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Prenatal Lipid-Based Nutrient Supplements Increase Cord Leptin Concentration in Pregnant Women from Rural Burkina Faso[1–3]

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Abstract

In developing countries, prenatal lipid-based nutrient supplements (LNSs) were shown to increase birth size; however, the mechanism of this effect remains unknown. Cord blood hormone concentrations are strongly associated with birth size. Therefore, we hypothesize that LNSs increase birth size through a change in the endocrine regulation of fetal development. We compared the effect of daily prenatal LNSs with multiple micronutrient tablets on cord blood hormone concentrations using a randomized, controlled design including 197 pregnant women from rural Burkina Faso. Insulin-like growth factors (IGF) I and II, their binding proteins IGFBP-1 and IGFBP-3, leptin, cortisol, and insulin were quantified in cord sera using immunoassays. LNS was associated with higher cord blood leptin mainly in primigravidae (+57%; $P = 0.02$) and women from the highest tertile of BMI at study inclusion (+41%; $P = 0.02$). We did not find any significant LNS effects on other measured cord hormones. The observed increase in cord leptin was associated with a significantly higher birth weight. Cord sera from small-for-gestational age newborns had lower median IGF-I (−9 μg/L; $P = 0.003$), IGF-II (−79 μg/L; $P = 0.003$), IGFBP-3 (−0.7 μg/L; $P = 0.007$), and leptin (−1.0 μg/L; $P = 0.016$) concentrations but higher median cortisol (+18 μg/L; $P = 0.037$) concentrations compared with normally grown newborns. Prenatal LNS resulted in increased cord leptin concentrations in primigravidae and mothers with higher BMI at study inclusion. The elevated leptin concentrations could point toward a higher neonatal fat mass. J. Nutr. 143: 576–583, 2013.

Introduction

Newborns with intra-uterine growth retardation (IUGR)[9] are at increased risk of morbidity and mortality during infancy (1,2) and chronic diseases in later life (3). Low-income countries are particularly affected by IUGR and represent 95% of all cases worldwide (4).

Endocrine factors, such as those in the insulin-like growth factor (IGF)-axis, play an important role in intrauterine growth regulation (5). Many studies have shown associations between birth size and cord blood concentrations of insulin, IGF-I, and to a lesser extent IGF-II (6–8). Whereas animal models have demonstrated that IGF-II is mainly associated with early embryonic and placental growth regulation (9), IGF-I appears to be the dominant growth regulator in late gestation. Specific IGF-binding proteins (IGFBP) influence the availability of free IGFs but also exhibit IGF-independent actions (10,11). Among the 6 identified IGFBPs, IGFBP-1 and IGFBP-3 correlate best with birth size. Concentrations of IGFBP-1 and IGFBP-3 are, respectively, higher and lower in cord blood of growth-retarded newborns (12). Cord leptin, produced by fetal adipocytes and placenta, has also been positively associated with birth size (13) and fetal fat mass (14,15). Moreover, growth-retarded newborns have higher cord cortisol (16,17) and lower cord insulin concentrations (12,18). Fetal insulin is related to nutrient availability and plays a mediating role in the IGF-axis (19).

Lipid-based nutrient supplements (LNSs) have been developed to complement the diet of vulnerable groups like young children and pregnant women in low-income countries (20). We previously reported that daily prenatal supplementation with LNS significantly increased birth length [4.6 mm (95% CI: 1.8, 7.3)] and placental weight [15.6 g (95% CI: 0.4, 30.7)] compared with multiple micronutrient supplements (MMNs) in a randomized, controlled trial including 1296 pregnant

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[3] This trial was registered at clinicaltrials.gov as NCT00909974.
[9] Abbreviations used: AGA, adequate-for-gestational age; Hb, hemoglobin; IGF, insulin-like growth factor; IGFBP-1, insulin-like growth factor binding factor; IUGR, intra-uterine growth retardation; LNS, lipid-based nutrient supplement; MMN, multiple micronutrient supplement; MUAC, mid-upper arm circumference; SGA, small-for-gestational age.
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women in Burkina Faso (21). The treatment effects of LNS on birth size showed important modifications by baseline maternal anemia [hemoglobin (Hb) <11.0 g/dL] and undernourishment (BMI <18.5 kg/m^2) with greater effects on birth length in anemic or undernourished gravids. We hypothesize that LNS supplementation increased birth size by endocrine regulation of fetal growth. This study had 2 objectives: *1*) to assess if LNS had an effect on IGF-I, IGF-II, IGFBP-1, IGFBP-3, leptin, cortisol, and insulin cord blood concentrations concomitant to the previously reported effects on birth length and placental weight, we further assessed if the previously reported effect modifications of LNS by maternal nutritional status and primgravidity are reflected by changes in cord hormone concentrations; and *2*) to examine associations between birth size anthropometry and cord hormone concentrations in a sub-Sahara African ecology.

