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Nutrient : Women Need Most

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SUMMARY

The body needs calcium to maintain strong bones and to carry out many important functions. Osteoporosis, reflected only by a low bone mineral density (BMD), is increasingly becoming a major public health problem in Asian countries. Genetic factors along with environmental factors are responsible for substantial variation in bone density and bone mass. Women whose calcium intake is inadequate before the age of 20-25 and do not achieve their ideal peak bone mass, have a higher risk later on in life of developing osteoporosis, because calcium is drawn from the bones as a reserve. In pregnancy, very high circulatory concentrations of estrogens and progesterone alter the concentration of many substances including calcium in the maternal blood. There is an increased demand for calcium and inorganic phosphate for fetal development during pregnancy. All these factors which negatively affect bone mass consistently persist in premenopausal as well as postmenopausal period.

A high incidence of vitamin D deficiency in pregnant and non-pregnant women has been reported from developing countries including India. Vitamin D deficiency and hypocalcemia have been associated with a variety of pregnancy complications such as preeclampsia, gestational diabetes and prematurity.

It has been observed that daily intakes of energy (1563.4 ± 267.2 kcal), protein (48.7 ± 8.7 g) and calcium (543.7 ± 161.3 mg) were below the recommended dietary allowance of women. The major part of this dietary calcium came from plant sources, which are known

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to have low bioavailability. The diets were typically cereal-based with a very low intake of protective foods and animal protein. Insufficient intakes of calcium do not produce obvious symptoms in the short term because the body maintains calcium levels in the blood by taking it from bone. But in the long run, it causes osteopenia and increases the risks of osteoporosis and bone fractures.

Daily dietary energy, protein, and calcium intakes were correlated with BMD at the lumbar spine. Age, BMI, and physical activity were significant predictors for BMD at all sites. Dietary pattern coupled with higher education levels and greater physical activity favored bone health. There is need to change dietary pattern and habits by improving education and socio-economic level. Calcium and vitamin D supplementation, especially in second and third decade are the first-line strategy for the prevention of osteoporosis.

Calcium is the most abundant inorganic element in human body and makes up about 1.5% to 2% of the total body weight and 40% of the total body minerals. Approximately 99% is present in bones and teeth, 1% in blood & extracellular fluid. Calcium is essential to all vital functions and an intracellular messenger indispensable for all cell functions. Serum calcium is the most precisely controlled biological constant. Apart from maintaining the structure & functions of bone, calcium is essential for nerve impulse transmission and immune function. It regulates muscle contraction as well as blood pressure & also essential for blood clotting.

The regulation of plasma calcium depends on the interplay of three organs i.e. bone, kidney and intestines. The adaptive mechanisms involved in the maintenance of homeostasis during the periods of increased calcium demands involve greater intestinal calcium absorption, decreased urinary calcium losses and increased bone resorption. These adaptations are mediated by increased secretion of the various calcitropic hormones such as 1,25-dihydro-xyvitamin D3 (1, 25(OH)2 D3), parathormone, calcitonin and parathyroid hormone related peptide (PTHrP). In all of these adaptive mechanisms, vitamin D is involved directly or indirectly.

A key adaptive change i.e increased 1, 25 dihydroxyvitamin D production during pregnancy, leads to a marked increase in intestinal calcium absorption and as a consequence, hypercalciuria. This is an independent phenomenon, occurring as early as 12 weeks of gestation and may allow the maternal skeleton to store calcium in advance of peak fetal demands later in third trimester.

The concentration of calcium in plasma is closely maintained at between 8.5-11 mg/dl. To maintain these titres, if diet does not provide enough calcium, then body steals it from the bones (1).

Usually low serum levels of calcium are not suspected clinically. Symptoms of hypocalcaemia therefore generally represent an extreme aberration in the homeostasis. Hypocalcaemia becomes symptomatic when its level falls below 5 mg/dL.

A recent study confirmed that serum ionic calcium levels remain normal during pregnancy (2). The decrease in total calcium level was because of hemodilution and hence decrease in albumin bound calcium fraction. True ionic hypocalcemia may occur in pregnant women with vitamin D deficiency.

Calcium Intake and Serum Calcium :

It has been demonstrated that 57.8% of consecutive non pregnant healthy young women (82/142), attending gynecological OPD with minor gynecological ailments, had hypocalcemia (serum calcium level below 8.5 mg/dL). However in none of them, the calcium levels were below 5 or resulted in symptoms pertaining to hypocalcemia. Serum calcium (mean±SD;mg/dL) was 7.5±0.9 and 9.3±0.6 in hypocalcemic and eucalcemic women respectively. There was no difference among the women with or without hypocalcemia with respect to their nutritional status or consumption of calories, protein or dietary calcium intake. Majority of women were well nourished signifying that malnutrition may not have been a determining factor (unpublished data).

