ORIGINAL ARTICLE

Leptin, malnutrition, and immune response in rural Gambian children

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Arch Dis Child 2002;**87**:192–197

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Accepted 6 March 2002

Background: The adipocyte derived hormone, leptin, has cytokine like function and may mediate the effects of starvation on immunity. Mice with congenital leptin deficiency (*ob/ob*) have small hypocellular thymuses and impaired cellular immunity. In humans leptin influences the differentiation of naïve and memory cells in vitro, and genetic leptin deficiency has been associated with an ill defined susceptibility to infection.

Aims: To describe the in vivo relation of leptin and immune function in children.

Methods: Fasting plasma leptin concentrations, immune function (T and B cell mediated vaccine responses and delayed type hypersensitivity), and mucosal function (dual sugar permeability test and salivary sIgA concentrations) were measured in a cohort of 472 moderately undernourished rural Gambian children.

Results: Leptin concentrations correlated with body fat assessed by mid upper arm circumference or BMI for age Z scores, and were very low compared to well nourished European norms (males 1.8 *v* 11.1 ng/ml; females 2.4 *v* 13.8 ng/ml). No detectable relations were found between leptin concentrations and any of the measures of immune or mucosal function.

Conclusions: The data confirm that leptin acts as a peripheral signal of energy restriction, but do not support an association between fasting plasma leptin levels and immune function in children of this age.

Recent data have suggested that leptin may mediate the effects of malnutrition on T cell function.[1 2] Leptin, the product of the *ob* gene, is a hormone derived primarily from adipocytes. The central function of leptin is to regulate energy balance and fat stores. Circulating leptin concentrations are highly correlated with adiposity and act as a peripheral signal providing an integrated measure of energy stores and flux to the central nervous system and other organs.[3] Starvation induces a notable fall in leptin concentrations. Leptin has structural homology with the long chain helical cytokine family, and the leptin receptor (in its constitutively active long form) resembles the gp130 family of cytokine receptors on lymphocytes, which includes the interleukin 6 (IL-6) receptor.[4] Leptin and TNFα can act as mutual secretagogues,[5 6] and leptin is increased by inflammation.[7] These observations suggest a role for leptin in modulating immune function.

Abnormalities of the myeloid lineage have been described in rodents defective in either leptin (*ob/ob*) or its receptor (*db/db*),[4 8–10] but they are not reported to be prone to infection and consistent immunodeficiency has not been shown.[11–16] However, leptin activity has been strongly linked to T cell function in promoting thymopoiesis and proliferation, Th1 responses, and adhesion of spleen cells in *ob/ob* and *db/db* mice.[1] In normal mice preadministration of exogenous leptin reversed the inhibitory effects of starvation on delayed type hypersensitivity (DTH) responses,[1] and prevented the massive reduction of the cortical $CD4^+$ $CD8^+$ thymocyte population induced by 48 hours starvation.[2]

Humans with genetic deficiency of leptin do not have consistent abnormalities of immune function, opportunistic infections, or severe lymphoid atrophy, though minor deficiency of immunity has been described.[17–19] In vitro leptin also regulates proliferation, Th1/Th2 balance, and adhesion with greater effects on human CD45RA (naïve) than CD45RO (memory) CD4 T cells, enhancing primary but not secondary immune responses.[1 20] In rheumatoid arthritis patients an association has been observed between low leptin concentrations following restricted calorie intake and reduced T cell numbers and activation.[21] However, low leptin concentrations induced by a diet only low in carbohydrate were not associated with diminished CD4 or CD8 T cell numbers or activation.[22]

This study investigated whether the in vivo T cell abnormalities in mice and in vitro findings in human T cells translated into an in vivo effect on immunity in a large cohort of rural Gambian children with moderate protein energy malnutrition. The relation of leptin concentrations with immune function was assessed by a range of functional tests, including seroconversion to vaccines designed to test B cell and T cell mediated responses.

