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Estimation of protein requirements in Indian pregnant women using a whole-body potassium counter

Rebecca Kuriyan[1,7*], Saba Naqvi[1,7], Kishor G Bhat[1], Tinku Thomas[2], Annamma Thomas[3], Shirley George[3], Sheela C Nagarajarao[3], Harshpal Singh Sachdev[4], Thomas Preston[5], Anura V Kurpad[6]

[1]Division of Nutrition, St. John's Research Institute, Bengaluru, India

[2]Division of Epidemiology and Biostatistics, St. John's Research Institute, Bengaluru, India.

[3]Department of Obstetrics and Gynecology, St. John's Medical College Hospital, Bengaluru, India

[4]Sitaram Bhartia Institute of Science and Research, New Delhi, India

[5]Stable Isotope Biochemistry Laboratory, Scottish Universities Environmental Research Centre, Glasgow, Scotland, UK

[6]Department of Physiology, St. John's Medical College, Bengaluru, India

[7]These authors contributed equally to this article and shared first authorship

Names for PUBMED indexing:

Kuriyan, Naqvi, Bhat, Thomas, Thomas, George, Sheela, Sachdev, Preston, Kurpad

***Corresponding Author**

Rebecca Kuriyan,

Professor, Division of Nutrition, St. John's Research Institute,

Bengaluru – 560034, India.

Phone/Fax: 91-80-49467000; Mobile No: +91 9886002210

Email: rebecca@sjri.res.in

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Running head: Protein requirements of Indian pregnant women

Abbreviations (in alphabetical order):

BCM- Body Cell Mass

BMI- Body Mass Index

CERN- Conseil Européen pour la Recherche Nucléaire

EAR- Estimated Average Requirement

GWG- Gestational Weight Gain

LMIC- Low to Middle Income Countries

PAL- Physical Activity Level

PE ratio- Protein Energy ratio

ROI- Region of Interest

SD- Standard Deviation

SGA- Small for Gestational Age

TBK- Total Body Potassium

TBN- Total Body Nitrogen

USDA- United States Department of Agriculture

WBKC- Whole-Body Potassium Counter

WHO/FAO/UNU- World Health Organisation/Food and Agriculture Organisation/United Nations University

Abstract

Background: The 2007 WHO/FAO/UNU recommendation for the Estimated Average Requirement (EAR) of additional protein during pregnancy for a gestational weight gain (GWG) of 12 kg (recalculated from a GWG of 13.8 kg) is 6.7 and 21.7 $g.d^{-1}$ in the 2^{nd} and 3^{rd} trimester respectively. This EAR is based on measurements of potassium accretion in high-income country (HIC) pregnant women. It is not known if low to middle income country (LMIC), but well-nourished, pregnant women have comparable requirements.

Objective: To estimate total body potassium (TBK) accretion during pregnancy in Indian pregnant women, using a whole-body potassium counter (WBKC), to measure their additional protein EAR.

Design: Well-nourished pregnant women (20-40 years, n = 38, middle socioeconomic stratum) were recruited in the first trimester of pregnancy. Anthropometric, dietary and physical activity measurements, and measurements of TBK using a WBKC, were performed at each trimester and at birth.

Results: The mid-trimester weight gain was 2.7 kg and 8.0 kg in the 2^{nd} and 3^{rd} trimesters, for an average 37 week GWG of 10.7 kg and a mean birth weight of 3.0 kg. Protein accretion was 2.7 and 5.7 $g.d^{-1}$, for an EAR of 8.2 and 18.9 $g.d^{-1}$ in the 2^{nd} and 3^{rd} trimesters, respectively. The additional protein EAR calculated for a GWG of 12 kg, was 9.1 and 21.2 $g.d^{-1}$ in the 2^{nd} and 3^{rd} trimester, respectively.

Conclusions: The additional protein requirements of well-nourished Indian pregnant women for a GWG of 12 kg in the 2^{nd} and 3^{rd} trimester were similar to the recalculated 2007 WHO/FAO/UNU requirements for 12 kg.

Keywords: Pregnancy, Protein Requirements, Total Body Potassium, Gestational Weight Gain, Whole-Body Potassium Counter

Introduction

Adequate protein intake during pregnancy is needed for optimal tissue accretion in the fetus and maternal support tissues. The additional protein requirement during pregnancy is measured as the mean of the requirement observed in healthy, well nourished, pregnant women. This is called the Estimated Average Requirement (EAR), and has been estimated from total body potassium (TBK) measurements in high-income country (HIC), well-nourished mothers, using a factorial method, as defined by 2007 WHO/FAO/UNU Expert Committee on Protein and Amino Acid Requirements (1). The TBK method, which measures whole body activity of naturally radioactive potassium (^{40}K), is independent of changing hydration status during pregnancy and free of radiation exposure from imaging techniques, and is ideal to evaluate the protein requirements of pregnancy (1). It provides an accurate measure of the metabolically active body cell mass (BCM) and protein (2,3), since the BCM contains more than 98% of the body's potassium content (2). In the factorial method, the EAR is first derived from the mean protein accretion ($g.d^{-1}$) during different trimesters of pregnancy, as measured by TBK accretion rates. The protein intake required to meet this deposition rate is derived by adjusting the latter for the efficiency of utilization of dietary protein (the proportion that would be deposited). To this was added the maintenance dietary protein requirement ($0.66\ g.kg.d^{-1}$) to support the mean mid-trimester gestational weight gain (GWG). The estimated EAR of additional protein was thus derived to be 7.7 and 24.9 $g.d^{-1}$ in the 2^{nd} and 3^{rd} trimester respectively, for a GWG of 13.8 kg.