Calcium Intake and Serum Calcium during pregnancy :

A total of 545 consecutive women in second trimester of pregnancy without any obstetric and medical risk factor attending the antenatal clinic were recruited (3). Nearly two thirds of women (66.4%) had hypocalcemia (362/545). None of the women had symptoms pertaining to hypocalcaemia. Daily intake of calcium was grossly inadequate in both the groups. Nearly half of the participants in both groups consumed less than 300 mg of calcium per day (Table 1). There was no correlation between daily calcium intake and the serum calcium levels (correlation coefficient: -0.8). Urinary calcium excretion was lower in women with hypocalcaemia versus those without hypocalcaemia but it did not reach significance (p value 0.08). On linear regression, serum calcium was not found to be dependent on any baseline variables included in this study. The pregnancy outcome was not significantly different in two groups (3).

Such a high incidence of hypocalcaemia has not been reported from a low income country like India. In a study by Ainy et al, 19% of 48 pregnant women had hypocalcaemia (i.e. serum calcium level below 8.5 mg/dL) (4). Bhalala et al has reported that 88% (37/42) mothers from Western India had serum calcium in normal range (8.1-10.5 mg/dL) and remaining 12% had serum calcium values from 7.1 to 7.7 mg/dL (5).

Table 1- Baseline characteristics of the pregnant study women

Characteristics	Group A Women with hypcalcemia (n=362)	Group B Women without hypocalcemia (n=183)	P value
Age* (years)	21.8±2.4	22.0±2.7	0.26
Participants' education			
Illiterate	42(11.6%)	16(8.8%)	
Up to metric	220(60.9%)	108(59.3%)	0.43
12th standard	53(14.7%)	32(17.6%)	
graduate	41(11.4%)	20(10.9%)	
Postgraduate	5(1.4%)	6(3.3%)	
Husband education			
Illiterate	31(8.6%)	8(4.4%)	
Up to metric	216(59.8%)	104(57.1%)	0.13
12th standard	48(13.3%)	37(20.3%)	
graduate	58(16.1%)	28(15.4%)	
Postgraduate	8(2.2%)	5(2.8%)	
Monthly per capita income* (Indian rupees)	1338±159	1422±101	0.35
Less than 500	12(6.6%)	14(3.9%)	0.16
More than or equal to 500	171(93.4%)	348(96.1%)	
Height * (cm)	149±6.2	149±6.1	0.31
Weight* (Kg)	51.9±8.6	51.7±9.4	0.81
Body mass index (Kg/M^2)			
Underweight: <18.5	24(6.6%)	18(9.8%)	
Normal: 18.5-24.99	229(63.3%)	120(65.6%)	0.31
Overweight: 25-29.99	84(23.2%)	32(17.5%)	
obese ≥30	25(6.9%)	13(7.1%)	
Gestation* (weeks)	18.2±3.4	17.6±3.6	0.06
Hemoglobin* (g/dL)	10.8±1.4	10.7±1.5	0.31
≤6: severe	1 (0.3%)	0	0.14
7-10: mod	68 (18.8%)	49 (26.8%)	
10-<11 mild	83 (22.9%)	34 (18.6%)	
Daily calcium intake* (mg)	323±203	327±193	0.81
<300	172(47.5%)	85(46.5%)	0.74
300-599	138(38.1%)	76(41.5%)	
600-1199	51(14.0%)	21(11.5%)	
≥1200	1(0.3%)	1(0.6%)	
Serum calcium level* (mg/dL)	7.2±0.8	9.8±0.8	0.00
Urinary calcium excretion * (mg/24 hr)	125±69	136±62	0.08

Data expressed as *mean±SD or number (%)

The principal maternal adjustment during pregnancy is an increasing parathyroid hormone secretion which maintains the serum calcium concentration in the face of a falling albumin level, an expanding extracellular fluid volume, an increasing renal excretion, and placental calcium transfer. The placenta transports calcium ions actively, making the fetus hypercalcemic relative to its mother, which in turn stimulates calcitonin release and perhaps suppresses parathyroid hormone secretion by the fetus.

