Original Article

Circulating IGF-1 may mediate improvements in haemoglobin associated with vitamin A status during pregnancy in rural Nepalese women

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Pregnancy exacerbates vitamin A (VA) deficiency and anaemia among women in developing countries. Improving circulating haemoglobin (Hb) requires erythrocyte production and availability of iron. Insulin-like growth factor-1 (IGF-1) functions in erythropoiesis, but its association with VA status and pregnancy-associated anaemia has not been studied. The aim of this study was to examine the relationship between serum retinol, IGF-1, and Hb among pregnant women in extant samples collected during a placebo-controlled trial of VA and beta-carotene (BC) supplementation in rural Nepal conducted from 1994 to 1997. Mid-pregnancy serum IGF-1 was measured in serum from n=589 randomly selected women of n=1186 in whom anthropometric, VA (retinol) and iron (Hb, erythrocyte zinc protoporphyrin (ZP), and ferritin) status data were available. Associations of IGF-1 with retinol, Hb or anaemia, and iron status were determined using multiple linear and logistic regression. Path analysis was used to explore the role of IGF-1 as a mediator between retinol and Hb, accounting for iron status. A 2.6 g/L increase in IGF-1 was observed per 0.1 mol/L increment in retinol (p<0.0001). Hb increased with each quartile of IGF-1, and odds of anaemia declined 68.8% from the 1st to 4th quartile. Improved iron status indicators explained only 29.1% of the association between IGF-1 and Hb, while IGF-1 explained 25.6% of the association between retinol and Hb. Increasing IGF-1 was likely one mechanism by which retinol improved circulating Hb in pregnant women of rural Nepal, although IGF-1 worked primarily through pathways independent of improved iron status indicators, perhaps by stimulating erythrocyte production.

Key Words: vitamin A, insulin-like growth factor-1, anaemia, pregnancy, haemoglobin

INTRODUCTION

Adequate vitamin A (VA) status is essential for reproductive health and successful pregnancy outcomes. Yet, it is estimated that globally over 19 million pregnant women are VA deficient, as assessed by low serum retinol, with over 9 million experiencing night blindness, and some of the highest regional prevalence in South-East Asia.[1] In Nepal, where maternal vitamin A deficiency (VAD) is endemic, weekly supplements from perinatal through to 3 month postpartum of VA or beta-carotene (BC) reduced maternal mortality by 40% and 49%, respectively, relative to a placebo (PL) in a randomized supplementation trial.[2] In that setting, VA supplementation reduced the prevalence of night blindness during pregnancy by ~40% (10.6 to 6.6%),[3] as well as the number of reported morbidities, particularly in late pregnancy.[4] Finally, VA and BC supplementation reduced the prevalence of anaemia and low iron stores by 10% among women in that trial,[5] from which this current investigation is derived.

South-East Asia also experiences some of the highest regional prevalence of anaemia during pregnancy, at nearly 50% of women affected.[6] Although iron-deficiency accounts for the majority of anaemia during pregnancy worldwide, it is exacerbated by VAD.[7,8] Pregnancy increases the requirement for iron in part to enhance erythropoiesis to meet the needs of both mother and fetus for tissue oxygenation. Erythropoiesis requires an increase in both erythrocyte cell production as well as the availability of iron to support adequate circulating haemoglobin (Hb) concentrations.[8,9] VAD may contribute to anaemia by adversely affecting iron absorption, storage, release or transport into the marrow, and may also be associated with inflammation-induced sequestration of iron.[7,8] Additionally, VAD may affect differentiation of erythrocyte precursors or their maturation.[7,8]

Insulin-like growth factor (IGF-1) also functions in erythropoiesis, particularly in the stimulation of erythro-

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cyte maturation, a complex and multi-step process.[10] Receptors for IGF-1 are found in both erythrocyte precursors and mature erythrocytes,[11] and studies have demonstrated erythropoietic activity of IGF-1 that is independent of erythropoietin.[11,12]

Although no data currently exist to link VA or its metabolites to circulating IGF-1 during pregnancy, associations between VA status and IGF-1 have been observed in infants[13] and adults.[14,15] The release of IGF-1, primarily from the liver, is typically stimulated by the release of growth hormone from the pituitary gland. During pregnancy circulating IGF-1 may be responsive to placental as well as pituitary growth hormone production.[16] The growth hormone/IGF-1 axis is sensitive to protein-energy nutritional status[17,18] and may also be influenced by VA status. Retinoids, the active metabolites of VA, stimulate growth hormone secretion from pituitary cells in experimental animals.[19-21]

In this study, we examined IGF-1 concentrations in archived serum samples in relation to VA, iron, and anaemia status of pregnant women of rural Nepal who had participated in a randomized, placebo-controlled trial of VA or BC supplementation from 1994-1997.[2] We first determined whether IGF-1 was associated with VA status. Second, we examined the association of maternal iron status, Hb, and anaemia with IGF-1. Finally, we explored the extent to which IGF-1 might function in the pathway through which improved VA status could enhance circulating Hb.