However, it is not known if nutrient requirements for a healthy pregnancy are similar across populations. While some studies suggest that the GWG and estimated fetal growth in pregnant women with optimal health, nutrition, education, and socioeconomic status are similar in different countries (4), others suggest otherwise, and specifically in Indian pregnancies, show that the estimated fetal growth is slower towards the end of pregnancy (5). The GWG could also be lower, and given the uncertainty of the occurrence of racial or ethnic differences (6,7) and the variability in fetal growth imposed by possible biological, socioeconomic, and cultural factors, it is important to evaluate the pregnancy protein requirement in LMIC populations, starting with women who might be assumed to be at no risk of nutritional deficiency.

Another area of uncertainty relates to the source of protein for fetal growth. If an undernourished mother met the requirement of the growing fetus by mobilizing her tissue protein, this would result in a net loss of metabolically active body cell mass (BCM) after pregnancy, with implications for her future health and subsequent pregnancy. While this does not occur in well-nourished HIC pregnancies (8), it is not known whether this applies globally. For example, the digestion and absorption of plant protein is low in healthy Indian men and women (9), and intestinal permeability was shown to be higher in healthy, well-nourished Indian women (10). Indians also have low protein reserves in terms of their muscle mass (11).

The objective of the present study was to measure the TBK and GWG in well-nourished, middle socioeconomic-class Indian pregnant women to arrive at estimates of their additional protein requirement in the 2nd and 3rd trimesters.

Subjects and Methods

Pregnant women between 18-40 years, identified at the Obstetrics Department of St. John's Medical College Hospital, Bengaluru, India, were recruited at ≤13 weeks gestational age (as judged by the date of the last menstrual period and confirmed by an ultrasonography scan). Mothers who anticipated moving out of the area before study completion, with twin or multiple pregnancies, had positivity for hepatitis B (HBsAg), HIV or syphilis (VDRL) infections, or were on daily vitamin supplements in addition to folate and iron, and those who had serious pre-existing medical conditions, were excluded from the study. Fifty eligible pregnant women were recruited, of which two were diagnosed to have gestational diabetes (12), when screened at 24 weeks gestation, and counselled for diet control. The experimental protocol was approved by the Institutional Ethics Committee and every participant provided an informed written consent. The study was conducted from April 2016 to October 2017.

At the 1^{st} trimester (~13 weeks), 2^{nd} trimester (14-26 weeks), 3^{rd} trimester (27-40 weeks) and at birth (≤7 days) visits, anthropometric measurements of body weight (nearest 0.1 kg, Salter, Avery Weigh-Tronix, India), height (nearest 0.1 cm, Seca 213, USA), abdominal circumference and hip circumference (nearest 0.1 cm) were recorded in duplicates using standard methodology (13,14). These were measured by the same trained person throughout the study, and intra-observer differences were ≤ 0.1% for all anthropometric parameters. Skinfold thickness, measured with Holtain calipers (nearest 0.2 mm, Crymych, UK), at three sites (biceps, triceps and subscapular) (15) were measured in triplicates (average CV of 1.1%) to obtain estimates of body fat (16). Intra-observer differences were within 0.1%. Sociodemographic details were recorded with an interviewer-administered questionnaire. Three separate 24-hour diet recalls (2 weekdays and 1 weekend) were also administered to assess the dietary intake during the different visits. Energy and nutrient intakes were computed using cooked food recipes and raw food nutrient databases (17,18). A previously validated physical activity questionnaire was used to assess the physical activity level (PAL) of the subjects (19).

The TBK was estimated from the naturally radioactive isotope (^{40}K) at the four time points referred above, using a whole-body potassium counter (WBKC) with a shadow shield design (20). Briefly, four 406.4 mm x 101.6 mm x 101.6 mm thallium-doped sodium iodide (NaI(TI)) detectors (Saint-Gobain Crystal and Detectors, Hiram, USA) were placed within a shielded detector box on top of the shadow shield. The gamma ray spectroscopy system associated with each detector included single units of photomultiplier, preamplifier, amplifier and multi-channel analyser to convert the gamma photon flux to a digital signal. In order to read the maximum signal of the corporeal gamma rays, the detectors were strategically placed to have a desired line of sight below and enable an unabridged count of the gamma rays (1.46 MeV) emanating from the subject lying beneath on the moveable bed of the WBKC (20). The peak associated with ^{40}K was identified in a specific region of interest, using the CERN ROOT package (21). A linear fit function was used to estimate the background counts underneath the ^{40}K peak. The peak was then fitted to a Gaussian curve, the area of which, after the subtraction of background, gave the true value of counts for each detector. Counts were then scaled to the time interval (in seconds) to get an average number of counts per second (20). Phantoms containing deionised water and known concentrations of potassium chloride solution were constructed in varying sizes to calibrate the WBKC. The phantoms were also used to account for the different detector efficiencies associated with varying body geometries. Monte-Carlo calculations were then applied to the different geometries to simulate the phantoms and human bodies of different shapes and sizes (22–24). The accuracy error of the WBKC was 2.8%. The mean precision was noted to be 1.9% of TBK and the mean counting error ranged from 0.8 to 2.7% for the phantoms (20).