Materials and Methods

Subjects and intervention. A subsample of umbilical cord sera was randomly selected from 1020 singleton pregnancies of women from the MISAME2 study, a randomized, controlled trial that compared the effects of prenatal LNS and MMN on birth size. This study was conducted from March 2006 to June 2008. The trial details and the composition of LNS and MMN are described elsewhere (21). Briefly, 1296 pregnant women from the catchment areas of 2 health centers from the Houndé district in Burkina Faso were randomized to receive either a daily tablet supplying 1 RDA of 15 micronutrients for pregnancy (MMN) or LNS supplying the same dose of multiple micronutrients (LNS). This locally produced LNS supplement consisted of peanut butter, soy flour, sugar, palm oil, and multiple micronutrients. One daily LNS dose (72 g) provided 372 kcal and 67 and 16% energy from fat and protein, respectively. Pregnant women were also randomized to 2 or 3 doses of sulfadoxine-pyrimethamine. The results of the malaria prophylaxis are reported elsewhere (22,23).

The number of analyzed samples was limited to 200 because of budgetary constraints. This allowed the detection of a difference of 0.4 SD between study groups, considered a medium effect size, with 80% statistical power and a 5% type I error.

Sample collection. At delivery, cord blood was sampled in a dry tube without preservative (60.610.001; Starstedt), allowed to clot at 4°C, and centrifuged at 3000 × *g* revolutions/min for 10 min. Samples were shipped to Belgium on dry ice, where they were stored at −20°C. After explaining the study's objectives and procedures, informed consent from the participants was obtained before study inclusion. The study was approved by the ethical committees of Centre Muraz, Bobo-Dioulasso, Burkina Faso, and the Institute of Tropical Medicine, Antwerp, Belgium.

Anthropometry. Maternal and newborns' anthropometry, gestational length, and Hb measurement procedures were described elsewhere (21,24). Rohrer's ponderal index was defined as 100 × birth weight (g)/birth length^3(cm^3). Small-for-gestational age (SGA) was considered a consequence of IUGR. SGA was defined as a birth weight for gestational age <10th percentile of a well-nourished reference population (25). Other newborns were classified as adequate-for-gestational age (AGA). Placentas were weighed untrimmed without removing the cord. Cords were clipped before measurement.

Cord hormone assays. Samples were analyzed in January 2009 at the clinical laboratory of Liège University Hospital in Belgium. Serum IGF-I was analyzed by chemiluminescence immunoassay on a Liaison autoanalyzer (DiaSorin). IGF-II and IGFBP-1 were analyzed by sandwich enzyme immunoassay using Webster, TX). IGFBP-3 was analyzed by sandwich enzyme immunoassay (ELISA) using the Quantikine human IGFBP-3 kit (R&D Systems). Leptin was analyzed by sandwich enzyme immunoassay using the Quantikine human leptin kit (R&D Systems). Insulin and cortisol were analyzed by electrochemiluminescence immunoassay on a Modular Analytics E170 autoanalyzer (Roche Diagnostics). The sensitivity of the assays was 3 ng/mL for IGF-I, 0.25 ng/mL for IGF-II, 0.25 ng/mL for IGFBP-1, 0.05 ng/mL for IGFBP-3, 7.8 pg/mL for leptin, 0.20 μU/mL for insulin, and 0.18 μg/L for cortisol. The intra-assay CV varied from 1.2 to 3.9% and was somewhat higher for IGF-1 (3.1–4.5%). All inter-assay CVs ranged from 1.7 to 5.5%.

Statistical analysis. Baseline variables are presented as means ± SDs for normally distributed data. All hormone concentrations showed positively skewed distributions and were $\log_{10}$-transformed to satisfy the assumption of homoscedasticity of the residuals from the multiple regression models with hormone concentrations as dependent variables.

Pearson's correlations were used to examine relationships among log-transformed hormone concentrations. Linear relationships were assessed by scatter plots.

For each hormone, we assessed the intervention effect and the relationship between prenatal variables on $\log_{10}$-transformed hormone concentrations. As a first step, we conducted bivariate analysis to assess the crude associations between all variables. Subsequently, we assessed multivariate associations by linear regression models including all variables with at least a modest ($P < 0.20$) relation with cord concentrations, after which a backward elimination procedure was performed using likelihood ratio testing ($P < 0.05$). Reported regression coefficients of the $\log_{10}$-transformed concentrations were back-transformed to the original scale and expressed as percentage change in concentration by one unitary change in the independent variable. Finally, we assessed if any imbalances in prenatal variables between study groups affected the intervention effect on cord hormone concentrations and results from adjusted analysis were reported in case the crude effect was modified by at least 10%.

