

Does High Protein Intake Cause Glomerular Injury in Very Preterm Neonates?

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Research Article

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Abstract

Background: Very preterm birth rate was 10.8% of all preterm in Asia. Early aggressive nutritional strategies in very preterm neonates is important for catching up growth; however, preterm kidneys have fewer, immature functional nephrons. Studies have showed that high protein intake induces nephron hypertrophy, proteinuria, and glomerular sclerosis through single nephron glomerular hyperfiltration (SNGHF), which leads to glomerulotubular injury.

Aim: to analyse the correlation between protein intake and glomerulotubular injury in very preterm neonates.

Method: A prospective cohort study was conducted in neonatal units of two hospitals in Jakarta.

Urine samples were taken three times at post-natal ages 0-48 hours (T1), 72 hours (T2), and 21 days (T3) for determining the urinary neutrophil gelatinase-associated lipocalin to creatinine (uNGAL/Cr) ratio. Protein intake were given in accordance with local guideline while considering the clinical condition of participants. Protein levels from formula milk were recorded daily from 14-21 days of age, while breastmilk protein was measured twice by using a human milk analyser. Urinary NGAL (uNGAL) was tested with an ELISA. Glomerulotubular injury was defined as a uNGAL/Cr ratio ≥ 1 SD (22.74 ng/mg) at post-natal age 21 days. High protein intake was defined as average protein intake ≥ 3 g/kg/day.

Results: Fifty-nine very preterm neonates were recruited, of which 39 completed the study. Glomerulotubular injury was found in 9 of 39 participants (23%). The proportion of glomerulotubular injury in very preterm neonates who had received high protein intake vs low protein intake was 5 of 29 vs 4 of 10 participants, respectively. The median of uNGAL/Cr ratio was not significantly different in the high vs low protein intake group (3.54 (range: 0.69-89.16) ng/mg vs (6.88 (range: 0.32-66.64)) ng/mg, respectively. The uNGAL/C ratio was not correlated with protein intake. However, it was inversely correlated with gestational age and birth weight.

Conclusions: The proportion of glomerulotubular injury in very preterm neonates given high protein diet was 5 of 29. The uNGAL/Cr ratio was increased at the post-natal age of 72 hours and decreased in 21 days in both high and low protein intake groups. High protein intake was not correlated with glomerulotubular injury.

Introduction

Indonesia has the fifth highest rate of preterm birth in the world. In 2014, the preterm birth rate was 10.4%.¹ Data from a tertiary referral hospital in Jakarta reported that the absolute preterm birth rate in 2018 was 507, of which 112 were very preterm. Very preterm neonates are defined as infants born at 28–32 weeks of gestational age; the survival rate in our centre was 58.9%.² The survival rates of very preterm neonates have been increasing as advancements in neonatology care continue to be made. Hence, morbidity caused by organ dysfunction is also increasing, including kidney dysfunction.

Very preterm neonates are born with a low number of functional nephrons, which results in a decrease in glomerular filtration surface area and an increase in glomerular arterial pressure.^{3,4} This mechanism increases the risk of hypertension and chronic kidney disease (CKD) later in life.⁴ Moreover, tubular immaturity, dysfunction of glomerular vasoregulation, low kidney perfusion, renal thrombosis, exposure to nephrotoxic agents and other events leading to acute kidney injury (AKI) are often found during the care of very preterm neonates.⁵ Acute kidney injury is always preceded by injury of kidney tubules and glomeruli. This injury can be detected early by measuring AKI biomarkers, such as urinary neutrophil gelatinase-associated lipocalin (uNGAL).⁶ A non-invasive method of obtaining urine samples used in uNGAL examination is beneficial for very preterm neonates since blood sampling is highly restricted due to their low blood volume. In order to control the dilutional factor in urine upon uNGAL testing, normalisation by using the uNGAL to creatinine (uNGAL/Cr ratio) is commonly performed.⁷