METHODS

NNIPS trial protocol

The data for this analysis were derived from a subsample of women enrolled in a double-blind, placebo controlled, cluster randomized trial conducted in the District of Sarlahi in the southern plains of Nepal from 1994 to 1997 to assess the efficacy of a continuous weekly dose of either VA or C on reproductive outcomes (Nepal Nutrition Intervention Project - Sarlahi, or NNIPS). The trial was carried out in a contiguous area of ~400 km^2 with a total population of approximately 176,000 divided into 270 wards. A total of 44,646 women of reproductive age were recruited into the supplementation and pregnancy surveillance activities as described previously.[2] Participating women received a weekly supplement containing VA (7,000 g retinol equivalents), BC (42 mg) or placebo based on the random assignment of treatment by ward, with the dosage intended to provide the equivalent of one daily recommended dietary allowance during pregnancy and lactation.[2] The participants' menstrual status, pregnancy status and vital events were tracked on a weekly basis. Newly pregnant women were enrolled into a protocol that included a mid-pregnancy, home-based assessment, and women were followed through 3 months postpartum.

A subsample of pregnant women, derived from about 10% of the study area, was selected for additional mid-pregnancy measures, including anthropometry and blood collection, which were conducted in a centrally located research clinic.[2,22,23] A total of 1,431 women were enrolled in this substudy, and an analyzable blood sample was collected from 1,186 women during pregnancy, forming the sampling frame for the study of IGF-1 concentrations reported here, from which n=589 samples were then randomly selected based on available resources for the IGF-1 analysis.

The study protocol was reviewed and approved by the Nepal Health Research Council in Kathmandu, Nepal, and the Committee on Human Research at the Johns Hopkins School of Public Health, Baltimore, MD, and consent was obtained prior to the collection of data.

Blood collection

Blood was collected into 7-mL trace metal-free vacuum blood collection tubes (Vacutainer, Becton Dickinson Company, Franklin Lakes, NJ, USA). Prior to processing, whole blood was used for haemoglobin (Hb) (Hemocue Hb, Sweden) and erythrocyte zinc protoporphyrin (ZP) (AVIV Biomedical, Inc., Lakewood, NJ, USA) assessment using rapid testing devices. Blood was then centrifuged at 750 x g for 20 mins to separate the serum. Aliquots of serum were stored in liquid nitrogen tanks in trace element-free cryotubes (Nalgene Company, Sybron International, New York, NY, USA) and shipped to the Johns Hopkins Bloomberg School of Public Health, Centre for Human Nutrition in Baltimore, MD, where they were stored at -80°C until later analysis.

Laboratory analysis

A random sample of approximately half of the available serum samples was selected for this study, such that IGF-1 concentrations were available for n=589 Nepalese women in mid-pregnancy. Serum IGF-1 was measured using an automated solid-phase chemiluminescent immunometric assay (Immulite 1000®, Siemens, Los Angeles, CA, USA) in the Centre for Human Nutrition laboratory at Johns Hopkins University. Inter-assay C.V. for the assay was 6.7%. Serum ferritin had previously been measured by fluorometric immunoassay (Delfia System, Wallac, Inc., Gaithersburg, MD, USA). Intra- and inter-assay CV's for ferritin were within 7.9% and 11.5%, respectively.[22] Retinol and BC were measured by reverse-phase high performance liquid chromatography (Beckman, System Gold, Columbia, MD, USA) with a Spherisorb ODS23, 150 x 4.6 mm column (Alltech Associates, Deerfield, IL, USA), and detected at wavelengths of 325 nm and 450 nm respectively, as previously described.[23]

Statistical analysis

Data on baseline and biochemical characteristics of the study subjects combined and by supplementation group were summarized as mean±SD, geometric mean and SD, or percent of affected individuals. Anaemia was defined as Hb <110 g/L, and further sub-classified as mild (90-109 g/L), or moderate to severe (<89 g/L). Cut-offs used to define iron deficiency were ZP >70 mol/mol heme, as ZP accumulates in red blood cells when iron is unavailable to produce Hb, and serum ferritin <10 g/L, indicative of exhausted body iron stores. Mid-upper arm circumference <22.0 cm was considered indicative of undernutrition.[24] Gestational age was estimated using information on the last menstrual period.

Differences in subject characteristics, including IGF-1

concentration, by assigned intervention group were initially explored by regression, with intervention groups expressed as dummy variables and robust standard errors calculated to account for randomization unit clusters. Ultimately, however, biological associations between VA status and IGF-1 were explored by combining intervention groups and examining serum retinol itself in association with IGF-1. This approach was justified by the substantial overlap in the distributions of serum retinol observed among intervention groups, the normal distribution of retinol when examined among intervention groups combined, and an assumption that any changes to IGF-1 were driven by a biological relationship between serum retinol and the growth hormone axis. This assumption was substantiated by examining regression models that included the intervention groups to demonstrate their lack of influence on the strength of associations between retinol and outcomes (IGF-1 and iron status), as well as examining interactions between intervention groups and serum retinol on outcomes to show that associations of retinol with outcomes did not differ by intervention status. These additional models are not shown. For ease of interpretation of regression models, outcomes were scaled to correspond to increments of 0.1 mol/L retinol or 10 g/L IGF-1, where appropriate. Associations of other maternal characteristics with IGF-1 and iron status were determined using correlation analysis, and those significantly (p<0.05) associated with IGF-1 were included as covariates when examining the association of retinol with IGF-1.