Differences in median hormone concentrations between SGA and AGA were tested by the Mann-Whitney test. We examined the associations between each hormone concentration and birth anthropometry by multiple linear regression. Models were adjusted for gestational length, newborn sex, intervention, primigravidity, and malaria prophylaxis (model 1). The combined influence of hormone concentrations on birth anthropometry was explored by multiple linear regression (model 2). All hormone concentrations were included in a saturated regression model. The model was reduced using a backward elimination procedure by likelihood ratio testing at $P < 0.05$ significance. Collinearity was assessed by inspecting the variance inflation factors of each model

The effect modification of the intervention by primigravidity, maternal nutritional status [BMI and mid-upper arm circumference (MUAC) tertiles], and anemia (Hb <11 g/dL) at study inclusion on cord hormone concentrations was tested. The reason to do so was based on the described interactions in the main intervention trial (21). The results of these subgroup analyses are presented only at significance.

Significance of all tests was set at $P < 0.05$, except for interaction ($P < 0.10$). All analyses were performed in Stata 11.2 (StataCorp).

Results

Three of 200 samples were hemolized and excluded from analysis. **Table 1** presents baseline maternal characteristics and newborns' anthropometry of 197 singleton pregnancies. The maternal characteristics of this subsample were similar as in the main intervention study, except for higher baseline prevalence of anemia and lower mean Hb in the MMN group.

LNS resulted in higher birth weight, birth length, and placental weight by 182 g, 8 mm, and 37 g, respectively. IGF-I, IGF-II, IGFBP-3, insulin, and leptin were positively related to each other but negatively related to IGFBP-1 and cortisol (**Table 2**).

Results from the bivariate analysis showed that primigravidae had lower cord IGF-I ($P = 0.004$), IGF-II ($P < 0.001$), IGFBP-3 ($P = 0.003$), insulin ($P = 0.002$), and leptin ($P = 0.026$) and higher cortisol ($P < 0.001$) concentrations compared with multigravidae (**Table 3**). Maternal height ($P = 0.048$) and age ($P < 0.001$) were positively associated with cord IGF-II. Gestational length was positively associated with cortisol concentrations ($P = 0.049$). Cord leptin was positively associated with maternal MUAC ($P = 0.005$) and maternal Hb concentration at baseline ($P = 0.049$).

TABLE 1 Mother and newborn characteristics for the MMN and LNS study groups[1]

Characteristic	MMN (n = 101)	LNS (n = 96)
Maternal characteristics		
Three doses of sulfadoxine-pyrimethamine, *n (%)*	43 (42.6)	47 (49.0)
Age, *y*	24.1 ± 6.2	25.1 ± 6.3
Pregnancy trimester at enrolment, *n (%)*		
First	42 (41.6)	47 (49.0)
Second	55 (54.4)	43 (44.8)
Third	4 (4.0)	6 (6.2)
Primigravidae, *n (%)*	22 (21.8)	18 (18.8)
Weight, *kg*	55.5 ± 6.4	55.7 ± 7.7
Height, *cm*	162.6 ± 5.5	162.9 ± 6.3
BMI, kg/m^2	21.0 ± 2.2	20.9 ± 2.2
BMI <18.5 kg/m^2, *n (%)*	10 (9.9)	11 (11.5)
MUAC, *cm*	25.9 ± 2.2	26.1 ± 2.2
Hb, *g/dL*	10.9 ± 1.5	11.4 ± 1.2
Anemic (Hb <11 g/dL), *n (%)*	50 (49.5)	31 (32.6)
Newborn characteristics		
Male, *n (%)*	53 (52.5)	50 (52.1)
Gestational age at delivery, *wk*	39.5 ± 1.7	39.3 ± 2.1
Weight, *g*	2914 ± 374	3096 ± 404
Length, *mm*	478 ± 23	486 ± 23
Ponderal index, $100 \times g/cm^3$	2.68 ± 0.39	2.71 ± 0.32
Placental weight, *g*	563 ± 109	600 ± 117
Low birth weight, *n (%)*	13 (12.9)	6 (6.3)
SGA, *n (%)*	41 (41.6)	26 (27.1)
Preterm, *n (%)*	9 (8.9)	10 (10.4)
Head circumference, *cm*	33.7 ± 1.4	33.7 ± 1.5
Chest circumference, *cm*	32.0 ± 1.8	32.5 ± 1.7
MUAC, *cm*	10.2 ± 0.9	10.5 ± 0.9
Umbilical cord Hb,[2] *g/dL*	15.1 ± 2.0	15.5 ± 2.4
Cord serum		
IGF-I, *μg/L*	41.2 (31.6, 61.3)	44.1 (33.2, 59.7)
IGF-II, *μg/L*	424 (321, 555)	456 (345, 600)
IGFBP-1, *μg/L*	145 (87, 326)	149 (73, 326)
IGFBP-3, *μg/L*	6.09 (4.70, 7.66)	6.37 (4.94, 7.92)
Cortisol, *μg/L*	107 (84, 145)	113 (90, 144)
Insulin, *IU/L*	2.88 (1.63, 4.87)	2.97 (1.31, 5.37)
Leptin, *μg/L*	4.25 (2.87, 6.13)	4.63 (3.14, 8.11)

[1] Values are means ± SDs, median (IQR), or *n* (%). Hb, hemoglobin; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; LNS, lipid-based nutrient supplement; MMN, multiple micronutrient supplement; MUAC, mid-upper arm circumference; SGA, small-for-gestational age.
[2] Hb analysis available for 189 cords (98 in MMN group and 91 in LNS group).

