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Effect of nucleotide intake and nutritional recovery on insulin-like growth factor I and other hormonal biomarkers in severely malnourished children

Edgar Vásquez-Garibay[1]*, Katja Stein[1], Juergen Kratzsch[2], Enrique Romero-Velarde[1] and Gerhard Jahreis[3]

[1]*Institute of Human Nutrition, University Center of Health Sciences, University of Guadalajara, Unit of Infant Nutrition Studies, Civil Hospital of Guadalajara 'Dr. Juan I. Menchaca', Guadalajara, Jalisco, Mexico*
[2]*Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnosis, University Hospital, Leipzig, Germany*
[3]*Institute of Nutrition, Friedrich Schiller University, Jena, Germany*

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The objective of the present study is to demonstrate the effect of nucleotide intake and intensive nutritional support on the concentration of insulin-like growth factor I (IGF-I) and other hormonal biomarkers in severely malnourished children. Twenty-six severely malnourished children <48 months of age received formula without lactose via enteral feeding for 2 weeks and *ad libitum* for an additional 2 weeks. Anthropometrical measurements were performed and serum concentrations of IGF-I, insulin-like growth factor binding protein-3 (IGFBP-3), leptin, soluble leptin receptor (sOB-R), as well as the estimated molar excess of sOB-R over leptin were obtained. Two groups were formed. One group received formula with nucleotides (NT + ; *n* 13) and the other without nucleotides (NT − ; *n* 13). A control group was included (*n* 13). Parametric and non-parametric tests as well as ANOVA models were used. Nutritional recovery, nucleotides intake, type of malnutrition, age and the interaction between gender and malnutrition influenced the concentration of IGF-I ($P<0{\cdot}001$). Nutritional recovery, nucleotides intake, gender and type of malnutrition had an effect on IGFBP-3 ($P<0{\cdot}001$). Nutritional recovery had a significant effect on serum leptin ($P=0{\cdot}001$). Age and nutritional recovery had an effect on sOB-R ($P<0{\cdot}001$); all variables included affected the molar excess of sOB-R over leptin ($P<0{\cdot}001$). In conclusion, nucleotide intake and nutritional recovery had a notable effect on IGF-I, IGFBP-3 and other hormonal biomarkers. This outcome could stimulate the catch-up growth of severely malnourished infants and toddlers during the nutritional recovery period.

Children: Malnutrition: Nucleotides: Nutritional recovery: Insulin-like growth factor I: Leptin

Milk-based infant nucleotide-supplemented formulas have been designed for normal infants because of their potentially beneficial effects on different human organic functions (Uauy *et al.* 1994; Carver, 2003) and these could be good alternatives to nutritional support for infants with severe and primary protein energy malnutrition (PEM; Vásquez-Garibay *et al.* 2004). Accumulative evidence from recent animal and clinical studies supports the original idea that nucleotides are semi-essential dietary nutrients, especially in hyper-catabolic or rapid growth states (Uauy *et al.* 1990; Gil, 2002). The great amount of nucleotides and their precursors in breast milk, the beneficial effects on intestinal flora, and the lipoproteins profile, their effect on intestinal growth and development, and the catch-up growth of infants small for gestational age support this theory (Uauy *et al.* 1990, 1994; Quan *et al.* 1991; Cosgrove *et al.* 1995; Gil, 2002). In fact, a dietary source of nucleotides may be particularly important for infants whose tissue requirements are greater than before (pre-term neonates, infants suffering from malnutrition or chronic diarrhoea) (Boza, 1998).

Insulin-like growth factor (IGF) I regulation with nutrients links diet and growth, bringing an interface between nutrients and hormones acting together to stimulate growth, while illustrating the cardinal role that nutrients play in the control of gene expression (Thissen *et al.* 1994). Circulating IGF-I probably represents the most meaningful serum index for adequate nutrient intake because of its regulatory mode, its growth-promoting effect, and its close relationship to N balance. Serum IGF-I level is positively related to nutritional status, and affected by other hormones like insulin (Jahreis *et al.* 1992).