The dietary intake of calcium was very low in all the participants and nearly half of women consumed calcium less than 300 mg daily (3). Another study revealed daily dietary calcium intake range from 800 to 1500 mg besides supplemental calcium intake of 250-500 mg (5). There was no relationship between the daily intake of calcium and the occurrence of hypocalcaemia. It is most likely that faulty dietary habits or vitamin D deficiency are responsible for it. The major part of this dietary calcium came from plant sources, which are known to have low bioavailability. The inhibitors of calcium absorption such as phytates and oxalates are abundant in the vegetarian diet and retard the absorption of dietary calcium. Moreover, absorption of calcium could be hampered by vitamin D deficiency as it is the major factor influencing absorption of calcium from the gut. A study by Vupputuri *et al* revealed that 60% of healthy adults living in Delhi city had 25 hydroxy vitamin D values ≤9 ng/ml which is significantly lower than the recommended vitamin D levels (6). In a study by Ainy *et al*, 20% of women had vitamin D levels <10 ng/ml in first trimester (4).

Devine *et al* showed that the fractional absorption of calcium was significantly negatively correlated with years since menopause ($r = -0.15$; $P < 0.05$) (7). The foods rich in calcium such as milk and dry fruits are expensive and not available to this population. Therefore, age related falling absorptive power along with poor quality of diet aggravates the situation.

It was observed that high calcium intake combined with adequate protein intake based on a high ratio of vegetable to animal protein may be protective against osteoporosis (8). Protein-energy under nutrition is a risk factor for bone loss, osteoporosis, and fracture, and the elderly in particular are at risk for protein under nutrition (9). Low levels of serum albumin negatively affect transport of serum calcium. It has been observed that women with higher calcium and better dietary intakes from high-income groups have better bone densities (10). There are reports of high prevalence of suboptimal dietary calcium intake and 25(OH) D insufficiencies in South Indian populations (11) and North Indian healthy subjects (12, 13).

Calcium and Pregnancy Complication :

A very low prevalence of pre-eclampsia had been reported from Ethiopia where the diet contained high levels of calcium. These observations were supported by other epidemiological and clinical studies and led to the hypothesis that an increase in calcium intake during pregnancy might reduce the incidence of high blood pressure and pre-eclampsia among women with low calcium intake. Low calcium intake may cause high blood pressure by stimulating the release of parathyroid hormone and/or renin, thereby increasing intracellular calcium in vascular smooth muscle and leading to vasoconstriction. Calcium supplementation is useful in pregnancy because it reduces parathyroid release and intracellular calcium, thereby reducing smooth muscle contractility and promoting vasodilatation. It reduces uterine smooth muscle contractility and prevents preterm labor and delivery. Calcium may also inhibit endothelial damage.

A total of 524 primigravida women with 12 to 25 weeks were randomly assigned into calcium (2 g of elemental calcium) and placebo group and followed-up until delivery (14). It was concluded that daily supplementation of 2 gram elemental calcium is associated with a reduction of 66.7% risk of pre-eclampsia and 44.9% risk of preterm delivery (Table 2, 3).

The greatest reduction in risk of preeclampsia after calcium supplementation was for women at high risk and those with low baseline dietary calcium intake (15,16). The study population (14) itself has low calcium intake (85.71-910.71 mg/day) compared with the recommended dietary allowances of 1000mg/day throughout pregnancy and lactation (17).

A Cochrane review of trials found that calcium supplementation during pregnancy is a safe and relatively inexpensive and cost effective means of reducing the risk of pre-eclampsia in women at increased risk and also in women from communities with low dietary calcium. It included 13 studies of good quality (involving 15,730 women) (18). One of these trials is from Northern India (14). The average risk of high blood pressure was reduced with calcium supplementation rather than placebo (12 trials, 15,470 women: risk ratio (RR) 0.65, 95%confidence interval (CI) 0.53 to 0.81). There was also a reduction in the average risk of pre-eclampsia associated with calcium supplementation (13 trials, 15,730 women: RR 0.45,95% CI 0.31 to 0.65). The effect was greatest for women with low baseline calcium intake (eight trials, 10,678 women: RR 0.36, 95% CI 0.20 to 0.65).

Calcium and Vitamin D during pregnancy :

Now, it is being observed that, not only dark-skinned but even Caucasian women tend to go into vitamin D deficiency during pregnancy. Studies have reported a prevalence that ranges from 18-84%, depending on the country of residence and local clothing customs. Global prevalence of vitamin D deficiency

Table 2: Baseline characteristics of pregnant subjects recruited and completed the study