To determine whether iron status indicators or Hb were associated with increased circulating IGF-1, women were categorized by quartiles of IGF-1 concentration, and trends in Hb, ZP and ferritin (both ln-transformed) were examined using regression analysis with dummy variables for increasing quartiles of IGF-1 or chi^2 analysis for categorical data. Logistic regression was used to determine odds ratios for anaemia, mild anaemia (versus absence of anaemia), moderate-to-severe anaemia (versus absence of anaemia), and iron deficiency defined by accumulated erythrocyte ZP (>70 mol/mol heme) or exhausted iron stores (ferritin <10 g/L), with respect to increasing categories of IGF-1. Again, robust standard errors were estimated in these regression models to account for randomization unit clusters since both the VA and BC interventions had the potential to affect iron status indicators and IGF-1.

To examine the extent to which retinol worked through IGF-1 to affect Hb concentration, and the extent to which IGF-1 affected changes in Hb through iron status indicators versus alternative, unassessed pathways (assumed to be the production of erythropoietic precursors), path analysis was conducted using the "pathreg" command in STATA 12.0 (UCLA Academic Technology Services. Stata FAQ: Path Analysis. Internet: http://www.ats.ucla.edu/stat/stata/faq/pathreg.htm, accessed 10 March 2012).[25] Regression equations were developed for pathways describing Hb, ferritin, ZP, and IGF-1 as outcomes, with retinol working through both direct and indirect (eg. via IGF-1 or iron status indicators) pathways to affect Hb. The strength of direct associations between variables was represented by standardized -coefficients, while indirect associations were calculated as the product of all standardized-coefficients in the path from the variable of interest to haemoglobin as the outcome.[26]

All analyses were completed using STATA 12.0 (STATA Corp, College Station, TX).

RESULTS

Subject characteristics for the sample of women are shown in Table 1 in total and by assigned intervention. Women tended to be undernourished as assessed by low height, weight, and skinfold measures. Nearly half (44.1%) of the women had mid-upper arm circumferences indicative of undernutrition. There were no differences in maternal age, gestational age at enrollment, parity, or anthropometric indicators across intervention groups, with the exception of literacy rate, which was lowest among the PL group (8.6%) but was low among all groups (14.9% of all women literate; not shown). Gestational age at the time of sample collection averaged 19.0±6.6 weeks, with a range from the 5th to 95th percentile of 10.3 to 32.6 weeks.

Among the biochemical variables, retinol differed by intervention group (p<0.0001), with highest concentrations of circulating retinol among women receiving VA (Table 1). This was expected and previously observed in the larger group of women from whom the participants in this study were sampled.[2,23] However, retinol distributions overlapped substantially, such that ranges for serum retinol by supplementation group were: placebo, 0.11-2.33 mol/L; VA, 0.38-2.19 mol/L; BC, 0.20-3.07 mol/L.

IGF-1 concentrations ranged from 26-335 g/L among all women, with a modest suggestion of an intervention effect as it increased across groups from PL to BC to VA (p=0.09) (Table 1). Conversely, mean values for iron status indicators did not differ by intervention group in this sample. Iron deficiency and anaemia were common. In this sample, mild anaemia occurred in 50.0% of women, with moderate-to-severe anaemia in 21.6% of women. The proportion of women who were anaemic was slightly lower in the VA (69.2%) and BC (68.4%) groups compared with placebo (76.2%), (p=0.09 for logistic regression). Iron deficiency was common, with 65% of women having elevated ZP and 55% with serum ferritin <10 g/L, although these proportions did not differ by intervention status.

Correlation analyses showed that IGF-1 was positively associated with serum retinol (r=0.22, p=0.0001). Serum IGF-1 declined with increasing age (r=-0.30, p<0.0001) and parity (r=-0.31, p<0.0001). It also increased in association with maternal body weight (r=0.16, p=0.001) and other measures of maternal anthropometry, including mid-upper arm circumference (r=0.12, p=0.0002), triceps (r=0.13, p=0.0002) and subscapular skinfolds (r=0.17, p=0.0001), but less so for arm muscle area (r=0.07, p=0.07) and maternal height (r=0.09, p=0.13). IGF-1 was not associated with gestational age (r=-0.01, p=0.8). When the significant determinants of IGF-1 were examined simultaneously in a multiple linear regression model (with weight having the strongest association and thus used as a proxy for nutritional status), each variable retained statistical significance (Table 2). However, among all variables, retinol was most strongly associated with IGF-1, such that each 0.1 mol/L increment in circulat-