Recent studies related to the IGF-I–growth hormone axis have suggested a possible link between leptin and the IGF-I system in the regulation of body composition (Palacio *et al.* 2002). Diminished leptin levels are crucial for metabolic adaptation to starvation, including a decrease in the metabolic rate that allows survival for longer periods (Fried *et al.* 2000). The hypothesis is that, in the context of the positive correlation between IGF-I and leptin levels, the marked reduction of circulating leptin concentration and IGF-I in both forms of severe PEM (marasmus and kwashiorkor) suggest that IGF-I plays an important role in controlling leptin secretions in these children (Soliman *et al.* 2000). Others (Llopis *et al.* 1998) have considered that low leptin levels in undernourished states might

Abbreviations: IGF, insulin-like growth factor; IGFBP-3, insulin-like growth factor binding protein-3; NT + , formula with nucleotides; NT − , formula without nucleotides; PEM, protein energy malnutrition; sOB-R, soluble leptin receptor.

* **Corresponding author:** Dr Edgar Vásquez-Garibay, fax +52 33 3618 9667, email inhu@cucs.udg.mx

account for decreased production of IGF-I and growth hormone binding protein. Likewise, re-feeding reverses the malnourished state, increases serum IGF-I, insulin-like growth factor binding protein-3 (IGFBP-3) and leptin levels, restoring normal growth (Palacio *et al.* 2002).

In order to fully appreciate the anabolic action of IGF-I in various clinical settings it is important to explore the whole nutritional environment (Thissen *et al.* 1994). The purpose of the present study was to test the hypothesis that children with severe and primary PEM fed an infant formula with added nucleotides over a period of 4 weeks with controlled nutritional support (Díaz-Gómez *et al.* 1997; Vásquez-Garibay & Romero, 2000) would show a significant improvement in IGF-I concentration and other hormonal biomarkers.

Subjects and methods

A clinical intervention study design was used. Subjects were assigned by convenience, forming two groups until the sample was completed. One group was fed a milk-based, lactose-free infant formula without nucleotides (NT −), and with corn syrup added to increase energy density from 2·8 to 3·35 kJ/ml. The other group was fed a similar formula and energy density with nucleotides added (NT +).

Infants were included only if they were full term, of normal birth weight (>2500 g), between the ages of 3 and 48 months and had primary and severe PEM. They were admitted if the weight to age or weight to length indices were below −3 standard deviations from the median (World Health Organization, 1996; Secretaría de Salud, 1999) or when the subject presented oedema and a clinical picture of kwashiorkor or marasmus–kwashiorkor independently of the weight/age or weight/length score. PEM was defined as primary when the cause of malnutrition was an inadequate and insufficient diet commonly associated with repeated upper respiratory infectious disease and/or frequent episodes of diarrhoea.

Children clinically free of infection, diarrhoea or any disease that might alter nutritional recovery were admitted into the study. Thirty-four subjects were included between March 1999 and September 2000. In group NT − (*n* 16) three were excluded for the following reasons: cystic fibrosis (one); deterioration of malnutrition (one); death (one). In group NT + (*n* 18) five were excluded for the following reasons: sepsis (one); milk protein intolerance (two); chronic pulmonary disease (one); voluntary discharge (one). Twenty-six subjects completed the study. A 'physiologically normal control group' was used to compare the serum hormonal biomarkers. They were taken from the hospital outpatient clinic and included thirteen healthy subjects, aged 12–48 months. In order to facilitate admission, this group was significantly older than group NT + (25·7 *v.* 13·5 months).

Variables

Dependent variables. Dependent variables were IGF-I (ng/ml), IGFBP-3 (mg/ml), leptin (ng/ml), soluble leptin receptor (sOB-R; ng/ml) and molar excess of sOB-R over leptin.

Independent variables. Independent variables were starting infant formula with nucleotides added; free nucleotides in infant formula (non-lactose, Nestle®) were closer to human milk content (μmol/l): (1) pyrimidines: UMP 31, CMP 47·4; (2) purines: AMP 9·5, GMP 5·5 and without nucleotides added (non-lactose, Nestle®).

Intervenient variables. Intervenient variables were age (months); gender (male/female); time of the nutritional recovery period; type of malnutrition: with oedema and weight/age or weight/length > −3 SD (kwashiorkor), with oedema and weight/age or weight/length < −3 SD (marasmus–kwashiorkor) and without oedema and weight/age or weight/height < −3 SD (marasmus).

Administration of infant formulas

The formula was placed in a feeding bag of 500 ml (Pisa®) then introduced into a feeding tube (D-731 o 732; Desvar de Mexico, S.A., Mexico City, Mexico) and administered to infants by continuous infusion pump (Braun®). After the fifth day the volume was increased to provide an energy intake of 837 kJ/kg per d and 4 g/kg proteins per d. At the beginning of the third week, they were fed *ad libitum* by bottle and children older than 12 months of age were started with complementary pap foods (cereal and/or vegetables mixed with the same formula used, Nestle®). Before and after each feeding the bottle and the container with the complementary food were weighed on a triple beam balance (Ohaus®). Every day, the total amount of formula, complementary foods, protein and energy intake were registered.

