

## Nutrient Recommendations for Pregnant and Lactating Women and Older Individuals

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### Energy

#### 1. Pregnancy

It is recommended that pregnant women increase their energy intake by 300 kcal/d to provide a theoretical gross need for energy during pregnancy. 300 kcal/d is gross energy need (80,000 kcal) divided by duration of pregnancy (250 d). Only about 14% of the 80,000 kcal is the energy need for fetal and uterine tissues gained during pregnancy. About 46% of it or 37,000 kcal is due to basal metabolic need for newly synthesized tissues. About 40% of the energy need is associated with fat that mother gained during pregnancy(Fig. 1).

However, the amount of weight gained during pregnancy varies widely among women. We observed that weight gain ranged from 10 to 55 lbs and there was no number that stands out as what most women gain. Weight is counting for the amount of tissues that has been synthesized and the amount of fat that has been stored. If weight gain is that variable

|  |     |
|--|-----|
| ~80,000 kcal   |     |
| Maternal Fat Gain<br>(32,000 kcal)                                       | 40% |
| Basal Metabolic Need for<br>Newly Synthesized<br>Tissue<br>(37,000 kcal) | 46% |
| Obligatory Need for Fetal<br>and Uterine Tissue                          | 14% |

Fig. 1. Theoretical need for energy during pregnancy.

during pregnancy, is energy need also variable during pregnancy?

Thus we carried out a longitudinal study to determine if energy expenditure varies among healthy pregnant women and if so, what accounts for this variation. We recruited ten pregnant women with similar BMI (19.5–26.0 kg/m<sup>2</sup>), age (20–37 yrs), educational level (0–5 yrs college), exercise (0–3 sessions/wk) and parity(1-2). All of them were married and did not smoke. Nine of them were Caucasian and one was Phillipino. We measured dietary intake, RMR (resting metabolic rate), body weight, total body water(TBW), body density, bone mineral, body protein, DIT (dietary induced thermogenesis) and work energy expenditure. All the measurements except bone mineral were carried out before conception, in the first trimester (8–10 wk of pregnancy), in the second trimester (24–26 wk), in the third trimester (34–36 wk) and 4–6 wk postpartum. Bone mineral was measured before conception and after delivery since the measurement required exposing to a source of radiation X-ray(Fig. 2). RMR and body fat change are two major components of energy requirements during pregnancy.

We developed a new method to determine body composition during pregnancy since composition of lean tissue gained during pregnancy is different from that of "standard" lean tissue. Normally FFM (fat-free mass) in non-pregnant adults is about 73% water, 20% protein, and 7% bone mineral. But the composition of FFM gained during pregnancy is highly

Nutrient Recommendations for Pregnant and Lactating Women and Older Individuals

|              | Pre-PG | Conception | 8-10 wks | 24-26 wks | 34-36 wks | Birth | 4-6 wks |
|--------------|--------|------------|----------|-----------|-----------|-------|---------|
| Diet         | ×      | ×          | ×        | ×         | ×         | ×     | ×       |
| RMR          | ×      | ×          | ×        | ×         | ×         | ×     | ×       |
| Body weight  | ×      | ×          | ×        | ×         | ×         | ×     | ×       |
| Body water   | ×      | ×          | ×        | ×         | ×         | ×     | ×       |
| Body density | ×      | ×          | ×        | ×         | ×         | ×     | ×       |
| Bone mineral | ×      | ○          | ○        | ○         | ○         | ○     | ×       |
| Body protein | ○      | ○          | ○        | ○         | ○         | ○     | ○       |
| DIT          | ×      | ×          | ×        | ×         | ×         | ×     | ×       |
| Work expend  | ×      | ×          | ×        | ×         | ×         | ×     | ×       |

× = measured  
 ○ = estimated or extrapolated

Fig. 2. Experimental design.

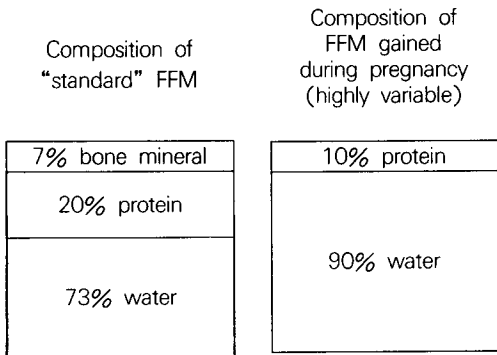


Fig. 3. Body composition.

variable. On the average it is 90% water and 10% protein(Fig. 3). We directly measured TBW using deuterium dilution and then body density using underwater weighing. Once we knew the amount of TBW and predicted changes in protein during pregnancy, we were able to calculate a new density of

FFM for each woman at each stage of pregnancy. Then we could calculate body fat(Fig. 4).