Parameter	Subjects recruited in the Study			Subjects who completed the Study		
	Placebo (n= 262)	Calcium (n= 290)	p value	Placebo (n = 251)	Calcium (n= 273)	p value
Age (yrs)	21.94±2.51 (18-31)	21.81±2.52 (17-35)	0.538	21.91±2.47 (18-31)	21.83±2.51 (17-35)	0.725
Height (cm)	149.24±6.17 (130-165)	149.26±6.17 (130-166)	0.961	149.14±6.22 (130-165)	149.32±6.22 (130-162)	0.747
Weight (kg)	51.97±8.74 (36-91)	51.69±8.85 (35-87)	0.717	51.86±8.75 (36-91)	51.68±8.91 (36-87)	0.822
BMI (kg/m^2)	23.37±3.89 (15.79-42.69)	23.25±3.98 (15.61-37.65)	0.710	23.35±3.92 (15.79-42.69)	23.35±3.92 (15.79-42.69)	0.688
Dietary Calcium intake (mg/day)	313.04± 203.75 (85.71- 873.43)	314.92± 201.59 (85.71– 910.71)	0.913	312.84± 204.63 (85.71– 873.43)	314.83± 201.87 (85.71– 910.71)	0.911
Serum calcium (mg/dl) ‡	7.93±1.39 (5.1-11.9)	8.14±1.49 (5.1-11.9)	0.093	7.92±1.38 (5.1-11.9)	8.13±1.49 (5.1-11.9)	0.105
Urine calcium (mg/dl) §	128.43±67.79 (40.5-400)	130.21±65.82 (40.5-387)	0.754	129.84±68.34 (40.5-387)	131.80±66.55 (40.5-387)	0.805
Gestational age at recruitment (Weeks)	17.80±3.53 (12-25)	17.85±3.63 (12-25)	0.869	17.80±3.51 (12-25)	17.86±3.51 (12-25)	0.854
Haemoglobin (gm %)	10.76±1.48 (7-14)	10.76±1.43 (6-14)	0.976	10.76±1.48 (7-14)	10.76±1.42 (6-14)	0.974
Systolic BP at recruitment (mmHg)	113.16±8.47 (90-130)	113.23±8.23 (90-130)	0.925	113.15± 8.47 (90-130)	113.23± 8.17 (90-130)	0.913
Diastolic DBP at recruitment (mmHg)	74.25±6.45 (50-90)	73.92±6.56 (50-92)	0.552	74.23± 6.51 (50-88)	73.78± 6.65 (50-92)	0.442

Data are given as mean ±SD
†Range in parentheses
‡Normal range: 8.5-10.5 mg%
§ Normal range: 100-400 mg/24hrs

Table 3: Maternal and Neonatal outcome in the study population

	Placebo group (n= 251) n (%)	Calcium group (n =273) n (%)	p value
Maternal outcome measures			
Pre-eclampsia	30(12)	11(4.0)	**0.001***
Preterm delivery	32(12.7)	19(7.0)	**0.026***
Induction of labor	12(4.8)	10(3.7)	0.524
Caesarean delivery	27(10.8)	41(15)	0.147
Fetal distress in labor	5(2.0)	6(2.2)	0.870
Meconium in labor	5(2.0)	10(3.7)	0.249
Neonatal outcome measures			
Mean period of gestation at delivery ± SD, weeks	38.27 ± 2.04	38.59 ± 1.67	0.050
Period of gestation at delivery in weeks, n (%) < 32 32-36 37-40 >40	 4(1.6) 28(11.2) 204(81.3) 15(6.0)	 1(0.4) 18(6.6) 233(85.3) 21(7.7)	0.112
Mean birth weight ± SD, gm	2685.35 ± 338.00	2696.27 ± 304.10	0.697
Birth weight in Kg, n (%) <2.0 2.0-2.5 2.5-4.0	 6(2.4) 65(25.9) 180(71.7)	 4(1.5) 60(22.0) 209(76.6)	0.398
Small for gestational age, n (%)	21(8.4)	17(6.2)	0.346
Stillbirth, n (%)	5(2.0)	6(2.2)	0.618

* significant p value.

in pregnant women has been estimated to be 5-50% (19).

In one of our studies of 418 primigravida attending the antenatal clinic of Lok Nayak Hospital, New Delhi, the prevalence of vitamin D deficiency (serum levels of 25 hydroxy vitamin D < 32 ng/ml) was found to be 95.4% (399/418). Most of the pregnant women (61%, 255/418) had insufficient levels of 25 hydroxy vitamin D (10-32ng/ml) and 34.4% (144/418) had severe deficiency of vitamin D (serum 25 hydroxy vitamin D level <10ng/ml). The mean maternal serum calcium levels improves significantly with increase in maternal serum 25 hydroxy vitamin D levels ($p<0.001$). A positive correlation was found between maternal serum 25 hydroxy vitamin D and maternal serum calcium ($r=0.651$; $p=0.000$) (Unpublished data).

Adverse outcomes such as preeclampsia, low birth-weight, neonatal hypocalcemia, poor postnatal growth, bone fragility, and increased incidence of autoimmune diseases have been found to be associated with low vitamin D levels during pregnancy and infancy.