METHODS

Study design

A total of 472 children (aged 7–9 years; mean 8.00 (SD 0.68) years) were recruited from rural villages in the West Kiang region of The Gambia, West Africa. The subjects were the oldest children from a cohort studied from birth in the West Kiang Maternal Dietary Supplementation Trial.[23] Children were drawn from both the intervention and control arms of the trial. We have shown that the prenatal intervention had no detectable effect on the immune function in this cohort. The nutritional status of these children followed the characteristic pattern for the region. Low birth weight (~2850 g) is followed by centile catch up in the first three months of life when fully breast fed. There follows a precipitate deterioration in nutritional status to about −2 Z scores for weight-for-age by 12

Abbreviations: ACT, α_1 antichymotrypsin; BMI, body mass index; CMI, cell mediated immunity; DTH, delayed type hypersensitivity; IL, interleukin; MUAC, mid upper arm circumference; sIgA, secretory immunoglobulin A; TNFα, tumour necrosis factor alpha

Table 1 Mean (SD) anthropometry and plasma leptin concentrations by gender

	Males (n=251)	Females (n=221)	p value
Age (years)	8.01 (0.69)	8.00 (0.68)	0.823
Weight (kg)	20.9 (2.71)	20.7 (2.70)	0.409
Height (cm)	121.7 (8.94)	121.6 (5.99)	0.169
BMI (kg/m^2)	14.00 (1.01)	13.89 (1.04)	0.266
MUAC (mm)	160.4 (15.1)	165.6 (13.3)	≤0.0001
Weight for age Z score	−1.63 (1.01)	−1.53 (0.83)	0.255
Height for age Z score	−1.00 (0.86)	−0.89 (0.94)	0.184
BMI for age Z score	−1.44 (0.92)	−1.42 (0.77)	0.822
Plasma leptin (ng/ml)	1.83 (0.48)	2.36 (0.91)	≤0.0001

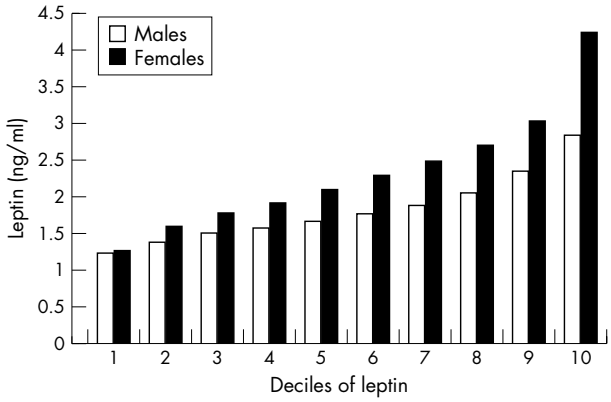

Figure 1 Deciles of plasma leptin by gender. Data are presented as the mean per decile group of the cohort.

months. A very gradual recovery occurs in later childhood (see table 1 for nutritional status when studied).

At baseline subjects were brought to the field laboratory in the early morning after an overnight fast. Weight, height, and mid upper arm circumference (MUAC) were measured. The dual sugar (lactulose–mannitol) test was performed as a measure of small intestinal integrity (permeability).[24 25] This has been shown to correlate with a chronic gastrointestinal inflammation and an excessive mucosal Th1 response (DI Campbell, personal communication). A sample of saliva was collected for measurement of salivary concentrations of secretory IgA (sIgA), and a venous blood sample was collected for the analysis of plasma leptin, plasma α_1 antichymotrypsin (ACT), and serum prevaccination antibody titres. Plasma ACT levels were assessed as an indication of intercurrent infections in the children. Samples were processed immediately and aliquots of saliva, plasma, and serum frozen at −40°C before transportation to the UK on dry ice for analysis. DTH was assessed to seven recall antigens (proteus, trichophyton, candida, tetanus, diphtheria, streptococcus, and tuberculin) using the Multitest cell mediated immunity (CMI) kit (Marcel Mérieux, Lyon, France) applied to the volar surface of the forearm.

On day 2 subjects were visited at home. A positive CMI response was recorded for diameters of induration over 2 mm, and data are presented as the total number of positive responses. Failure to respond to any of the seven test antigens was considered indicative of reduced immunocompetence or anergy. All children were also given a single injection of the 23 valent pnuemococcal capsular polysaccharide vaccine, Pneumovax (Merck, Sharpe and Dohme) and a preliminary dose of the human diploid cell rabies vaccine (HDCV, Pasteur-Merieux). These two vaccines were chosen to assess both a primarily T cell dependent (rabies) and a primarily T cell independent (pneumococccal) vaccine response. In addition, the use of the rabies vaccine in this population provided a means to explore the seroconversion to a naïve antigen.