The formula fulfilled the total requirements of water, energy, proteins and other nutrients during the first 2 weeks of enteral feeding, no other foods were offered during this period because infants, even those older than 12 months, had severe PEM. From the first day, all subjects received daily oral vitamins (1 ml; 1500 μg RAE vitamin A, 25 μg vitamin D, 50 mg vitamin C, 1 mg thiamin, 0·8 mg riboflavin, 6 mg niacin) and folic acid (0·5 mg). From the sixth day elemental Fe was given in daily doses of 3 mg/kg. Depending on the new weight, the energy and protein intake by kg was adjusted to 837 kJ/kg per d and 4 g/kg per d.

Anthropometric measurements

At the beginning of the study and once per week for 4 weeks, two observers (E. V. G. and K. S.) were responsible for the following measurements. Weight: subjects were weighed without clothes in a calibrated scale (model 440, Bame, Mexico City, Mexico; with a minimum of 5 g). Length: each subject was measured by two observers with an infant measuring board read to the nearest 0·1 cm as described by Fomon (1977). Weight/age, length/age and weight/length indices were calculated and expressed as *Z* scores. Measurements of the head and the arm circumference were also taken. Triceps, subscapular, subcostal and supra iliac skin fold thicknesses were obtained with a Lange Skinfold Caliper (Cambridge Scientific Industries, Cambridge, MD, USA). The upper arm muscle area and the upper arm fat area (Frisancho, 1990) were estimated. The percentage of body fat was calculated with Slaughter's equation (Slaughter *et al.* 1998):

for girls: = (sum triceps and subscapular skinfolds) − 0·013 (sum triceps and subscapular skinfolds)^2 − 2·5;

for boys: = (sum triceps and subscapular skinfolds) − 0·008 (sum triceps and subscapular skinfolds)^2 − 1·7.

Laboratory analysis

Blood samples were collected by antecubital venopuncture at 07.00 hours in the following way: before the beginning of the intervention, then after 2 weeks of enteral feeding (2 weeks) and again at the end of the *ad libitum* feeding (4 weeks).

Serum aliquoted was stored at −80°C until assayed in Leipzig, Germany. IGF-I levels were determined by RIA (Mediagnostic, Reutlingen, Germany) with a sensitivity of 0·02 ng/ml, and an intra- and inter-assay CV below 8 %. The IGF-I assay involved an extraction step (acidification and IGF-II excess) to measure total IGF-I. IGFBP-3 levels were determined by ELISA (DSL, Sinshein, Germany) with a sensitivity of 0·004 mg/l, and an intra- and inter-assay CV below 12 %. Leptin was measured by ELISA (R&D Systems, Wiesbaden, Germany), with a sensitivity <7·8 pg/ml, and an intra- and inter-assay CV below 6 %.The sOB-R concentration was determined by an in-house ligand-immunofunctional assay (Kratzsch *et al.* 2002). The molar excess of sOB-R over leptin was calculated and equalled sOBR, then divided by leptin content and multiplied by 0·13.

Statistical analysis

After the descriptive statistics were calculated an unpaired Student's *t* test and a non-parametric Mann–Whitney *U*-test were taken, respectively, to compare the geometric means of anthropometrical measurements and the ranks of hormonal biomarkers between the groups NT − *v.* NT + as well as for each group *v.* the control group at different stages of the study. A univariate ANOVA with a corrected model for each hormonal biomarker as a dependent variable was used; gender, age, group (NT − or NT +), time of nutritional intervention (at the start, at 2 and 4 weeks), type of malnutrition, and interaction among independent variables were included. In addition, a general linear model: multiple comparisons with repeated measures for time, individually on both hormonal biomarkers and fat incorporation indicators within each group were calculated. Excel (Microsoft Corp., Redmond, WA, USA), Epi-Info 2000 version 1.0 (CDC, Atlanta, GA, USA) and SPSS version 10 for Windows (SPSS Inc., Chicago, IL, USA) programs were used to process data and analyse information. Null hypothesis was rejected with $P \leq 0{\cdot}05$.

Ethical considerations

The protocol was submitted and approved by the Ethics Committee of the Hospital Civil 'Dr. Juan I. Menchaca' and the University of Guadalajara, Mexico. Parents or legal guardians were informed of the study and a signed consent form was requested. Once a consent form was signed by a parent or legal guardian, candidates for the study were admitted to the metabolic ward. Specialized personnel took care of the subjects for the duration of the study.