During the TBK measurements, subjects lay supine for 30 minutes on the moveable bed of the WBKC. The bed was then rolled under the detectors, to measure the entire body (from superior to inferior) in 3 segments, at counting intervals of 10 minutes each. To account for the discomfort of lying supine for 30 minutes especially in the 3rd trimester, the software of the WBKC was designed to allow the measurement to be paused and restarted. This feature, along with the moving bed with precise stops, gave the subject the option to change her posture to lateral or sitting position between the three 10-minute intervals. The TBK content was estimated using the constant proportion of ^{40}K to its major stable isotopes. From this, total body nitrogen (TBN) was calculated, assuming a TBK to nitrogen ratio of 2.15 mmol K.g^{-1}N (25). Total body protein was then estimated as 6.25 x TBN (g) (26). The TBK was also used to calculate BCM, where BCM (kg) = 0.0092 x TBK (mmol) (27). The EAR of additional protein at each trimester was calculated from the sum of the mean protein deposition value adjusted for the efficiency of utilization of dietary protein (1), and the additional maintenance requirement of the mean mid-trimester GWG. The safe level of the additional protein requirement was calculated assuming a coefficient of variation of 12.5% (28). These values of the EAR were with reference to the observed GWG in this study and could also be recalculated for a theoretical GWG of 12 kg, assuming linearity of the relation between protein deposition and GWG. The theoretical GWG of 12 kg was chosen because it was defined as the average GWG for Indian women (29); this also allowed for comparisons with the 2007 WHO/FAO/UNU report (1), where similar assumptions were made for protein deposition with a GWG of 12 kg. However, Indian women, many of whom have a low body weight at the start of pregnancy, may have an even lower GWG (29) with otherwise normal pregnancy outcomes, and therefore, the EAR for a theoretical GWG of 10 kg was also calculated.

Body fat and fat free mass (FFM) were also calculated from a cellular model of the body (26). The Fat Free Mass (FFM) was calculated from the measured BCM and the Total Body Water (TBW) derived from previous literature on hydration in pregnant women (30). Body fat was then calculated as the difference between body weight and FFM.

Data are presented as mean and standard deviation (SD). The distribution of TBK, BCM and body weight at each trimester measurement were checked for normality using Quantile-Quantile plots. The change in TBK and weight across trimesters was examined using Repeated Measures ANOVA, with pairwise comparison of trimesters using Bonferroni-adjusted post-hoc tests. Similar analyses were carried out for the dietary intake, energy expenditure and physical activity levels during pregnancy. A sample size of 34 was estimated for a 6.5 g increment in TBK (8) observed from 1st to 3rd trimester of pregnancy, with twice the value as SD for the increment. Assuming a 30% drop out rate (loss to follow up and miscarriages), the total sample size was calculated to be 50. A sensitivity analysis of GWG, TBK accretion and birth weight was performed, excluding the women with gestational diabetes, as compared to entire sample.

Correlations between BMI, accretion rates, GWG, and birth weight were also carried out. Paired t test and Mann Whitney U test analyses were performed where relevant. All analyses were performed using Stata version 14 (Statistical Software: Release 14. College Station, TX: StataCorp LP) and $p<0.05$ was considered statistically significant.

Results

Of the recruited 50 pregnant women, 7 dropped out of the study. Five of the remaining 43 subjects did not come for one of the TBK measurements across the trimesters and 8 did not come after delivery. The participant flow chart is presented in **Figure 1.** The lost to follow up subjects were not different from the rest, as their mean body mass index (BMI) at recruitment was 23.1 ± 4.4 kg.m^{-2}, which along with their socioeconomic status, was not different to the rest of the women. All subjects belonged to the middle socio-economic stratum, scored according to the modified Kuppuswamy's criteria, that included occupation, education and income of the family (31). The physical characteristics of the subjects are presented in **Table 1**. The age of the subjects ranged from 20-40 years and the body weight at recruitment ranged from 34.5-88.4 kg. The mean BMI of the subjects at the 1st trimester was 23.4 ± 4.6 kg.m^{-2}. Nineteen of the women had normal BMI, while 5 were underweight and 14 were overweight/obese according to the WHO classification (32). The mean percent body fat was 31.9 ± 5.7% as calculated from skinfolds. The mean percent body fat estimated from the cellular model was 31.9 ± 2.0%, which was not statistically different from the skinfold estimate (p = 0.97). The mean birth weight was 3.0 ± 0.4 kg, ranging from 2.3 to 4.1 kg. The mean gestational age at birth was 39.3 ± 1.0 weeks. Seventy per cent of the babies were classified as appropriate for gestational age, as per the intergrowth newborn size standards (33), which was similar to the value observed in a previous study from Bengaluru, India (34). Most babies were male (70%) in this study.