Aggressive nutritional intervention by administering a high protein diet has been used in our NICU to accelerate growth and improve the neurodevelopmental outcomes of very preterm neonates.⁸ This strategy is started by administering 2.5 g/kg/day protein soon after birth and gradually increasing to 4 g/kg/day by post-natal age day 3.⁹ Experimental animal and human studies showed that a high protein diet induces kidney hypertrophy, proteinuria, and glomerulosclerosis via single nephron glomerular hyperfiltration (SNGHF). Hypothetically, SNGHF is an early developmental process of chronic kidney disease in adulthood.¹⁰ Since preterm neonates need high protein intake to catch up to healthy growth percentiles, the impact of high protein intake on preterm nephrons as well as the potential long term effects of this diet requires additional research. A previous study reported that very preterm infants receiving a protein diet of up to 7 g/kg/day showed an increase in the estimated glomerular filtration rate (eGFR).¹¹ However, another study showed that protein intake of up to 3.6 g/kg/day in very preterm infants did not increase the levels of urea, creatinine, cystatin C serum and urinary β 2 microglobulin.¹² The purpose of this study was to examine the correlation between protein intake and glomerulotubular injury in very preterm neonates.

Methods

Study population

This study was a prospective cohort study from birth to 21 days of age involving 59 very preterm neonates admitted to the NICU of two referral hospitals, Cipto Mangunkusumo General Hospital (CMGH) and Bunda Menteng Hospital (BMH), between June 2019 and May 2020. Participants were recruited consecutively and excluded if there were major congenital abnormalities, intrauterine growth restriction (IUGR), maternal exposure to nephrotoxic drugs during pregnancy, difficulties in collecting urine samples, and parental refusal of consent to participate in the study. Sample size was estimated based on a formula for correlation and was determined to be 39 subjects.

This study measured glomerulotubular injury, protein intake, and other variables. The primary outcome was the correlation between protein intake and glomerulotubular injury. Protein intake level, prevalence of glomerulotubular injury, weight increment, weight to age z-score (WAZ), comorbidities, and factors associated with glomerulotubular injury were secondary outcomes measured in this study.

Glomerulotubular injury

Spot urine samples were collected three times during the study, at post-natal age 0–48 hours (T1), 72 hours (T2), and 21 days (T3). At least 5 mL of urine was collected each time with a urine collector or urethral catheter for uNGAL and urine creatinine. Samples were kept in a refrigerator at a temperature of 13-15°C for a maximum of 12 hours and then were sent to Prodia Laboratory in a cooler box at the same temperature. Samples were centrifuged, and some were tested randomly for urine creatinine (uCr). Supernatants were then frozen at -70°C until further analysis. Urinary NGAL (uNGAL) was tested with an ELISA method using Quantikine® Immunoassay (R&D Systems, Minneapolis, USA; NGAL Immunoassay). The urinary NGAL to creatinine ratio was produced by dividing uNGAL (ng/mL) by uCr (mg/dL). The distribution of the uNGAL/Cr ratio was statistically analysed, and the standard deviation was determined. Glomerulotubular injury was defined as a spot urinary NGAL to creatinine (uNGAL/Cr) ratio ≥ 1 SD at day 21.

Protein intake

Protein intake included the total parenteral protein in the form of amino acids and oral/enteral protein found in breastmilk or formula milk administered to the participants. The protein intake level was administered according to NICU guidelines for very preterm neonates, which were 2.5 g/kg/day of protein in the first 24 hours; 3.5 g/kg/day in the next 24 hours; and 4 g/kg/day thereafter. Neonates were then divided into two groups based on protein intake. Low protein intake was defined as < 3 g/kg/day and high protein intake was defined as ≥ 3 g/kg/day. Protein intake was recorded from the medical chart at 0–48 hours, 72 hours, and 21 days. From 14 to 21 days of age, the total fluid and total protein intake from both parenteral and enteral sources were recorded. Full feed was defined as consumption of at least 100 mL/kg body weight/day of milk orally/enterally. Enteral diet sources included breast milk, formula milk, and fortified breast milk. Protein intake from formula milk was calculated by dividing the total protein in 100 mL of milk and body weight. Protein intake from breastmilk was calculated by taking the average breastmilk protein content at day 14 and 21. The protein content of breastmilk was measured by sampling 5 mL of breastmilk and testing it in a human milk analyser (Miris®, Uppsala, Sweden; infrared spectroscopy). Protein content from a human milk fortifier (HMF) was also measured based on the volume added to the breastmilk.

