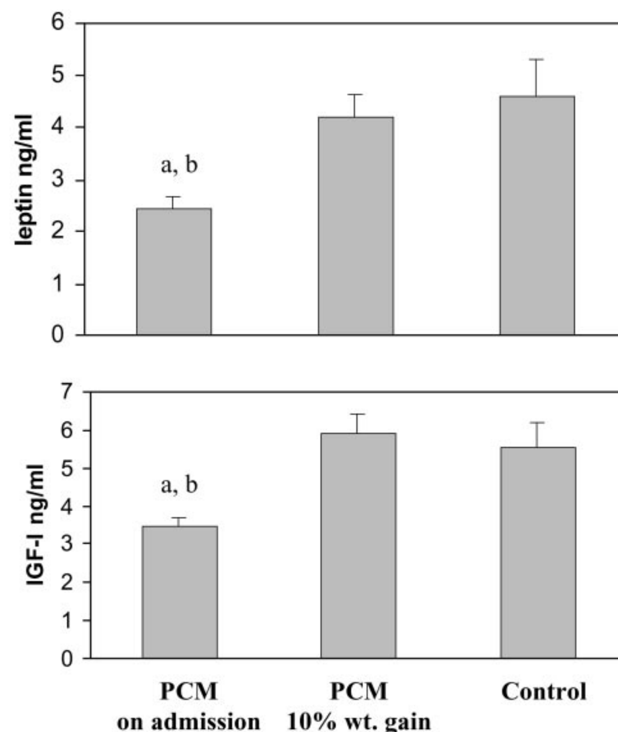

FIG. 1. Leptin and IGF-I levels in PCM infants. Results are expressed as the mean ± SEM. a, $P < 0.05$ *vs.* control; b, $P < 0.05$ *vs.* PCM after 10% weight gain.

Proinflammatory cytokine production by PBMC

TNFα production by LPS-stimulated PBMC of malnourished infants was similar to that in the control group on admission, increasing significantly after the 10% weight gain and reaching higher levels than in controls ($P < 0.05$, by *t* test;

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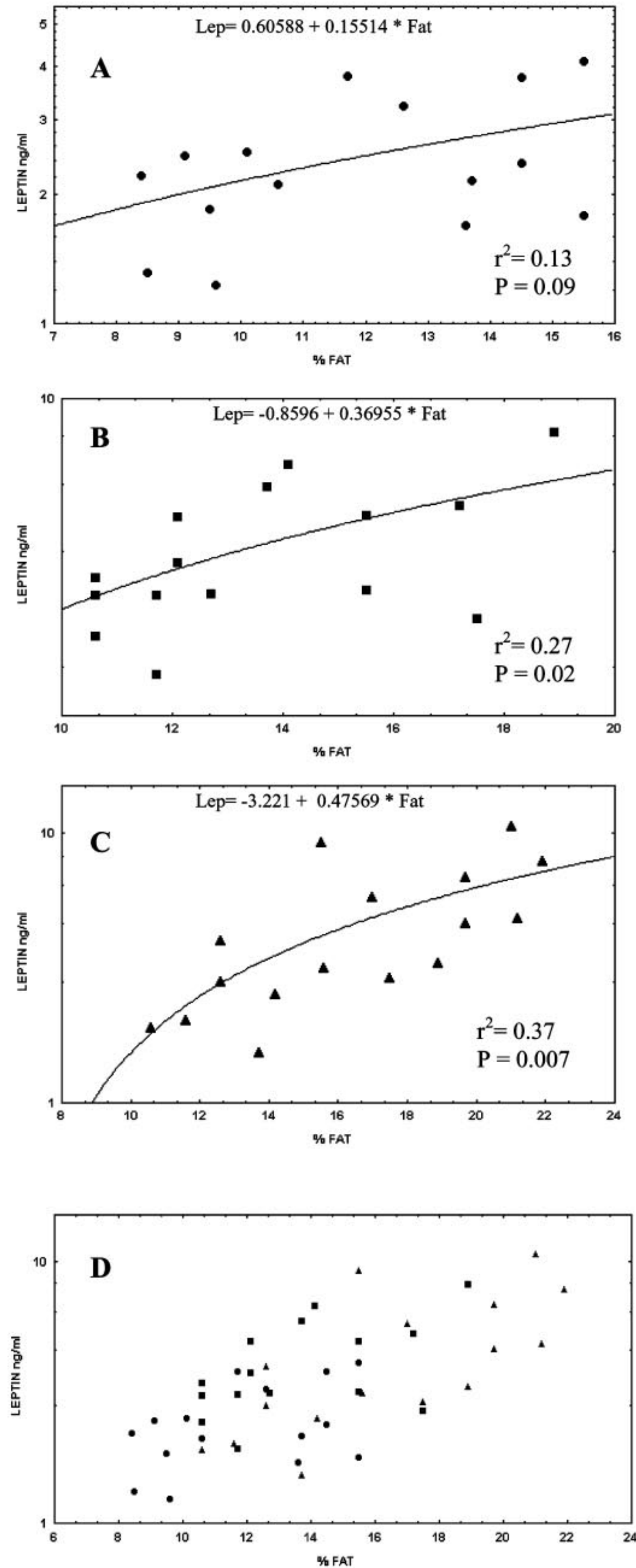