Results

The mean age was 20·9 (SD 9·8), 13·5 (SD 10·1) and 25·7 (SD 12·1) months in group NT − , group NT + and the control group, respectively. Group NT − had a higher initial mean weight and a lower weight for age (−3·99 *Z*) than group NT + (−3·64 *Z*), attaining an important weight gain ($P<0{\cdot}05$) and a not so important improvement in weight/length at 2 and 4 weeks (Table 1). Throughout the nutritional

Table 1. Physical characteristics of the subjects: anthropometrical indicators‡

(Mean values and standard deviations)

	Group NT − (*n* 13)		Group NT + (*n* 13)		Control group (*n* 13)	
Indicator	Mean	SD	Mean	SD	Mean	SD
Age (months)	20·9	9·8	13·5	10·1	25·7	12·1
Weight (g)						
At the start	6522	1297	5449	1673	12 006	3460
Two weeks	8041*	1532	6698	1742		
Four weeks	9126*	1743	7365	2067		
Length (cm)						
At the start	72·5	6·4	66·2	9·1	83·9	11·3
Two weeks	73·0	6·0	66·7	8·0		
Four weeks	74·1	6·2	67·8	7·6		
Length/age (*Z* score)						
At the start	−3·23	1·04	−2·75	1·07	−0·60	0·86
Two weeks	−3·18	1·09	−2·73	1·10		
Four weeks	−2·95	1·02	−2·55	1·21		
Weight/age (*Z* score)						
At the start	−3·99	0·74	−3·64	0·85	−0·31	1·12
Two weeks	−2·81	0·96	−2·59	0·74		
Four weeks	−2·04	0·84	−1·84	1·35		
Weight/length (*Z* score)						
At the start	−3·00 †	0·76	−2·39	0·71	0·14	1·18
Two weeks	−1·35	0·98	−0·94	0·53		
Four weeks	−0·44	0·91	−0·50	0·69		

Group NT + , formula with nucleotides; group NT − , formula without nucleotides.
Mean values were significantly different from those of group NT + : *$P<0{\cdot}05$.
Mean value was significantly different: $P<0{\cdot}05$.
‡ For details of procedures, see p. 684.

recovery period both groups demonstrated similar and considerable fat increase and muscle incorporation ($P<0{\cdot}001$; Table 2). No significant differences between the groups at any stage of the study were found.

The IGF-I and IGFBP-3 concentration in each group registered a noticeable increase ($P<0{\cdot}001$) at 2 weeks; however, the IGF-I concentration observed was significantly higher in the second week than in the fourth week in group NT + *v.* group NT − ($P=0{\cdot}03$ and $P=0{\cdot}06$, respectively; Fig. 1). The leptin concentration in each group also showed a substantial increase, meanwhile the sOB-R concentration and the molar excess of sOB-R over leptin were significantly decreased ($P<0{\cdot}001$; Fig. 2).

Subjects in group NT − were more malnourished and older than subjects in group NT + . At the start, IGF-1, IGFBP-3 and leptin concentration were considerably lower in group NT − and NT + than in the control group. The IGF-I concentration continued to be lower throughout the study in group NT − *v.* group NT + and control group ($P<0{\cdot}04$ and $P=0{\cdot}006$, respectively). IGFBP-3 concentration was higher at 2 and 4 weeks in group NT − *v.* control ($P<0{\cdot}04$ and $P<0{\cdot}005$). There was a lower concentration of leptin in group NT + than in group NT − ($P<0{\cdot}04$) at 4 weeks, which is probably related to the final weight obtained in group NT − , although the middle and the final percentage of fat obtained were similar in both groups. Differing from the leptin tendency at 2 and 4 weeks, the sOB-R concentration in both groups was lower than the concentration in the control group ($P<0{\cdot}001$) The molar excess of sOB-R over leptin was higher at the beginning in both groups *v.* control ($P<0{\cdot}001$; Table 3). This indicator was extremely high at the start, much higher than previously described for children during the first years of life (Zastrow *et al.* 2003), but declined sharply after 2 weeks of nutritional recovery.