The dietary intake of the pregnant women across the trimesters are presented in **Table 2.** The pregnant women's mean reported daily energy intake at recruitment was 7.8 ± 1.8 $MJ.d^{-1}$, with a protein intake of 57.7 ± 16.5 $g.d^{-1}$ (~12.3 ± 1.8% Protein: Energy (PE) ratio). In comparison to 1st trimester, the energy and protein intakes increased by 18 and 20%, and 15 and 18% in the 2nd and 3rd trimesters, respectively. As the energy and protein intakes increased proportionately across the trimesters, the PE ratio remained about the same (~12%) throughout the pregnancy. Dietary carbohydrate and fat intakes were 61.0 ± 5.3% and 27.5 ± 5.3% of the total energy intake and the distribution of these macronutrients also remained similar in all the trimesters of pregnancy. The subjects were predominately non-vegetarians (86.8%) and consumed non-vegetarian foods twice a week. The mean daily energy expenditure was 8.2 ± 1.2 MJ at recruitment, which increased by 0.8 MJ at the 2nd trimester and then remained essentially the same in the 3rd trimester. The physical activity records yielded a mean PAL of 1.50 ± 0.1, remaining essentially unchanged throughout the pregnancy.

The mean body weight, TBK and BCM of the subjects increased significantly across the trimesters (**Table 3**). The body weight increased significantly for each trimester from the previous (all $p<0.001$). The TBK and BCM measurements in the 3rd trimester were significantly higher than measurements in both the 1st and the 2nd trimesters (all $p<0.05$ after Bonferroni adjustment for multiple comparisons). The paired t tests performed on post-delivery measures of body weight, TBK and BCM, with corresponding measures at 1st trimester showed a significant difference only for body weight ($p<0.001$). The sensitivity analysis of GWG, TBK accretion and birth weight which excluded pregnant women with gestational diabetes, showed no significant difference compared to the entire sample. BMI was not correlated with TBK accretion in any of the trimesters, when considered within BMI groups of underweight, normal and overweight (32). The birth weight of the babies of low BMI women did not significantly affect the overall birth weight of the sample. Since the number of subjects were few in each BMI category, interpretation of BMI specific protein accretion rates could not be made. Additionally, there was no correlation between parameters of protein accretion, GWG and birth weight.

The calculated protein deposition rates based on the mean TBK accretion in the 2nd and 3rd trimester of 0.04 g.d^{-1} and 0.08 g.d^{-1}, were 2.7 g.d^{-1} in the 2nd trimester and 5.7 g.d^{-1} in the 3rd trimester respectively. This deposition rate was adjusted for an efficiency of dietary protein utilization of 42% (1). To this were added the additional maintenance protein requirement of the GWG in each trimester, calculated as the additional protein intake required to support the mid-trimester weight gain. The EAR thus calculated was 8.2 g.d^{-1} and 18.9 g.d^{-1} in the 2nd and 3rd trimester respectively **(Table 4)**, for an observed GWG of 10.7 kg. The safe level of intake (or recommended daily allowance, RDA) was based on an assumed variability in the requirement of 12.5%, and was 10.2 g.d^{-1} and 23.6 g.d^{-1} in the 2nd and 3rd trimesters, respectively.

The calculated EAR of additional protein requirement for a GWG of 12 kg was 9.1 g.d^{-1} and 21.2 g.d^{-1}, corresponding to a safe intake of 11.4 g.d^{-1} and 26.3 g.d^{-1} in the 2nd and 3rd trimester respectively. Similarly, for a GWG of 10 kg, the EAR of additional protein was calculated as 7.6 g.d^{-1} and 17.6 g.d^{-1} in the 2nd and 3rd trimester respectively. A visual comparison of the EAR estimates from the present study for a GWG of 12 kg, with those of the 2007 WHO/FAO/UNU Expert Committee (1), also recalculated for a GWG of 12 kg, is presented in **Figure 2**.

Discussion

The estimates of the average additional protein requirements in pregnancy obtained from the present study, based on measurements of protein accretion using a WBKC, is the first from India, and to our knowledge, from any LMIC. The mean TBK gain during pregnancy, accounted for by the fetus, placenta, amniotic fluid, uterus, plasma, and red blood cells, in the present study at the 37th week, was 9.1 g, which was similar to the TBK gain (8.23 g) observed in HIC women (8). Earlier studies have estimated similar, if slightly higher, amounts of TBK gains of 11.4 g and 9.4 g (35,36), with the latter study (36) having a GWG of 10.4 ± 2.7 kg at the 37th week of pregnancy, which was similar to the present study. The GWG of 10.7 kg at the 37th week of gestation (11.7 kg on extrapolation to 40 weeks of gestation) was associated with a reasonable mean birth weight of 3.0 ± 0.4 kg (range 2.3-4.1 kg). The total body protein accretion observed in the present study was 674 g and was comparable to the accretion estimates found in HIC pregnant women (8,29).