On day 16 a finger prick blood sample was collected for assessment of antibody response to the first rabies vaccination. On day 30 a second rabies vaccination was given. At the same visit, a second finger prick blood sample was obtained for assessment of antibody response to the Pneumovax vaccine. On day 60 a final finger prick blood sample was collected for the second measurement of rabies antibodies.

Laboratory analysis

Plasma leptin concentrations were determined using a well characterised commercially available human leptin radioimmunoassay kit (Linco Research Inc., St Charles, Missouri).[26] The limit of sensitivity was 0.5 ng/ml, the intra-assay CV was 5.1%, and the interassay CV was 2.8%. Urinary lactulose and mannitol were measured by an automated enzymatic assay using the Cobas-Bio centrifugal analyser, as described previously.[27–30] The interassay CVs were 5.83% for the lactulose and 6.63% for the mannitol. Salivary concentrations of sIgA were measured by ELISA using an adapted method of the procedure developed for determination of breast milk antimicrobial factors.[31 32] The interassay CV was 5.7%. Plasma ACT was measured by nephelometric assay using the Cobas-Bio centrifugal analyser (Roche Diagnostica Instruments, Basle, Switzerland). All assays were performed at MRC Human Nutrition Research, Cambridge.

Antibody levels against the pneumococcal polysaccharide vaccine were measured at the Department of Immunobiology, Institute of Child Health, London. Antibody responses were tested against four polysaccharides (types 1, 5, 14, and 23). Antirabies antibody titres were determined at the Central Veterinary Laboratories, Surrey, UK, using the Rapid-Focus Fluorescence Inhibition Test (RFFIT) of the WHO.[33]

Ethical approval

Ethical approval for the study was obtained from the joint Gambian Government/MRC The Gambia ethical committee. Informed consent was obtained from all parents/guardians of the participating children.

Statistical analyses

Weight for age, height for age, and body mass index (BMI) for age standard deviation scores were calculated using Cole's LMS method,[34] and the stature, weight, and BMI reference curves for the UK, 1990.[35 36] Associations between leptin and immune endpoints were tested by ANOVA and multiple regression (DataDesk, version 6 for Windows, Ithaca, New York, USA). Data have been presented graphically using the point means of the exposure variables divided into either groups of four (lowest to highest; "quartiles") or ten (lowest to highest; "deciles") plotted against the corresponding outcome variable for each group.

RESULTS

Table 1 shows the anthropometric characteristics of the children, and plasma leptin concentrations by gender. There