Male newborns also had lower cord leptin concentrations (P = 0.001).

LNS supplementation increased cord leptin by 18% (P = 0.045) compared with MMN (Table 3). This difference decreased slightly to 16% (P = 0.059) after adjustment for covariates significantly associated with leptin concentrations (maternal MUAC, primigravidity, and sex). We observed that there was a baseline imbalance in maternal Hb between study groups; however, adjusting for this imbalance did not alter the LNS effect on leptin concentrations (difference <10%). There were no effects of LNS on other hormone concentrations.

The subgroup analysis described in the main study (21) was repeated to confirm whether factors modifying the effect of LNS on birth anthropometry influenced cord hormone concentrations. We therefore tested interactions of the intervention with primigavidity, baseline maternal anemia, and baseline maternal BMI (tertiles) on cord hormone concentrations. There was an interaction between intervention and primigravidity on cord leptin only (P-interaction = 0.007). In primigravidae, LNS increased cord leptin by 90% (P = 0.004) compared with MMN, whereas there was no difference in multigravidae (**Fig. 1**). This parallels the increase in ponderal index by LNS only in primigravidae (P-interaction = 0.057).

Compared with the multigravid mothers, the primigravid mothers were younger (18.2 ± 1.5 vs. 26.2 ± 5.9 y) but did not differ in height (162.6 ± 5.8 vs. 162.8 ± 5.9 cm), BMI (21.1 ± 2.4 vs. 20.9 ± 2.1 kg/m^2), or MUAC (25.9 ± 2.4 vs. 26.1 ± 2.2 cm).

There was an interaction between LNS and maternal BMI tertiles at study inclusion on cord leptin (P-interaction = 0.065), with the strongest positive effect in the highest maternal BMI tertile (**Fig. 2**). This mirrored the variations of the differences in ponderal index across maternal BMI tertiles, although this interaction did not reach significance (P-interaction = 0.125). The same analysis was performed with maternal MUAC tertiles at baseline and showed

TABLE 2 Associations between hormone concentrations in cord sera from pregnant women supplemented with MMN or LNS[1]

	IGF-I (μg/L)	IGF-II (μg/L)	IGFBP-1 (μg/L)	IGFBP-3 (μg/L)	Cortisol (μg/L)	Insulin (IU/L)
IGF-II (μg/L)	0.51[b]					
IGFBP-1 (μg/L)	−0.50[b]	−0.32[b]				
IGFBP-3 (μg/L)	0.58[b]	0.17[a]	−0.30[b]			
Cortisol (μg/L)	−0.30[b]	−0.32[b]	0.17[a]	−0.22[b]		
Insulin (IU/L)	0.50[b]	0.30[b]	−0.36[b]	0.39[b]	−0.35[b]	
Leptin (μg/L)	0.46[b]	0.29[b]	−0.33[b]	0.34[b]	−0.22[b]	0.44[b]

[1] All correlations were computed on $\log_{10}$-transformed values. [a]$P < 0.05$; [b]$P < 0.0001$. IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding factor; LNS, lipid-based nutrient supplement; MMN, multiple micronutrient supplement.

similar effect modifications of the intervention (results not shown), with higher cord leptin in the highest MUAC tertile (+46%; P = 0.002). There were no significant interactions between intervention and maternal nutritional status at baseline on any other cord hormone (data not shown). There were also no significant interactions between intervention and maternal anemia at baseline on any of the cord hormones (results not shown).

SGA newborns (n = 68) had lower median cord serum IGF-I (38 vs. 48 ng/mL; $P < 0.001$), IGF-II (384 vs. 480 μg/L; $P < 0.001$), IGFBP-3 (5.9 vs. 6.6 μg/L; $P = 0.005$), and leptin (4.0 vs. 5.0 μg/L; $P = 0.007$) and higher median cortisol (125 vs. 102 μg/L; $P = 0.01$) concentrations than AGA newborns (n = 129). Cord serum IGFBP-1 (209 vs. 138 μg/L; $P = 0.07$) and insulin (3 vs. 3 IU/L; $P = 0.60$) concentrations were similar in SGA and AGA newborns.

Table 4 shows the results of multiple regression analysis of cord hormones on birth anthropometry. Fitting models with one hormone as predictor (model 1) showed that all cord hormones were associated with birth weight. However, when the joint influence of the hormones was assessed (model 2), only IGF-I, IGF-II, and leptin demonstrated independent associations. Birth length was only positively associated with IGFBP-1. Ponderal index was significantly associated with all hormones except IGFBP-1. However, only IGF-II, cortisol, and leptin were significantly associated with ponderal index in model 2. Placental weight was associated with IGF-II and negatively associated with IGFBP-1 when all hormones were included in the regression model. Finally, we did not find evidence that LNS altered the associations between cord hormones and birth size anthropometry for model 1 and 2 outcomes.