Other variables

Birth length, birth weight and head circumference at birth were recorded. Weight increments were monitored at day 14 and 21 and plotted to an INTERGROWTH post-natal growth (IPNG) curve, producing a weight to age z-score (WAZ). During hospitalisation, secondary data from medical records were collected. Those data included nephrotoxic medication exposure, necrotizing enterocolitis (NEC), patent ductus arteriosus (PDA), intraventricular haemorrhage (IVH) and sepsis. These variables were recorded secondarily from medical records.

Statistical analysis

Clinical characteristics and other variables were described by the median and range and the mean and standard deviation. Correlation of protein intake and the uNGAL/Cr ratio at T1, T2 and T3 were analysed with Spearman's analysis. A chi square test was performed to examine the association between protein intake and glomerulotubular injury. A bivariate analysis was performed to describe factors associated with glomerulotubular injury. A Fisher's exact test was carried out for nephrotoxic medication exposure and sepsis association to glomerulotubular injury. An independent t test was performed for birth weight, gestational age and mean protein intake associated with glomerulotubular injury. The correlation of birth weight with the uNGAL/Cr ratio was then analysed with Spearman's test. Statistical analysis was performed using SPSS version 17.

This study was approved by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia under the protocol number KET-400/UN2.F1/ETIK/PPM.00.02/2019.

Results

Urine samples were collected consecutively from 59 very preterm neonates. Thirty-nine (66.1%) participants completed the cohort study but 20 (33.9%) participants died as described in Fig. 1. One hundred and fifty-one urine samples were available for analysis.

Participant characteristics are shown in Table 1. Data for nephrotoxic medication exposure and comorbidities were only recorded for participants at CMGH. Echocardiography and head ultrasound were performed to 44 and 41 participants. The median protein intake at the age of 0–48 hours, 72 hours, and 21 days were 2.09 (range: 0.72–3.48), 3.12 (range: 0.87–4), and 3.44 (range: 2–5.18) g/kg/day, respectively. The median uNGAL/Cr ratios at post-natal age 0–48 hours, 72 hours, and 21 days were 7.75 (range: 0.45–104.11), 12.58 (range: 0.39–63.78), 4.22 (range: 0.32–89.16) ng/mg, respectively. We found no difference in uNGAL/Cr ratios between the high protein intake group and the low protein intake group across post-natal ages 0–48-hours, 72 hours, and 21 days (Table 2).

Table 1
Participant characteristics

Characteristics		N	
Birth weight (g)	mean (SD)	59	1236.2 (237.1)
Gestational age (weeks)	median (range)	59	30 (28–32)
Weight increment (g/kg/day)	median (range)	59	
Post-natal age 0–14 days		45	0 (-13–3.2)
Post-natal age 0–21 days		59	6.5 (-95.4–62.9)
Weight to age z-score (WAZ)	median (range)	59	
WAZ at birth		59	0.3 (-2.5–3.6)
WAZ at post-natal age 21 days		59	1.3 (-2.9–2.8)
Sex	n (%)	59	
Male		37	37 (62.7)
Female		22	22 (37.3)
Nephrotoxic medication	n (%)	56	44 (78.6)
Necrotizing enterocolitis/NEC	n (%)	56	33 (58.9)
Patent ductus arteriosus/PDA	n (%)	44	19 (43.2)
Intraventricular haemorrhage/IVH	n (%)	41	13 (31.7)

Table 2
uNGAL/Cr level in relation to protein intake

Time	uNGAL/Cr level (ng/mg)		p
	Low protein intake (n = 11) median (range)	High protein intake (n = 28) median (range)	
T1	9.06 (0.45–39.27)	11.40 (0.63-104.11)	0.62
T2	19,79 (0.97-45,70)	12.47 (039-63.78)	0.38
T3	6,88 (0.32–66.64)	3.54 (0.6-89.16)	0.30
T1: post-natal age 0–48 hours; T2: 72 hours; T3: 21 days			

Protein intake and glomerulotubular injury

The correlation between protein intake and uNGAL/Cr ratios is shown in Fig. 2. An association between protein intake and glomerulotubular injury is shown on Table 3. Glomerulotubular injury was defined as a uNGAL/Cr ratio above 1 SD (22.74 ng/mg) at post-natal age 21 days. The prevalence of glomerulotubular injury in very preterm neonates was 9/39 (23%). In the high protein intake group, 5 of 29 participants had glomerulotubular injury. In addition, 4 of 10 participants had glomerulotubular injuries in the low protein intake group. The prevalence of glomerulotubular injury in both groups was not statistically different ($p = 0.2$).