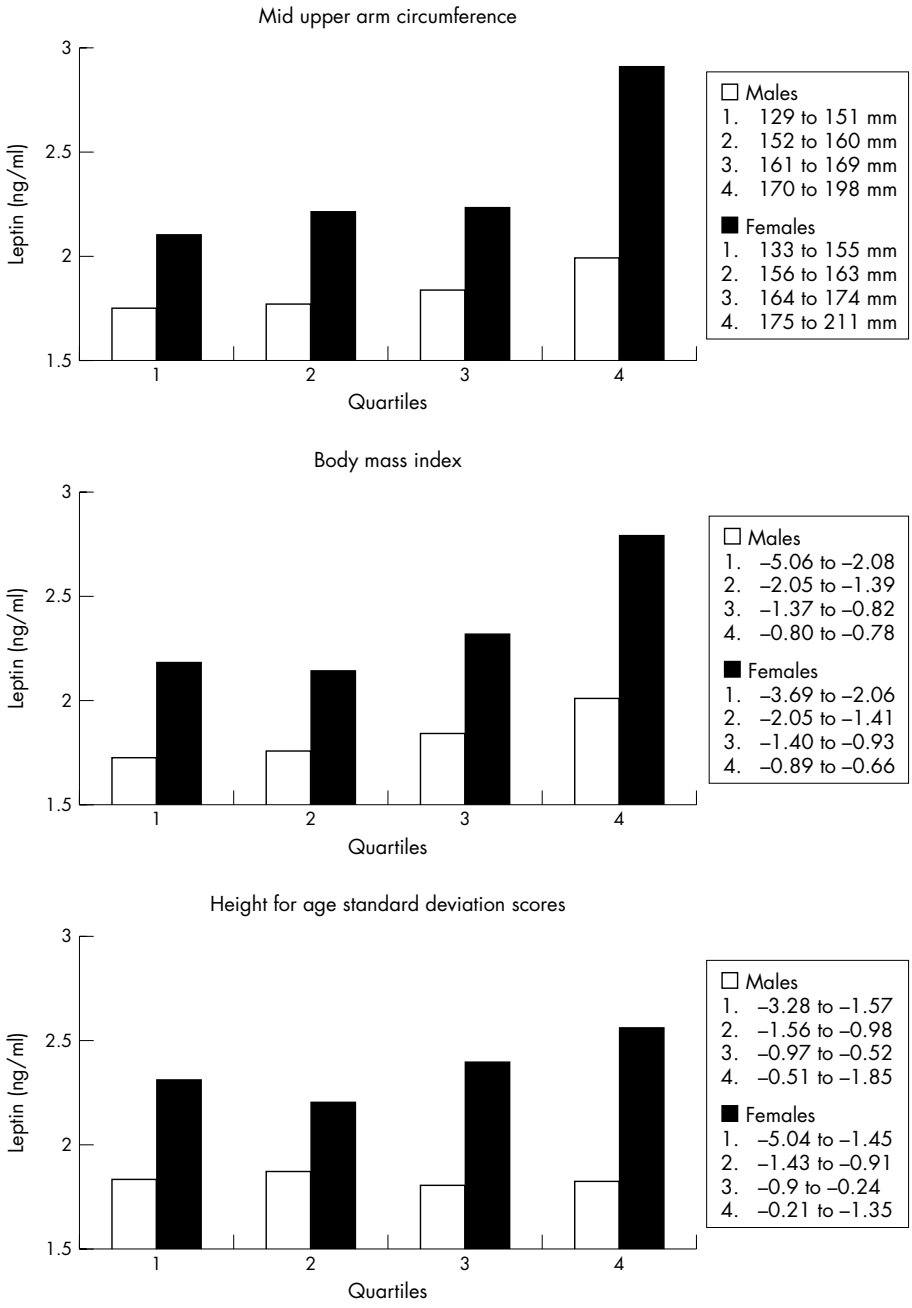

Figure 2 Plasma leptin by quartiles of MUAC, BMI, and height for age SD scores.

were no significant differences between the males and females for weight and height, but the MUAC was significantly greater in the female children. The mean (SD) leptin concentration was 2.08 (0.76) ng/ml. Leptin concentrations were significantly greater in the female children (2.36 (0.91) *v* 1.83 (0.48), $p = 0.0001$). Figure 1 shows the leptin concentrations by deciles of leptin for the genders separately. The female children in the study showed a significantly greater range of values than the males (females 0.86–9.31 ng/ml; males 0.97–3.47 ng/ml).

Leptin concentrations were positively associated with MUAC ($p \leqslant 0.0001$), positively associated with BMI for age Z scores ($p \leqslant 0.0001$), but not associated with height for age Z scores ($p = 0.079$) (fig 2). The observed gender difference remained significant after adjustment for MUAC and BMI for age standard deviation scores ($p \leqslant 0.0001$). Plasma leptin concentrations were not significantly associated to the age of the children within the narrow age range studied ($p = 0.24$).

Leptin and immune function

None of the measures of immune or mucosal function were significantly related to plasma leptin concentrations for either the male, or the female children in the study. Results are illustrated by deciles of leptin concentration for female subjects only, as they showed the greater range of leptin. Figure 3 shows the vaccination responses (rabies response after the first dose of the vaccine, and serotypes 1, 5, and 23 responses to the pneumococcal vaccine). Figure 4 shows the CMI response, lactulose/mannitol ratio, salivary sIgA, and ACT levels. There was a similar absence of any relation in the boys and when the sexes were combined.