When the additional protein requirements from the present study were recalculated for a GWG of 12 kg, they were reasonably similar to the recalculated 2007 WHO/FAO/UNU recommendation for a similar GWG; 6.7 g and 21.7 g additional protein per day in the 2nd and 3rd trimester respectively (1). The difference between the two recalculated requirements was marginal, with additional protein EAR recalculated from the present study being slightly higher (by 2.4 $g.d^{-1}$) in the 2nd trimester and slightly lower (by 0.5 $g.d^{-1}$) in the 3rd trimester (Figure 2). These finding thus suggest that, when a similar GWG is considered, the 2nd and 3rd trimester EAR values from the present study are similar to those in the 2007 WHO/FAO/UNU report (1). Maternal height is an important factor in GWG and birth outcome (37,38), and the additional protein requirement, while nominally for a GWG of 12 kg, might also need recasting in terms of the height and BMI of the Indian population and therefore their expected GWG of 10 kg (29). This also relates to the concern of overfeeding during pregnancy, given that the median height of non-pregnant, non-lactating women in India (39) is 152.4 cm (149.0 and 156.4 cm at the 25th and 75th percentile respectively). In contrast, most of the women (82%) in the present study were >153 cm tall, and 80% of them were from the upper sub-stratum of the middle class socioeconomic status.

The EAR for additional protein has also been measured by the indicator amino acid oxidation method (IAAO), which measures the total protein requirement. This was carried out in healthy Canadian pregnant women, at 11–20 weeks (early) and 31–38 (late) weeks of gestation, and were found to be much higher (40) than the estimates from the present study. The IAAO is based on the measurement of the oxidation of an indicator or 1-^{13}C–labelled indispensable amino acid (IAA), which reflects the adequacy of protein or other IAA in the diet. In a dose response measurement, the indicator oxidation falls to a nadir as the protein or IAA intake approaches an adequate value. This can be mathematically defined on this dose response curve to reflect the protein or IAA requirement (41). In the Canadian study (40), the requirements increased by 32 and 63% over the non-pregnant EAR, in comparison to the ~18 and 35% increase observed in the present study at the 2nd and 3rd trimester over the 1st trimester. The difference might be related to differences in the habitual protein intake, which was 93 and 105 g.d^{-1} (1.44 and 1.47 g.kg^{-1}.d^{-1}) for the 2nd and 3rd trimester in the Canadian study, in comparison to 68 and 70 g.d^{-1} (1.08 and 1.03 g.kg^{-1}.d^{-1}) in the present study, as well as to differences in the GWG (12.4 kg at the 35th week compared to 10.7 kg at the 37th week in the present study).

The TBK after delivery (measured within 7 days of delivery) in the present study, did not differ significantly from the first trimester, supporting the existing literature from a HIC population (8,36) that there is no net accretion in protein during pregnancy. Using the observed increment in dietary protein intake (10.0 g.d^{-1} of quality protein, obtained after adjusting for the protein digestibility corrected amino acid score of 80% (42) and the average rate of protein deposition (4.1 g.d^{-1}), from the 1st to 3rd trimester, the efficiency of utilization of protein was calculated to be ~41%. While this is a crude estimate, given the high variability (~30%) of dietary data estimation by questionnaire, it is similar to the value of efficiency of dietary protein utilization of 42% that is currently used (1) to adjust the measured protein deposition value, to obtain the EAR.

The increase in protein intake during pregnancy was more marked in the 2^{nd} than in the 3^{rd} trimester and this finding was consistent with earlier studies in Bengaluru (43,44), which have also observed that there was no significant increase in food and nutrient intake from the 2^{nd} to 3^{rd} trimester. This pattern of a plateau in the dietary intake at 3^{rd} trimester by Indian women, rather than an increase to meet the additional requirement, could be due to sociocultural beliefs, practices and perceived symptoms of acidity, breathlessness and heaviness (45). It thus presents challenges of translating the increasing EAR of protein and other nutrients in the 3^{rd} trimester into practice, without the use of high-protein supplements, The EAR of additional protein of 8.2 $g.d^{-1}$ and 18.9 $g.d^{-1}$, along with the recommended extra energy intake of 1464 kJ (29) in the 2^{nd} and 3^{rd} trimester, for the observed GWG can be achieved, for example, by consuming an additional 250 mL and 600 mL of milk per day, respectively. This would translate to 300 mL and 650 mL of milk for a GWG of 12 kg. Various food combinations can be made in the diet of a pregnant woman to achieve the additional amounts of protein intake needed to meet their requirements, by using foods with high quality protein content, such as milk and milk products, lentils, rice and lentil blends, eggs and meat. Very high intakes of protein are not recommended during pregnancy, and the recommendation for additional protein intake should be viewed in the context of the expected GWG and the prenatal nutritional status of the mother (46). The total protein intake should also be viewed in relation to the energy intake as the PE ratio; as observed in the present study, this was about 12% and well within safe limits.

The strength of the current study is that it used an accurate TBK measurement to define the EAR for additional protein in healthy well-nourished urban Indian women, with good pregnancy outcomes. The high accuracy and precision of the counter (>97% and <2% respectively) in relation to standards (phantoms) of different potassium content, sizes and geometries (20), along with appropriate adjustments for body geometry by Monte-Carlo simulations, give confidence that the results are robust. Limitations were the small sample size, wide range in bodyweight (from underweight to overweight), loss to follow-up (24%) and predominantly male births (70%). In addition, the small sample size also made it difficult to infer the specific effect of BMI on TBK accretion. Since most Indian women are relatively small-statured, more studies are required to define their protein requirements, particularly related to optimal pregnancy outcomes.

