ENERGY METABOLISM DURING THE MENSTRUAL CYCLE, PREGNANCY AND LACTATION IN WELL NOURISHED INDIAN WOMEN

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ABSTRACT

Energy metabolism during the menstrual cycle, pregnancy and lactation in well nourished Indian women

Thesis by
Leonard Sunil Piers

The measured basal metabolic rates (BMR) of present day Indian women were found to be comparable to published BMRs, measured in Indian women over 50 years ago, but 7 percent lower than present European/American women. This can possibly be due to differences in body composition or climate. Schofield's equation, used to predict the BMR of women in the 18-30 age group from body weight, was found to over-estimate the BMR of young Indian women by approximately 9 percent. This commonly used equation also overestimates the BMR of young American women by almost 7 percent (Chapter 2). The BMR and the thermic effect of a meal (TEM) was measured in the pre- and post-ovulatory phases of a single menstrual cycle. BMR was not significantly different, however, the TEM was 18.5 percent higher ($p < 0.05$) during the post-ovulatory phase, as compared to the pre-ovulatory phase of the same menstrual cycle. This increment in the TEM is small in absolute terms and is unlikely to affect the energy requirements of these individuals (Chapter 3). Both pregnancy and lactation are energetically expensive and would appear to require substantial increments in energy intake. However, the measured increment in energy intake rarely appears to be adequate to meet all the costs of pregnancy and lactation. Therefore, saving of energy by an enhancement of the efficiency of maternal energy metabolism was postulated as a possible mechanism by which the costs of pregnancy and lactation are met. Therefore, the BMR and TEM were measured at 12, 24, and 34 weeks of gestation (Chapter 4), as well as, 12 and 24 weeks of lactation (Chapter 5), in a group of well nourished Indian women and compared to a group of non-pregnant, non-lactating controls. No energy saving was associated with either the BMR or TEM during pregnancy or lactation. However, the dietary energy intake, estimated at the same time as the metabolic measurements, appeared to be substantially increased during the last two trimesters of pregnancy and during the initial 24 weeks of lactation; and was apparently adequate to meet the extra energy expenditure associated with pregnancy and lactation.

Propositions

1. Basal metabolic rates of well nourished Indian women in the reproductive age group are comparable to other well nourished populations.

This thesis

2. The thermic effect of a meal is significantly higher during the post-ovulatory phase of the menstrual cycle, as compared to the pre-ovulatory phase.

This thesis

3. It is conceivable that the higher luteal phase noradrenaline is causally related to the higher estradiol levels, leading to incomplete inactivation by reducing tissue uptake or competitive inihibition of catechol-*O*-methyl transferase.

Davidson et al. *Clin & Exp Pharmac & Physiol* 1985;12:489-493

4. There is no energy saving associated with either basal metabolism or diet induced thermogenesis during pregnancy or lactation.

This thesis

5. Well nourished Indian women have a pregnancy performance comparable to well nourished women from developed countries.

This thesis

6. The total energy needs of pregnancy amount to 335 MJ or about 1.2 MJ/d.

FAO/WHO/UNU 1985.

7. The extra energy requirements for tissue deposition and milk production during pregnancy and lactation respectively are largely met by a commensurate increase in the energy intake.

This thesis

8. Present recommended energy intakes for lactation are too high for healthy Filipino women who show adequate lactational performance.

Guiiermo-Tuazon et. al., *Am J Clin Nutr* 1992; **56**:874-880.

9. The chance that a player on his first throw of a pair of dice will get a double six is about 0.03, so an observer who sees this happen might believe that he had "statistically significant" evidence that the dice were loaded.

Garrow in *Energy balance and obesity in man*, 1978.

10. As you sow so shall you reap.

11. Necessity is the mother of invention.

12. You cannot make an omelette without breaking eggs.

Contents

Chapter 1

INTRODUCTION

The determination of the influence of ethnicity, climate, and environmental conditions on the requirements of populations in different parts of the world is becoming increasingly important. The possible adaptations that may allow an individual to survive in a particular environment needs to be established and benefits or disadvantages of such adaptations, when present, needs to be assessed.

Nutritional requirements of individuals and population groups are matters of considerable importance to individuals as diverse as health care professionals, physiologists, economists, planners, policy makers, farmers, the food industry and bankers. It is often difficult to differentiate clearly between physiological and social requirements, and the requirements recommended by official bodies frequently tend to be influenced by the various interest groups. Even if physiological needs can be precisely estimated, it is not uncommon to add on an arbitrary safety allowance and perhaps adjust them to suit local dietary habits and practices.

This thesis attempts to address the question of energy requirements of Indian women in the reproductive age group from a physiological and nutritional point of view. It is now been accepted by the Food and Agriculture Organization /World Health Organization /United Nations University (FAO/WHO/UNU) that estimates of energy requirements of individuals or populations groups, worldwide, should be based on measures of energy expenditure rather than estimates of energy intake[1]. The underlying reasoning being that both in developed and less developed countries actual intakes are not necessarily those that maintain a desirable body weight or optimal levels of physical activity, and hence health in its broadest sense.

There are several physiological processes that account for energy expenditure over a day. In a sedentary individual the largest component (approximately 70%) is basal metabolism, which can be studied by measuring the basal metabolic rate (BMR).

It is the rate of energy expenditure measured in the post-absorptive state under highly standardized conditions[2]:

- at complete rest (immobile), lying down, shortly after awakening
- in thermoneutral state
- 12 - 14 hours after the last meal (viz. post-absorptive)
- awake, at sexual repose and emotionally undisturbed
- without disease or fever

The BMR does not correspond to the minimal energy expenditure as erroneously reported in the literature. Energy expenditure can be further lowered by drowsiness, sleep, and meditation. The term resting metabolic rate (RMR) is the rate of energy expenditure at rest under *non standardized* conditions, i.e. not in a post-absorptive state, either lying down or comfortably sitting down, possibly postprandial (after meals of variable composition), with possible previous physical activity. Therefore, the RMR is usually greater than the BMR. It is, however, often used interchangeably with BMR, a practice that should be avoided.

The component of energy expenditure associated with the ingestion, digestion, absorption, transport, storage and oxidation of food is termed diet induced thermogenesis (DIT), and can be studied by measuring the thermic effect of a meal (TEM). An additional component of this process is the stimulation of the sympathetic nervous system by the food substrates and hormones secreted in response to feeding. However, even after taking into account all possible processes associated with food intake the associated energy expenditure is seldom more than 10 percent of the total 24-hour energy expenditure.

The third component of the 24 hour energy expenditure is the energy expenditure associated with physical activity (PA). This component is, perhaps, the most variable and accounts for an increasingly greater share of the total 24-hour energy expenditure, as individuals become more physically active.

The basal metabolic rate

The basal metabolic rate (BMR) forms the basis for assessing the 24-hour energy expenditure using the factorial method[1]. Schofield et. al.[3] compiled the available BMR data world wide and developed a series of predictive equations for BMR from a simple anthropometric measure such as body weight. These age and sex specific equations are being increasingly used and recommended for the estimation of energy expenditure and by extension energy requirements, both at the community and population level[4]. In the analysis of Schofield et. al.[3] it was observed that the BMR of Indian males and females of all ages, in the database, were almost 13 percent lower than their age, sex and weight matched European/American counterparts. This was thought to constitute an *ethnic* feature of this population.

Henry and Rees[5] reviewed the BMR measurements made in the tropics and using data not previously evaluated by the Schofield report[3] demonstrated that the BMRs of people living in the tropics were significantly lower than that predicted by Schofield's[3] equations. However, the deviation seen in tropical women in the 18-30 age group (-3.8%, n=350) was smaller than that seen in tropical men of the same age group (-10.8%, n=1174). However, the phase of the menstrual cycle at the time of the BMR measurement was not taken into account. These discrepancies in results between the older Indian data analyzed by Schofield et. al. and current estimates by Henry & Rees[5], are not merely academic since they have important implications in assessing energy requirements of this population.

In this thesis a study on the BMR of Indian women in the 18-30 age group is presented *(Chapter 2)*. All subjects being prospectively measured during the same phase of the menstrual cycle. This data is then compared to values already published in the literature for European and American women.

The menstrual cycle

Women in the reproductive age group are a particularly vulnerable group. In a world that is rapidly changing, the role of the woman in society is being altered dramatically. In addition to the stress and strain of motherhood women are entering the work force

in ever growing numbers. Therefore, it is becoming increasingly important to keep their special requirements in mind when arriving at norms for this particular population.

Relatively little is known about how the menstrual cycle affects energy and substrate metabolism. It is, therefore, an area of considerable interest at the present. Changes in nutritional requirements in individuals at different time points was listed as a priority area of research in the most recent report of the FAO/WHO/UNU joint expert consultation[1]. Cyclical changes in energy expenditure and intake, associated with the different phases of the menstrual cycle, would not only have a significant influence on the design of nutritional and physiological studies requiring a pre-menopausal control group, but also in the determination of the energy requirements in these individuals, as well as, the population as a whole.