FIG. 2. Correlation between log leptin and body fat percentage. A, PCM infants on admission; B, PCM infants after 10% weight gain; C, control infants; D, all three groups.

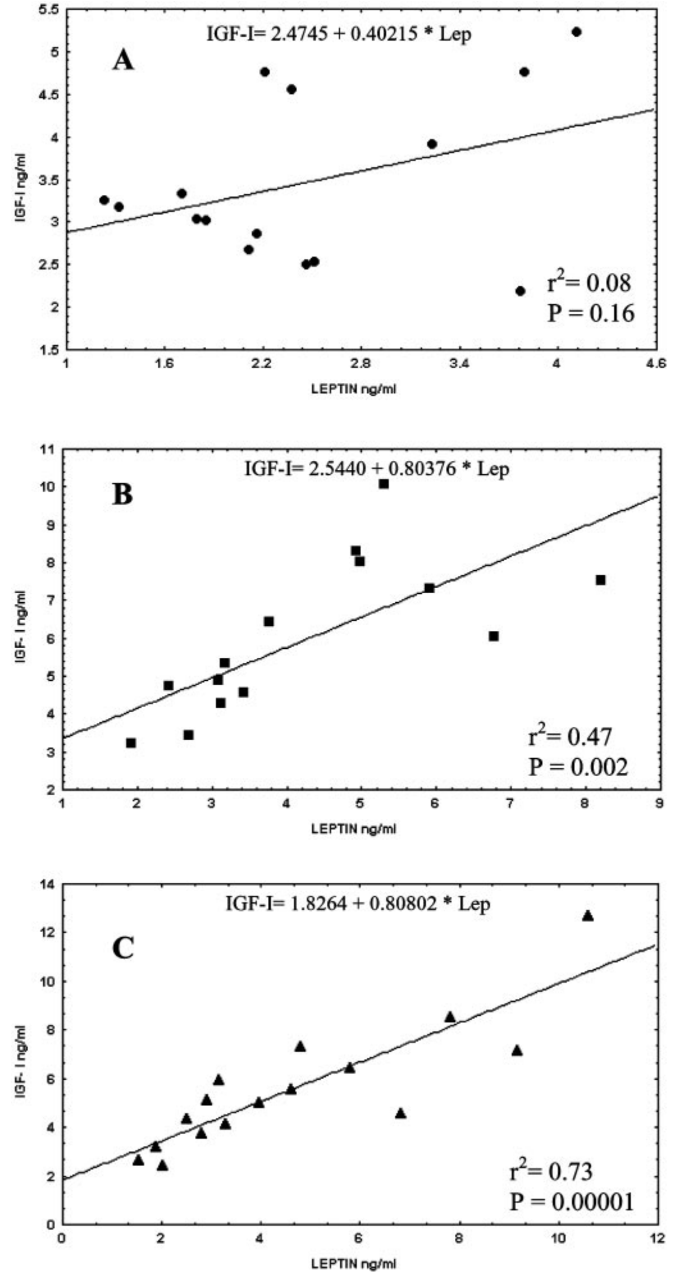

FIG. 3. Correlation between leptin and IGF-I levels. A, PCM infants on admission; B, PCM infants after 10% weight gain; C, control infants.