Table 3
Association of protein intake and glomerulotubular injury

	Glomerulotubular injury		Total
	No, n (%)	Yes, n (%)	
Protein intake			
Low (< 3 g/kg/day)	6 (60%)	4 (40%)	10
High (≥ 3 g/kg/day)	24 (82.8%)	5 (17.2%)	29

Factors associated with glomerulotubular injury

Factors associated with glomerulotubular injury included birth weight ($p = 0.065$, 95%CI -10.7;338.6)) and mean protein intake at post-natal age 21 days ($p = 0.03$, 95%CI 0.05; 1.18). Sepsis ($p = 0.61$, 95%CI -0.85; 3.1), nephrotoxic medication ($p = 0.49$, 95%CI 1.10; 1.61) and gestational age ($p = 0.44$, 95%CI -0.95; 2.2) were not associated with glomerulotubular injury. Birth weight was inversely correlated with the uNGAL/Cr ratio at post-natal age 0–48 hours ($r = -0.31$, $p = 0.016$), 72 hours ($r = -0.42$, $p = 0.002$) and 21 days ($r = -0.36$, $p = 0.025$).

Discussion

Our study found no correlation between protein intake and uNGAL/Cr ratios as a biomarker for glomerulotubular injury at post-natal age 48 hours, 72 hours, and 21 days. This correlation was illustrated in Fig. 2. In the first 48 hours of life, some very preterm neonates received a protein level that was lower compared to the guideline, with a median of 2.09 (range: 0.72–3.48) g protein administered per day. This protein intake level was not significantly correlated with the uNGAL/Cr ratio. However, the pattern in Fig. 2A shows that the uNGAL/Cr ratio increased proportionally with the increase in protein intake. During the first 48 hours, very preterm neonates experience physiological and haemodynamic stress in their new extra-uterine environment, which impacts uNGAL/Cr ratio. Furthermore, tubular immaturity due to

immature resorptive function during the early neonatal period causes an increase of the uNGAL/Cr ratio along with an increase in protein intake.

In contrast to post-natal age 0–48 hours, there was no significant correlation between protein intake and glomerulotubular injury at post-natal age 72 hours and 21 days. At these points, the higher the protein intake, the lower the uNGAL/Cr ratio was (Fig. 2B and 2C). Tubular maturation process that occurs with age contributes to the improvement of tubular resorptive function of uNGAL.

Our results support previous studies which also showed no impairment of tubules and glomeruli in very preterm infants with protein intake levels up to 3.6 g/kg/day at the age of 14 days.¹² This study measured serum urea, creatinine, cystatin C and urinary β 2 microglobulin as biomarkers for glomerular and tubular injury. The protein intake level given to neonates in our study at post-natal age 0–48 hours, 72 hours, and 21 days were in the range of the study, which was 3 to 3.6 g/kg/day.¹² Another study reported a decrease in eGFR, sodium clearance, and osmolality with the administration of breastmilk with supplemental protein up to 7 g.¹¹

We found that the proportion of very preterm neonates experiencing glomerulotubular injury at the age of 21 days was 9/23 (23%) with no difference between the high protein intake group and the low protein intake group. Glomerulotubular injury occurred in 5 of 9 participants receiving high protein intake (> 3 g/kg/day) and in 4 of 10 subjects receiving low protein intake (≤ 3 g/kg/day). The prevalence of acute kidney injury in our study was similar to the prevalence observed in a previous study involving 2,110 preterm neonates in the USA. Acute kidney injury prevalence was 28% in neonates at a gestational age of 22–28 weeks and 14% in neonates at a gestational age of 29–35 weeks based on the modified KDIGO criteria. Acute kidney injury in very preterm neonates was associated with an increased risk of death (adjusted odds ratio (OR) 2.8; IK 95% 1.7–4.7) and prolonged hospitalisation. Factors found to be associated with AKI in very preterm birth were birth outside the hospital, the use of epinephrine in resuscitation, hyperbilirubinemia on admission, hereditary metabolic diseases, surgical intervention, more frequent renal function surveillance and admission to a paediatrics hospital.¹³ Those factors were not addressed in our study.