TABLE 3 Associations between pregnancy characteristics and hormone status in cord sera from pregnant women supplemented with MMN or LNS[1]

	Dependent variables [coefficient (95% CI)][2]						
Independent variables	IGF-I (μg/L)	IGF-II (μg/L)	IGFBP-1 (μg/L)	IGFBP-3 (μg/L)	Cortisol (μg/L)	Insulin (IU/L)	Leptin (μg/L)
LNS vs. MMN							
Crude	3.9 (−8.8, 19)	7.8 (−3.4, 20)	−8.3 (−27, 15)	0.05 (−12, 14)	0.98 (−10, 14)	−0.78 (−28, 37)	18 (0.34, 39)[a]
Adjusted							16 (−0.57, 35)
Height (cm)							
Crude	0.4 (−0.7, 1.6)	0.94 (0.01, 1.9)[a]	−0.57 (−2.5, 1.4)	−0.03 (−1.1, 1.1)	−0.64 (−1.6, 0.39)	0.96 (−1.8, 3.8)	0.55 (−0.84, 2.0)
Adjusted		0.79 (−0.11, 1.7)					
BMI (kg/m^2)							
Crude	−0.6 (−3.6, 2.4)	0.02 (−2.5, 2.6)	−2.0 (−7.1, 3.3)	−0.25 (−3.2, 2.8)	0.69 (−2.1, 3.6)	−1.5 (−8.7, 6.2)	2.8 (−1.0, 6.7)
Adjusted							
MUAC (cm)							
Crude	1.6 (−1.4, 4.7)	1.5 (−1.00, 4.1)	−3.4 (−8.3, 1.8)	1.3 (−1.6, 4.3)	−1.5 (−4.2, 1.2)	−2.4 (−9.3, 5.1)	5.4 (1.6, 9.2)[b]
Adjusted							5.7 (2.1, 9.6)[b]
Age (y)							
Crude	0.3 (−0.8, 1.4)	1.8 (0.90, 2.6)[c]	−0.55 (−2.4, 1.3)	0.61 (−0.42, 1.7)	−0.78 (−1.7, 0.19)	1.1 (−1.6, 3.7)	1.1 (−0.22, 2.4)
Adjusted		0.93 (−0.07, 1.9)					
Hb (g/dL)							
Crude	2.2 (−2.5, 7.2)	1.5 (−2.50, 5.6)	−2.1 (−9.9, 6.3)	0.78 (−3.8, 5.6)	1.1 (−3.3, 5.6)	−4.5 (−15, 7.4)	6.1 (0.03, 13)[a]
Adjusted							5.7 (−0.01, 11)
Primigravidity[3]							
Crude	−21 (−33, −7.6)[5]	−25 (−34, −15)[c]	15 (−13, 53)	−21 (−33, −8.0)[a]	33 (15, 53)[c]	−47 (−64, −21)[5]	−20 (−35, −2.7)[a]
Adjusted		−19 (−31, −6.1)[c]			36 (18, 57)[c]		−18 (−32, −1.1)[a]
Sex[4]							
Crude	−8.2 (−19, 4.6)	−3.5 (−14, 7.9)	5.2 (−16, 32)	−6.0 (−17, 6.9)	5.7 (−6.3 19)	−26 (−47, 1.7)	−23 (−35, −10)[b]
Adjusted							−25 (−36, −12)[b]
Gestational length (wk)							
Crude	−2.4 (−5.7, 1.0)	−0.87 (−3.8, 2.1)	−0.56 (−6.4, 5.7)	−0.78 (−4.1, 2.7)	3.2 (0.02, 6.6)[a]	−3.2 (−11, 5.5)	3.0 (−1.4, 7.5)
Adjusted					4.2 (0.97, 7.4)[b]		

[1] Values are mean, n = 197. [a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.001$. Hb, hemoglobin; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; LNS, lipid-based nutrient supplement; MMN, multiple micronutrient supplement; MUAC, mid-upper arm circumference.
[2] Concentrations were $\log_{10}$-transformed for linear regression.
[3] Binary variable, reference class is multigravidae.
[4] Reference class is female.