Fig. 4). IL-1 production by LPS stimulated PBMC from PCM infants tended to be low on admission compared with that in well nourished controls ($0.05 > P < 0.1$, by t test; Fig. 4). When 10% weight had been achieved, IL-1 production was comparable to that in the control group, with a tendency to become significant compared with the production on admission ($0.05 > P < 0.1$, calculated by paired t test). Malnourished infants released comparable quantities of IL-6 as well nourished controls on admission and after the 10% weight gain. No differences in spontaneous production of IL-1, TNFα, and IL-6 from PBMC were detected between the groups in any period of time.

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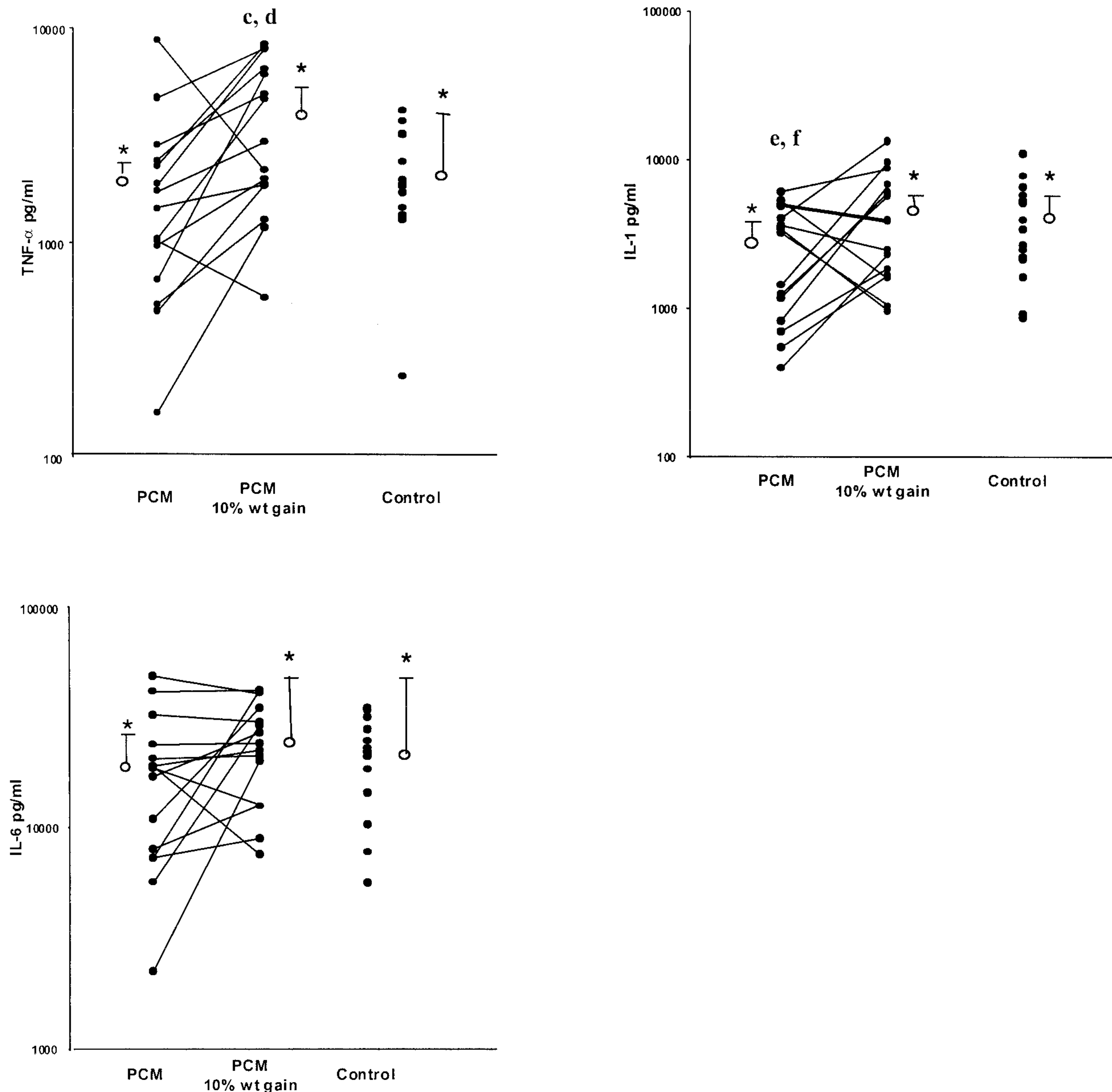

FIG. 4. Proinflamatory cytokine production by stimulated PBMC of 15 PCM and 16 control infants. *, Mean ± 1 SD. c, $P < 0.05$ *vs.* control; d, $P < 0.05$ *vs.* PCM on admission; e, $0.05 > P < 0.1$ *vs.* control; f, $0.05 > P < 0.1$ *vs.* PCM after 10% weight gain.