The fat gain at the end of the first trimester was little, but fat gain at the end of the second and third trimester were highly variable among subjects. The fat gained at the end of third trimester was 0 to 10 kg. Energy cost associated with fat gain also varied widely among women. Three women gained less but seven gained more than 32,000 kcal, a theoretical energy cost of fat gain during pregnancy. The energy cost ranged from 0 to 106,000 kcal and the average was 47,000 kcal(Fig. 5). There was a significant correlation between total gestational weight gain and fat gain(Fig. 6)( $\gamma=0.69$ ,  $P<0.05$ ). We did not find any relationship between fat gain and infant birth weight.

Individual changes in RMR at the end of first trimester were little. Increase in RMR by the second

|              |  |
|--------------|--|
| Water        | Total Body Water : Deuterium dilution  |
| Fat          | Total Body Fat : Hydrostatic weighing with a adjustments for changes in density of FFM due to water retention.                         |
| Protein      | Total Body Protein : By difference pre-pregnancy changes during pregnancy estimated from protein content of weight gain(Hytten, 1971). |
| Bone Mineral | Total Bone : DEXA pre-prenancy ; changes during pregnancy estimated from gains in fetal bone mineral(Widdowson, 1964).                 |

Fig. 4. Four compartment model.

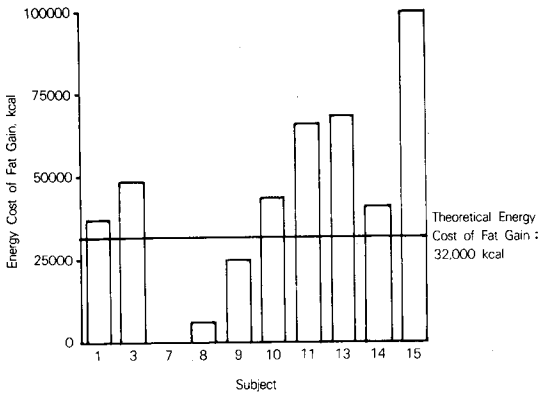


Fig. 5. Energy cost of fat gain.

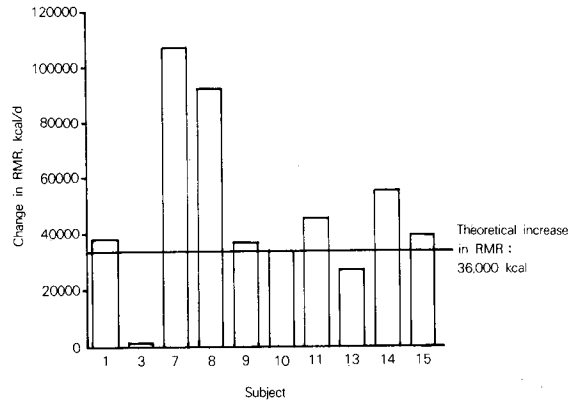


Fig. 7. Cumulative energy cost of changes in RMR.

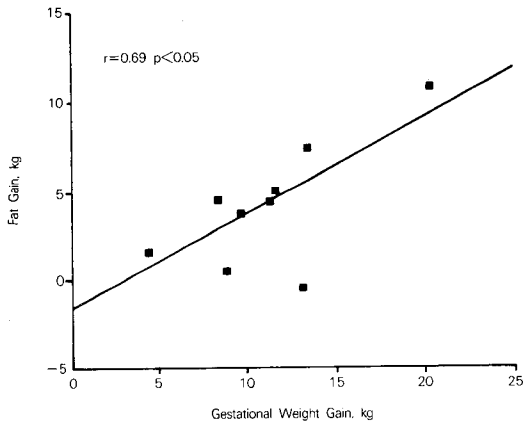


Fig. 6. Relationship between total gestational weight gain and fat gain.

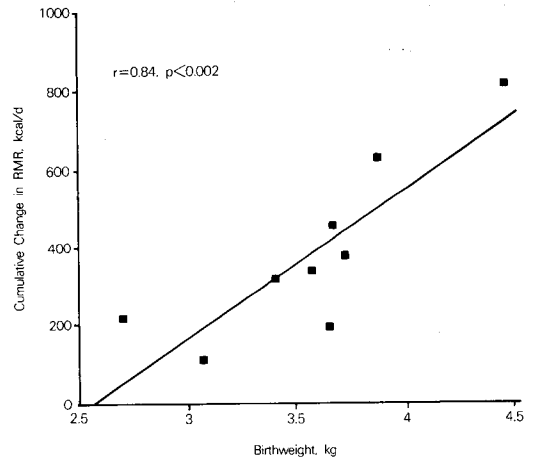
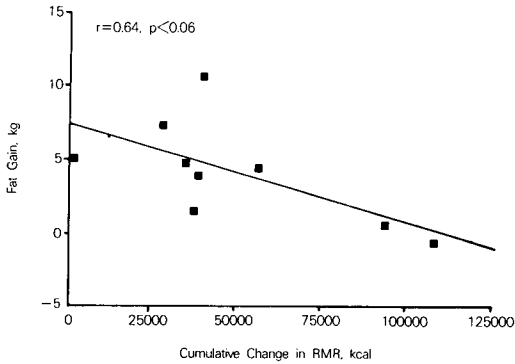