DISCUSSION

The children in this study were suffering from moderate to moderately severe protein energy undernutrition (being both underweight and short), with mean BMI for age standard deviation scores of –1.44 and –1.42 for the males and females

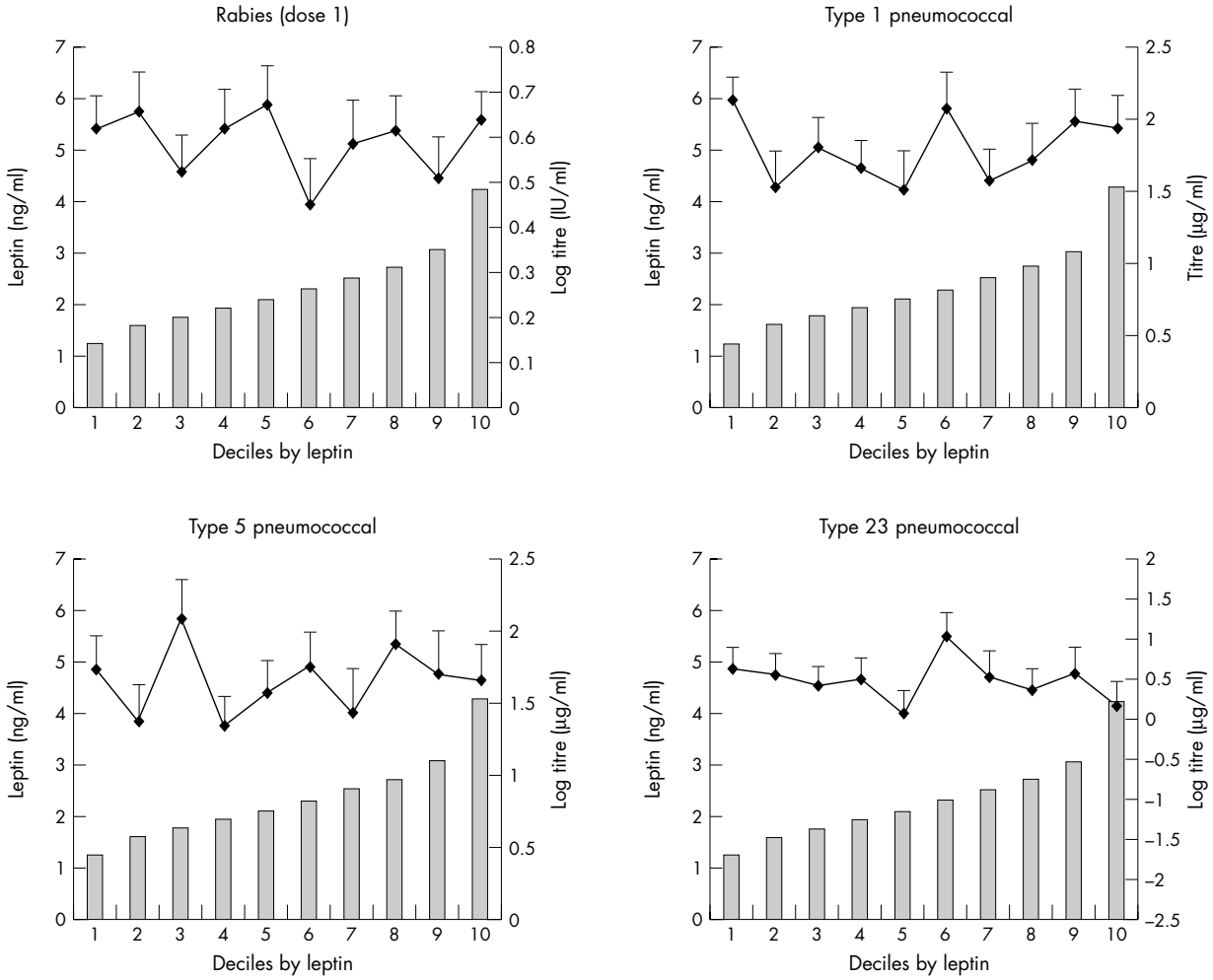

Figure 3 Vaccine response by deciles of plasma leptin. Data are presented as the mean per decile group of the cohort. Error bars represent the standard error of the mean.

respectively. A quarter of the children had a BMI for age below −2 Z scores. Such patterns are quite typical for rural children in many areas of the developing world. The corresponding plasma leptin concentrations were 1.83 ng/ml for the males and 2.36 ng/ml for the females. Consistent with previous studies, plasma leptin concentrations were significantly related to the measures of body fat (MUAC and BMI for age Z scores), but not to the measure of stature (height for age Z scores). The Gambian leptin concentrations were considerably lower than those reported in previous studies from well nourished children of a similar age.[37–42] For example, the median plasma leptin concentrations of 8–10 year old Italian children (also assayed using the Linco kit) were sixfold higher at 11.1 and 13.8 ng/ml for males and females respectively.[39] Corresponding BMI Z scores (also calculated using the LMS method of Cole) for the Italian children were +1.06 and +0.82.