In a systematic review and meta-analysis, vitamin D insufficiency is found to be associated with an increased risk of gestational diabetes, pre-eclampsia, and small for gestational age infants. Pregnant women with low 25-hydroxy vitamin D levels had an increased risk of bacterial vaginosis and lower birth weight infants, but not delivery by caesarean section (20).

The serum 25-hydroxy vitamin D [25(OH) D] level is thought to reflect the vitamin D nutritional status accurately and has been used widely for this purpose. Sunlight exposure and dietary intake are the main determinants of serum 25 (OH) D levels.

Calcium Intake and Bone Mineral Density :

Osteoporosis is a silent disease reflected only by a low bone density. Osteoporosis is increasingly becoming a major public health problem in Asian countries, due mainly to the rapid aging of the population.

A cross-sectional study of 255 women (20-70 years) was conducted to find out the relation of dietary nutrients and bone mineral density (BMD) in North Indian women (21). The study subjects (participants) were healthy normal relatives of the patients being admitted in the obstetrical and gynecological wards of the hospital. The mean age, height, weight and body mass index (BMI) were 40.5 ± 12.6 years, 153.4 ± 4.9 cm, 57.3 ± 12.0 kg and 24.3 ± 4.8 kg/m2 respectively. The daily dietary intakes of the subjects were: energy, 1563.4 ± 267.2 kcal; protein, 48.7 ± 8.7 g; fat, 31.3 ± 9.3 g and calcium, 543.7 ± 161.3 mg. The diets were typically cereal-based with a very low intake of protective foods such as milk and milk products, flesh foods, fish, fruits and vegetables. Animal sources of protein were consumed irregularly. The daily intakes of energy, protein and calcium of postmenopausal women were significantly lower than that of premenopausal women (1487.8±259.6 kcal, 46.4 ± 9.3 gm, 496.1± 169.3mg and 1608.9±262.0 kcal, 50.1± 8.1 gm, 572.5 ± 149.6 mg respectively; p <0.001). Baseline characteristics according to various age groups are shown in Table 4.

BMI, physical activity and educational level was positively correlated with BMD. The daily intakes of energy (1563.4 ± 267.2 kcal) and protein (48.7 ± 8.7 g) were below the recommended dietary allowance. Daily dietary total energy, protein and calcium intake had significant correlations with BMD lumbar spine. Serum calcium levels were found to have positive correlation with BMD femoral neck and Ward's triangle.Stepwise multiple linear regression analyses showed that age, BMI and physical activity were the significant predictors for BMD at all sites. In addition, energy intake was also a predictor for BMD at lumbar spine (21).

Genetic factors seem to account for substantial variation in bone density and bone mass and environmental factors also influence the quality and durability of bone (22, 23). The effect of environmental factors on bone is likely to vary across the lifespan and length of exposure to exercise, diet, alcohol, caffeine and smoking may have increasing impact in older women.

Indians from low-income groups subsist on diets that have inadequate calcium coupled with too few calories, proteins and micronutrients (24). The mean daily intakes of energy and protein were below the recommended dietary allowance (RDA) of 2225 kcal/day and 50 g/day respectively for these women (Table 4). The calcium intake of the participants was only low as compared to the RDA of 800–1,000 mg/day, which is accepted worldwide (25).

The premenopausal females were more osteopenic than postmenopausal females (26) but osteoporosis was more prevalent in postmenopausal females (Table 5). It represents that the factors which negatively affect bone mass consistently persisted in premenopaual as well as postmenopausal period. These factors combined with estrogen deficiency then made these women osteoporotic after the attainment of menopause. Osteoporosis not only affects elderly but young adults also. This is determined by ideal peak bone mass. The peak bone mass is usually achieved in thirties and than it declines (27). If a young adult does not achieve their ideal peak bone mass, they develop osteoporosis at much earlier age.

It was found that calcium intake is affected by various demographic factors like educational level, socioeconomic status. Approximately 71% of the subjects had less than 10 years of education and had lower intake of calcium (296.24mg/day). Total 85% of the subjects had their per capita income below 2000 Indian rupees and had lower intake of calcium (376.92mg/day). The mean calcium intake improves with the improvement of educational levels of the

Table 4: Baseline Characteristics, daily dietary intake and biochemical parameters* of study population