Alterations in energy expenditure over the course of the menstrual cycle have been reported by several workers[6-9]. All these studies report an increase in the basal metabolic rate (BMR) or sleeping metabolic rate (SMR) in the post-ovulatory (luteal) phase of the menstrual cycle. However, other reports have failed to demonstrate any increase in the BMR[10]. Most of these studies, however, were carried out over the course of more than one menstrual cycle, therefore the observed differences may have been influenced by the between-cycle variability, rather than a true difference between the pre- and post-ovulatory phases of a single menstrual cycle.

The study presented in this thesis was carried out to establish if there were changes in the Basal Metabolic Rate (BMR), the Thermic Effect of a Meal (TEM), and dietary energy intake over the course of a single menstrual cycle, as well as, between the same phase of two consecutive menstrual cycles, in well nourished Indian women in the reproductive age group *(Chapter 3)*.

Pregnancy

Women are under tremendous physiological stress during pregnancy. Practically every organ system is affected and pre-pregnant norms are no longer applicable. For the assessment of maternal nutritional status during pregnancy, weight gain has been used

as an indicator. Weight gain during pregnancy has been positively correlated to the birth weight of the infant[11-13] and the chances of having an infant with a low birth weight are reduced with adequate weight gain during this period. On the other end of the spectrum is the fact that excessive weight gain is related to pathological conditions such as toxaemia[14] and obesity[15-17]. It, therefore, becomes important to establish the norm for a given population as weight gains at both the extremes could have undesirable effects, both on the mother and the foetus.

The weight gain in pregnancy is accounted for by the weight of the foetus, placenta, amniotic fluid, uterus, breasts, blood, maternal fat stores, and extracellular extravascular fluid[18]. In energetic terms the cost of laying down this tissue, as well as, the energy content of the protein and fat making up the tissue has to be assessed. This assessment is best made by studying, longitudinally, women in the reproductive age group, who are not pregnant or lactating, who subsequently become pregnant. Studies in normal, well nourished, women living in industrialized countries have demonstrated that they do not increase their energy intake by large amounts during pregnancy[19,20]. It is also claimed that women in many developing countries achieve a successful pregnancy outcome in spite of being chronically undernourished[21]. This has prompted investigators the world over to look for energy savings associated with the physiological processes that constitute the 24 hour energy expenditure, such as basal metabolism, diet induced thermogenesis and physical activity.

The energy cost of pregnancy is assessed from the cumulative increase in the BMR over non-pregnant, non-lactating values or values obtained very early in pregnancy; in addition to the maternal fat stores, and deposition of protein and fat in maternal tissues. The increase in energy intake is also assessed from the cumulative increase in energy intake over either non-pregnant, non-lactating or early pregnancy values.

There is some evidence that the BMR is reduced during early and mid-pregnancy[22-27], enabling the mother to save energy that could, possibly, be channelled towards meeting the cost of pregnancy. However, reports on Asian women[28], and well nourished Western women[20,29-33] have not been able to demonstrate these changes. No mechanism has been

postulated for the reduced BMR in any of the studies that have reported these reductions. In their review on human energy metabolism Schoeller & Fjeld[34] are of the opinion that the BMR is not reduced to compensate for the added energy costs of pregnancy. However, what seems to be a common factor in all the above mentioned studies is the existence of large between individual variability. This would give the impression that any single recommended figure would have very little value to an individual but would only be useful for population based recommendations.

The study presented in this thesis (Chapter 4) was designed to assess, longitudinally, the changes in anthropometry, BMR, TEM and energy intake in a group of well nourished Indian, women over the course of a normal pregnancy. Comparison with a group of non-pregnant, non-lactating controls provides an assessment of energy sparing that may be associated with the components of the 24-hour energy expenditure studied, which could possibly account for the discrepancy observed between the energy cost of pregnancy and the extra energy intake over the corresponding period.

Lactation

Lactation is also thought to impose a substantial stress on energy metabolism and the FAO/WHO/UNU joint expert consultation[1] estimated the energy cost to be almost 3.1 MJ/d. There is, however, some evidence that this may be an overestimate of the true requirement, as adaptive changes in energy metabolism may occur during lactation that may reduce its cost. The increment in energy intake during lactation does not appear to be large enough to account for its entire cost[35-39]. However, the BMR appears to be reduced in lactating women, as compared to non-lactating postpartum subjects, by approximately 210 kJ/d (50 kcal/d), at 9 weeks postpartum[37]; or remain unchanged when compared to measurements made after lactation had stopped[39-40], thus indicating a possible enhancement of metabolic efficiency during this period. However, other longitudinal studies have demonstrated a rise in energy expenditure of approximately 5 percent above the pre-pregnant baseline[41,42]. An association was found between postpartum body mass index (BMI), (but not the breast milk output), and the increase in BMR. Indicating that the increment in BMR associated with breast milk production is lower in thinner, as compared to fatter women. Once again there seems to be a large

between individual variability, making it difficult to recommend a single value.

The other possible source of energy during lactation is from maternal fat stores that are built up during pregnancy. Several studies have demonstrated fat mobilization during lactation[35,36,38,39]. Reduction in the cost of physical activity may also have a role in subsidizing the cost of lactation[36,38]. The study presented in this thesis (Chapter 5) attempts to uncover energy sparing associated with the BMR and TEM that could, possibly, help in subsidizing the energy cost of lactation.

Outline of thesis

This thesis presents the results of studies on the BMR conducted on non-pregnant, non-lactating Indian women in the reproductive age group in **Chapter 2.** A comparison of the BMR, TEM and energy intake is made between the phases of a single menstrual cycle and between the same phase of two consecutive menstrual cycles in **Chapter 3.** Changes in the BMR, TEM, anthropometry and energy intake over the course of a normal pregnancy, in a group of well nourished Indian women are compared to a non-pregnant, non-lactating control group, together with an assessment of the energy cost of pregnancy in **Chapter 4.** In **Chapter 5** the same group of women are followed through the first six months postpartum, and changes in BMR, TEM and energy intake are compared to the pregnant state, as well as, to a group of non-pregnant, non-lactating controls. A general discussion of the work carried out, results and conclusions are presented in **Chapter 6.**

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Chapter 2

BASAL METABOLIC RATES OF INDIAN WOMEN[1]

Leonard S. Piers and Prakash S. Shetty.

ABSTRACT

Basal Metabolic rates (BMRs) in 60 Indian women resident in Bangalore and aged 18-30 years were measured during the mid-follicular phase of the menstrual cycle. The data were used to obtain a predictive equation for BMR from body weight. The BMR measurements were comparable to BMRs of Indian women reported more than 50 years ago, while they were 9.2 percent lower than Schofield's equation and 4.2 percent lower than that predicted by the equation of C.K.J.Henry & D.G.Rees (1991) *Eur J Clin Nutr.* **45**, 177-185. The equation obtained in this study predicts closely the BMRs of American women but underestimates BMRs of European women compared with figures recently reported in the literature.

[1]*European Journal of Clinical Nutrition* 1993; **47**: 586-591.

INTRODUCTION

It is now well accepted that estimates of energy requirements of individuals or population groups worldwide, are to be based on measures of energy expenditure rather than energy intake[1]. For this purpose measures or estimates of basal metabolic rate (BMR) form the basis for assessing 24-h energy expenditure using the factorial method. Schofield, Schofield & James[2] had compiled the available BMR data world wide, over the last 60 years and developed predictive equations for BMR from a simple anthropometric measure like body weight. These age and sex specific predictive equations are being increasingly used and recommended for the estimation of energy expenditure and hence energy requirements, both at the community and population level[3].

During this exercise it was observed that the BMRs of Indians (which comprised a proportion of the total database) were 12.8 percent lower in males and 12.9 percent lower in females of all ages, as compared to their age, sex or weight matched European/American counterparts[2]. It was implied that this reflected an *ethnic* feature of this population although an extension to other populations living in the tropics was not made. Prospective measurements made by us, had revealed that Indians males in the 55-60 kg body weight range were only 5-6 percent lower in their BMR from Schofield's predictive equation[4]. Henry & Rees[5] reviewed BMR measurements made in the tropics and using data not previously evaluated by the FAO/WHO/UNU[1] report, demonstrated that the BMR of people living in the tropics were significantly lower than that predicted by Schofield's equations[2]. However, the deviation seen in tropical women (-3.8%, n=350) aged 18-30 years was smaller than that seen in tropical men (-10.8%, n=1174) of the same age. This disparity may be explained on the basis of the cyclical changes in BMR recorded during the menstrual cycle, which would account for a difference of up to 8.5 percent between the pre and post-ovulatory phases[6]. If the BMR of female subjects from tropical and temperate regions, used in the analysis were not all measured at exactly the same stage of their menstrual cycles the variations due to the menstrual cycle would make the detection of any difference between the two groups more difficult. These discrepancies in results, between the older Indian data analyzed by Schofield et. al.[2] and

current estimates by Henry & Rees[5], are not academic since they have important implications in assessing the energy requirements of this population. In this article we reexamine this question by comparing the BMRs, all prospectively measured during the same phase of the menstrual cycle, of Indian women with BMRs of Indian women reported in the literature[7] and with BMRs of European and American Women, in the 18-30 age group.