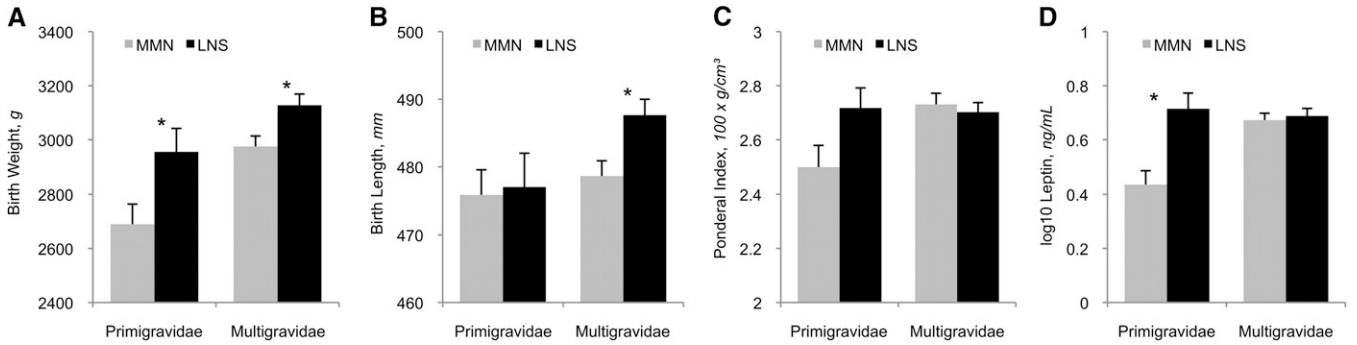

FIGURE 1 Estimated birth weight (*A*), birth length (*B*), ponderal index (*C*), and cord $\log_{10}$ leptin (*D*) by multiple linear regression adjusted for newborn's sex, malaria prevention, catchment area, and gestational length and stratified by primigravidity and intervention to pregnant women (LNS vs. MMN), $n = 22$ (primigravidae/MMN), 18 (primigravidae/LNS), 79 (multigravidae/MMN), and 78 (multigravidae/LNS). Values are adjusted means and SEMs. *Mean differences between LNS and MMN groups, $P < 0.05$. LNS, lipid-based nutrient supplement; MMN, multiple micronutrient supplement.

Discussion

The findings of this study indicate that prenatal LNS was modestly associated with higher leptin concentrations. However, significant, positive LNS effects on cord leptin were noted in primigravidae and women from the upper BMI tertile at study conclusion.

Prenatal LNS increased cord leptin by 18%. This effect was slightly reduced after adjustment for possible confounders. To our knowledge, there are no human studies that have assessed the effect of this type of nutritional intervention on cord hormones. Animal studies demonstrated that maternal overnutrition (155% of habitual diet) increases leptin gene expression in fetal adipose tissue (26,27). Cord leptin is produced by both placenta and fetus, but previous studies point out that cord leptin reflects fetal fat mass (14,15). We also observed that cord leptin was consistently positively associated with newborn ponderal index.

Prenatal LNS significantly increased cord leptin in primigravidae and also resulted in an increased newborn ponderal index. First pregnancies are characterized by lower birth weight (28,29) and adiposity (30). This is reflected by the significantly lower cord leptin in these pregnancies. No intervention effect on any of the dosed cord hormones was found in multigravidae, where we previously reported the largest LNS effect on birth length (21).

Prenatal LNS also increased cord leptin in mothers from the highest baseline BMI and MUAC tertiles. LNS supplementation of these mothers also resulted in a higher fetal ponderal index. In addition, cord leptin was associated with maternal MUAC at enrolment, newborn weight, and ponderal index. We therefore speculate that LNS increases birth weight by primarily adding fetal fat mass in pregnancies of mothers well nourished at baseline. The positive associations of leptin with maternal nutritional status and newborn ponderal index emphasize the role of cord leptin as a marker of a positive energy balance.

The results of these subgroup analyses confirm our previous observation that LNS supplements have differential effects on fetal growth according to early-pregnancy maternal nutritional status and primigravidity (21). This could lead to the hypothesis that prenatal LNS supplementation results in 2 distinct body size phenotypes at birth. LNS supplementation results in a greater average length in newborns with a lower ponderal index from mothers that start gestation with a suboptimal nutritional status. LNS supplementation in mothers who initiate their pregnancy well nourished translates into increased fetal fat mass. The latter finding would be striking for a rural sub-Saharan population. Yet it is difficult to conclude if the additional fetal fat mass should be interpreted as a negative endocrine profile or an additional energy reserve that could bridge the first difficult months of growth. Ong et al. (31) reported an inverse relation in British infants between cord leptin and weight gain from birth to 24 mo, which suggests a regulatory role for leptin in postnatal growth. Contrary to this finding, Collinson et al. (32) did not find any association between cord leptin concentrations and growth up to 52 wk in Gambian infants.