Age Group (years)	20-29	30-39	40-49	50-59	60-69	20-69
Number (%)	67 (26.3)	63 (24.7)	54 (21.2)	46 (18.0)	25 (9.8)	255 (100)
Age (Years)	25.51 ± 2.82	34.95± 2.62	43.87 ± 3.08	53.22 ± 2.48	63.72± 3.68	40.47 ± 12.64
Height (cm)	153.55± 5.26	153.49 ± 5.29	153.00± 4.91	154.13± 4.68	152.40±3.25	153.41 ± 4.92
Weight (kg)	56.52 ± 12.06	57.06± 11.47	60.89± 13.89	58.74± 10.31	49.44± 8.51	57.29 ± 12.05
BMI (kg/m^2)	23.94 ± 4.66	24.25± 4.35	25.98 ± 5.63	24.51± 4.07	21.22± 3.66	24.28 ± 4.76
Per capita income (INR)	4800.00 ± 3047.60	5058.73 ± 3523.77	3967.35 ± 4207.42	4634.91 ± 5162.09	3588.64 ± 4266.24	4539.05 ± 3977.91
Parity	1.21 ± 1.17	2.48 ±1.23	3.15 ± 1.65	3.78 ± 1.78	4.64 ± 2.32	2.73 ± 1.89
Energy (kilocalories/ day)	1636.00 ± 214.40	1660.22 ± 299.09	1447.76 ± 192.67	1488.39 ± 249.82	1512.20 ± 340.52	1563.36 ± 267.16
Dietary Protein (gm/day)	50.83± 9.47	50.15 ± 6.86	49.26 ± 9.09	46.82 ± 7.41	41.52 ± 8.56	48.70 ± 8.73
Dietary Calcium (mg/day)	577.85 ± 132.81	594.14 ±156.06	488.56 ± 145.35	523.46 ±168.60	481.56 ± 205.95	543.71 ± 161.31
Dietary Fat (gm/day)	31.75 ± 5.63	32.67 ± 6.71	29.70 ± 7.31	30.80 ± 7.56	30.60 ± 21.71	31.26 ± 9.28
Serum calcium (mg/dl)	8.42 ± 1.15	8.06 ± 1.16	8.43 ± 1.39	8.45 ± 1.37	8.54 ± 1.21	8.35 ± 1.26
Serum Phosphorus (mg/dl)	3.85 ± 0.56	3.96 ± 0.64	4.13 ± 0.83	4.06 ± 0.97	3.82 ± 0.59	3.97 ± 0.73
Serum albumin (g/L)	4.26 ± 0.70	4.35± 0.64	3.83 ± 0.67	4.09±0.69	4.54 ± 0.66	4.19 ± 0.70
Alkaline phosphatase (U/L)	173.43 ± 45.02	155.44 ± 41.64	171.93 ± 91.75	158.11 ± 46.55	155.80 ± 39.12	164.18 ±57.47

* Values are mean ± S.D. BMI = Body Mass Index, INR = Indian Rupees
Normal Range:
Serum calcium 8.0-11.0 mg%, Serum Phosphorus 2.7-4.5 mg%, Serum Alkaline phosphatase 95-226 U/L, Serum albumin 3-5 g/L

subjects and their husbands (subjects: p = 0.000, husband: p=0.000) (28). It has been observed that Indian women with low education level have low calcium intake (29).

There were significant differences across all educational groups with respect to weight, BMI, dietary intakes of energy, protein, calcium and BMD at the lumbar spine and Ward's triangle (Table 6). The protective role played by educational level could be due to other overall determinants, such as a better health status and nutrition, a more positive attitude to taking drugs or preventive measures, and a more efficient use of health care resources. In a complex manner, under nutrition might affect the positive relationship between occupational activities and bone parameters (30). With improved educational level and per capita income, the population have better living condition and then better nutritional intake.

Table 5: Prevalence of osteopenia and osteoporosis in all participants. Data are presented as percentages with osteopenia (> -2.5 SD to < 1.5 SD below young adult BMD) and osteoporosis (< 2.5 SD below young adult BMD)

Age (Number)	20-29 years (67)	30-39years (63)	40-49years (54)	50-59years (46)	60-69 years (25)	20-69 years (255)
Lumbar Spine (L2-L4):						
Osteopenia	32.8%	19.0%	35.2%	21.7%	24.0%	27.0%
Osteoporosis	4.5%	0%	18.5%	17.4%	60.0%	14.1%
Femur Neck:						
Osteopenia	31.3%	39.7%	38.9%	34.8%	8.0%	33.3%
Osteoporosis	4.5%	0%	11.1%	4.3%	56.0%	9.8%
Femoral Ward's triangle:						
Osteopenia	31.3%	42.9%	33.3%	32.6%	8.0%	32.5%
Osteoporosis	0%	3.2%	18.5%	6.5%	60.0%	11.8%

Conclusion :

Assessment of dietary intake revealed that the daily intake of pregnant subjects comprises of the local vegetables regularly and few dairy products. There is an urgent need for measures to improve the nutritional status, dietary calcium intake and thus the bone health of women. It can be achieved largely by improving dietary pattern and habits and by improving education and socio-economic level. There are evidences suggesting that Vitamin D supplementation should also be recommended. These steps along with awareness towards bone health especially in second and third decade will go a long way to improve morbidity of osteoporosis.