In conclusion, the present study is the first to estimate the protein requirements of Indian pregnant women using TBK estimates, where it found fairly similar values for the EAR in the 2nd and 3rd trimester to those defined in the 2007 WHO/FAO/UNU report (1) extrapolated to GWG of 12 kg. This puts special emphasis on the quality of food that must be eaten during pregnancy in LMIC, particularly with reference to protein.

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Conflict of Interest

All authors report no conflict of interest.

Authors' contributions

The authors' responsibilities were as follows - AVK, RK, HSS, TP designed the research; AT, SG and SCN recruited and provided obstetric care to pregnant women; SN conducted research and analysed data; KGB contributed to data analysis using Monte Carlo simulations; TT provided guidance for statistical analysis; AVK, RK, SN and KGB were involved in the interpretation of results; RK, SN and AVK wrote the paper; AVK had primary responsibility for final content. All authors read and approved the final manuscript.

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Table 1: Characteristics of the pregnant women at the time of recruitment

Variable	Mean ± SD
Age (years)	27.3 ± 4.9
Weight (kg)	57.8 ± 12.6
Height (cm)	157.3 ± 4.7
BMI ($kg.m^{-2}$)	23.3 ± 4.6
% Fat[1]	31.9 ± 5.7
% Fat[2]	31.9 ± 2.0

Values are mean ± standard deviation (SD)

n = 38 (pregnant women who completed all three trimester measurements)

BMI- Body Mass Index; % Fat- Fat as percentage of body weight

[1] Measured from skinfolds

[2] Estimated from body cell mass measurement from the whole-body potassium counter and the derived estimates of total body water

[1] and [2] showed no statistical difference using paired t test analysis ($p = 0.97$)

Table 2: Dietary intake and physical activity data of the pregnant women across trimesters

Variable	1st Trimester	2nd Trimester	3rd Trimester	p-value
Energy (MJ.d^{-1})	7.8 ± 1.8^{1}	9.5 ± 2.1^{2}	9.8 ± 2.9^{2}	<0.001
Protein (g.d^{-1})	57.7 ± 16.5^{1}	67.9 ± 16.1^{2}	70.3 ± 24.0^{2}	0.002
Carbohydrate (g.d^{-1})	282.8 ± 60.4^{1}	338.8 ± 77.8^{2}	359.7 ± 94.5^{2}	<0.001
Fat (g.d^{-1})	57.0 ± 20.4^{1}	71.5 ± 24.7^{2}	71.8 ± 30.5^{2}	0.002
Protein (%.d^{-1}) or PE ratio	12.3 ± 1.8	12.1 ± 1.5	12.0 ± 1.6	0.579
Carbohydrate (%.d^{-1})	61.0 ± 5.3	60.3 ± 5.9	63.3 ± 13.3	0.570
Fat (%.d^{-1})	27.5 ± 5.3	28.2 ± 5.3	27.1 ± 6.0	0.266
Energy Expenditure (MJ.d^{-1})	8.2 ± 1.2^{1}	9.0 ± 1.5^{2}	9.0 ± 1.6^{2}	<0.001
PAL	1.5 ± 0.1^{1}	1.6 ± 0.2^{2}	1.5 ± 0.2^{1}	0.016

n = 38; Values are mean ± standard deviation (SD)

MJ- Megajoules; PAL- Physical Activity Level

%.d^{-1}- Percentage of total energy intake per day

PE ratio- Ratio of Protein to Energy

Different superscripts indicate statistical significance with post-hoc Bonferroni adjusted $p<0.05$

Table 3: Measurements of body weight, total body potassium and body cell mass across pregnancy and post-delivery of the baby.

Variable	1st Trimester	2nd Trimester	3rd Trimester	Post Delivery[1]	p-value
Weight (kg)	57.8 ± 12.6[2]	63.2 ± 13.2[3]	68.5 ± 13.8[4]	65.1 ± 14.2[5]	<0.001
TBK (g)	110.2 ± 21.7[2]	113.4 ± 22.6[2]	119.2 ± 22.3[3]	111.3 ± 32.3	0.0002
BCM (kg)	25.9 ± 5.1[2]	26.7 ± 5.3[2]	28.1 ± 5.3[3]	26.2 ± 7.6	0.0002
Mean PD ($g.d^{-1}$)	-	2.7	5.7	-	

n = 38; Values are mean ± standard deviation (SD

TBK- Total Body Potassium

BCM- Body Cell Mass

PD- Protein Deposition: Calculated from the difference between the measured TBK values at each trimester. The TBK (mmol) was converted to total body nitrogen (TBN, g) assuming a TBK to N ratio of 2.15 mmol $K.g^{-1}N$ (25). Total body protein was estimated as 6.25 x TBN (g) (26). Mean PD ($g.d^{-1}$) was estimated after adjusting for mean difference in number of days between the TBK measurements at each trimester