Table 2. Fat and muscle incorporation*

(Mean values and standard deviations)

	Group NT − (*n* 13)		Group NT + (*n* 13)	
Indicator	Mean	SD	Mean	SD
Sum of four skinfolds (mm)				
At the start	12·3	3·2	13·0	3·9
Two weeks	19·3	5·0	20·3	4·8
Four weeks	26·2	5·4	26·2	4·8
Body fat (%)†				
At the start	7·0	2·6	7·7	3·6
Two weeks	11·6	3·8	11·6	2·8
Four weeks	16·0	3·9	14·9	2·2
Upper arm fat area (cm^2)				
At the start	2·1	0·88	2·3	1·5
Two weeks	3·6	1·6	3·4	1·04
Four weeks	5·2	1·7	4·7	1·04
Upper arm muscle area (cm^2)				
At the start	5·6	1·05	4·8	1·16
Two weeks	6·6	1·1	6·1	1·45
Four weeks	7·3	1·35	6·8	1·65

Group NT + , formula with nucleotides; group NT − , formula without nucleotides.

* For details of procedures, see p. 684. There were no significant differences between groups.

† Slaughter's equation (Slaughter *et al.* 1998).

At 2 weeks gender had a clear and important effect on the IGF-I concentration, favouring females (*n* 15) over males (*n* 11) (59 (SD 36·4) *v.* 44 (SD 44·4) ng/ml, respectively; $P<0{\cdot}03$) and at 2 weeks the IGFBP-3 concentration between females and males (2·07 (SD 0·48) *v.* 1·15 (SD 0·52) mg/l, respectively; $P<0{\cdot}02$) and at 4 weeks (2·17 (SD 0·40) *v.* 1·33 (SD 0·52) mg/l, respectively; $P<0{\cdot}02$). After 2 weeks, IGFBP-3 concentration was lower in marasmus (*n* 10) *v.* kwashiorkor (*n* 10) (1·40 (SD 0·25) *v.* 2·22 (SD 0·48) mg/l, respectively; $P<0{\cdot}03$) and in marasmus–kwashiorkor (*n* 6) *v.* kwashiorkor (*n* 10) (1·02 (SD 0·42) *v.* 1·44 (SD 0·78) mg/l, respectively; $P=0{\cdot}039$). At the start, the leptin concentration was lower in marasmus than in kwashiorkor (0·08 (SD 0·04) *v.* 0·66 (SD 0·44) ng/ml, respectively; $P<0{\cdot}03$).

During the nutritional recovery period it was observed that the IGFBP-3 concentration (1·36 (SD 0·77) *v.* 0·98 (SD 1·08) mg/l, respectively; $P=0{\cdot}006$) and the sOB-R concentration (71·8 (SD 27·9) *v.* 48·5 (SD 57·3) ng/ml, respectively; $P=0{\cdot}035$) were higher in those under 24 months than those over 24 months of age.

Univariate ANOVA

Insulin-like growth factor I. In the corrected model four variables and two interactions had an effect on IGF-I (adjusted R^2 0·661, $P<0{\cdot}001$). The nutritional recovery period showed a major impact ($P<0{\cdot}001$) with higher levels at 2 and 4 weeks ($P<0{\cdot}001$). Group NT + showed higher levels of IGF-I than group NT − with a group effect ($P<0{\cdot}001$). The type of malnutrition showed an overall effect on IGF-I ($P=0{\cdot}013$). Children with kwashiorkor had higher levels than children with marasmus ($P=0{\cdot}008$). Regardless of the type of malnutrition, young infants (3–12 months) had higher levels of IGF-I ($P=0{\cdot}008$), which could be related to accelerated linear growth at this age. We have no explanation as to why females with kwashiorkor had higher concentrations of IGF-I after 2 weeks and females with kwashiorkor and marasmus–kwashiorkor had higher concentrations of IGF-I after 4 weeks, with its associated interaction effect ($P=0{\cdot}017$).

Insulin-like growth factor binding protein-3. Four variables in the corrected model had an effect on IGFBP-3 (adjusted R^2 0·633, $P<0{\cdot}001$). The nutritional recovery time showed a gradual increase of IGFBP-3 ($P<0{\cdot}001$). Group NT − presented small but higher levels of IGFBP-3 than group NT + ($P<0{\cdot}024$) and females had higher concentrations of IGFBP-3 ($P=0{\cdot}001$). Children with kwashiorkor had the highest concentrations of IGFBP-3 and those with marasmus–kwashiorkor had the lowest ($P<0{\cdot}001$). Age played an important role on IGFBP-3 ($P=0{\cdot}003$), exhibiting higher concentrations at earlier ages (12–24 months; $P<0{\cdot}001$).