SUBJECTS AND METHODS

All BMR's were measured in duplicate, under standard conditions[8], using a Hartmann & Braun Metabolator that was validated against other indirect calorimetry systems[9]. All measurements were made between 6:30 and 8:30 a.m. in a room where temperatures ranged from 24 to 29°C and barometric pressure from 679 to 690 mm Hg. Each BMR was measured over ten minutes with a ten to twenty minute break between measurements. The BMR was considered to be technically valid if the two consecutive BMR measurements were within ± 3 percent of their mean. On an average replicates were within 0.7 percent in this study. For comparative purposes we also predicted BMR from the following equations for women aged 18-30 years:

1. Schofield et. al.[2]

BMR (kJ/d) = 54.5 x Body weight + 2513.5

2. Henry & Rees[5]

BMR (kJ/d) = [{0.048 x Body weight (kg)} + 2.562] x 1000

Comparisons were made with estimates of BMR using the equation derived from the prospective measurements made in this study, along the lines earlier reported[10].

Subjects

For the purpose of this study, we selected only those subjects with access to *ad libitum* energy and protein intakes, in the 18-30 age group. All subjects were non-smokers and were not on medication of any kind. They were free from disease and were weight stable for the previous 6 months (± 1 kg of current weight). The Current Indian Women (CIW)

in this study were all residents of Bangalore. All BMR's were measured on the 7th or 8th day of the menstrual cycle (The first day of menstruation being taken as Day 1).

The BMR and anthropometric data on Past Indian Women (**PIW**, n=52) was obtained from published values[7]. The BMR and anthropometric data on Current European/American Women (**CEAW**, n=52) was obtained from publications over the last decade (Ravussin et. al.[11], n=5; Morgan[12], n=6; Schutz et. al.[13], n=8; Owen et. al.[14], n=15; Bingham et. al.[15], n=2; Weststrate[16], n=16). No mention was made of the phase of the menstrual cycle at the time of BMR measurement in these studies.

Statistical Analysis

Comparisons between 'Measured BMR' and 'Predicted BMR' were made on the basis of 'paired t-tests'. Comparisons between CIW, PIW and CEAW were made using a 'One-way Analysis of Variance' (ANOVA). An 'Analysis of Co-Variance' (ANCOVA)[17], to adjust for differences in body weight between groups was used to compare the BMR among the groups. The data on American and European women were compared by 'unpaired t-tests'. A regression of the measured BMR of CIW on their body weight was obtained, using the method of least squares. Differences were considered to be significant at $p \leq 0.05$ level for all statistical procedures.

RESULTS

There were significant differences ($p < 0.01$) in age, height, weight, BMR on a one-way analysis of variance (ANOVA) between all three groups i.e. CIW, PIW and CEAW (Table 1). The BMIs of the CEAW were significantly higher ($p < 0.01$) than either the CIW or the PIW, while, there were no difference between the CIW and PIW. On an analysis of co-variance (ANCOVA) of the BMR, in order to adjust for differences in body weights between groups, there was no significant difference between the CIW and PIW. The adjusted BMR of the CEAW, however, remained significantly higher ($p < 0.01$) by 6.7 and 9.1 percent, as compared to CIW and PIW respectively.

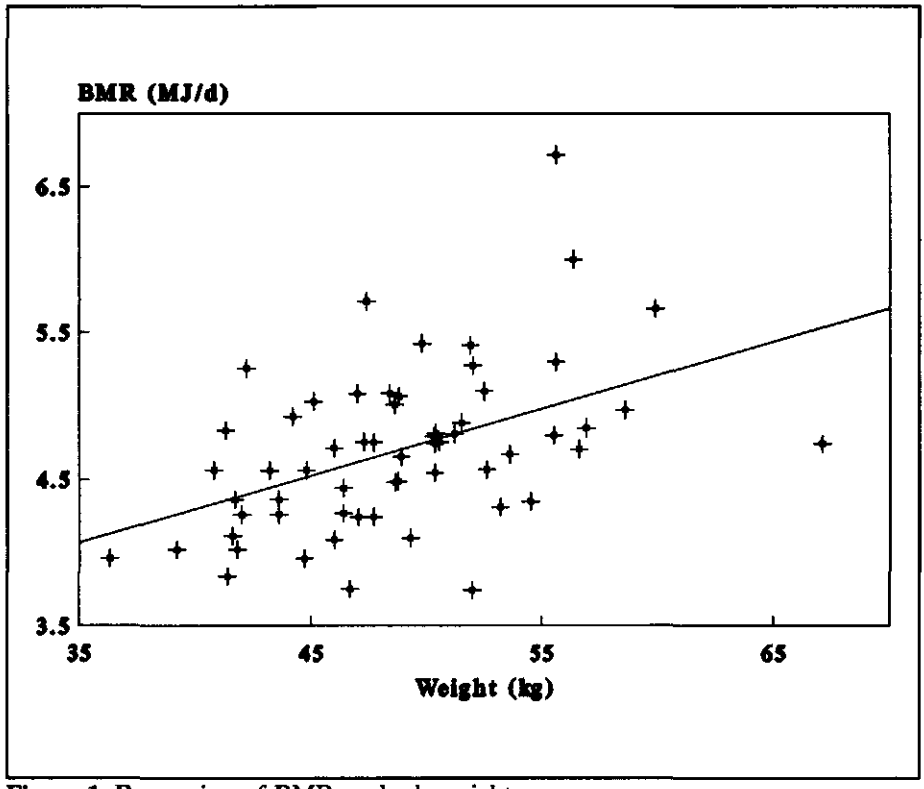

Figure 1. Regression of BMR on body weight

A regression equation of BMR on body weight was also generated using those CIW subjects with BMI ≥ 18.5 (n=38). BMR was then predicted for all CIW subjects (n=60) using this equation. The mean difference of the predicted BMR from the measured BMR was not statistically significantly between the two groups (**BMI ≥ 18.5**, 7.2 kJ/d vs **BMI < 18.5**, 12.3 kJ/d). Also, an ANCOVA of the BMR, to adjust for the differences in weight between CIW with BMI ≥ 18.5 (n=38) and those with BMI < 18.5 (n=22), showed no significant differences. Since the BMRs were comparable when adjustments for the differing body weights were made, all the 60 data points (Fig. 1) were used in the generation of the following prediction equation:

BMR (kJ/d) = Weight x 45.46 [± 11.36] **+ 2479.7** [± 557.4]

Table 2 shows that the equations of Schofield et. al.[2], and Henry & Rees[5] over-predict BMR of CIW by 9.2, and 4.2 percent respectively. The BMR of PIW[7] were similarly over predicted by these equations. On selecting women with BMI's ≥18.5 from the CIW (n=38) and PIW (n=26), the BMR was still significantly over predicted ($p<0.01$) by the equations of Schofield et. al.[2], and Henry & Rees[5] (CIW: +9.5% and +4.4% respectively, PIW: +11.6% and +6.9% respectively).

The current equation closely predicts the BMR of PIW but significantly under predicts ($p<0.05$) the BMR of the CEAW by 6.3 percent. The estimates of BMR for CEAW using the equation of Schofield et. al.[2], were significantly higher ($p<0.05$) than the measured BMR reported in the publications. However, there was no statistically significant difference between the measured BMR, of European and American women, and that predicted by the equation of Henry & Rees[5]. The body weights of the **American** women[11,14] (Mean ± SD, 56.6 ± 7.2 kg) were not significantly different from those of the **European** women[12,13,15,16] (59.3 ± 6.6 kg) on an independent t-test. The BMR of the American women[11,14], however, were significantly lower than that of the European women[12,13,15,16] ($p=0.05$)(Mean ± SD; **American**: 5.2 ± 0.8 vs **European**: 5.6 ± 0.6 MJ/d). The equation of Schofield et. al.[2] significantly over predicted ($p<0.05$) the BMR of American women[11,14] (Mean ± SD, 6.8 ± 10.7%) while accurately predicting the BMRs of the European women[12,13,15,16]. The equation of Henry & Rees[5] closely predicted the BMR's of both European[12,13,15,16] and American women[11,14], while the current Indian equation significantly under predicts ($p<0.05$) European women's[12,13,15,16] BMR (Mean ± SD, -8.3 ± 12.0%) but closely predicted the BMR of the American women[11,14].

DISCUSSION

The requirements of energy for individuals, communities or population groups worldwide, necessitate the assessment of 24 hour energy expenditure[1]. Towards this end BMR's are being increasingly measured or predicted, since they now form the basis of the factorial approach for estimating total energy expenditure. The prediction of BMR from a simple

Table 1. Comparison of age, height, weight, BMI, BMR and adjusted BMR between Current Indian Women (CIW), Past Indian Women (PIW) and Current European/American Women (CEAW)[+].

	CIW (n=60)	PIW (n=52)	CEAW (n=52)
Age (years)	21.6 ± 3.1	22.0 ± 2.4	23.5 ± 2.7[ab]
Height (m)	1.59± 0.5[bc]	1.53± 0.6[ac]	1.64± 0.6[ab]
Weight (kg)	48.7 ± 5.7[bc]	44.4 ± 6.4[ac]	58.2 ± 6.9[ab]
BMI (kg/m^2)	19.3 ± 2.2	19.0 ± 2.6	21.3 ± 2.1[ab]
BMR (MJ/d)	4.7 ± 0.6[bc]	4.4 ± 0.4[ac]	5.5 ± 0.7[ab]
Adjusted BMR (MJ/d)	4.9 ± 0.1	4.8 ± 0.1	5.3 ± 0.1[ab]

[+]Mean ± SD

Significant differences ($p<0.01$) between groups for all variables on a One-way ANOVA, multiple comparison tests using the method of Least Significant Differences (LSD) used for identifying between-group differences.