[1] n = 30

Different superscript (2-4) indicate statistical significance with post-hoc Bonferroni adjusted $p<0.05$

[5] Significant difference ($p<0.001$) between 1st trimester and post-delivery visits using paired t test analysis

Table 4: Calculated additional protein requirement during pregnancy in the present study and for a theoretical GWG of 10 and 12 kg

Trimester	Mid-trimester weight gain (kg)	Additional protein for maintenance (g.d^{-1}) [1]	Protein deposited (g.d^{-1})	Dietary protein requirement for deposition (g.d^{-1}) [2]	Mean extra protein requirement or EAR (g.d^{-1}) [3]	Safe intake (g.d^{-1}) [4]
Women gaining average 10.7 kg during gestation (this study)						
2nd (14-26 weeks)	2.7	1.8	2.7	6.4	8.2	10.2
3rd (27-40 weeks)	8.0	5.3	5.7	13.6	18.9	23.6
Women gaining average 12.0 kg during gestation (theoretical)						
2nd (14-26 weeks)	3.0	2.0	3.0	7.2	9.1	11.4
3rd (27-40 weeks)	9.0	5.9	6.4	15.2	21.2	26.3
Women gaining average 10.0 kg during gestation (theoretical)						
2nd (14-26 weeks)	2.5	1.6	2.5	6.0	7.6	9.5
3rd (27-40 weeks)	7.5	4.9	5.3	12.7	17.6	22.0

n = 38

[1] Midterm increase in weight x estimated average requirement for maintenance for adults 0.66 $g.kg.d^{-1}$

[2] Protein deposited, adjusted for a 42% efficacy of utilization

[3] Estimated Average Requirement; sum of extra maintenance plus protein deposited

[4] Safe intake = Mean extra protein requirement + 1.96 x Standard Deviation extra protein requirement (corresponding to a coefficient of variation of 12.5%). This requirement (which refers to high quality protein that meets criteria for digestibility and amino acid score) is that protein intake at which the risk of deficiency is <2.5%

Legends for figures

Figure 1: Participant Flow Chart

Figure 2: Assuming a linear relation between protein deposition and gestational weight gain (GWG), the figure depicts a comparison of the recalculated Estimated Average Requirement (EAR) of additional protein for the present study (n = 38) for a theoretical GWG of 12 kg with the EAR for a similar GWG recalculated from the EAR for 13.8 kg GWG as observed by the 2007 WHO/FAO/UNU Expert Committee (1)