Leptin. Only the nutritional recovery time had an effect on leptin ($P=0{\cdot}011$). A higher level was found at 4 weeks *v.* at the start ($P=0{\cdot}001$).

Soluble leptin receptor. In the corrected model two variables produced an effect on sOB-R (adjusted R^2 0·495, $P<0{\cdot}001$); the nutritional recovery time ($P<0{\cdot}001$) and age both had an effect on sOB-R ($P=0{\cdot}006$), where high levels were seen in younger infants (3–12 months).

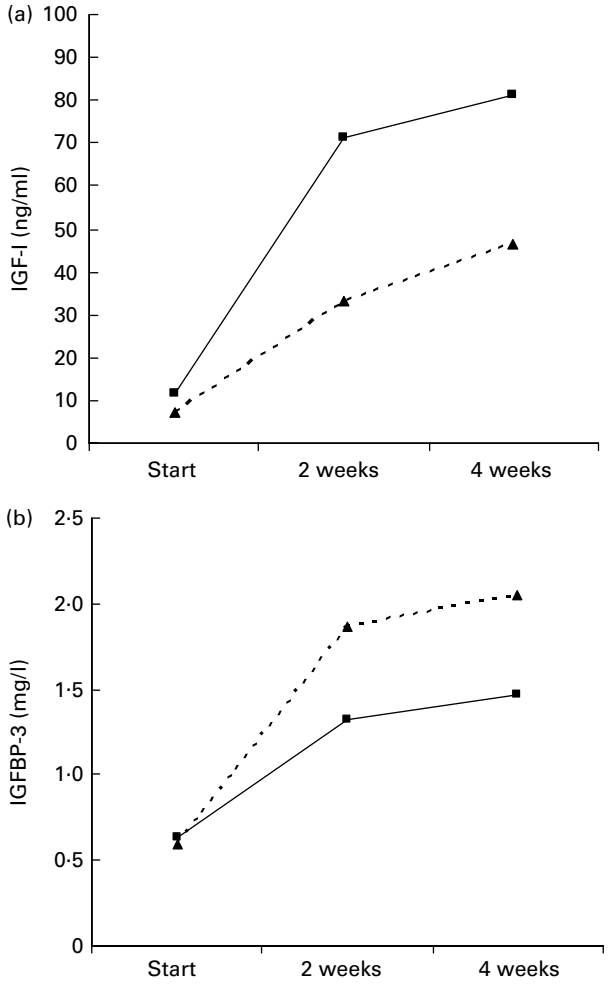

Fig. 1. The insulin-like growth factor I (IGF-I; a) and insulin-like growth factor binding protein-3 (IGFBP-3; b) concentration in both groups (group NT +, formula with nucleotides, ■; group NT −, formula without nucleotides, ▲) showed an increase ($P<0·001$), especially in the first 2 weeks. A major increase in the IGF-I concentration in the second week in group NT + *v.* group NT − was also observed ($P=0·03$). For details of procedures, see p. 684.