All participants who had glomerulotubular injury in both intake groups were exposed to nephrotoxic antibiotics, gentamicin and/or amikacin. Four participants in the low protein intake group who experienced glomerulotubular injury received two types of nephrotoxic drugs, gentamicin and amikacin, during hospitalisation. Gentamicin and amikacin cause glomerulotubular injury by damaging segment S1 and S2 (proximal tubules), whilst NGAL is mostly produced as a result of damage of the proximal tubule.¹⁴ The “baby NINJA” study reported positive associations between the number of nephrotoxic agents administered in the NICU and the increase risk of AKI in very preterm infants.¹⁵ Nonetheless, bivariate analysis of several factors such as nephrotoxic drug exposure, sepsis, gestational age and birth weight showed no association with glomerulotubular injury at the age of 21 days in our participants. However, it is also important to mention that this study was not designed to analyse those factors, and the number of participants may have affected the results of the statistical analysis.

Importantly, the uNGAL/Cr ratio was affected by gestational age and birth weight. We found that the uNGAL/Cr ratio was inversely related to gestational age in very preterm neonates. As reported in the previous study, gestational age is the only parameter affecting the uNGAL/Cr ratio in the mixed model regression analysis compared with other parameters, such as sex, race, prevalence of AKI, and the insertion of an umbilical artery catheter.¹⁶ In the first 48 hours, participants with higher gestational ages showed lower uNGAL/Cr ratio baseline levels. In very preterm neonates, gestational age is a critical factor that affects the quantity of functional nephrons, the ability of the nephrons to filtrate and the maturation of renal tubules.¹⁷ In contrast, the uNGAL/Cr ratio has been negatively correlated with birthweight.^{16,18} A significant correlation between a decrease in the uNGAL/Cr ratio and an increase in birthweight was shown by our study. Multivariate regression analysis at a logarithmic scale of the uNGAL/Cr ratio showed a significant effect of birthweight, gestational age, and post-natal age on uNGAL/Cr ratio. The level of uNGAL/Cr ratio decreases by 13.4% with a 100 g increase in birthweight (95% CI: -16,6;(-10,1)), and increases by 11.3% with a 1 week increase in gestational age (95% CI: -15,3; (-7,1)). Nonetheless, uNGAL/Cr ratio increases by 4.4% with a 1 day increase in post-natal age (95% CI: -5,6; (-3,2)).¹⁹

We also found that the median protein intake received by participants at post-natal age 0–48 hours (2 (range: 0.72–3.48)) g/kg/day, 72 hours (3.12 (range: 0.87- 4)) g/kg/day, and 21 days (3.44 (range: 2–5.18)) g/kg/day were lower than our NICU guidelines. Even though the protein intake was lower than expected, the weight to age z-score (WAZ) at 21 days post-natal age according to the IPNG curve showed that 32 of 39 very preterm neonates had an increase in body weight compared to their birth weight. This supports a previous systematic review that found that 3 g/kg/day protein administration is needed for making up growth in very preterm neonates.²⁰

The main strength of our study is the adequate number of samples used to show the correlation between protein intake and glomerulotubular injury. We also included only very preterm neonates with appropriate gestational ages, which excludes the confounding effect of low birth weight on glomerulotubular injury. Some limitations of this study include that the cut-off point of the uNGAL/Cr ratio to determine the glomerulotubular injury was based on the standard deviation of our data. Urine samples were not collected on a daily basis; therefore, variation in the post-natal period could have been missed in this study. We were unable to control the exposure to nephrotoxic drugs and sepsis in the very preterm neonates during our study observation. However, we included those factors in our analysis.

Conclusion

In conclusion, the present study shows no correlation between high protein intake and glomerulotubular injury in very preterm neonates. The proportion of glomerulotubular injury experienced by very preterm neonates in the high protein diet group was 5 of 29. In very preterm neonates, the uNGAL/Cr ratio was increased at 72 hours of age and decreased in 21 days in both the high and low protein intake groups. Our findings also support the current nutritional guideline for very preterm neonates, which recommends administering high protein levels to promote growth. A longer follow-up period and combination of glomerulotubular injury biomarkers should be studied further to monitor the long-term effects of high

protein intake. In addition, other factors that contribute to glomerulotubular injury in very preterm neonates should be further evaluated.