Fig. 8. Relationship between the cumulative change in RMR and gain in fat tissue.

and third trimester were diverse and RMR return to baseline levels in the postpartum period. The average increase in RMR energy expenditure in the third trimester was 375 kcal/d. The range was from 100 to 800 kcal/d. Cumulative energy cost of changes in RMR was also diverse (Fig. 7). Only five subjects showed similar increase in RMR to 36,000 kcal, a theoretical increase in RMR. Four subjects expended more energy than 36,000 kcal. There was a significant correlation between changes in RMR and birth weight ( $\gamma=0.84, P<0.002$ ) (Fig. 8). The larger the change in RMR is, the larger the birth weight is. The fetus is metabolically active and contributes a lot to the total RMR. There was relationship between

the cumulative changes in RMR and gain in fat tissue ( $\gamma=0.64, P<0.06$ ), although the relationship was not significant. High increase in RMR is associated with smaller gain of fat and vice versa (Fig. 9).

There are several ways in which energy metabolism can adapt during pregnancy. First, physiological changes could occur to reduce amount of fat she gained and conserve energy which is needed for the fetus. Second, behavioral changes could occur to reduce spontaneous activity and increase food intake. Third, metabolic changes could occur to modify efficiency in energy utilization (Table 1). We believe that



**Fig. 9.** Relationship between changes in RMR and birth weight.

**Table 1.** Adaptations in energy metabolism during pregnancy

- Physiological
  - Marginal or excessive fat reserves at conception
  - Reduced gain in body fat
- Behavioral
  - Reduced spontaneous activity
  - Increased food intake
- Metabolic
  - Modified efficiency in energy utilization

the changes in body fat and in RMR may be influenced by body weight of mother at conception. In the study of women in Gambia, pregnant women, who were underweight, carried out hard agricultural work and did not have much fat and did not increase food intake, decreased RMR during pregnancy. Total energy cost of those women for the entire pregnancy was only 1000 kcal. RMR of U.S. obese pregnant women, when it is expressed as kcal/kg FFM, was 25% higher than that of normal weight pregnant women.

Thus pregnant woman might need individualized counselling. Probably different energy RDAs should be recommended to women who are underweight and overweight. 300 additional kcal/d may not be needed by all women if they reduce their physical activity. The best we can do is to advise normal weight and overweight women to eat a carbohydrate-

and nutrient-rich diet to appetite. One of U.S. dietary guideline for all non-pregnant adults is to limit fat intake to 30% of energy. It might be better to consider to make similar recommendation for pregnancy. We also hypothesized the amount of fat in the diet is related to the amount of fat gained during pregnancy since it is more efficient to store fat than to convert carbohydrate to fat and glucose is a preferred fuel of the fetus. It is encouraged to increase food intake (about 500 kcal/d) for pregnant women who are underweight and undernourished (whose protein intake is less than a half of RDA)(Table 2).

**2. Lactation**

Current energy RDA for lactation is + 500 kcal/d. The lactation energy recommendation assumed that the efficiency of milk production is 80%. Also for every 100 ml of milk produced 85 dietary kcal are required. And the average milk volume is 750 ml/d in the first six months and 600 ml/d in the second

**Table 2.** Recommendations for energy intake during pregnancy

- Individualize dietary counseling
- Allow variable energy intakes ; 300 additional kcal/d may not be needed by all women
- Advise normal weight and overweight women to eat a carbohydrate- and nutrient-rich diet to appetite
- Encourage increased food intake(about 500 kcal/d) for pregnant women who are :

Underweight Undernourished

**Table 3.** Lactation energy recommendation

Assumes :

- Efficiency of milk production is 80%
- 85 dietary kcal are required for every 100ml milk produced
- Average milk volume is 750ml/d in first 6 months ; 600ml/d in second 6 month
- Coefficient of variation is 12.5%
- 100 to 150kcal/d available from maternal fat stores

RDA : + 500kcal/d

six months. Coefficient of variation is 12.5% and 100 to 150 kcal/d was available from maternal fat stores to supplement energy in the diet. So these assumptions lead to +500 kcal/d for lactation (Table 3).