In this study antibody responses to both the pneumococcal and the rabies vaccine were significantly greater in the female children (data not presented). Their greater fat mass and higher circulating leptin may have a role to play in this improved response. However, this did not emerge from correlation analysis of plasma leptin with the range of in vivo indices of immune function used, all of which are variably dependent on T cell function, but also include significant elements of B cell and macrophage function. Most responses were probably secondary but there was also no association with the first dose of rabies vaccine, which can be assumed to be a novel antigen to most, if not all, of the subjects. The lack of association was not accounted for by the confounding effect of infection in stimulating leptin secretion, as there was no relation with an acute phase reactant (α_1 antichymotrypsin). A type II statistical error can also be eliminated as the sample size was very large and the precision of the vaccine responses was sufficient to detect significant age trends for vaccine responses, even within the narrow age group studied (data not presented here).

This suggests that in humans leptin concentrations as low as one sixth of those found in well nourished children do not significantly suppress protective cognate immunity; a finding that is consistent with the majority of published data on the physiology of leptin secretion and its relation to infection and immunity.

Humans and mice genetically deficient in leptin do not have major susceptibility to infection or opportunistic infections. One human kindred shows an excess of death from infections in childhood, but no consistent immunodeficiency was detected[18] or shown in mice.[11–16] However, there are convincing data that leptin has direct activity on thymocytes and circulating mature T cells. Low concentrations of leptin are responsible for the reduced size and cellularity of the thymus in the *ob/ob* mouse, and starvation induced thymic involution in normal mice.[1 2] In both cases this can be shown to be caused by increased apoptosis in $CD4^+8^+$ thymocytes, which is reversed by exogenous leptin.[1 2] mRNA of the leptin receptor isoform responsible for intracellular signalling is expressed in highly purified resting human $CD4^+$ T cells. This is reflected in effects of leptin (sometimes at supraphysiological concentrations) on