[a]significantly ($p<0.05$) different vs Current Indian Women (CIW)
[b]significantly ($p<0.05$) different vs Past Indian Women (PIW)
[c]significantly ($p<0.05$) different vs Current European/American Women (CEAW)

[*]Adjusted for differences in weight using Analysis of Co-Variance. Fisher's Highly Significant Difference procedure used to identify differences between groups.

Table 2. Difference between Measured and Predicted BMR of Indian women in the 18-30 years age group[+]

	CIW (n=60)	PIW (n=52)	CEAW (n=52)
SS&J[a] (kJ/d)	-499.9±495.1*	-519.5±294.4*	-236.2±593.8*
(%)	(-9.2± 9.3)	(-10.5± 5.6)	(-4.1± 10.5)
H&R[b] (kJ/d)	-206.1±491.0*	-277.0±284.2*	96.1±590.0
(%)	(-4.2± 9.8)	(-5.9± 5.8)	(1.8± 11.1)
Present Equation (kJ/d)	0.0±490.8	-84.1±280.0	324.2±590.7*
(%)	(0.0± 10.3)	(-1.8± 6.1)	(6.3± 11.6)

[+]Mean ± SD

Absolute mean difference (kJ/d) = Measured BMR - Predicted BMR

Values in parentheses are: Percent Mean difference = [(Measured BMR - Predicted BMR)/Predicted BMR] x 100

*significantly ($p<0.05$) different vs Measured BMR on a paired t-test

[a]Schofield, Schofield & James (1985)

[b]Henry & Rees (1991)

anthropometric parameter like body weight, was an attempt to estimate BMR, when actual measurements were unavailable[1].

The suggestion of Schofield et. al.[1] that both Indian men and women had lower BMR's than their European/American counterparts was a restatement of an often reported observation[18-22]. Since these observations were made over 60 years ago, we were interested in reassessing this *ethnic* feature. On adjusting for differences in body weight, current Western women (both European and American) have significantly higher BMRs than Indian women in this series. This difference was of the order of 6.7 percent, even after adjustments for differences in body weight were made. Differences in the BMR measured during the different phases of the menstrual cycle, possibly play an important role since they can contribute to as much as 8.5 percent of the variation in the same subject[6]. All the women in our study were measured in the mid-follicular phase (on the 7th or 8th day following the onset of the menstrual period). The exact time point of the BMR measurement is not available for the studies used for the compilation of European/American data, as such details were not provided.

It is not unlikely that the Western women have a greater proportion of their body weight made up of muscle and viscera, with their inherently higher energy expenditure, as compared to, their Indian counterparts. Indian women thus may have lower fat free mass (FFM) as compared to their Western counterparts. This would, perhaps, explain in part the current findings of a lower BMR in them. Banerjee[23] had earlier concluded that on correcting for active tissue mass or cell solids, there were no differences between Indians and Western subjects in their BMR. As data was not available on the body composition of the CIW and PIW this comparison has not been made here.

The equation of Henry & Rees[5] closely predicts the BMR of European and American women, while significantly over predicting the BMRs of Indian women (both current and past) (Table 2). This may well be due to the fact that no consideration was given to the period in the menstrual cycle when the measurement of the BMR was made in the data collated by Henry & Rees[5]. The equations of Schofield et. al.[2] significantly over predict the BMR of Indian women (both current and past), as well as, European and American

women in the data set. Although several factors may be involved in producing such differences, it is not unlikely that such results are an artefact of the sample used to derive the equations; an explanation proposed by Schofield himself though not emphasized strongly[24]. The over-prediction suggests that current BMRs of both Indian and Western populations have are lower to BMRs of Western subjects of identical body weights, measured over 60 years ago. This could signify that well nourished females in the 18-30 age group have a lower active tissue mass now for the same body weight world wide as compared to sixty years ago. Alternatively, as suggested by Soares & Shetty[25], if the active tissue masses are similar, then overall, there must be a lowering of its metabolic activity. The latter could be due in turn, to either a lowering of the activity per se, or to a relatively greater proportion of active tissue mass being comprised of organs with a relatively lower activity at rest such as muscle mass, as compared to visceral tissue, in both Indian and Western individuals as compared to their counterparts 60 years ago. Another factor that may be responsible for the over prediction could be that the BMR's of American women in this data set were 4.7 percent lower than their European counterparts, though this difference did not reach statistical significance. On separating the American from the European data the equation of Schofield et. al.[2] accurately predicts the BMR of the European women while significantly over estimating the BMR of the American women by almost 7 percent. This pattern of a lower BMR as compared to estimates based on Schofield's equation, is also seen in American males of the same age group, when compared to Europeans[10].

These possibilities though interesting to examine in their own right, should not distract us from the fact that with the use of the generally accepted Schofield or FAO/WHO/UNU[1] equations, we could well be over-estimating energy requirements. The results of this study indicate that the BMR of CIW are only about 6-7 percent lower than CEAW. Thus an *ethnic* feature, as suggested by Schofield et. al.[2] seems to be unlikely, as this lower BMR may be explained by several factors such as climatic influences, diet, or perhaps due to differences in the amount and/or composition of the active tissue mass. When the American data was separated from the European, the current equation, as well as, the equation of Henry & Rees[5] accurately predicted the BMR for Americans while the European data appeared to be significantly higher than

that predicted, an observation made earlier for men[10]. There is reason to believe that the currently recommended predictive equations for BMR of Schofield, Schofield & James[2] would significantly over-estimate the energy requirements of well nourished populations all over the world. International agencies that deliberate on these matters should review these suggestions, since they are important for all future food policy decisions. Further studies with accurate measures of body composition would go a long way in determining the existence of any true difference in the BMR of Indian women from their Western counterparts. For the prediction of BMRs of well nourished Indian women, in the 18-30 years age group, we propose the use of the present equation.

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Chapter 3

THE BASAL METABOLIC RATE AND THERMIC EFFECT OF A MEAL IN THE PRE- AND POST-OVULATORY PHASES OF THE MENSTRUAL CYCLE IN WELL NOURISHED INDIAN WOMEN

Leonard S. Piers, Sumita N. Diggavi, Juliet Rijskamp, Joop M. A. van Raaij, Prakash S. Shetty and Joseph G. A. J. Hautvast

ABSTRACT

The basal metabolic rate (BMR), thermic effect of a standard test meal (TEM) were measured on two occasions, in each phase (pre-ovulatory and post-ovulatory, as confirmed by plasma levels of progesterone and estradiol) of a single menstrual cycle, using indirect calorimetry, in a group of 13 well-nourished Indian women. A single measurement of the BMR and TEM was also made in the pre-ovulatory phase of the following menstrual cycle. There was a significant ($p < 0.05$) increase in the TEM (Pre-ovulatory: 6.72 ± 1.57 vs post-ovulatory: 7.96 ± 1.48 %) of 18.5 percent during the luteal phase of the menstrual cycle. This amounted to a 23 kJ difference over 5 hours. There was no significant difference in the BMR. Fasting plasma glucose, insulin, thyroid stimulating hormone (TSH), tri-iodothyronine (T_3), thyroxine (T_4) measured in 5 of the subjects in both phases of the same menstrual cycle were found to be comparable. Dietary energy and macro-nutrient intakes measured in all subjects in both phases of the menstrual cycle were also found to be similar. Changes in the BMR between consecutive menstrual cycles were small, as was the total energy expenditure following ingestion of the test meal. We conclude that the changes in energy expenditure in relation to the BMR and TEM over the course of a single menstrual cycle, and between two consecutive cycles, are small. In nutritional terms the significant rise in post-ovulatory TEM is small and is unlikely to alter energy requirements in well-nourished women from the reproductive age group.

INTRODUCTION

Alterations in energy expenditure over the course of the menstrual cycle have been reported by several workers[1-4]. All these studies report an increase in the basal metabolic rate[1,4] (BMR), sleeping metabolic rate (SMR)[3], or total energy expenditure (TEE)[2], in the post-ovulatory (luteal) phase of the menstrual cycle. However, other reports have failed to demonstrate any increase in the BMR or the thermic effect of a meal (TEM)[5]. Reported increases of between 7-16 percent of the TEE[2,6] during the post-ovulatory period would require a significant increase in energy intake during this period.

Changes in nutritional requirements in individuals at different time points and whether these requirements have wide validity or are influenced by factors such as ethnicity, climate, or adaptation were listed as priority areas of research in the report of the joint Food and Agricultural Organization /World Health Organization /United Nations University (FAO/WHO/UNU) joint expert consultation[7]. Cyclical changes in energy expenditure and intake, associated with the different phases of the menstrual cycle, would not only have a significant influence on the design of nutritional and physiological studies requiring a pre-menopausal control group, but also in the determination of the energy requirements in these individuals, as well as, the population as a whole. However, before these differences are extrapolated to all population groups more evidence from different parts of the world is necessary.