We did not find significant effects of LNS on other cord hormones concomitant with the previously reported effect on birth length and placental weight. Besides the possibility that the study

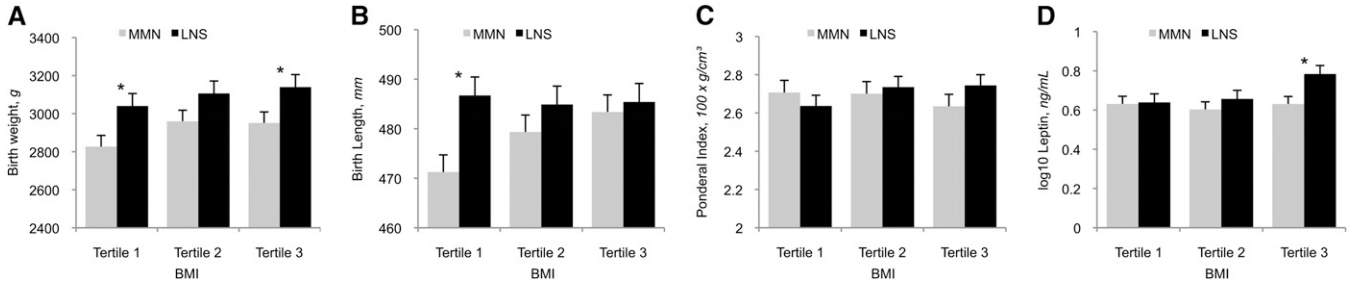

FIGURE 2 Estimated mean birth weight (*A*), birth length (*B*), ponderal index (*C*), and cord $\log_{10}$ leptin (*D*) adjusted for newborn's sex, malaria prevention, primigravidity, and gestational length and stratified by maternal BMI at study inclusion and intervention to pregnant women (LNS vs. MMN), $n = 33$ (lower tertile/MMN), 34 (middle tertile/MMN), 34 (upper tertile/MMN), 32 (lower tertile/LNS), 32 (middle tertile/LNS), and 32 (upper tertile/LNS). Values are adjusted means and SEMs. The ranges for the BMI tertiles were lower (15.8–19.7 kg/m^2), middle (19.7–21.8 kg/m^2), and upper (21.8–28.1 kg/m^2). *Mean differences between LNS and MMN groups, $P < 0.05$. LNS, lipid-based nutrient supplement; MMN, multiple micronutrient supplement.

TABLE 4 Associations between cord serum hormone concentrations and birth anthropometry in pregnant women supplemented with MMN or LNS[1]

	Model 1[2]		Model 2[3]	
	Coefficient (95% CI)	*P* value	Coefficient (95% CI)	*P* value
Birth weight (g)				
IGF-I (μg/L)	7.57 (5.47, 9.67)	<0.0001	4.71 (2.27, 6.95)	<0.0001
IGF-II (μg/L)	0.78 (0.47, 1.08)	<0.0001	0.42 (0.11, 0.72)	0.008
IGFBP-1 (μg/L)	−0.90 (−1.26, −0.54)	<0.0001		
IGFBP-3 (μg/L)	41.8 (19.7, 63.9)	<0.0001		
Cortisol (μg/L)	−1.44 (−2.33, −0.56)	0.001		
Insulin (IU/L)	15.9 (2.3, 29.4)	0.022		
Leptin (μg/L)	42.3 (28.2, 56.3)	<0.0001	28.0 (14.1, 41.9)	<0.0001
Birth length (mm)				
IGF-I (μg/L)	0.09 (−0.05, 0.23)	0.20		
IGF-II (μg/L)	0.001 (−0.02, 0.02)	0.93		
IGFBP-1 (μg/L)	−0.03 (−0.05, −0.01)	0.009	−0.03 (-0.05, -0.01)	0.0088
IGFBP-3 (μg/L)	0.53 (−0.81, 1.87)	0.43		
Cortisol (μg/L)	0.01 (−0.04, 0.07)	0.62		
Insulin (IU/L)	−0.31 (−1.11, 0.49)	0.44		
Leptin (μg/L)	0.23 (−0.66, 1.12)	0.61		
Ponderal index (100 × kg/cm^3)				
IGF-I (μg/L)	5.27 (3.03, 7.39)	<0.0001		
IGF-II (μg/L)	0.71 (0.40, 1.01)	<0.0001	0.52 (0.22, 0.82)	0.001
IGFBP-1 (μg/L)	−0.30 (−0.67, 0.07)	0.11		
IGFBP-3 (μg/L)	30.0 (8.1, 51.8)	0.007		
Cortisol (μg/L)	−1.51 (−2.36, −0.65)	<0.0001	−0.94 (−1.76, −0.12)	0.025
Insulin (IU/L)	18.8 (5.8, 31.9)	0.005		
Leptin (μg/L)	34.2 (20.2, 48.3)	<0.0001	28.6 (15.0, 42.1)	<0.0001
Placental weight (g)				
IGF-I (μg/L)	1.14 (0.39, 1.90)	0.003		
IGF-II (μg/L)	0.15 (0.05, 0.25)	0.005	0.11 (0.00, 0.22)	0.034
IGFBP-1 (μg/L)	−0.18 (−0.30, −0.06)	0.004	−0.14 (−0.27, −0.02)	0.027
IGFBP-3 (μg/L)	8.30 (0.98, 15.62)	0.027		
Cortisol (μg/L)	−0.29 (−0.54, −0.00)	0.049		
Insulin (IU/L)	3.48 (−0.90, 7.85)	0.12		
Leptin (μg/L)	4.22 (−0.67, 9.11)	0.09		