However, these assumptions might not be valid to all lactating women. There may be changes in efficiency of milk production. We measured milk volume and found the volume ranged from 450 to 1000 ml/d. Not every woman has the same amount of fat reserve which can be used during lactation. Thus a number of homeostatic adjustments could occur to meet the energy need for lactation. A woman could increase energy intake, decrease physical activity, mobilize her body fat or change metabolic efficiency with which she uses energy in her diet (Table 4). We did not see an increase in energy intake in all cases, although we saw the increase in some cases. Several people have measured the efficiency of milk synthesis. They divided milk energy content (MEC) by MEC plus difference between BMR in lactation and BMR in non-pregnant, non-lactating state. The efficiency ranged from 94% in Gambia to 100% in England (Table 5). The measured efficiency of milk production was higher than 80% which was used as the basis for the calculation of RDA. In the study of the effect of dieting on milk production it was found that as long as the energy intake was 1500 kcal/d or higher there was no effect on milk volume. Thus future energy RDAs for well-fed women should continue to be less than the estimated need to allow a slow rate of weight loss after childbirth.

### 3. Older Individuals

Current energy RDA for old age (51+yr) is 2300 kcal/d for men and 1900 kcal/d for women. The cut-up point of old age that has been suggested was 65, although some people suggested 70. It might be better to consider physical conditioning and other physiological measurements of health in addition to age as criteria for old age.

**Table 4.** Potential homeostatic adjustments to meet lactation energy needs

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- Increase energy intake
- Decrease physical activity
- Mobilize body fat
- Change metabolic efficiency

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**Table 5.** Efficiency of milk synthesis

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Efficiency of milk synthesis, %

$$= \frac{\text{MEC}}{\text{MEC} + (\text{BMR}_L - \text{BMR}_{\text{NPNL}})}$$

MEC = Milk energy content

- Sweden 97 %
- England 100 %
- Gambia 94 %
- Scotland 97 %

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Energy RDA was calculated from RMR and a factor for physical activity (1.5×RMR). This calculation has several assumptions. RMR and physical activity were assumed to be two primary determinants of energy expenditure and this assumption is probably true. RMR can be adequately predicted from age, sex, and body weight and that is pretty true as long as the individual does not have large deviations in body composition. We also assumed that RMR declines after age 50 and physical activity declines gradually with aging and the efficiency of dietary energy use does not vary among individuals. These last three assumptions may not be true for everyone. We should consider these assumptions in depth before recommendations for energy intake for older individuals is established. It was assumed that energy needs declined with aging partly due to the expected decrease in RMR which is associated with decrease in lean body mass as one gets older which is primarily due to decrease in physical activity. However, there are some very active old folks who probably do not lose lean tissue at the same rate as they age. Therefore, RMR do not decline accordingly. Future energy RDAs need to consider the wide variability in active patterns of older individuals.

## Calcium

### 1. Pregnancy and Lactation

Current calcium RDA is 1200 mg/d for pregnant and lactating women, irrespective of age. 1200 mg/d of calcium is also recommended for 15–24 yr old girls. This recommendation is based on the assumption that newborn accumulates about 30 g calcium and daily milk secretion contains about 240 mg calcium. There are several potential adjustments in calcium metabolism during pregnancy and lactation to provide fetal and lactation needs. The women could increase calcium intake which could lead to increase in absorption. There could be decrease in endogenous secretion into the gut. Bone resorption could increase or resorption of calcium by the kidney could increase.

We carried out a longitudinal study to follow calcium homeostasis during pregnancy and lactation and after weaning in women consuming RDA of calcium. The measurements were carried out at six time points, prior to conception, in the first, second and third trimester, early lactation (7–9 wks postpartum) and post-menses (2–6 mos after the onset of menstruation). We measured dietary calcium intake using weighed food record, true absorption of calcium using stable isotopes of calcium, urinary calcium excretion, milk calcium content, and bone mi-

neral content(Fig. 10).