This study was carried out to establish if there were changes in the basal metabolic rate, thermic effect of a meal and dietary energy intake over the course of a single menstrual cycle, as well as, to establish if there were any large between-cycle differences in the BMR and TEM, in well nourished Indian women from the reproductive age group.

SUBJECTS AND METHODS

Study design (Table 1)

Twenty healthy non-pregnant non-lactating women in the reproductive age group were recruited for the study. The study consisted of two parts. The first part consisted of a paired comparison of anthropometry, basal metabolic rate (BMR), thermic effect of a meal (TEM), substrate oxidation rates, fasting plasma hormone and substrate levels and dietary intakes between the pre-ovulatory phase of the menstrual cycle (i.e. between days 6-10 of the menstrual cycle, the first day of menstruation being considered as day 1) and the post-ovulatory phase of the same menstrual cycle (i.e. between days 21-25 of the menstrual cycle). All metabolic measurements were made on two occasions in each phase of the menstrual cycle. The second part consisted of a comparison of the anthropometry, BMR, TEM and substrate oxidation rate in the pre-ovulatory phase of two consecutive menstrual cycles (i.e. between days 6-10 of the menstrual cycle). As metabolic measurements were only made on a single occasion in the pre-ovulatory phase of the second menstrual cycle (i.e. between days 6-10 of the menstrual cycle) , these were compared to the measurements made on the first occasion during the corresponding phase of the previous cycle.

Subjects

Subjects were either from the middle or upper socio-economic class, as assessed by Kuppuswamy's[8] urban socio-economic assessment scale; were non-vegetarians with *ad libitum* access to food. All subjects were non-smokers, in good health, weight stable, and were not on any kind of chronic medication or oral contraceptives. The subjects were recruited from amongst the staff of St. John's Medical College and Hospital, or by personal approach.

Table 1. Study Design

Phase of menstrual cycle	**Pre-ovulatory** phase of first menstrual cycle (day 6-10)		**Post-ovulatory** phase of first menstrual cycle (day 21-25)		**Pre-ovulatory** phase of next menstrual cycle (day 6-10)
Occasion	1	2	1	2	1
Height	x	-	-	-	-
Weight	x	x	x	x	x
Mid-arm circumference	x	-	x	-	x
Skinfold thicknesses	x	-	x	-	x
BMR	x	x	x	x	x
TEM	x	x	x	x	x
Fasting blood sample	-	x	-	x	-
Dietary intakes[a]	x	-	x	-	-

[a]Measured intakes recorded for 5 consecutive days prior to metabolic measurements in each phase of the menstrual cycle

Methods

Energy expenditure was measured by respiratory gas exchange measurements using a 'ventilated hood'. The ventilated hood measurement system consisted of a plastic hood which surrounded the subject's head and a soft plastic collar surrounding the neck and shoulders. A fixed flow (50 l/min) of room air was maintained through the hood by connecting the outlet of the hood through a calibrated rotameter (Fischer Controls, UK) to a suction pump. A small sample of air (1 l/min) was drawn off from the outlet of the rotameter for minute to minute estimation of oxygen (O_2) and carbon-dioxide (CO_2) concentrations using a paramagnetic oxygen analyzer (Servomex 540 A, Taylor Instruments, UK) and an infra-red carbon-dioxide analyzer (Type SSI, Analytical Development Co., U.K.) respectively. O_2 and CO_2 concentrations of the room (reference) air were monitored every hour for 10 minutes, prior to the start of a metabolic measurement. Moisture content and temperature were measured by means of a dew-point hygrometer of the optical condensing type (General Eastern Systems, 1100 DP, USA), and a mercury thermometer placed in the air stream emerging from

the rotameter, respectively. Atmospheric pressure was measured by means of a mercury barometer at the start of each measurement protocol. Oxygen consumption (VO_2) and carbon-dioxide production (VCO_2) rates (calculated for STPD conditions, standardized for temperature, pressure and dryness), Respiratory Quotient (RQ = VCO_2/VO_2), and energy expenditure[9] were calculated from this data and recorded every minute using a computerized data acquisition and storage system[10]. The gas analyzers were calibrated before the start of each measurement using pure nitrogen (100%) (Indian Oxygen Ltd., Bangalore) for 'zero', and a mixture of carbon-dioxide (1%), oxygen (21%) and nitrogen (78%) (Bhoruka Gases Ltd., Bangalore), for the 'span' setting. These calibration gases were passed through the analyzers at the end of the measurement protocol, 6 hours after the initial calibration, to check for instrument drift. All data was subsequently corrected for instrument drift during this period. This measurement system had been validated against other methods of measurement of oxygen consumption[11]. Calibration using the N_2 infusion technique[12] yielded a net discrepancy of less than 0.4 percent in the estimation of oxygen consumption. Ethanol burns conducted at regular intervals over the duration of the study produced energy expenditures within ± 2 percent of expected values. Room temperatures were maintained between 24 and 29°C on all days of the study , while barometric pressures ranged from 681 to 692 mm Hg.

The measurement protocol employed was as follows (Figure 1). Subjects were instructed to complete their evening meal by 19:30 hours, and to be in bed by 22:00 hours on the night prior to the metabolic measurements. On the morning of the metabolic measurement they were instructed to wake between 6:00 and 6:30 hours, complete their toilet and empty their bladder while noting the exact time. They were then transported to the laboratory by the department vehicle. On entering the laboratory all subjects were instructed to rest in bed for 30 min. prior to the start of the BMR measurement. At the end of this mandatory rest period the basal metabolic rate was measured for 30 min, after an initial 10 min period to allow O_2 consumption to stabilize while within the ventilated hood. Basal metabolic rate (BMR) was calculated by obtaining the mean energy expenditure over this 30 min period.

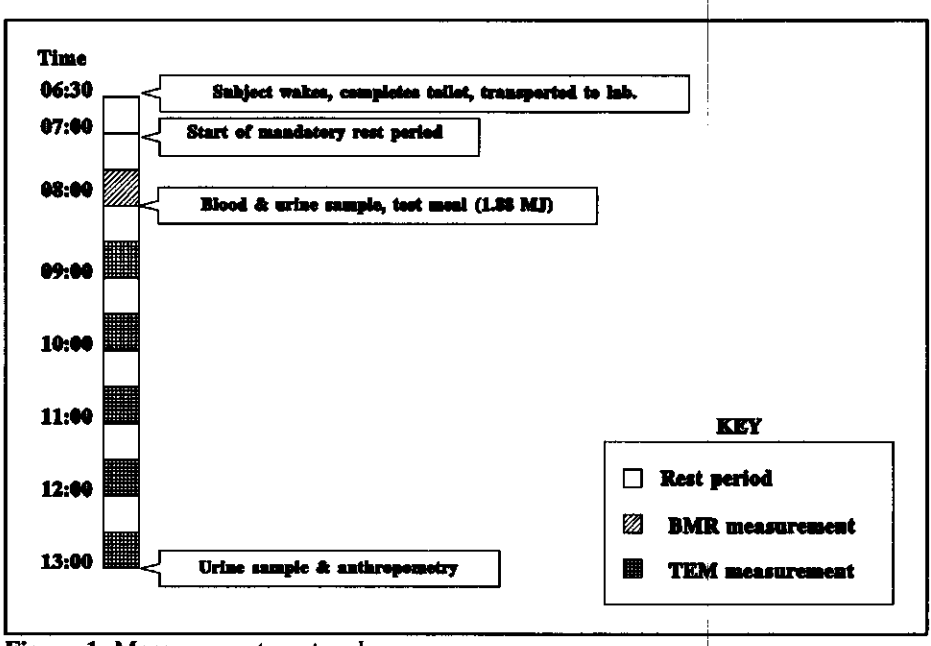

Figure 1. Measurement protocol

Following the measurement of the BMR the hood was removed. A fasting blood sample (10 ml) was collected from the antecubital vein using aseptic precautions for the estimation of glucose using the orthotoluidine method, insulin, triiodothyronine (T_3), thyroxine (T_4), thyroid stimulating hormone (TSH) using locally available RIA kits (Bhabha Atomic Research Centre, Bombay, India), while estradiol and progesterone were measured using DPC RIA kits (Diagnostic Products Corporation, Los Angeles, USA) in 5 subjects.

The subjects were then instructed to void. The voided urine volume was noted and then acidified with concentrated hydrochloric acid (1 ml acid per 100 ml urine). An aliquot was stored for estimation of total urinary nitrogen (TUN) excretion, over the preprandial period, by the micro-kjeldhal method[13]. (All urine voided during the postprandial period together with the final urine sample obtained at the end of the metabolic measurements, was collected in the same manner, for estimation of TUN excretion, over the postprandial period.)

Following the collection of the basal urine sample the subjects were served a standard

test meal which consisted of tinned milk powder, rice cereal and sugar, together with a glass of lemon juice. The cereal was mixed with the powdered milk and part of the sugar and made up to 200 ml with water. The remaining sugar was mixed with 200 ml of water to which lemon juice was added for flavour. Together this provided 1.88 MJ (450 kcal) of energy, with 10 percent of the energy being derived from protein (11.2 g/100 g), 15 percent from fat (7.6 g/100 g) and 75 percent from carbohydrate (84.2 g/100 g). Energy and nutrient composition was derived from the manufacturer's product information. All tinned products were used well before their date of expiry and produced by the same manufacturer.