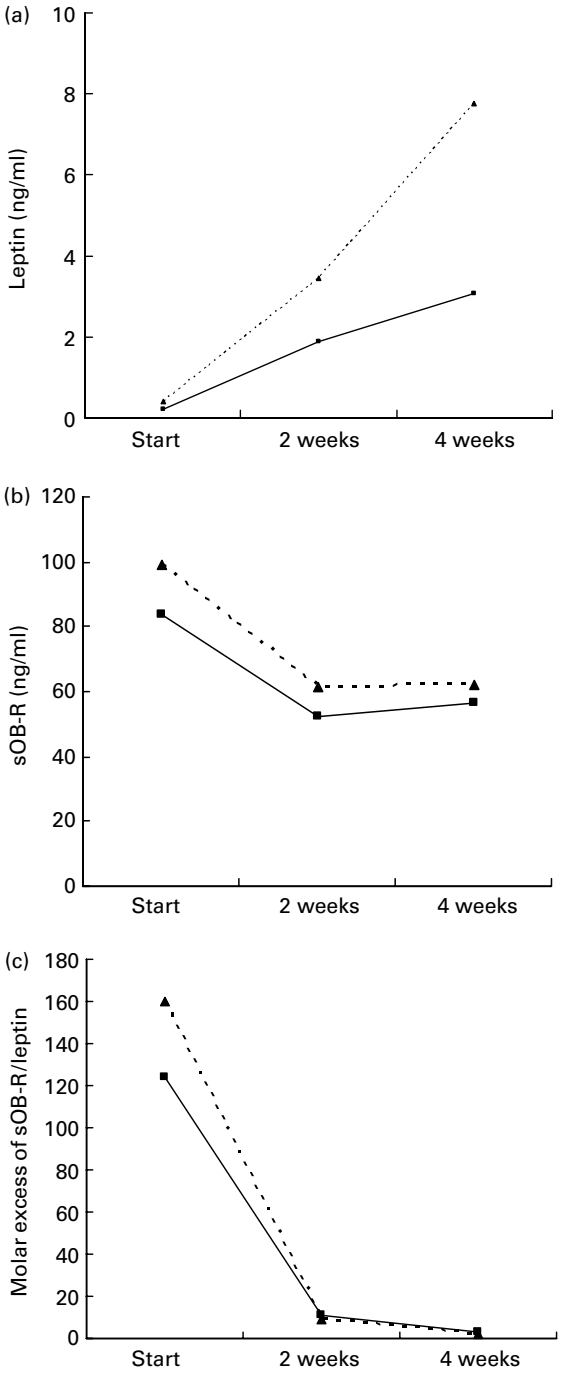

Fig. 2. The leptin concentration (a) in both groups (group NT +, formula with nucleotides, ■; group NT −, formula without nucleotides, ▲) showed an increase, while the soluble leptin receptor (sOB-R; b) concentration and the molar excess of sOB-R over leptin (c) decreased ($P<0·001$) especially in the first 2 weeks. A major increase in the leptin concentration in the fourth week in group NT − *v.* group NT + was observed ($P=0·03$). For details of procedures, see p. 684.

Discussion

After 2 weeks of enteral feeding nucleotide intake had a noteworthy effect on IGF-I production and at 4 weeks a non-significant but mild effect ($P=0·06$) was also seen. Malnutrition decreases mucosal growth and differentiation while re-feeding with a nucleotide-supplemented diet produces jejunum and ileum restoration in rats previously fed a nucleotide-free diet (Ortega *et al.* 1995). Dietary nucleotides can promote protein synthesis and growth, as well as normal development and function (Lopez-Navarro *et al.* 1996; Perez *et al.* 2004; Saez-Lara *et al.* 2004). Therefore, we can speculate that a formula with nucleotides added could have a stimulating effect on IGF-I, which is synthesized in most tissues, mainly in the liver, encouraging cellular growth.

The present results demonstrate that an intake of 837 kJ/kg per d and 4 g protein/kg per d given to children with primary and severe PEM during 4 weeks of nutritional recovery, with an initial 2 weeks of intensive nutritional support, produced profound changes in the concentration of hormonal biomarkers: IGF-I, IGFBP-3, leptin, sOB-R and in the molar excess of sOB-R over leptin, as well as in fat and muscle incorporation. We could also demonstrate that children fed an infant formula without lactose, but with nucleotides added, had a significantly higher concentration of IGF-I during the nutritional recovery, especially after the first 2

Table 3. Hormonal biomarkers by group‡
(Median values with their standard errors)

	Group NT − (*n* 13)		Group NT + (*n* 13)		Control group (*n* 13)	
Biomarkers	Median	SEM	Median	SEM	Median	SEM
Leptin (ng/ml)						
At the start	0·11**	0·17	0·14**	0·06	1·82	0·47
Two weeks	2·03	1·29	1·85	0·40		
Four weeks	5·03*†	1·94	2·63	0·37		
sOB-R (ng/ml)						
At the start	95·50	8·83	80·10	6·64	91·30	5·01
Two weeks	63·00**	4·41	50·20**	4·38		
Four weeks	64·50 **	4·36	52·80**	4·40		
IGF-I (ng/ml)						
At the start	5·08*	1·50	9·73**	2·45	64·00	5·91
Two weeks	32·00*†	5·25	55·40**	15·95		
Four weeks	46·77	5·44	62·90	12·01		
IGFBP-3 (mg/l)						
At the start	0·53**	0·08	0·61**	0·07	1·19	0·07
Two weeks	2·00	0·29	1·35	0·12		
Four weeks	2·29*	0·27	1·45*	0·10		
Molar excess of sOB-R over leptin						
At the start	82·77**	65·84	67·41**	35·93	7·06	9·07
Two weeks	3·69	3·53	4·23	4·52		
Four weeks	1·56**	0·80	2·10**	0·46		