Calcium intake of the 14 women prior to pregnancy was 1100 mg/d and it increased to 1500 mg/d during pregnancy. Absorption of calcium was 30% before pregnancy, and there was no difference between the absorption before pregnancy and in the first trimester. Calcium absorption increased to about 50% in the second trimester and third trimester and it went down to the pre-pregnant level in early lactation and post-menses. The urinary calcium increased during pregnancy probably due to increased blood flow to the kidney. During lactation urinary calcium levels were significantly lower than in the non-pregnant state or in pregnancy and they continued to be low at the onset of menstruation(Table 6, Fig. 11). There was no differences in mineral content in spine between pregnancy and portpartum. Bone mineral content of spine dropped by 5.6% from postpartum to lactation. Usual rate of spinal bone mineral loss after menopause is 1–2% per year. Here we saw 5.6% decrease in two months. There was remarkable mobilization of bone mineral in early lactation probably due to low estrogen levels. The decrease in spinal bone mineral between pregnancy and lactation was 6.8%. Spinal mineral content increased by 5.3% from lactation to post-menses(Table 7).

To summarize, physiological adjustments in calcium metabolism of women consuming about 1200

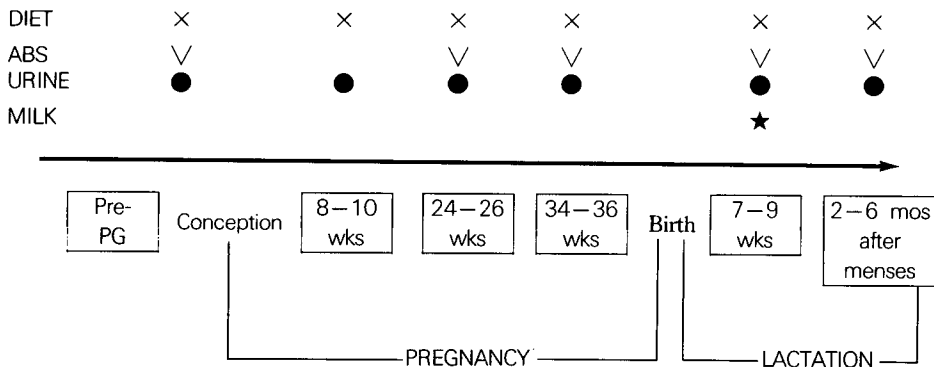


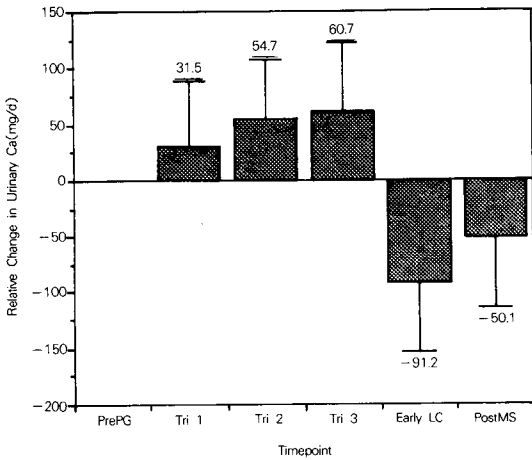
Fig. 10. Study design-timepoints.

**Table 6.** Results

| Time point         | Diet Ca (mg/d)           | True abs of Ca (% of dose) | Urine Ca (mg/d)         |
|--------------------|--------------------------|----------------------------|-------------------------|
| PrePG (N=13)       | 1091 <sup>a</sup> (313)  | 30.5 <sup>a</sup> ( 8.6)   | 163 <sup>a</sup> ( 83)  |
| Tri 1 (N=14)       | 1310 <sup>ab</sup> (180) | —                          | 202 <sup>bc</sup> ( 83) |
| Tri 2 (N=14)       | 1262 <sup>ab</sup> (253) | 49.3 <sup>b</sup> (10.4)   | 230 <sup>bc</sup> (116) |
| Tri 3 (N=14)       | 1401 <sup>b</sup> (290)  | 52.6 <sup>b</sup> (10.6)   | 249 <sup>c</sup> (149)  |
| Early LACT (N=14)  | 1179 <sup>ab</sup> (238) | 32.8 <sup>a</sup> (10.3)   | 75 <sup>a</sup> ( 49)   |
| Post-Menses (N=11) | 1071 <sup>a</sup> (337)  | 37.8 <sup>a</sup> ( 8.9)   | 103 <sup>ab</sup> ( 45) |

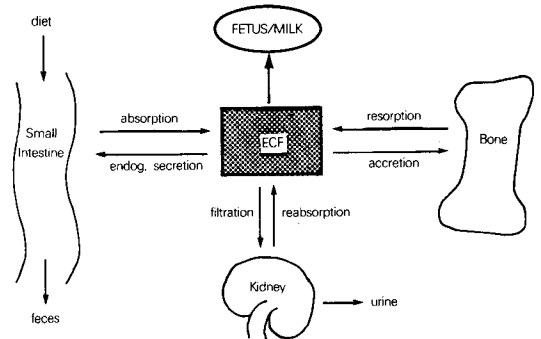
Means(SD)