After the ingestion of the meal, the ventilated hood was replaced and the O_2 consumption and CO_2 production were measured intermittently for the next 5 hours. The second thirty minutes in each hour were considered to be a 'measurement period' and representative of the energy expenditure for the entire hour; the initial 30 min being designated as a 'rest period'. The subject was instructed to lie awake and motionless in the recumbent position during the 'measurement periods'. Between the 'measurement periods', i.e. during the 'rest periods', they were also asked to lie quietly in bed though some movement and reading was permitted. Ten minutes prior to the start of each 'measurement period' they were instructed to rest motionless to allow their O_2 consumption to stabilize, while within the ventilated hood, in preparation for the next 'measurement period'.

The thermic effect of the standard test meal (TEM) was calculated by obtaining the mean increment in energy expenditure during the 'measurement period' (30 min) in each hour (which was considered representative for the entire hour) above pre-meal basal values. The cumulative increment in energy expenditure for the 5 hours following the ingestion of the test meal was expressed as a percentage of the energy content of the meal. The post-meal total energy output (PMTEO) was calculated by obtaining the total energy expenditure during the 5 hours after the ingestion of the test meal[14]. Substrate oxidation rates were calculated during the pre- and postprandial period in each subject, using total oxygen consumption (VO_2), carbon-dioxide (VCO_2) production rates and total urinary nitrogen (TUN) excretion during the pre and postprandial periods[15]. No

correction was made to the protein oxidation rate to take into account any change in the blood urea pool between the fasted and fed states. Non-Protein Respiratory Quotients (NPRQ) were calculated after subtracting the oxygen consumed (PVO_2) and carbon dioxide produced ($PVCO_2$) in protein oxidation, using the formulas of Jequier et. al.[16], from the measured VO_2 and VCO_2 of the subject $[(VCO_2\text{-}PVCO_2)/(VO_2\text{-}PVO_2)]$.

On completion of all metabolic measurements for the day, anthropometric measurements were made for assessment of nutritional status. These included height, weight, skinfold thicknesses (biceps, triceps, subscapular, supra-iliac) and mid-arm circumference (MAC). Height was measured using a moveable anthropometer (Nivotoise, France) and recorded to the nearest 0.1 cm. Body weight was measured on a digital weighing scale (Soehnle, Germany) and recorded to the nearest 100 g. Subjects stood upright with the head in the horizontal plane, in light indoor clothing. For the measurement of the skinfold thicknesses (biceps, triceps, subscapular, supra-iliac) a Holtain skinfold calliper (Crymmych, U.K.) was used. All the skinfolds were measured on the right side of the body and recorded to the nearest 0.2 mm. Every skinfold was measured in triplicate and the mean of these 3 values was used for calculation. Fat mass (FM) was calculated after estimating percent body fat from the sum of four skinfolds and applying the formula of Durnin & Womersley[17]. Fat free mass (FFM) was estimated from the difference of weight and FM.

A week prior to the metabolic measurements the subjects were visited at home and provided with two separate household measures of known volume, together with a tape measure, to measure the volume of all solid and liquid food consumed. In addition a booklet was provided on each occasion to record the time of the meal, a general description of the food/drink consumed, details of any unconventional food item eaten, the exact volume consumed as measured in the household measures provided and dimensions of solid food that could not be measured in the measures provided. All subjects were instructed by a trained dietitian as to the proper use of the household measures and the type of detail required in the description of the food items or beverages being consumed. The method of recording this information in the booklet provided was demonstrated in a simple example on the initial page of each booklet. No

special advice as to the type or quantity of food to be consumed was given, except to encourage all subjects to eat to the dictates of their appetite and not change their eating pattern during this period. This record was maintained for 5 consecutive days prior to the metabolic measurement. On the day of the metabolic and anthropometric measurements the dietitian checked the dietary record maintained by the subject and clarified any ambiguities, following the completion of the metabolic and anthropometric measurements. The volume of food consumed was converted to weight by weighing a similar food item, prepared in the laboratory using a standardized recipe, in the household measure used by the subject. Fruits, sweets, snacks etc were purchased based on the dimensions provided by the subjects and weighed in the laboratory. These data were then used for estimation of energy and macro-nutrient content of the diet by the use of standard food composition tables[18-20].

Statistical analysis

Data are presented as Mean ± SD. The mean of the two BMR, TEM, PMTEO and weight measurements, made in each phase of the cycle, were used for the 'within-cycle' comparison of the pre- and post-ovulatory phases of the menstrual cycle. The mean energy and macronutrient intakes measured over 5 days, in each phase of the same menstrual cycle, were used to assess the change in dietary intake between the two phases of the first menstrual cycle. A paired t-test was used to detect differences between the pre and post ovulatory phases of the same menstrual cycle, as well as, between the pre-ovulatory phases of the two consecutive menstrual cycles in the 'between-cycle' comparison of antropometric and metabolic data. Statistical significance was accepted at the 5 percent level. All data was analyzed using SPSS/PC+ (Version 4.0, SPSS Inc. 444 N.Michigan Avenue, Chicago, Illinois, USA) statistical package on an IBM-PC 386 compatible computer.

Ethical Approval

Ethical approval was obtained for the study from the Human Investigation Committee of the Medical School. Informed consent in writing was obtained from all subjects prior to the start of the measurements.

RESULTS

Of the 20 subjects recruited for the study 6 did not complete all planned measurements. One of the 14 subjects who did complete the study failed to ovulate, as demonstrated by an increase in the progesterone levels in the post-ovulatory blood sample to around 5 ng/ml in the mid-luteal phase of the menstrual cycle[21]. Hence what follows is data on the 13 subjects who ovulated and completed all planned measurements.

Table 2. Subject characteristics measured during the course of a single menstrual cycle[a].

Phase of menstrual cycle	**Pre-ovulatory** phase of first menstrual cycle (day 6-10)	**Post-ovulatory** phase of first menstrual cycle (day 21-25)
No. of subjects (n)	13	13
Age (years)	26.9 ± 6.6	
Height (m)	1.61 ± 0.06	
Weight (kg)	52.0 ± 7.7	51.8 ± 7.4
Body Mass Index (kg/m^2)	20.1 ± 2.5	20.0 ± 2.4
Sum of 4 skinfolds (mm)	54.4 ± 14.0	55.2 ± 15.8
Fat (%)	27.7 ± 3.6	27.8 ± 4.1
Fat mass (kg)	14.4 ± 3.3	14.5 ± 3.6
Fat free mass (kg)	37.5 ± 5.3	37.3 ± 4.7

[a]Mean ± SD

Within cycle comparisons

Subject characteristics are given in Table 2. There was no significant change in the body weight, body mass index (BMI), sum of four skin-fold thicknesses, percent body fat, fat mass (FM) or fat free mass (FFM), between the two phases of the menstrual cycle. A comparison of the metabolic measurements made in the two phases studied

are presented in Table 3. There were no significant differences in the BMR (Pre-ovulatory: 3.18 ± 0.21 vs Post-ovulatory: 3.16 ± 0.25 kJ/min) and PMTEO (Pre-ovulatory: 3.60 ± 0.20 vs Post-ovulatory: 3.66 ± 0.27 kJ/min) in the pre- and post-ovulatory phases of the menstrual cycle. The TEM, however, showed a significant ($p<0.05$) increase of 18.5 percent in the post-ovulatory (luteal) phase of the menstrual cycle (Pre-ovulatory: 6.72 ± 1.57 vs Post-ovulatory: 7.96 ± 1.48 percent) (Figure 2). In absolute terms this was a difference of 23 kJ over 5 hours (Pre-ovulatory: 127 ± 30 vs Post-ovulatory: 150 ± 28 kJ/5h). This difference was also reflected in the PMTEO, which was non-significantly higher in the post-ovulatory period (Pre-ovulatory: 1080 ± 60 vs Post-ovulatory: 1097 ± 80 kJ/5h), though only by 17.6 kJ/5h. With the exception of pre-ovulatory protein oxidation rates, there were significant increases ($p<0.05$) in the protein, fat and carbohydrate oxidation rates and non-protein respiratory qoutient (NPRQ) during the postprandial period, as compared to those calculated during the basal (BMR) period, in both phases of the menstrual cycle. There were no significant differences between the basal, or the postprandial substrate oxidation rates between the two phases of the menstrual cycle, with the exception of basal protein oxidation rates, which were significantly lower than pre-ovulatory basal protein oxidation rates.