Table 1. Characteristics of Nepalese pregnant women at study enrolment and by intervention group

		All	By intervention		
		(N=589)	PL (N=198)	BC (N=196)	VA (N=195)
Demographics	Age[†], y	24.3±5.3	24.7±5.0	24.4±5.6	23.9±5.3
	Gestational age[†], wk	19.0±6.6	18.9±6.9	19.1±6.2	19.1±6.8
	Parity[†], %				
	0	23.8	20.3	26.3	24.7
	1-3	50.9	53.3	49.5	50.0
	≥4	25.3	26.4	24.2	25.3
Anthropometry	Height, cm	150±5.2	150±5.2	150±5.6	150±4.8
	Weight, kg	43.5±5.4	43.5±5.2	43.3±5.4	43.7±5.8
	MUAC, cm	22.3±1.8	22.3±1.6	22.2±1.7	22.2±2.0
	Triceps skinfold, mm	8.6±2.7	8.5±2.5	8.7±2.7	8.8±3.0
	Subscapular skinfold, mm	11.9±3.6	12.0±3.7	12.0±3.5	11.9±3.8
	Arm muscle area[†], cm^2	24.1±4.3	24.5±4.1	23.8±4.6	23.8±4.6
Biochemistry	Retinol [†,‡], µmol/L	1.12±0.39	1.00±0.36[a]	1.08±0.40[a]	1.29±0.36[b]
	IGF-1, µg/L	130±50.3	122±49.5	131±52.8	137±47.6
	Hb[†], g/L	100±16	98±15	101±17	101±14
	ZP[†], µmol/mol heme	90 (54, 150)	88 (52, 151)	95 (56, 161)	87 (55, 140)
	Ferritin[†], µg/L	10 (4, 22)	9 (4, 20)	9 (4, 21)	10 (4, 24)

PL: placebo; VA: vitamin A supplement group; BC: beta-carotene supplement group; MUAC: mid-upper arm circumference; IGF-1: insulin-like growth factor-1; Hb: haemoglobin; ZP: zinc protoporphyrin; data expressed as mean±SD, geometric mean (-1SD, +1SD) for ZP and ferritin, or % for categorical variables.
[†]Missing data: age, n=3; gestational age, n=3; parity, n=8; retinol, n=1; Hb, n=1; ZP, n=3; ferritin, n=36.
[‡]Significant (p<0.05) between-group differences by Wald's test noted with superscripted letters.

Table 2. Serum retinol as a determinant of circulating IGF-1 and iron status indicators among pregnant Nepalese women

	IGF-1[†], g/L		Hb[‡], g/L		ZP[§], ln (mol/mol)		Ferritin[¶], ln (g/L)	
	(95% CI)	p value	(95% CI)	p value	(95% CI)	p value	(95% CI)	p value
Serum retinol, per 0.1 mol/L	2.6 (1.5, 3.7)	<0.001	0.70 (0.36, 1.03)	<0.001	-0.025 (-0.037, -0.013)	<0.001	0.014 (-0.006, 0.033)	0.16
Gestational age, wk	-0.1 (-0.8, 0.7)	0.8	-0.44 (-0.66, -0.23)	<0.001	0.012 (0.006, 0.020)	0.001	-0.027 (-0.039, -0.017)	<0.001
Maternal age, y	-1.4 (-2.6, -0.2)	0.03	---	---	---	---	---	---
Parity, rel to 0								
1-3	-18.9 (-32.5, -5.3)	0.008	---	---	---	---	---	---
≥4	-27.0 (-45.2, -8.8)	0.005						
Maternal weight, kg	0.8 (0.04, 1.6)	0.04	---	---	---	---	---	---

Based on multiple linear regression with any significant covariates in the model, and robust standard errors calculated to generate 95% confidence intervals; IGF-1: insulin-like growth factor-1; Hb: haemoglobin; ZP: zinc protoporphyrin; CI: confidence interval.
[†]N=574, F=24.0, R^2=0.18, p<0.0001; [‡]N=585, F=19.5, R^2=0.07, p<0.0001;
[§]N=583, F=16.3, R^2=0.07, p<0.0001; [¶]N=550, F=23.3, R^2=0.06, p<0.0001.

ing retinol was associated with a 2.6 g/L increase in circulating IGF-1 (p<0.0001).

Among these variables, only retinol and gestational age were associated with iron status indicators. Increments of retinol were significantly associated with improved iron status indicators, with the exception of ferritin, with adjustment for gestational age at the time of sample collection (Table 2).

IGF-1 and Hb, anaemia, and iron status

Iron status indicators and the percent of participants in given anaemia and iron status categories are shown by increasing quartile of IGF-1 in Table 3. Mean values for all indicators improved over increasing quartiles of IGF-1, although not significantly so for ferritin. Similarly, the prevalence of anaemia and iron deficiency as assessed by ZP>70 mol/mol heme declined over increasing quartiles of IGF-1, while the decline in iron deficiency as assessed by ferritin <10 g/L was not significant. The decline in anaemia with increasing quartiles of IGF-1 was dramatic for moderate-to-severe anaemia, the prevalence of which declined from 36.7% to 13.6% from the 1st to 4th quartile of IGF-1, but not for mild anaemia.