List Of Abbreviations

AKI	acute kidney injury
BMH	Bunda Menteng Hospital
CKD	chronic kidney disease
CMGH	Cipto Mangunkusumo General Hospital
eGFR	estimated glomerular filtration rate
IPNG	INTERGROWTH post-natal growth
IUGR	intrauterine growth restriction
IVH	intraventricular hemorrhage
NEC	necrotizing enterocolitis
NICU	neonatal intensive care unit
PDA	patent ductus arteriosus
SD	standard deviation
SNGHF	single nephron glomerular hyperfiltration
uCr	urine creatinine
uNGAL	urine neutrophil gelatinase-associated lipocalin
uNGAL/Cr	urine neutrophil gelatinase-associated lipocalin to creatinine ratio
WAZ	weight to age z-score

Declarations

Ethics approval and consent to participate

All methods in this study were carried out in accordance with relevant guidelines and regulations. The study protocol was approved by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia, Indonesia (number of approval decision: KET 400/UN2.F1/ETIK/PPM.00.02/2019 for the prospective study). All parents provided informed consent as legal guardians of the patients participating in the study.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author.

Competing interests

The authors declare no competing financial interests.

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Author contributions

The authors made the following contributions: conception and design of the study (HAP, PPT), data collection (HAP), statistical analysis (HAP), interpretation of the data (HAP, PPT, PAW), article writing (HAP) and manuscript review and modification (HAP, PPT, PAW). All authors read and approved the final version of the manuscript.

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Sample size estimation

Sample size was estimated by formula for correlation:

$$n = \left[\frac{(Z_{\alpha} + Z_{\beta})}{0,5 \ln \left[\frac{(1+r)}{(1-r)} \right]} \right]^2 + 3$$

$$n = \left[\frac{(1,96 + 1,64)}{0,5 \ln \left[\frac{1 + 0,59}{1 - 0,59} \right]} \right]^2 + 3$$

n = 31

n = sample size

α = type I error, $Z\alpha = 1,96$

β = type II error, 10%

r = correlation (0,59)³⁶

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Figures

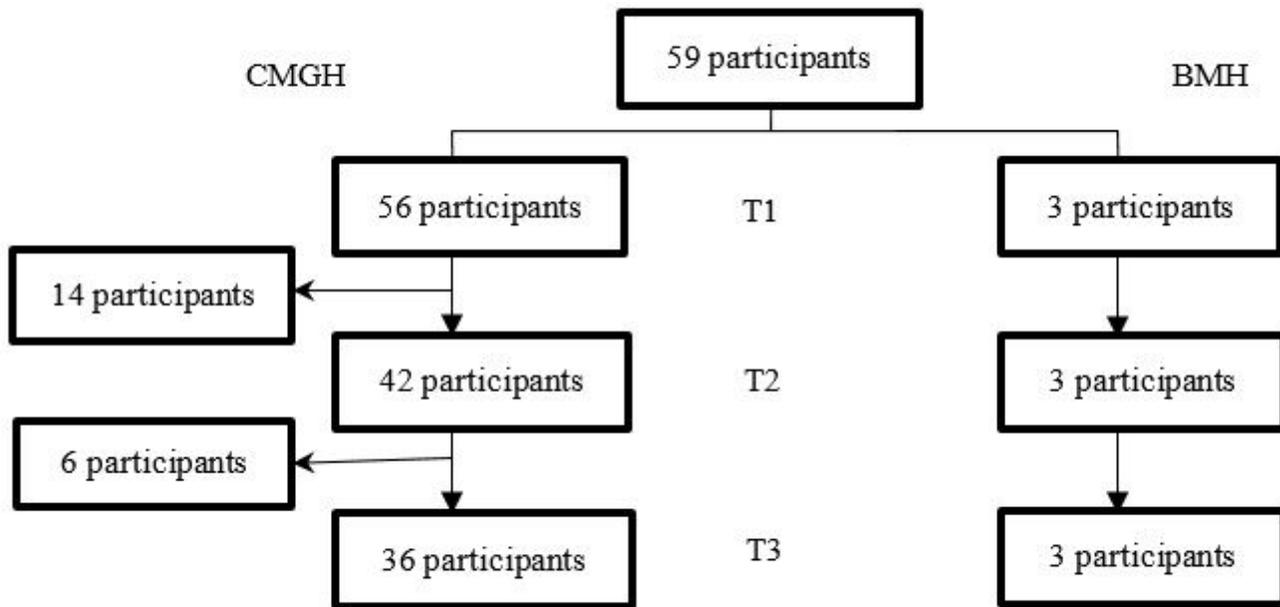


Figure 1

Study population. A total of 59 participants were recruited. The first sample was collected at post-natal age 0-48 hours (T1). Of the 59 participants, 45 participants had a second sample collected at post-natal age 72 hours (T2), and 39 subjects had a third sample collected at post-natal age 21 days (T3) in both hospitals, Cipto Mangunkusumo General Hospital (CMGH) and Bunda Menteng Hospital (BMH).