Th1 and Th2 cytokine production by PBMC

Interferon-γ (IFNγ) production by mitogen-stimulated PBMC from PCM infants was similar to that in the control group on admission. However, a significant increase ($P < 0.05$, by *t* test and paired *t* test) was observed after the 10% weight gain compared with well nourished infants and with PCM on admission, respectively (Fig. 5). On the other hand, a significantly higher production of IL-4 was detected in PCM subjects compared with well nourished controls on admission ($P < 0.05$, by *t* test). After recovery of 10% of initial weight, a significant decrease in the production of this cytokine was shown ($P < 0.05$, by paired *t* test). No differences in spontaneous production of IFNγ and IL-4 by PBMC were detected between the groups in any time period.

Lymphoproliferative response to mitogens

Malnourished infants showed a significant decrease in the lymphoproliferative response to PHA and Con A on admission compared with controls ($P < 0.05$). After gaining 10% of initial weight, this response increased to the levels observed in the well nourished group (Table 2).

Discussion

A significant reduction in leptin levels and a trend toward a decrease in proinflammatory and Th1 cytokine production was observed in PCM infants on admission. However, when the infants recovered 10% of their initial weight, circulating

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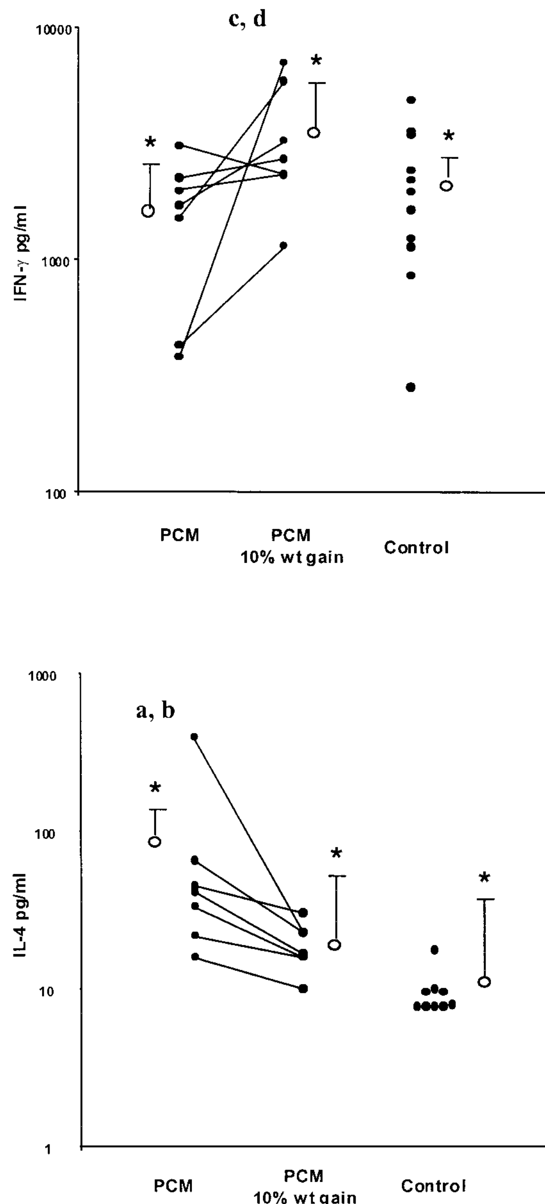

FIG. 5. Th1 and Th2 cytokine production by stimulated PBMC of 7 PCM and 12 control infants. *, Mean ± 1 SD. c, $P < 0.05$ *vs.* control; d, $P < 0.05$ *vs.* PCM on admission; a, $P < 0.05$ *vs.* control; b, $P < 0.05$ *vs.* PCM after 10% weight gain.

hormone levels became normal, associated with a significant increase in cytokine production.