Annexure

Dietary evaluation of all the participants was done by the following method (21). The dietary evaluation of all the subjects was done by using the 24-hour recall method (11). All the subjects were asked for everything they consumed in the last 3 days including 2 consecutive working days and one holiday. Time of the day, food, quantity, and recipes of composites dishes were recorded. The self-prepared standardized portion sized models in terms of households measures (cups, glasses, spoons, bowls, etc.) were used to accurately quantify the intake of both raw and cooked food items. Portion sizes were quantitated by the participants to which standard weights were assigned. Completeness, creditability of the reported number of servings and consistency in reported consumption frequencies were checked. The validity and repeatability of the dietary assessment was rechecked at random over the period of the study. For analyses, food consumption data were converted into energy and nutrients. The intakes of total

Table 6: Daily dietary intakes and BMD of participants by educational levels

Variables *	No education (n = 85)	Less than 10 years (n = 102)	10 to 12 years (n = 30)	More than 12 years (n = 38)	P value
Energy (kcal)	1485.4 ± 311.7	1607.6 ± 233.5	1636.2 ± 295.6	1661.5 ± 168.6	0.006**
Protein (g)	45.7 ± 7.9	50.2 ± 7.8	50.9 ± 12.5	49.5 ± 7.8	0.002**
Fat (g)	29.3 ± 13.2	31.8 ± 6.1	32.3 ± 7.7	33.3 ± 5.6	0.103
Calcium (mg)	497.5 ± 174.2	557.4 ± 146.5	583.3 ± 172.9	578.9 ± 140.5	0.009**
BMD at Lumbar spine (g/cm^2)	0.996 ± 0.210	1.152 ± 0.187	1.192 ± 0.186	1.198 ± 0.185	0.0001**
BMD at Femur neck (g/cm^2)	0.885 ± 0.208	0.933 ± 0.152	0.966 ± 0.163	0.968 ± 0.184	0.092
BMD at Ward's triangle (g/cm^2)	0.752 ± 0.257	0.811± 0.205	0.817 ± 0.184	0.876 ± 0.225	0.036**

* =Values are mean ± S.D
** = Significant P value
BMD = Bone mineral density

energy, calcium, fat and protein were calculated with the use of food composition table, detailing the nutritive value of Indian foods, developed by Indian Council of Medical Research (31). The daily amount of total calories, calcium, fat and protein consumed by the subjects, were calculated by taking the mean value of the 3 days for each foodstuff.

REFERENCES

1. Bushinski and Monk (1998). Electrolyte quintet: Calcium. *Lancet* **352**: 306-311.

2. Kovacs CS (2012). The role of vitamin D in pregnancy and lactation: insights from animal models and clinical studies. *Annu Rev Nutr* **32**:97-123.

3. Kumar A, Agarwal K, Devi SG, Gupta RK, Batra S (2010). Hypocalcemia in Pregnant Women. *Biol Trace Elem Res* **136(1)**:26-32.

4. Ainy E, Ghazi AA, Azizi F (2006). Changes in calcium, 25(OH) vitamin D3 and other biochemical factors during pregnancy. *J Endocrinol Invest* **29(4)**:303-307.

5. Bhalala U, Desai M, Parekh P, Mokal R, Chheda B (2007). Subclinical Hypovitaminosis D among exclusively breastfed young infants. *Indian Pediatr* **44(12)**:897-901.

6. Vupputuri MR, Goswami R, Gupta N, Ray D, Tandon N, Kumar N (2006). Prevalence and functional significance of 25-hydroxyvitamin D deficiency and vitamin D receptor gene polymorphism in Asian Indians. *Am J Clin Nutr* **83**:1411–1419.

7. Devine A, Prince RL, Kerr DA, *et al* (1993). Correlates of intestinal calcium absorption in women 10 years past the menopause. *Calcif Tissue Int* **52(5)**:358-360.

8. Weikert C, Walter D, Hoffmann K,

Kroke A, Bergmann MM, Boeing H (2005). The relation between dietary protein, calcium and bone health in women: results from the EPIC-Potsdam cohort. *Ann Nutr Metab* **49(5)**:312-318.

9. Bonjour JP (2005). Dietary protein: an essential nutrient for bone health. *J Am Coll Nutr* **24(6 Suppl)**:526S-536S.