These associations were confirmed by examining the likelihood of anaemia and iron deficiency as expressed by odds ratios (Figure 1). Odds of having anaemia declined 68.8% from the first to the 4th quartile of IGF-1. This was primarily associated with declines in moderate-to-severe anaemia, whose odds were reduced by 53.3% from the first to second quartile, and by 84.0% from the first to

Table 3. Indicators of iron status by quartile of IGF-1 concentration

	IGF-1				*p*-value†
	Q1	Q2	Q3	Q4	
IGF-1, g/L	76±13	106±9	139±9	199±38	-----
Hb, g/L	93±19	99±15	102±13	105±12	<0.001
Hb <110 g/L	82.3%	77.0%	67.1%	59.2%	<0.001
Hb ≥90 and <110 g/L	45.6%	54.7%	53.4%	45.6%	
Hb <90 g/L	36.7%	22.3%	13.7%	13.6%	<0.001
ZP, µmol/mol heme	103 (60, 175)	92 (55, 155)	86 (53, 142)	80 (50, 129)	0.002
ZP >70 mol/mol heme	72.6%	68.7%	65.1%	56.5%	0.03
Ferritin, g/L	8.2 (3.6, 18.8)	8.9 (4.2, 18.9)	10.2 (4.40, 23.9)	11.0 (4.7, 25.9)	<0.06
Ferritin <10 g/L	61.0%	55.4%	53.7%	50.0%	0.3

Q: quartile; IGF-1: insulin-like growth factor-1; Hb: hemoglobin; ZP: zinc protoporphyrin.
†For continuous data, multiple linear regression with robust standard errors was used to test for differences among groups, with quartiles expressed as dummy variables and Q1 as the reference group; for categorical data, chi^2 analysis was used to test for differences among groups, including a 3 x 4 table of no, mild, and moderate anaemia by IGF-1 quartile.

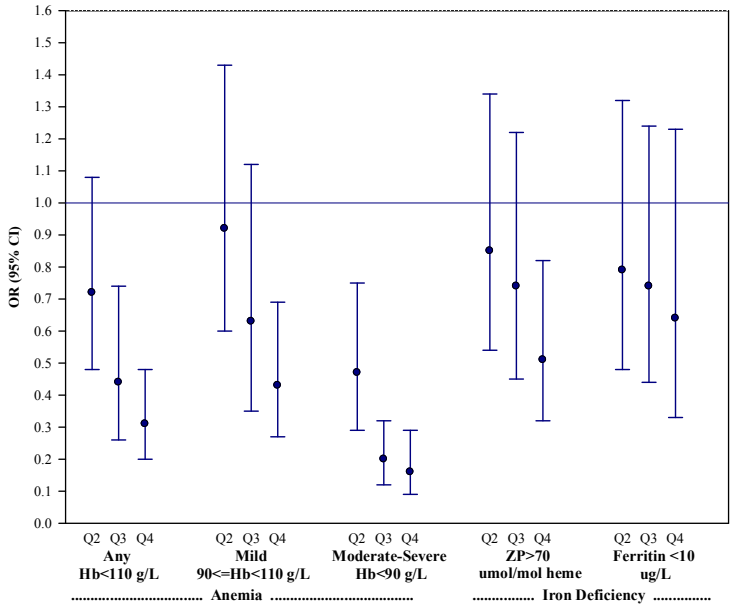

Figure 1. Odds ratios and 95% CI for risk of any anaemia, or mild or moderate anaemia (versus no anaemia), or iron deficiency by elevated zinc protoporphyrin (ZP) or low ferritin (versus not) with increasing quartile of IGF-1 concentration. The figure depicts comparisons relative to the lowest quartile of IGF-1 concentration as the referent group. Odds ratios and 95% CI's were determined by logistic regression with robust standard errors calculated.

fourth quartile. Odds of iron deficiency defined by elevated ZP declined by 51.1% in the fourth quartile of IGF-1, but the effect on low ferritin was not significant.

IGF-1 in the path between retinol and Hb

Coefficients associated with the variables in the path analysis are shown in Table 4, with corresponding biological pathways presumed to exist illustrated with the standardized-coefficients in Figure 2. Pathways to explain Hb concentration included those directly from retinol, ZP, and IGF-1. ZP and IGF-1 retained their significant association with Hb in the path regression model. In turn, retinol, ferritin (a proxy for body iron availability), and IGF-1 were all significantly associated with ZP, while IGF-1, but not retinol, was only modestly associated with ferritin.

Table 5 summarizes the strength of the various cumulative paths from IGF-1 or retinol to Hb. IGF-1 did not appear to improve Hb through iron status indicators to a great extent, with the pathway through ferritin yielding the lowest explained association (0.023 or 8.9% of the total IGF-1 effect on Hb), and with an intermediary role through ZP (0.052 or 20.2% of the total IGF-1 effect on Hb). The "direct" path from IGF-1 to Hb yielded the strongest association (0.182), explaining 70.8% of the total association of IGF-1 with Hb.