Different superscripts denote significant difference,  $p < 0.05$

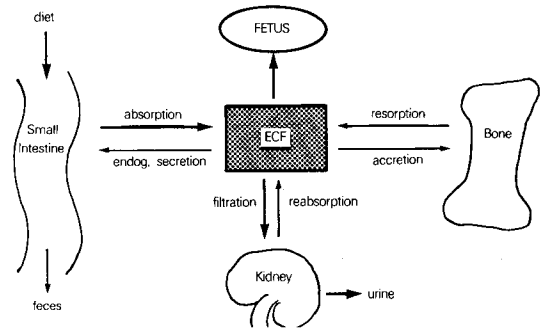


**Fig. 11.** Mean relative change from prePG in daily urinary Ca.

mg calcium/d during pregnancy, early lactation and late lactation differ (Fig. 12). During pregnancy the main way to provide calcium needs for the fetus is to increase calcium absorption (Fig. 13). During early lactation the need for calcium for milk synthesis is provided primarily by increase in reabsorption



**Fig. 12.** Sources of Ca for PG & LACT.



**Fig. 13.** Sources of Ca for pregnancy.

**Table 7.** DEXA bone mineral content % changes

| Comparison periods   | Arms  | Legs  | Trunk | Spine | Total body |
|----------------------|-------|-------|-------|-------|------------|
| PrePG vs PP (N=10)   | -0.3% | -0.4% | -0.2% | -0.9% | +0.1%      |
| PP vs LACT (N=13)    | -1.0% | +0.5% | -1.3% | -5.6% | -0.4%      |
| PrePG vs LACT (N=10) | -1.5% | +0.5% | -2.8% | -6.8% | -0.4%      |
| LACT vs PM (N=9)     | -0.3% | -0.2% | +1.0% | +5.3% | -0.1%      |
| PrePG vs PM (N=6)    | -1.6% | +0.8% | -4.3% | -4.5% | -0.5%      |

$P < 0.05$ , 2 dependent sample T-test

PrePG=prior to conception

PP=2 wks postpartum

LACT=early (2 mos) lactation

PM=2-6 mos post-menses

of calcium by the kidney and resorption of the bone (Fig. 14). During late lactation (after the onset of menses) calcium reabsorption by the kidney increased while bone mineral is replaced (Fig. 15). If the mother is still breast-feeding, calcium absorption may rise.

We carried out a study in Hmong women in California to investigate calcium metabolism of pregnant women consuming low calcium in the diet. We recruited 10 non-pregnant and 11 pregnant women who consumed less than 600 mg/d of calcium (Table 8). We collected dietary intake information, fasting blood sample and urine sample from non-pregnant women at one time point and from pregnant women at the second and third trimester and 6 wks postpartum. There was an increase in urinary calcium excretion during pregnancy and it dropped at the postpartum period (Fig. 16). Urinary excretion of pyridinium crosslinks, a metabolite of collagen in bone increased significantly in the third trimester (Fig. 17). There-

fore, it appeared that bone calcium reserves might be mobilized in women consuming less than 600 mg calcium/d during pregnancy.

Institute of Medicine suggested supplemental calcium is given to pregnant women if their calcium intake is less than 600 mg/d to prevent bone resorption. It has been suggested that a low maternal calcium intake may decrease fetal bone mineral. Thus women consuming less than 600 mg calcium/day should increase their intake by selecting calcium-rich foods or by supplementation (Table 9).

2. Older individuals

Current calcium recommendation for old individuals

Table 8. Subject characteristics (Mean ± SD)

|                    | Nonpregnant | Pregnant  |
|--------------------|-------------|-----------|
| Age, yrs.          | 27 ± 6      | 27 ± 6    |
| Height, inches     | 58 ± 2      | 59 ± 2    |
| Weight, lbs.       | 121 ± 20    | 124 ± 17  |
| Parity             | 4.7 ± 2.3   | 4.3 ± 2.6 |
| Time in U.S., yrs. | 10 ± 1      | 12 ± 2    |
| Birthweight, kg    |             | 3.5 ± 0.6 |

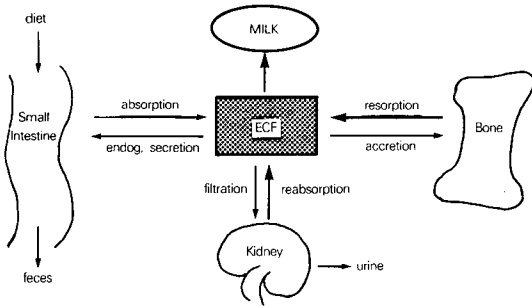


Fig. 14. Sources of Ca for early lactation (pre-menses).