Figure 1

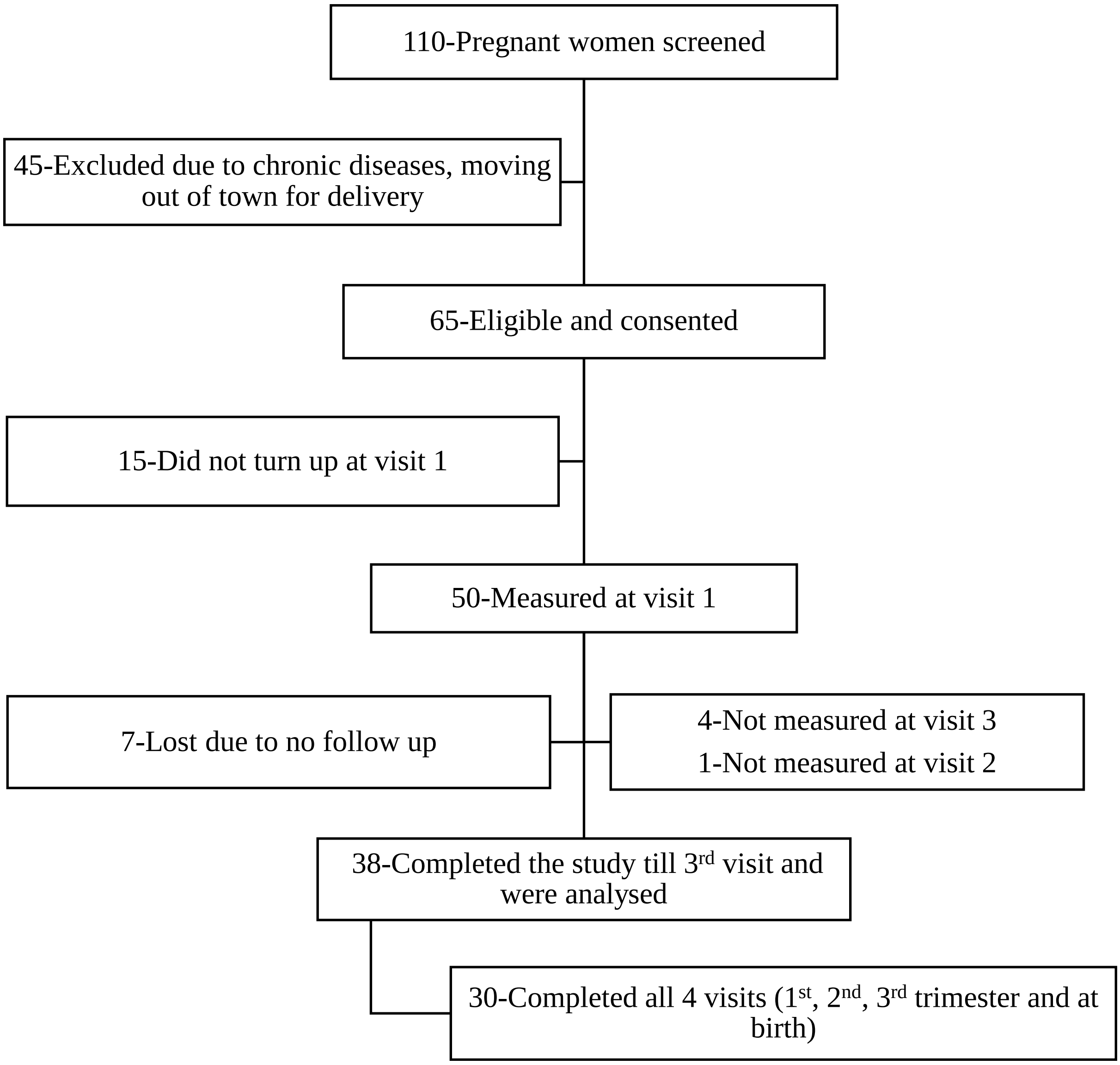
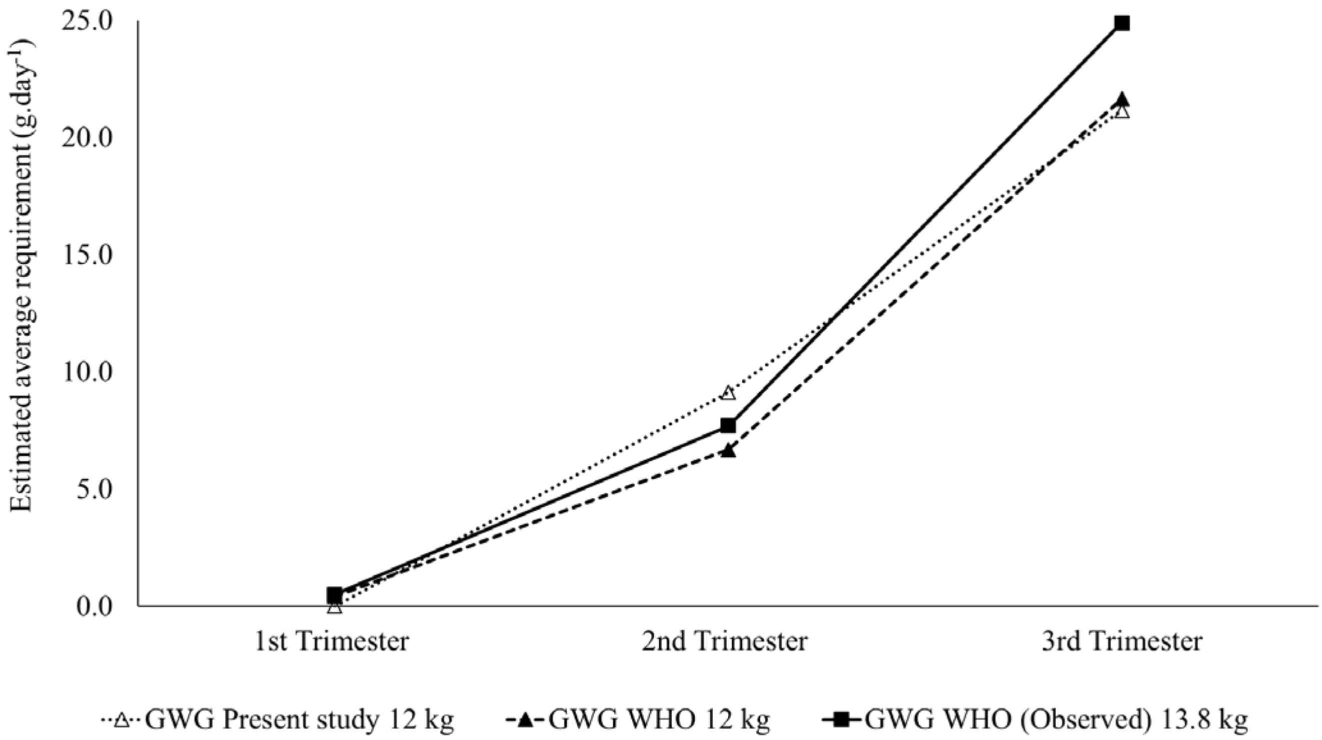

110-Pregnant women screened
45-Excluded due to chronic diseases, moving out of town for delivery
65-Eligible and consented
15-Did not turn up at visit 1
50-Measured at visit 1
7-Lost due to no follow up
4-Not measured at visit 3
1-Not measured at visit 2
38-Completed the study till 3rd visit and were analysed
30-Completed all 4 visits (1st, 2nd, 3rd trimester and at birth)

Figure 2


25.0
20.0
15.0
10.0
5.0
0.0
Estimated average requirement (g.day-1)
1st Trimester
2nd Trimester
3rd Trimester
GWG Present study 12 kg
GWG WHO 12 kg
GWG WHO (Observed) 13.8 kg