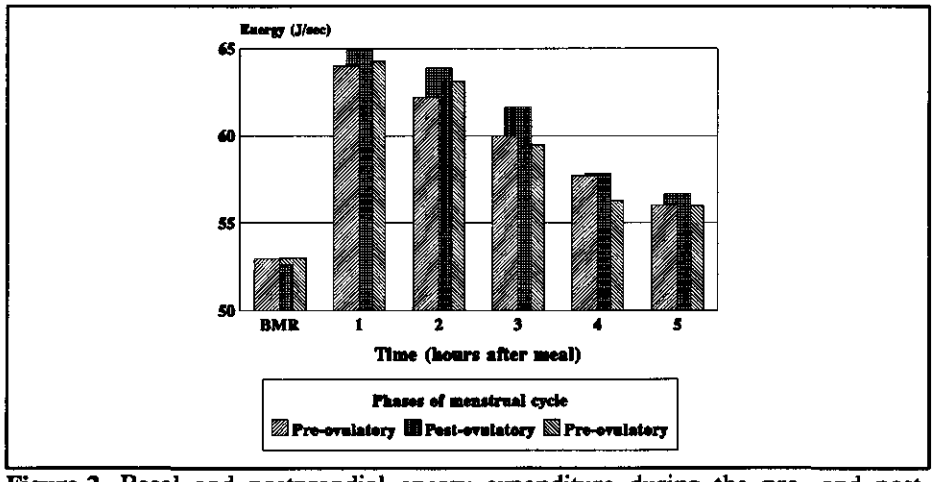

Figure 2. Basal and postprandial energy expenditure during the pre- and post-ovulatory phase of a single menstrual cycle and the pre-ovulatory phase of the following menstrual cycle.

Table 3. Basal and postprandial metabolic parameters measured during the course of a single menstrual cycle[a]

Phase of menstrual cycle	**Pre-ovulatory** phase of first menstrual cycle (day 6-10)	**Post-ovulatory** phase of first menstrual cycle (day 6-10)
No. of subjects (n)	13	13
Basal Metabolic Rate (BMR)		
(kJ/min)	3.18 ± 0.21	3.16 ± 0.25
(MJ/day)	4.57 ± 0.37	4.55 ± 0.37
(kJ/kg wt/day)	89.5 ± 12.1	88.8 ± 8.9
(kJ/kg FFM/day)	123.0 ± 15.7	123.0 ± 12.3
Thermic Effect of the Meal (TEM)		
(% energy in test meal)	6.72 ± 1.57	7.96 ± 1.48[b]
Post Meal Total Energy Output (PMTEO)		
(kJ/min)	3.60 ± 0.20	3.66 ± 0.27
(kJ/5 hours)	1079.6 ± 59.9	1097.3 ± 80.1
Non Protein Respiratory Quotient		
Basal	0.82 ± 0.04	0.84 ± 0.05
Postprandial	0.96 ± 0.03[c]	0.97 ± 0.05[c]
Basal Substrate Oxidation Rate (mg/min)		
Protein	31.6 ± 14.2	24.2 ± 9.0[b]
Fat	37.8 ± 9.5	35.4 ± 12.1
Carbohydrate	65.9 ± 21.5	80.1 ± 27.7
Postprandial Substrate Oxidation Rate (mg/min)		
Protein	34.1 ± 5.5	35.6 ± 9.6[c]
Fat	10.6 ± 6.6[c]	7.6 ± 11.1[c]
Carbohydrate	159.1 ± 16.9[c]	168.5 ± 14.5[c]

[a]Mean ± SD

[b]significantly ($p < 0.05$) different from pre-ovulatory value on a 'paired t-test'.

[c]significantly ($p < 0.05$) different from basal value obtained during BMR measurement on a 'paired t-test'

Table 4. Dietary energy and macronutrient intakes measured during the course of a single menstrual cycle[a].

Phase of menstrual cycle	**Pre-ovulatory** phase of first menstrual cycle (day 6-10)	**Post-ovulatory** phase of first menstrual cycle (day 21-25)
No. of subjects (n)	13	13
Energy (MJ/d)	7.12 ± 1.03	7.12 ± 1.38
(kcal/d)	1701 ± 246	1702 ± 330
Protein (g/d)	47 ± 11	49 ± 15
(% of total energy)	(11.0)	(11.6)
Fat (g/d)	72 ± 18	72 ± 26
(% of total energy)	(38.1)	(38.1)
Carbohydrate (g/d)	217 ± 29	215 ± 40
(% of total energy)	(50.9)	(50.6)

[a]Mean ± SD; Note: Measured records were maintained for 5 consecutive days prior to the metabolic measurements in each phase of the first menstrual cycle.

Table 5. Fasting plasma substrate and hormone levels measured during the course of a single menstrual cycle[a].

Phase of menstrual cycle	**Pre-ovulatory** phase of first menstrual cycle (day 6-10)	**Post-ovulatory** phase of first menstrual cycle (day 21-25)
No. of subjects (n)	13	13
Plasma glucose (mg/dl)(n=5)	65.0 ± 7.3	65.1 ± 10.1
Insulin (μU/ml)(n=5)	12.7 ± 5.8	9.8 ± 3.9
Tri-iodothyronine T_3 (ng/ml)(n=5)	1.5 ± 0.2	1.8 ± 0.7
Thyroxine T_4 (μg/dl)(n=5)	9.3 ± 1.6	9.1 ± 2.0
Thyroid Simulating Hormone TSH (μIU/ml)(n=5)	0.7 ± 0.3	0.6 ± 0.3
Estradiol (pg/ml)(n=13)	80.3 ± 43.2	155.9 ± 88.0[b]
Progesterone (ng/ml)(n=13)	0.2 ± 0.1	8.8 ± 4.4[b]

[a]Mean ± SD

[b]significantly ($p<0.01$) different vs pre-ovulatory levels on a paired 't-test'.

Table 6. Anthropometric and metabolic parameters measured in the same phase of two consecutive menstrual cycles[a]

Phase of menstrual cycle	**Pre-ovulatory** phase of first menstrual cycle (day 6-10)	**Pre-ovulatory** phase of second menstrual cycle (day 6-10)
No of subjects (n)	13	13
Body weight (kg)	52.1 ± 7.8	51.7 ± 7.6
Body Mass Index (kg/m^2)	20.1 ± 2.5	20.0 ± 2.5
Body Fat (%)	27.7 ± 3.6	27.4 ± 3.9
Fat Free Mass FFM (kg)	37.6 ± 5.31	37.4 ± 4.87
Basal Metabolic Rate BMR (kJ/min)	3.17 ± 0.19	3.13 ± 0.23
Thermic effect of meal TEM (% of energy contained in meal)	6.66 ± 1.83	7.34 ± 2.06
Post Meal Total Energy Output PMTEO (kJ/5h)	1076.9 ± 56.8	1076.8 ± 63.7
Non Protein Respiratory Quotient		
Basal	0.83 ± 0.05	0.83 ± 0.04
Postprandial	0.97 ± 0.04[b]	0.98 ± 0.03[b]
Basal substrate oxidation rates (mg/min)		
Protein	32.4 ± 14.6	27.4 ± 12.8
Fat	36.6 ± 13.1	36.0 ± 8.5
Carbohydrate	67.8 ± 27.1	72.9 ± 27.7
Postprandial substrate oxidation rates (mg/min)		
Protein	37.0 ± 8.7	34.7 ± 11.8
Fat	8.7 ± 8.6[b]	5.4 ± 7.0[b]
Carbohydrate	159.9 ± 20.3[b]	171.2 ± 24.6[b]

[a]Mean ± SD

[b]significantly ($p<0.05$) different vs basal value obtained during BMR measurement on a paired 't-test'.

There were no significant differences between pre- and post-ovulatory energy, protein, fat or carbohydrate intakes as estimated from the measured records, maintained over 5 consecutive days, in each phase of the menstrual cycle (Table 4). There were no significant differences in the plasma glucose, insulin, T_3, T_4 and TSH. There were, however, significant increases in the plasma estradiol and progesterone levels (Table 5).

Between cycle comparisons

There were no significant differences in anthropometry, and pre- or postprandial energy expenditure, between the pre-ovulatory phases of the two consecutive menstrual cycles in question (Table 6). The differences in the BMR and TEM though non-significant, were of the order of 1.3 and 10.2 percent, respectively. There was no significant increase in the protein oxidation rate in the postprandial period of either menstrual cycle as compared to basal values, however, fat oxidation rates were significantly ($p<0.05$) reduced and carbohydrate oxidation rates were significantly ($p<0.05$) increased, in both menstrual cycles. These changes were, however, comparable between the two cycles.

DISCUSSION

Alterations in energy expenditure over the course of the menstrual cycle have been reported by several workers[1-4]. There is also evidence to support changes in food intake associated with the menstrual cycle[22]. Estimates of energy requirements should, however, be based on energy expenditure, as recognized by the *Ad Hoc* committee of the FAO/WHO[23]. The increases in post-ovulatory (luteal) energy expenditure, if present, would therefore have to be taken into account when arriving at recommendations for energy intakes of women in the reproductive age group. However, before such a move is made more evidence from different parts of the world including different populations should be available on energy expenditure throughout the menstrual cycle.

Most of the studies reporting changes in energy expenditure associated with the different phases of the menstrual cycle appear to have been made over the course of more than one menstrual cycle[1-5], and therefore the observations would include the between-cycle variability, in addition to, within-cycle variability. Some of the studies observed increases in the sleeping metabolic rate (SMR)[3], while others in the basal metabolic rate (BMR)[1,4] or total energy expenditure (TEE)[2,6], during the post-ovulatory (luteal) phase of the menstrual cycle. This increase in energy expenditure being ascribed to the thermogenic effect of progesterone. Weststrate[5] measured BMR and thermic effect of a meal (TEM) in 23 women in the pre- and post-ovulatory periods, over the course of three menstrual cycles. No differences in the BMR or TEM responses were demonstrated between the two phases of the menstrual cycle.