Group NT +, formula with nucleotides; group NT −, formula without nucleotides; IGF-I, insulin-like growth factor I; IGFBP-3, insulin-like growth factor binding protein-3; sOB-R, soluble leptin receptor.
Median values were significantly different from those of the control group: *$P<0·05$; **$P<0·001$.
Median values were significantly different from those of group NT +: †$P<0·03$.
‡ For details of procedures, see p. 684.

weeks of continuous enteral feeding than children receiving a similar, but nucleotide-free formula. The effect of this could be beneficial in increasing linear growth velocity, cephalic circumference and weight gain as observed in other infants born with intrauterine growth retardation (Cosgrove *et al.* 1995). The impact of intensive nutritional support on IGF-I and other hormonal biomarkers seems to be more noticeable in man during the high growth velocity period typical of infants and toddlers. Kondrup *et al.* (1992) could not demonstrate any effect on serum IGF-I and IGFBP-3 concentration within a comparable time span (4 weeks) in adults with liver cirrhosis receiving a protein supplement.

Soliman *et al.* (2000) have pointed out that during prolonged PEM the decreased synthesis of IGF-I and low levels of insulin and/or its decreased effect due to an insulin-resistant status, ensure the deviation of substrate away from growth and toward supporting metabolic homeostasis. The protein and/or energy restriction and catabolic states reduce circulating concentrations of IGF-I and the amount of carbohydrate eaten is a major determinant of this IGF-I response to growth hormone stimulation (Snyder *et al.* 1989; Clemmons & Underwood, 1991). It has also been demonstrated that a diet without protein did not result in full restoration of IGF-I levels (Straus & Takemoto, 1990), showing that not only was energy required, but that protein was important for maintaining IGF-I levels. In rats, short-term protein restriction reduces hepatic IGF-I mRNA levels (Snyder *et al.* 1989). IGF-1 concentration is important to protein adaptation and energy metabolism to altered nutritional states (Freyburg *et al.* 1995). IGF-I and IGFBP provide a mechanism linking nutrition and growth because the nutritional status, the dietary energy and protein supply are critical for the hepatic production of circulating IGF and IGFBP (Thissen *et al.* 1994; Holt *et al.* 2000).

The present results agree with Prentice *et al.* (2002), who observed that severe childhood PEM displays two distinct clinical types, marasmus and kwashiorkor. It is feasible that a higher fat mass and consequently leptin levels in the pre-kwashiorkor condition could lead to a maladapted state by suppressing cortisol and growth hormone. Soliman *et al.* (2000) found, with results comparable to the present results, that children with severe PEM show greater triceps and subscapular skinfold thickness in kwashiorkor (possibly related to oedema) than in marasmus and that this is reflected in higher leptin levels.

Zastrow *et al.* (2003) have discovered that a molar excess of sOB-R inhibits leptin's bioactivity, most likely through its competition with the membrane receptor. They have speculated that the higher molar excess of sOB-R over leptin in children during the initial years of life may have a modulating impact on leptin action. Since food intake and energy storage are important in the first years of life, the bioactive hormone levels may be reduced in young children's circulatory systems during periods of marked growth velocity. It appears that under certain clinical conditions, the molar excess of sOB-R over leptin has a suppressive effect on leptin's action. In severely malnourished children this may be important, especially in situations requiring high energy intake, particularly in those with marasmus type of malnutrition in young infants aged 3–12 months (Kratzsch *et al.* 2002, 2004; Zastrow *et al.* 2003).

Conclusion

One conclusion, supported by the present results, is the premise that dietary nucleotides could be 'temporarily' essential nutrients in young children with severe and primary PEM. This means that nucleotides, as well as other physiological actions, could stimulate growth hormone activity via a significant IGF-I increase. This is especially true in infants under 12 months of age, when the need for high growth velocity exists and the demand to repair different tissues would be greater (Uauy *et al.* 1994, 1990; Gil, 2002; Carver, 2003).

There are other variables, such as gender (Martinez de Icaya *et al.* 2000), the moment and type of nutritional intervention (enteral *v.* oral feeding), the age and type of malnutrition, during nutritional recovery that could have a significant impact on changes in these hormonal indicators in severely malnourished children less than 48 months old. These hormonal biomarkers, especially IGF-I and the molar excess of sOB-R over leptin, might be used as indicators to evaluate the efficiency of dietary support in children presenting a nutritionally critical condition.

In closing, an infant formula fed to these children during a controlled 4-week period with supplemented nucleotides and an energy increase from 2·8 to 3·35 kJ/ml would have a favourable impact on hormonal growth indicators and on nutritional recovery.

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