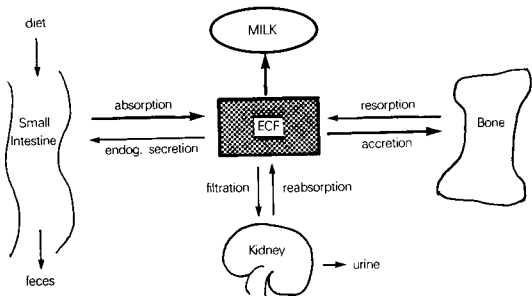


Fig. 15. Sources of Ca for late lactation (post-menses).

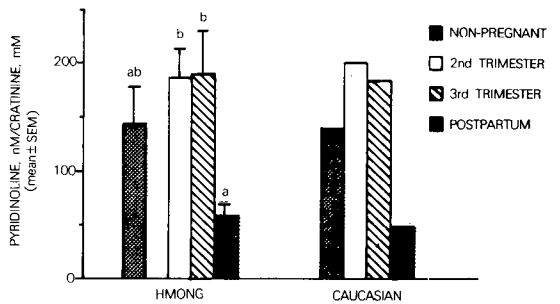


Fig. 16. Urinary calcium excretion.

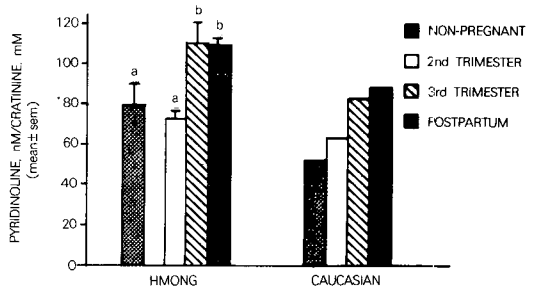


Fig. 17. Urinary pyridinium crosslinks.



**Table 9.** Conclusions and recommendations for calcium intake during pregnancy

- Homeostatic adjustments in calcium metabolism occur in women consuming the recommended 1200 mg calcium/day
- Bone calcium reserves may be mobilized in women consuming less than 600mg calcium/day during pregnancy
- A low maternal calcium intake may decrease fetal bone mineral
- Women consuming less than 600 mg calcium/day should increase their intake by selecting calcium-rich foods or by supplementation.

duals is 800 mg/d. No increased calcium intake is recommended for postmenopausal women to reduce the risk of osteoporosis. One of the major concerns for old age is the relationship between calcium intake and postmenopausal bone loss. Supplemental calcium (1–1.5 g/d) may attenuate the loss of some bones but not of all bones. The best treatment seems to be estrogen replacement particularly among those women who are at the risk of osteoporosis. Supplemental calcium should not be substituted for hormone replacement. In women unable to take estrogen, calcium intakes of 1–1.5 g/d may reduce postmenopausal bone loss (Table 10).

## Zinc

### 1. Pregnancy and Lactation

We recommend 15 mg zinc/d for adult males and

**Table 10.** Will high calcium intakes reduce postmenopausal bone loss?

- Supplemental calcium may attenuate the loss of some bones
- Supplemental calcium should not be substituted for hormone replacement
- In women unable to take estrogen, calcium intakes of 1–1.5 g/d may reduce postmenopausal bone loss

12 mg/d for adult females. Current RDAs of zinc are 15 mg/d for pregnancy and 19 and 16 mg/d for early and late lactation. The RDAs are established based on zinc content of tissue gained during pregnancy (100 mg) (Table 11) and the zinc content of milk output during lactation (Table 12). No allowance is made for any adjustments in maternal zinc homeostasis.

There are several ways in which additional zinc is provided during pregnancy and lactation. There could be increase in dietary intake of zinc, increase in absorption or decrease in endogenous excretion to the gut. There are a lot of zinc in bone and muscle. So there might be mobilization of zinc from the bone and the muscle. And also there might be increase in zinc reabsorption by the kidney (Fig. 18).

We studied zinc metabolism during pregnancy and lactation. Dietary zinc intake, fasting plasma zinc, urinary zinc excretion, % fractional zinc absorption, endogenous fecal zinc, and milk zinc level were measured prior to pregnancy, in the first, second, and third trimester and at 7–9 wks of lactation (Fig. 19).