[1] Values are mean, n = 197. IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; LNS, lipid-based nutrient supplement; MMN, multiple micronutrient supplement.
[2] Linear regression model adjusted for primigravidity, newborn's sex, malaria prophylaxis, catchment area, nutritional intervention, and gestational length.
[3] Linear regression model adjusted for retained hormone concentrations by backward elimination at likelihood ratio ($P < 0.05$), catchment area, nutritional intervention, malaria prophylaxis, primigravidity, newborn's sex and gestational length.

was underpowered to detect more subtle changes in hormone concentrations, it is possible that the presence of multiple micronutrients in both supplements might have leveled out an intervention effect. For instance, Roberfroid et al. (33) found that MMN increased cord IGF-I only in boys and cord cortisol in primiparae of the same population. This reported MMN effect modification by gender on cord IGF-I could not be demonstrated in this study, which suggests that cord IGF-I might be primarily affected by the micronutrients present in the prenatal supplements.

A second objective of this study was to provide insights into the associations between maternal factors and newborn's size in a sub-Sahara African population. In our study, heavier babies had higher cord IGF-I and leptin concentrations and lower IGFBP-1 and cortisol concentrations. This confirms the independent positive association between IGF-I and leptin, and birth weight, a finding that was already reported in the same population (33) and by other studies (8,34,35). This recurrent observation suggests that the IGF-axis and leptin do not share common pathways. To our surprise, IGF-II was also associated with birth weight independently of IGF-I. IGF-II has been more associated with embryonic growth, whereas the role of IGF-I is more important during the second half of gestation (36).

The negative association between IGFBP-1 and birth length is consistent with previous reports (35,37). A study in transgenic mice showed that overexpression of IGFBP-1 resulted in pleiotropic defects in several skeletal units with smaller bones as a result (38). Another study in transgenic mice showed that the association between IGFBP-1 and fetal growth retardation was independent of IGF-I concentrations (11). The negative association between IGFBP-1 and placental weight, observed in our study and in others (39), could also be part of the explanation. In vitro models suggest that maternal hypoxia leads to overexpression of IGFBP-1 and results in fetal growth restriction (40,41). However, the lack of association between birth length and IGF-I or leptin (7,35,37) was an unexpected finding.

Cord IGF-II, cortisol, and leptin were independently associated with newborn ponderal index. Leptin concentrations have been reported to correlate very well with ponderal index (42).

This finding seems quite consistent given the correlation between neonatal fat mass and leptin. The independent negative association between cord cortisol and ponderal index, along with the higher cord cortisol in SGA newborns, supports the role of the hypothalamic-pituitary-adrenal axis in fetal growth. Fetal glucocorticoid concentrations are much lower than those in maternal circulation in normal pregnancies. It was demonstrated in animal models and humans that fetal exposure to higher concentrations of maternal glucocorticoids, like cortisol, is associated with IUGR and lower birth weight (43). Cortisol is able to pass through the placenta, where it is converted into inactive 11-ketoforms by placental 11β-HSD2. Increased cord cortisol concentrations may therefore be a consequence of reduced 11β-HSD2 activity or expression (43). Cianfarani et al. (44) proposed that cortisol has a regulatory function in the IGF-axis, which might explain the absence of an association between cortisol and birth weight when IGF-I was included in the regression model.

Previous studies demonstrated that placental weight was positively associated with IGF-II and IGF-I (12). However, only IGF-II significantly explained the variation in placental weight when concomitantly analyzed by multiple regression. This finding is in line with experiments in mice, where IGF-II, but not IGF-I, mutants had much smaller placentas compared with the wild types (19).

This study has a number of limitations. The sample size might have been too small to identify small changes in the IGF hormone concentrations with sufficient statistical power. Major conclusions on the role of the IGF-axis in prenatal nutrition and fetal growth can therefore not be drawn. We also compared the effect of LNS with MMN, which is known to exert an effect on fetal growth in this study population (24). Moreover, it was reported that prenatal MMN, compared with iron/folic acid supplements, demonstrates sex-specific effects on IGF-I (33), which could have leveled out an intervention effect on the IGF-axis.

In conclusion, prenatal LNS supplementation was associated with higher cord leptin concentrations. Maternal nutritional status at early gestation and primigravidity modified the effect of LNS, which resulted in 2 apparent phenotypes of newborns. The public health value of these results needs to be assessed.

Acknowledgments

L.H., D.R., U.A., J.V.C., and P.K. designed the study; D.R., L.H., H.L., I.V., and N.M. oversaw the field work; L.H. analyzed data; L.H. and D.R. wrote the paper; Y.T. contributed substantially to the data interpretation; and L.H., P.K., and J.V.C. had primary responsibility for final content. All authors read and approved the final manuscript.

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