10. National Institute of Nutrition (2002). Annual Report. National Institute of Nutrition, Hyderabad, India.

11. Harinarayan CV, Ramalakshmi T, Venkataprasad V (2004). High prevalence of low dietary calcium and low vitamin D status in healthy South Indians. *Asia Pac J Clin Nutr* **13**:359–365.

12. Goswami R, Gupta N, Goswami D, Marwaha RK, Tandon N, Kochupillai N (2000). Prevalence and significance of low 25-hydroxy D concentrations in healthy subjects in Delhi. *Am J Clin Nutr* **72**:472–475.

13. Shatrugna V, Kulkarni B, Kumar PA, Rani KU, Balakrishna N (2005). Bone status of Indian women from low income group and its relationship to the nutritional status. *Osteop Int* **16**:1827–1835.

14. Kumar A, Devi SG, Batra S, Singh C, Shukla DK (2009). Calcium supplementation for the prevention of pre- eclampsia. *Intl J Gynecol Obstet* **104(1)** :32-36.

15. Hofmeyr GJ, Duley L, Atallah A (2007). Dietary calcium supplementation for prevention of pre-eclampsia and related problems: a systematic review and commentary. *BJOG* **114**:933-943.

16. Hofmeyr GJ, Atallah AN, Duley L (2006). Calcium supplementation during pregnancy for preventing hypertensive disorders and related problems Cochrane Database of Systematic Reviews. Issue 3. Art. No.: CD001059. DOI: 10.1002/14651858. CD001059.pub2.

17. Indian Council of Medical Research (ICMR), 1990. Recommended dietary intakes for Indians, New Delhi.

18. Hofmeyr GJ, Lawrie TA, Atallah ÁN, Duley L (2010). Calcium supplementation during pregnancy for preventing hypertensive disorders and related problems. Cochrane Database of Systematic Reviews. Issue 8. Art. No.: CD001059. DOI: 10.1002/14651858.CD001059.pub3.

19. Dawodu A, Wagner C (2007). Mother-child vitamin D deficiency: an international perspective. *Arch Dis Child* **92(9)**:737-740.

20. Aghajafari F, Nagulesapillai T, Ronksley PE, Tough SC, O'Beirne M, Rabi DM (2013). Association between maternal serum 25-hydroxyvitamin D level and pregnancy and neonatal outcomes:

systematic review and meta-analysis of observational studies. *BMJ* **26**; 346.

21. Kumar A, Mittal S, Orito S, Ishitani K, Ohta H (2010). Impact of dietary intake, education, and physical activity on bone mineral density among North Indian women. *J Bone Miner Metab* **28(2)**: 192-201.

22. Krall EA, Dawson-Hughes B (1993). Heritable and life-style determinants of bone mineral density. *J Bone Miner Res* **8**:1–9.

23. Jouanny P, Guillemin F, Kuntz C, *et al* (1995). Environmental and genetic factors affecting bone mass. Similarity of bone density among members of healthy families. *Arthritis Rheum* **38**:61–67.

24. National Nutrition Monitoring Bureau (2002). Diet and nutritional status of rural population. National Institute of Nutrition, Hyderabad, India .

25. Indian Council of Medical Research (ICMR), 1990. Recommended dietary intakes for Indians, New Delhi.

26. Mittal S, Kumar A, Gupta RK, Kapoor S, Gulati P, Shukla DK (2011). Comparison of bone mineral density and its variables between premenopausal and postmenopausal women. *J Obstet Gynecol India* **61(2)** : 200– 204.

27. Gilsanz V, Gibbens DT, Carlson M, Boechat MI, Cann CE, Schulz EE (1998). Peak trabecular vertebral density:A comparison of adolescent and adult female. *Calcif Tissue Int* **43**:260-262.

28. Kumar A, Meena M, Gyneshwori S, Gupta RK, Batra S (2009). Calcium in Midpregnancy. *Arch Obstet Gynecol* **279(3)**:315-319.

29. Mohapatra P, Mohapatra SC, Aggrawal DK, Agarwal KN, Gaur SD (1990). Nutritional status of antenatal women in rural areas of Varanasi, Uttar Pradesh. *Man India* **70(1)**:85–91.

30. Shatrugna V, Kulkarni B, Kumar PA, et al (2008). Relationship between women's occupational work and bone health: a study from India. *Br J Nutr* **99(6)**:1310-1315.

31. Gopalan C, Rama Sastri BV, Balasubramanian S C (2004). Nutritive value of Indian foods. First edition; Hyderabad, National Institute of Nutrition, ICMR. 47-58.