**Table 11.** Components of added zinc during pregnancy

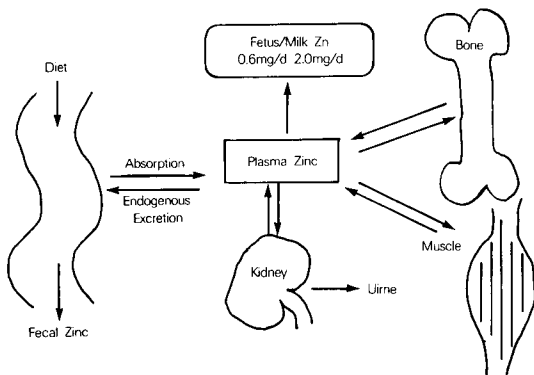
|                | Tissue weight<br>g | Zn concentration<br>ug/g wet tissue | Zn content<br>mg |
|----------------|--------------------|-------------------------------------|------------------|
| Fetus          | 3400               | 17                                  | 57.8             |
| Placenta       | 650                | 10                                  | 6.5              |
| Amniotic fluid | 800                | 0.2                                 | 0.5              |
| Uterus         | 970                | 25                                  | 24.3             |
| Mammary tissue | 405                | 13                                  | 5.3              |
| Blood          | 1250               | 5                                   | 6.3              |
| Total          |                    |                                     | 100.7            |

Nutrient Recommendations for Pregnant and Lactating Women and Older Individuals

**Table 12.** Calculated milk zinc output during lactation<sup>a</sup>

| Month of Lactation | Zn conc. |                     | Milk volume |                    | Zinc output (mg/day) |                        |
|--------------------|----------|---------------------|-------------|--------------------|----------------------|------------------------|
|                    | ug/ml    | (3.88) <sup>d</sup> | ml          | (850) <sup>c</sup> | mean <sup>b</sup>    | mean+1 SD <sup>c</sup> |
| 1                  | 2.83     | (3.88) <sup>d</sup> | 750         | (850) <sup>c</sup> | 2.12                 | 3.30                   |
| 2                  | 1.25     | (1.90)              | 750         | (850)              | 0.94                 | 1.62                   |
| 3                  | 0.91     | (1.23)              | 750         | (850)              | 0.68                 | 1.04                   |

- a. Personal communication, Krebs NF and Hambidge KM, 1987
- b. Mean zinc output : Mean [Zn]×750ml/1000
- c. Mean+1 S.D. : (Mean [Zn]+1S.D.)×850ml/1000
- d. Mean concentration (mean concentration+1 standard deviation)
- e. Assumed standard volume 750ml (assumed upper end of volume range 850) (Butte and Garza, 1985)

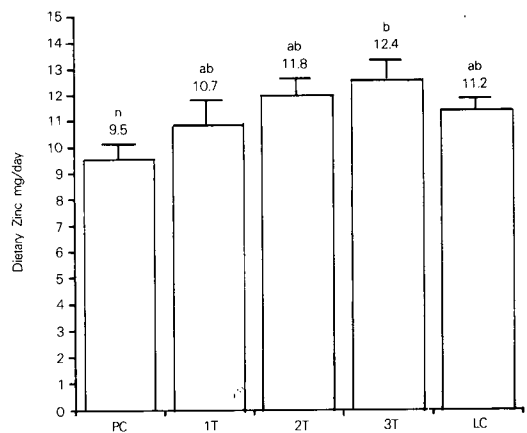


**Fig. 18.** Sources of zinc for pregnancy and lactation.

The women increased zinc intake significantly from 9.5 mg/d in pre-pregnancy to 12.5 mg/d in pregnancy (Fig. 20). Plasma zinc declined by 25% during pregnancy probably due to expansion of plasma volume (Fig. 21). The increase in urinary zinc excretion observed was probably due to an increase in the glomerular filtration rate (Fig. 22). It appeared that there was possibly small adjustment in zinc absorption,

although it was highly variable among women (Table 13). It is not completely understood how the need for zinc during pregnancy is met at this time point. There was no significant increase in zinc intake during lactation. Zinc absorption increased significantly in early lactation (Fig. 23).

Response in zinc homeostatis among women co-



**Fig. 20.** Dietary zinc (mean±SEM, n=13).

| Measured Variables         | Gestation      |          |           |           | Lactation |
|----------------------------|----------------|----------|-----------|-----------|-----------|
|                            | Pre-Conception | 8–10 wks | 24–26 wks | 34–36 wks | 7–9 wks   |
| Dietary zinc               | ×              | ×        | ×         | ×         | ×         |
| Fasting plasma zinc        | ×              | ×        | ×         | ×         | ×         |
| Urinary zinc excretion     | ×              | ×        | ×         | ×         | ×         |
| Fractional zinc absorption | ×              |          | ×         | ×         | ×         |
| Endogenous fecal zinc      | ×              |          |           | ×         | ×         |
| Milk zinc                  |                |          |           |           | ×         |
| (Bone mineral density)     | ×              |          |           |           | ×         |

**Fig. 19.** Study design.