It is well known that circulating leptin concentrations closely correlate with body fat mass and that higher levels of this hormone are observed in obese subjects (15, 17); a decline of leptin levels occurs during acute starvation and weight loss, with return to normal values after refeeding (18). In addition, leptin levels are low in many forms of malnutrition, including intrauterine growth retardation and untreated anorexia nervosa (19, 20). To date few data on leptin levels in protein calorie malnutrition have been reported. Our data show that PCM infants had low leptin serum levels; these results are in agreement with the data recently published by Soliman *et al.* (8). Interestingly, the recovery of only 10% of weight in our children, which took place approximately 30 d after refeeding, produced a significant increase in the levels of this hormone. The rise in leptin did not parallel the increase in body fat mass, a situation that was also observed in individuals subjected to acute fasting or energy restriction, in which the reduction of leptin levels was not concomitant to the diminution of fat body mass (19). These observations suggest that the leptin concentration is regulated by factors other than fat mass (21). Some researchers postulate that leptin behaves like a signal for energy stores, speculating that the rise of this hormone could represent an adaptive phenomenon necessary for growth development (19). Experimental evidence shows that low leptin levels could be a predictor of body weight gain based on the data of Jacquet *et al.* (19), who demonstrated that children born with intrauterine growth retardation had higher leptin levels at 1 yr of age compared with normal controls. These researchers (19) suggested that the higher leptin levels in their children could represent an adaptive response that stimulated catch-up growth. An alternative explanation could be that high serum leptin concentrations could reflect a defect in adipose tissue function that impairs the sensitivity of the regulatory system of leptin synthesis and secretion (19). The increasing risk of obesity during adult life of children born with intrauterine growth retardation supports this hypothesis (22).

TABLE 2. Lymphoproliferative response to mitogens in PCM infants

	n	PHA (cpm)	Con A (cpm)
PCM admission	15	62142 ± 21362[a,b]	50456 ± 26368[a,b]
PCM 10% wt gain	15	87498 ± 31743	74081 ± 22471
Control	16	84555 ± 29541	85131 ± 28185

PHA, Phytohemaglutinin. Results are expressed as the mean ± SD.
[a] $P < 0.05$ *vs.* control.
[b] $P < 0.05$ *vs.* PCM after 10% weight gain.

Recent evidence indicates that cytokines play an important role in the nutrition-infection complex (23). On one hand, an impairment of cytokine production has been reported in malnutrition, and furthermore, infections induce cytokine release, which is known to interfere with metabolic activity and cause appetite loss that alter nutritional status (13, 23, 24). We observed a trend toward reduced PBMC IL-1 production in PCM infants on admission, which agrees with the data reported in malnourished children and anorexia nervosa (12, 25, 26), and explains why these children have an impaired capacity to mount a febrile reaction as a response to infectious agents. TNFα production by PBMC from PCM infants on admission did not differ from that in normal controls, a finding in disagreement with the results reported by Keenan *et al.* (25) in adults with anorexia nervosa. On the other hand, IL-6 production in malnourished infants was similar to that in normal controls, results that differ from those of Doherty *et al.* (27), who reported a diminished production of this cytokine in severe PCM infants, a fact that could explain the difference in our cases with moderate PCM. Surprisingly, when 10% of weight had been gained, a significant increase in IL-1 and TNFα production, concomitant with the increase in circulating leptin levels, was observed in the PCM patients. Furthermore, Faggione *et al.* (28) reported that the decrease in leptin levels during fasting contributes

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to the increased susceptibility to LPS toxicity. These researchers showed that LPS induced a 5-fold greater increase in serum TNFα in fasted mice compared with fed animals (28). The TNFα increase was blunted by exogenous leptin replacement, suggesting that leptin could be protective by both inhibiting TNFα induction by LPS and by reducing TNFα toxicity. These researchers concluded that leptin exerts a regulatory role on the immune response during the nutritional recovery period (28).