The total association of retinol with Hb (0.203) was less than that of the IGF-1-Hb association (0.257). Retinol appeared to function through a variety of pathways to a modest extent, with the pathway through ZP being the strongest (0.089), at 43.8% of the total association of retinol with Hb, and the pathway through IGF-1 (0.052) accounting for 25.6%, or about one quarter, of the association of retinol with Hb. The "direct" pathway, that unexplained by any of the other variables examined here, was modest (0.045, or 22.2% of the total path from retinol to Hb), and the pathway through ferritin was weakest (0.017, or 8.4% of the total path from retinol to Hb).

Table 4. Nested regression analysis of pathways from retinol and IGF-1 to haemoglobin in Nepalese women (n=548)

Outcome	Variable	β-Coefficient	SE	*p*-value	R^2	Standardized β-Coefficient
Hb, g/L	Retinol, 0.1 µmol/L	0.170	0.122	0.161	0.47	0.045
	ZP, ln (µmol/mol heme)	-18.1	0.93	<0.001		-0.621
	IGF-1, per 10 µg/L	0.53	0.09	<0.001		0.183
ZP, mol/mol	Retinol, 0.1 µmol/L	-0.019	0.005	<0.001	0.20	-0.144
	Ferritin, ln (µg/L)	-0.238	0.024	<0.001		-0.386
	IGF-1, per 10 µg/L	-0.008	0.004	0.03		-0.083
Ferritin, g/L	Retinol, 0.1 µmol/L	0.015	0.009	0.10	0.02	0.071
	IGF-1, per 10 µg/L	0.015	0.007	0.03		0.096
IGF-1, per 10 g/L	Retinol, 0.1 µmol/L	0.261	0.054	<0.001	0.04	0.201

IGF-1: insulin-like growth factor-1; Hb: haemoglobin; ZP: zinc protoporphyrin; SE: standard error. Conventional-coefficients, SE, *p*-values, and R^2 were generated from multiple linear regression models for the outcomes and independent variables listed. Standardized-coefficients are generated by setting the distributions of each variable to a mean of 0 and a standard deviation of 1 in the regression models, and they represent the strength and direction of association between the variable and outcome with a maximum absolute value of 1. The standardized-coefficients are displayed on Figure 2 to demonstrate the strength of the path segments between retinol or IGF-1 and Hb. Data in the table were generated using the pathreg command in Stata (UCLA Academic Technology Services. Stata FAQ: Path Analysis. Internet: http://www.ats.ucla.edu/stat/stata/faq/pathreg.htm; accessed 2012/3/10).

Table 5. Pathways through which IGF-1 and retinol are associated with maternal Hb during pregnancy in Nepalese women (n=548)

Route	Pathway	Calculation	Strength of association	Contribution of path, %
IGF-1 to Hb	Direct		0.182	70.8
	Through ZP	-0.083 x -0.621	0.052	20.2
	Through Ferritin	0.096 x -0.386 x -0.621	0.023	8.9
	Total	0.182 + 0.052 + 0.023	0.257	100
Retinol to Hb	Direct		0.045	22.2
	Through ZP	-0.144 x -0.621	0.089	43.8
	Through Ferritin	0.072 x -0.386 x -0.621	0.017	8.4
	Through IGF-1	0.201 x 0.257 (total from above)	0.052	25.6
	Total	0.032 + 0.078 + 0.009 + 0.056	0.203	100

Hb: haemoglobin; IGF-1: insulin-like growth factor-1; ZP: zinc protoporphyrin. The total strength of association of IGF-1 with haemoglobin was calculated as the sum of the products of all the standardized -coefficients for each path between IGF-1 and Hb depicted in Figure 2. The contribution of each individual path to the total strength of association between IGF-1 and Hb could then be expressed as a percent. Pathways from retinol to Hb were similarly assessed.