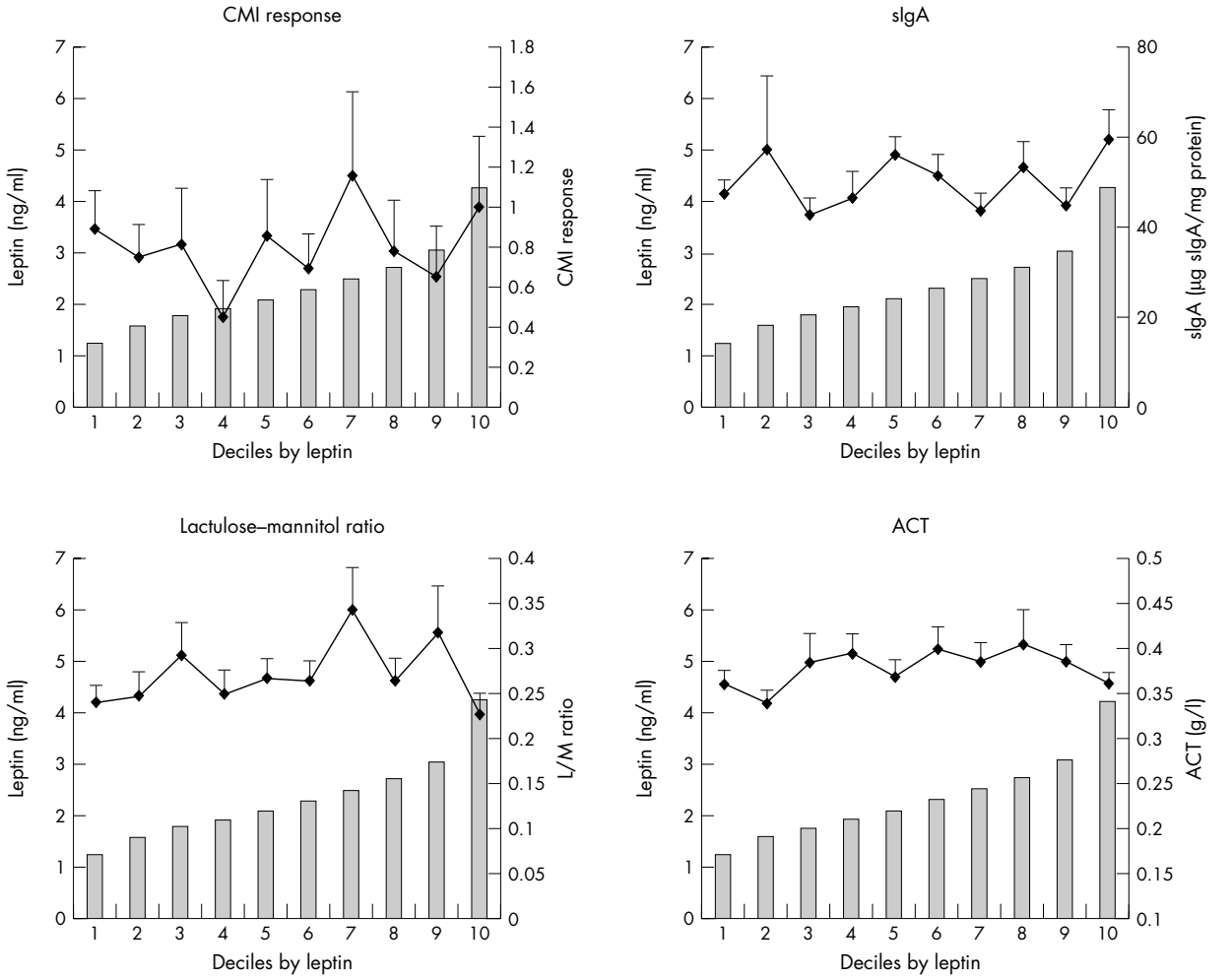

Figure 4 CMI response, lactulose–mannitol ratio, salivary sIgA, and plasma ACT by deciles of plasma leptin. Data are presented as the mean per decile group of the cohort. Error bars represent the standard error of the mean.

T cell function in vitro,[1 20] mainly confined to naïve cells and primary responses.[1]

The inhibitory effect of low leptin concentrations on murine thymopoiesis and human in vitro T cell responses does not translate into compromised specific immune responses in these malnourished children. This may partly be because leptin mainly affects the proliferation of naïve T cells,[1] and most of the subjects will have had exposure to pneumococcal polysaccharide and the antigens used to assess CMI. However, at least one primary specific response is also preserved as the first dose of rabies vaccine was a novel (naïve) antigen. Ninety four per cent of subjects made a protective response after the first dose and 99.6% after two doses.[43]

One interpretation consistent with these data and with the emerging role of leptin as a major regulator of energy expenditure is that malnutrition induced hypoleptinaemia inhibits non-essential physiological processes as suggested previously.[1] However, though this may include thymopoiesis, which in postnatal life is mainly concerned in maintaining an already established T cell repertoire in an energy inefficient way, it does not appear to include the inhibition of secondary immune responses. Secondary responses are relatively parsimonious in energy utilisation as they involve expansion of a limited number of clones of memory T and B cells. Even some primary responses requiring the expansion of a limited number of specific precursors are preserved. This supports the apparently inconsistent findings that thymic involution is an exquisite barometer of starvation[44] with the lack of evidence of a significant T cell immunodeficiency in malnutrition,[45] and is consistent with an immune system which has evolved during intermittent periods of starvation on a hunter-gatherer population.[46]

ACKNOWLEDGEMENTS

We thank staff at MRC Keneba for their help with this project. We are also grateful to Dr Paul Lowings, Central Veterinary Laboratories, Surrey, UK, Dr David Goldblatt, Department of Immunobiology, Institute of Child Health, London, UK, and Ms Dorothy Stirling, MRC Human Nutrition Research, Cambridge, UK, for their contribution to the analysis of samples.

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This work was funded by the UK Medical Research Council with additional financial support from the Nestlé Foundation and the Nutricia Research Foundation

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