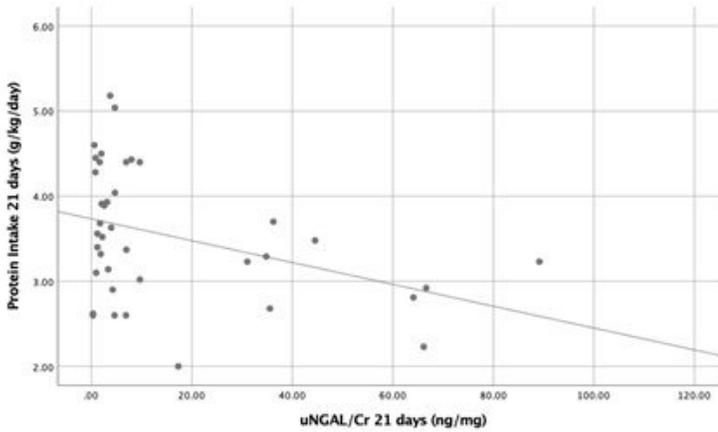
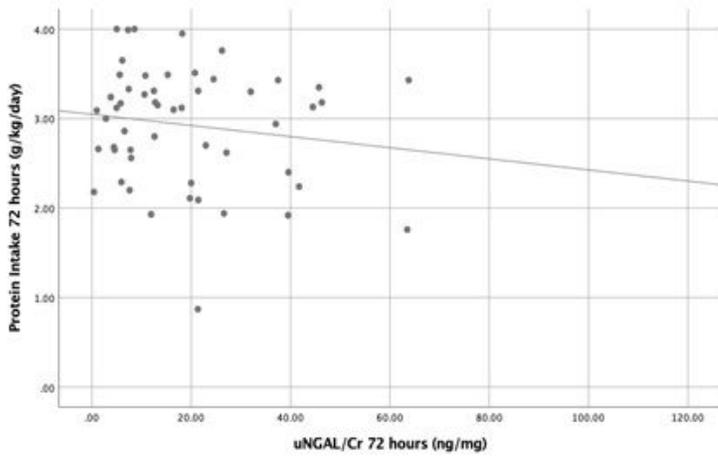
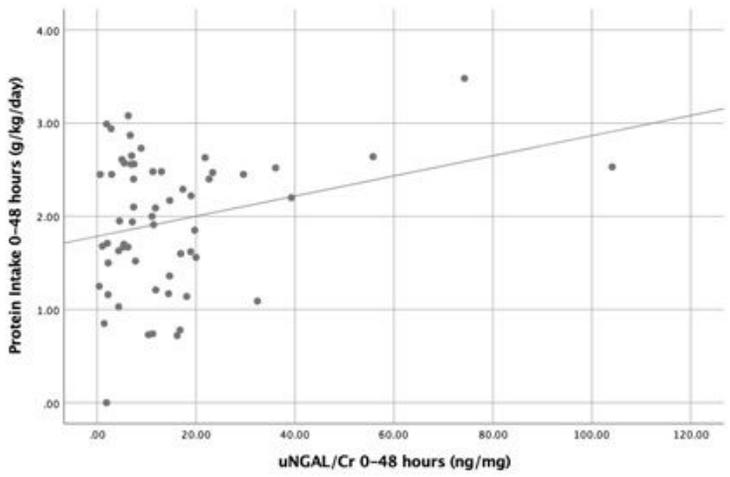


Figure 2

The urine NGAL creatinine ratio was not correlated with protein intake at post-natal age 0-48 hours (T1), 72 hours (T2), and 21 days (T3). However, uNGAL/Cr was increased in protein intake at T1 (A) and decreased at T2 (B) and T3 (C).