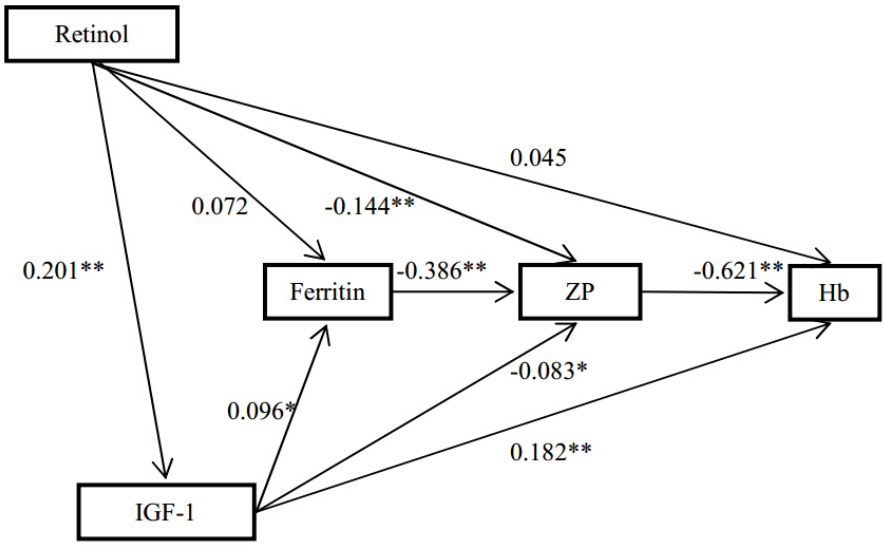

Figure 2. Proposed pathways and corresponding standardized-coefficients for associations through which circulating serum retinol and insulin-like growth factor-1 (IGF-1) may improve haemoglobin (Hb) among pregnant women of Nepal, accounting for maternal gestational age. ZP is zinc protoporphyrin. Statistical significance of the coefficients is noted by *, $p<0.05$, and **, $p<0.001$. The -coefficients were derived from related regression models as shown in Table 4, and strength of the various possible paths is shown in Table 5 as the product of the standardized -coefficients along the paths of interest.

DISCUSSION

This study of women of rural Nepal establishes a relationship between circulating retinol and IGF-1 during pregnancy; moreover, IGF-1 was associated with increased haemoglobin concentration and decreased risk of anaemia and iron deficiency as defined by elevated erythrocyte ZP. Enhancing Hb through an elevation in IGF-1 appears to be one of several biological paths by which improved VA status can function to reduce anaemia during pregnancy. We hypothesize that IGF-1 primarily functioned by stimulating erythrocyte production, consistent with experimental data in the literature, with the modest degree through which IGF-1 functioned through an indicator of iron availability (ie., ferritin), and the extent to which IGF-1 functioned through an indicator of erythropoietic activity (ZP) and other unexplained pathways.

Low circulating IGF-1 occurs commonly in protein-energy malnutrition, fasting states, and other clinical conditions that affect overall nutritional status.[18,27] Consistent with this, IGF-1 in this study was positively associated with maternal arm circumference, skinfolds, and body weight. Moreover, concentrations of IGF-1 among women in this study were lower than average values reported among women in developed countries, who have been shown to have mean IGF-1 concentrations of ~200 g/L in mid-pregnancy, with ranges of ~100-300 g/L.[16,28] Serum IGF-1 also declined with increasing age among the Nepalese women, with the highest values in the teenage years, as observed in healthy non-pregnant reference populations.[29] It also declined with increased parity regardless of age; an association of IGF-1 with parity has been reported in a developed country setting,[30] although not consistently.[28] No association of IGF-1 with gestational age was observed, although if one existed in this setting we would have expected to detect it based on a wide distribution of gestational ages and presence of expected gestational age effects on iron status indicators.

While an association of IGF-1 with protein-energy status is well accepted, our study demonstrated an association of IGF-1 with VA status during pregnancy. Although the association of IGF-1 with supplementation with VA or BC in the parent trial of this study was only suggestive, a strong association of IGF-1 with retinol persisted even after adjustment for other variables that were associated with IGF-1. Only recently have studies demonstrated an association between VA intake or serum retinol and IGF-1 in adult populations,[14,15] motivated by concern about the potential role of the proliferative activity of IGF-1 in cancer generation. On the other hand, in Indonesian infants, in whom IGF-1 is expected to promote appropriate growth, serum retinol and BC were both positively associated with circulating IGF-1, while zinc status was not.[13] The association of increased IGF-1 in relation to circulating retinol is consistent with findings relating retinoids with the stimulation of the growth hormone axis.[31] As well, studies in rodent and quail models have found a direct link between VA depletion and lower circulating IGF-1 concentrations.[32,33]

In humans during pregnancy, circulating IGF-1 is produced by both the liver and placental tissue[34] and may primarily reflect the production of placental rather than pituitary growth hormone.[16] VA metabolites have been shown to promote essential placental tissue and amniotic membrane development through a variety of retinoid signalling functions.[35-37] As such, enhanced IGF-1 in this population may serve as a marker of improved placental function among women whose VA status was improved, although no direct benefit of supplementation with either VA or BC was observed on outcomes such as birth weight, fetal loss, or infant mortality in the parent trial.[38]

We did, however, show a notable association of enhanced maternal IGF-1 concentration with improved circulating Hb and declines in the risk of anaemia, particularly moderate-to-severe anaemia. These outcomes improved incrementally with increasing quartiles of IGF-1 concentration. Similar associations of IGF-1 with hematologic status have been observed in other population groups. Cross-sectional studies have found positive associations of IGF-1 and Hb among female adolescents as well as the elderly.[39,40] Among children with Laron syndrome, which results from dysfunction of growth hormone signalling, treatment with IGF-1 resulted in a dramatic increase in Hb concentrations from 115 to 130 g/L,[41] and in anaemic haemodialysis patients, erythropoietin therapy increased serum concentrations of IGF-1; and IGF-1, but not erythropoietin, was associated with increased hematocrit.[42] More recently, a population-based study of healthy adults found a positive association between circulating IGF-1 and Hb, and found circulating IGF-1 to be protective against anemia.[43]