No information correlating leptin levels and cytokine production in human malnutrition is available at present in the current literature. The studies published evaluating the effect of leptin on immune function have been performed only in experimental animals and in *in vitro* experiments (6, 29). It has been reported that leptin-deficient *ob/ob* mice cannot clear and kill circulating *Escherichia coli* as efficiently as normal mice, suggesting that these genetically obese mice have an impaired immune system (29). The possibility that leptin-dependent signals regulate immune function is also supported by the observation that Zucker *fa/fa* rats, an obese and diabetic strain with deficient leptin receptors, produced suboptimal quantities of proinflammatory cytokines when challenged with LPS (29). Furthermore, studies of macrophages from *ob/ob* mice (which lack leptin) and *db/db* mice (which have dysfunctional leptin receptors) show an impaired ability to phagocytize and kill *Candida* (29, 30). Recombinant leptin restored the phagocytic function of cells from *ob/ob* mice, but not that of cells from *db/db* mice, indicating that leptin may regulate macrophage phagocytosis via mechanisms that require direct interactions with its receptor (29). The evidence that leptin can also increase phagocytic activity in macrophages isolated from lean, nondiabetic mice supports these findings and suggests that the phagocytic defect in *ob/ob* and *db/db* mice is a direct result of leptin deficiency rather than secondary to obesity-related diabetes (31). These researchers also have demonstrated that leptin is necessary for animals to mount an optimal cytokine response when challenged with endotoxin, a fact supporting our findings and suggesting that this hormone could stimulate proinflammatory cytokine production indirectly by a posttransductional mechanism (29).

Impaired cell-mediated immunity, demonstrated in our study by the decrease in the mitogen-stimulated lymphoproliferative response, is a feature of malnutrition that predisposes to infectious episodes; however, the mechanisms responsible for this alteration remain unknown. When the PCM infants recovered 10% of their weight, leptin levels and lymphoproliferative responses returned to normal, becoming similar to those of well nourished healthy controls. These results are consistent with the findings of Lord *et al.* (6), who demonstrated that the *in vitro* addition of leptin to mixed lymphocyte cultures enhanced their proliferative response, the increment being much higher when isolated CD4 cells were employed as responders, suggesting that T lymphocytes are the principal target for leptin action.

Until now, no information on Th1 and Th2 activity has been reported in malnutrition. Our study reveals an imbalance between lymphocyte subpopulations in PCM, demonstrated by the trend toward a decrease in IFNγ, and the increase in IL-4 production, concomitant with the presence of low leptin levels. When a 10% recovery of weight was achieved, a significant enhancement of IFNγ production associated with suppression of IL-4 was shown in parallel with the increase in circulating leptin levels. These findings are similar to those reported by Lord *et al.* (6), who observed that *in vitro* leptin addition to PBMC stimulated IL-2 and IFNγ production, indicating that leptin may favor a Th1 response, whereas its absence may promote Th2 activity.

It is possible to speculate that leptin could exert immunological modulation by acting directly on T cell receptors or indirectly, through the hypothalamic-pituitary-adrenal axis. Some time ago we demonstrated that central noradrenergic hyperactivity in malnourished rats could be one of the mechanisms responsible for immunosuppression in malnutrition (32). Leptin may also modulate central noradrenergic activity, and therefore, through this pathway it may regulate immune function (33).

A significant decrease in IGF-I and IGFBP-3 levels was observed in PCM infants on admission, in agreement with previous results (34). With the recovery of 10% of weight, these growth factors increased to levels similar to those in well nourished controls.

Many studies have shown that in rats central leptin infusion stimulates basal secretion of GH, indicating that leptin could act as a neuromodulator of the growth axis (35). We did not measure GH levels in our patients, but we assume that this may be increased. We also postulate that the increased leptin levels could induce an increase in GH sensitivity that contributes to stimulate IGF-I production.

Thus, leptin could play an important role in growth factor regulation as well as in modulation of the immune response. However, immunoregulation may not be attributed only to leptin, because growth factors function as immunomodulators (36, 37). It has been reported that hypophysectomy induces an immunodeficiency in rats that is reversed by administration of GH and PRL (37). Furthermore, GH and IGF-I receptors have been described on the surface of lymphocytes (37). However, in patients with Laron syndrome, a genetic disease characterized by low levels of IGF-I and IGFBP-3, similar to those observed in PCM, no immunological deficits have been reported (37). It is therefore possible that growth factors do not play a fundamental role in immune regulation in PCM.

In conclusion, our findings suggest an association between the increase in leptin and the immunological recovery observed after refeeding of malnourished infants. However, a therapeutic trial is needed as a proof that leptin could enhance Th1 activity in malnutrition.

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Address all correspondence and requests for reprints to: Dr. Liana Schlesinger, Institute of Nutrition and Food Technology, Casilla, 138-11 Santiago, Chile. E-mail: lschlesi@uec.inta.uchile.cl.

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