Mechanistically, substantial evidence links IGF-1 with the process of erythropoiesis. Experimental data have shown that IGF-1 demonstrates erythropoietic activity distinct from erythropoietin,[11,12] although it may require additional primary regulators of erythropoiesis to stimulate erythroid colony forming units.[12] The reported mechanism through which IGF-1 enhances erythropoiesis in hemopoietic cells is via intrinsic tyrosine kinase phosphorylation of IGF-1 and insulin receptors.[10,44]

A variety of data exist linking VAD to anaemia risk, although mechanisms to explain this association have not been well delineated. Our data show that while the link between serum retinol and improved anaemia status is modest in this population, retinol likely works in a variety of ways to improve circulating haemoglobin. The path through IGF-1 explained ~1/4 of the association of retinol with Hb. An association of either retinol or IGF-1 to Hb was only weakly explained by improvements in ferritin, suggesting mechanisms that are not strictly dependent on the availability or utilization of iron stores. Stronger evidence existed for the path from retinol through improved ZP, which declines when erythropoiesis functions properly. In turn, the majority (~2/3) of the pathway between IGF-1 and Hb was not explained by changes in either iron status indicator, consistent with a mechanism of action distinct from improving iron availability. The stronger association of IGF-1 with protoporphyrin compared with ferritin supports a supposition that IGF-1 works by enhancing erythopoietic activity, as erythrocyte ZP accumulates relative to heme, the product of the incorporation of iron with protoporphyrin, when iron is unavailable to maturing red blood cells.

A limitation of the study is that we do not have a direct measure of erythropoietic activity, such as the reticulo-

cyte production index.[45] Additionally, given the cross-sectional design of the analysis, we cannot dismiss the possibility that in this setting retinol-induced improvements in maternal Hb led to conditions (less fatigue and greater activity) allowing for the enhanced production of IGF-1 (through greater fitness and muscle mass), despite the consistency of our hypothesis with current available evidence. And finally, links between circulating retinol and IGF-1 concentrations during pregnancy were explored in an observational manner despite the context of a randomized trial, where weekly VA supplementation itself showed only a modest impact on IGF-1 concentrations in mid-pregnancy. Nonetheless, our results demonstrate that IGF-1 may be an important marker to consider for assessing maternal health during pregnancy, particularly in populations at risk of both VA and iron deficiencies. Our intriguing findings provide a look at a possible intermediary role for IGF-1 in alleviating the iron deficiency anemia that is enhanced by VAD. The links between VA status, the growth hormone/IGF-1 axis, improvements in anemia status, and their potential for improving the health of mothers and infants, warrant future study and refinement.

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AUTHOR DISCLOSURES

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Original Article

Circulating IGF-1 may mediate improvements in haemoglobin associated with vitamin A status during pregnancy in rural Nepalese women

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循环 IGF-1 可能介导了尼泊尔农村妇女怀孕期间的维生素 A 状况相关的血红蛋白的提高

怀孕加剧了发展中国家妇女维生素 A 的缺乏和贫血。提高循环血红蛋白（Hb）需要红细胞的生成和可利用的铁。胰岛素样生长因子-1（IGF-1）在红细胞生成中发挥其功能，但是尚没有其与维生素 A 状况和妊娠相关贫血之间关系的研究。本研究的目的是在 1994 年到 1997 年间在尼泊尔农村进行的补充维生素 A 和 β-胡萝卜素（BC）的安慰剂-对照试验现存的样本中，研究怀孕妇女血清视黄醇、IGF-1 和 Hb 之间的关系。从 1186 名有人体测量、维生素 A（视黄醇）和铁（血红蛋白、红细胞锌原卟啉和铁蛋白）的资料的妇女中，随机测定 589 名妊娠中期妇女的血清 IGF-1。采用多重线性回归和 logistic 回归来确定 IGF-1 和视黄醇、Hb 或贫血与铁营养状况之间的关系。采用路径分析探讨 IGF-1 作为视黄醇和 Hb 之间介物的作用来评估铁营养状况。观察到视黄醇每增加 0.1 mol/L，IGF-1 增加 2.6 g/L（p<0.0001）。Hb 随着 IGF-1 四分位的增加而增加，并且从第 1 个四分位到第 4 个四分位，贫血风险降低了 68.8%。铁营养状况改善的指标只能解释 IGF-1 和 Hb 之间关系的 29.1%，而 IGF-1 能够解释视黄醇和 Hb 之间关系的 25.6%。虽然 IGF-1 主要通过独立改善铁营养状态指标的途径发挥作用，增加的 IGF-1 通过视黄醇改善尼泊尔农村怀孕妇女的循环 Hb 也可能是机制之一，可能是通过刺激红细胞生成。

关键词：维生素 A、胰岛素样生长因子-1、贫血、妊娠、血红蛋白