This study provides data on the basal metabolic rate and the thermic effect of a meal, in well nourished Indian women, over the course of a single menstrual cycle. In addition, the between-cycle differences in these processes has also been measured. There was no significant difference, in the body weight or body composition over the course of the menstrual cycle in this study.

There was no significant change in the BMR or post meal total energy output (PMTEO), though the PMTEO did show a tendency to be higher by 1.6 percent (17.7 kJ/5h) in the post-ovulatory period. There was, however, a 18.5 percent increase ($p<0.05$) in the TEM. The within individual coefficient of variation (CV) for the BMR, TEM and PMTEO was 7.6, 22.7, and 6.7 percent respectively. This would permit a 90 percent (power = 0.90) chance of uncovering a true difference of 5, 15, and 4.5 percent or more, in the BMR, TEM, and PMTEO respectively, in this study[24]. These CVs are similar to those reported by Weststrate[5], of 6.0 and 27.5 percent for the BMR and TEM respectively. The postprandial energy expenditure was still significantly ($p<0.05$) higher 5 hours after the meal, as compared to basal values. This could well be due to the circadian rhythm observed in oxygen consumption in the basal state[25,26]. Weststrate[5], however, was not able to demonstrate any difference in the resting metabolic rate (RMR) or TEM measured in the morning and afternoon, which would suggest that the TEM response may have been incompletely measured in

this study. Earlier studies with male subjects in our laboratory, however, had demonstrated that the TEM response was complete six hours after a 2.5 MJ (600 kcal) test meal having the same composition as that used in the current study[14]. Another possible explanation for the significantly higher energy expenditure observed 5 hours following the meal could be due to restlessness brought on by six hours of confinement to bed[27].

It is more than likely that the observed increase in the TEM is a result of several inter-related metabolic processes stimulated by the ingestion of food. Insulin is a pre-eminent hormone that regulates energy balance. It is required for the uptake and storage of glucose in the cell. Insulin can increase energy expenditure in several ways. It has a direct effect on Na^+-K^+ pumping across the cell membrane[28], and increased Na^+ pump activity in skeletal muscle has been demonstrated following insulin administration[29,30]. Insulin also stimulates the sympathetic nervous system (SNS), enhancing the sympathetic tone[31,32]. Thus, it is thought to mediate thermogenesis by two mechanisms, an obligatory component increasing glucose uptake, and a facultative component enhancing catecholamine activity[33]. Elevation of catecholamines in the luteal phase of the menstrual cycle is also thought to be related to higher estradiol levels, due to incomplete inactivation, by reduction in tissue uptake[34], or competitive inhibition of catechol-*O*-methyl transferase[35]. It has recently been shown that plasma estradiol levels are positively correlated with carbohydrate oxidation, and inversely correlated with fat oxidation[36]. This phenomenon was explained by an increase in tissue sensitivity to insulin mediated by estradiol[37]. Therefore, the significant post-ovulatory increase in plasma estradiol ($p < 0.05$) could well be responsible for an increased tissue sensitivity to insulin. This would result in an increase in glucose entry into the cell and also an enhancement of the sympathetic tone, which in turn could possibly explain the higher TEM in the post-ovulatory phase of the menstrual cycle. The substrate oxidation rates also reveal a trend towards an increase in postprandial carbohydrate oxidation and decrease in fat oxidation, associated with higher non-protein respiratory quotients (NPRQ) in the post-ovulatory (luteal) phase of the menstrual cycle, thus lending support to this argument. The enhanced tissue sensitivity to insulin, mediated by estradiol, would have a very small

effect, if any, during the BMR; as plasma insulin and glucose levels are low in the fasting state. Adrenergic activation is also thought to stimulate ovarian progesterone production[38]. The elevated TEM observed in the luteal phase of the menstrual cycle may, therefore, be a consequence of this activation of the SNS, which is thought to account for between 30-70 percent of the TEM response[39,40]. The thermogenic effect of progesterone is also well documented[41] when administered either to normal or ovariectomized women[42,43]. The mechanism of this effect, however, is still unclear. Earlier studies on energy expenditure and the menstrual cycle have suggested that the higher progesterone levels in the post-ovulatory phase of the menstrual cycle are responsible for the 8-16 percent increase in TEE[2]. Another interesting observation is the significantly lower basal protein, and apparently higher carbohydrate oxidation rates accounting for 13 and 44 percent of the energy expenditure respectively, during the post-ovulatory phase of the cycle, as compared to 17 and 36 percent of the energy expenditure due to protein and carbohydrate oxidation respectively during the pre-ovulatory phase. Postprandial substrate oxidation rates, however, appear to be comparable between the two phases.

There was no change in the dietary energy or macronutrient intake over the course of the menstrual cycle, similar to results of Fong and Kretsch[44] but in contrast to the findings of Dalvit[22]. If dietary intakes are thought to be representative of TEE then physical activity levels (PAL) can be estimated from the energy intakes and BMR. Estimated PALs were not significantly different between the two phases of the menstrual cycle (Pre-ovulatory: 1.56 ± 0.26 vs Post-ovulatory: 1.57 ±0.32). These suggest that subjects in our study were sedentary and physical activity was unlikely to have had a confounding effect on the observations.

The between-cycle differences in the BMR appears to be small, but seems to cause a large differences in the TEM (10.2%), these differences in BMR and TEM appear to be reciprocal as there appears to be no difference in the PMTEO observed in the pre-ovulatory phase of the two consecutive menstrual cycles. This is because the TEM is calculated as an increment in energy expenditure over the BMR measured prior to the administration of the test meal.

In conclusion, the changes in energy expenditure in relation to the BMR and TEM over the course of a single menstrual cycle are small, in contrast to other reports. In nutritional terms the significant rise in post-ovulatory TEM is also small and is unlikely to alter energy requirements in well nourished women from the reproductive age group. This elevation of the TEM during the post-ovulatory (luteal) phase of the menstrual cycle may be due to several inter-related factors, such as estradiol enhancing the sensitivity of tissues to insulin, and/or its effect on circulating levels of catecholamines with their inherent thermogenic effect, which in addition are known to enhance progesterone production, another thermogenic hormone. It is likely that all these factors contribute, albeit to differing degrees, in effecting a rise in the post-ovulatory TEM. There were no significant differences in anthropometry or energy intake observed between the two phases of the menstrual cycle. Changes in the BMR between cycles though being small can affect the estimation of the TEM. However, the PMTEO is very reproducible and would suggest that energy expenditure in relation to the ingestion of a meal is fairly constant between consecutive menstrual cycles.

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Chapter 4

CHANGES IN ENERGY EXPENDITURE, ANTHROPOMETRY AND ENERGY INTAKE DURING THE COURSE OF A NORMAL PREGNANCY IN WELL NOURISHED INDIAN WOMEN: AN ASSESSMENT OF THE ENERGY COST OF PREGNANCY

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ABSTRACT

The basal metabolic rate (BMR), thermic effect of a meal (TEM), anthropometry, and dietary intakes were measured in 18 non-pregnant, non-lactating controls, and in 18 pregnant women at 12, 24 weeks and 34 weeks of gestation, in order to uncover any enhancement of metabolic efficiency during pregnancy that may result in a saving of energy. The BMR of the pregnant subjects at 12 weeks of gestation was significantly ($p < 0.05$) higher, as compared to the controls. The BMR, in absolute terms, rose with the progression of pregnancy. However, when expressed per kg body weight there were no significant differences between trimesters. There was no significant difference in the TEM between the controls and pregnant subjects at 12 weeks of gestation, nor were there any differences with the progression of pregnancy. These results suggest that there is no energy saving associated with the BMR or TEM during pregnancy. The mean weight gain in the pregnant subjects from 12 weeks to term was 11.4 ± 3.7 kg, mean birth weight of the infants was 3.06 ± 0.41, mean placental weight was 0.63 ± 0.15. The mean estimated gain in adipose tissue and fat mass were 3.1 ± 3.6 and 2.2 ± 2.9 kg, respectively. The mean energy cost of pregnancy, estimated from the sum of: energy content of fat stores (100 MJ); an estimate of fat and protein deposited in the infant (45 MJ); and the cumulative increase in basal metabolism (143 MJ), was 288 ± 171 MJ, close to the energy intake recommended by the FAO/WHO/UNU. The energy intake of pregnant subjects at 12 weeks of gestation was not significantly different from the non-pregnant, non-lactating, controls. The cumulative increase in energy intake over that measured at 12 weeks of gestation, in the pregnant subjects was 290 ± 280 MJ; apparently meeting the entire cost of pregnancy. However, there was no significant

correlation between the energy cost of pregnancy and the cumulative increase in energy intake.

INTRODUCTION

The Food and Agriculture Organization/ World Health Organization/ United Nations University[1] recommendations for energy intake during pregnancy in women who maintain their pre-pregnant level of activity is an extra 1.2 MJ/d, which amounts to 335 MJ over the 40 weeks of gestation. This figure is reduced to 235 MJ (0.84 MJ/d) for those who reduce their physical activity below pre-pregnant levels. These estimates are based on theoretical costs of protein and fat accumulation in the mother and the foetus, together with the increment in energy expenditure for its maintenance. There is evidence available now that maternal energy intake is not substantially altered to meet this estimated increase in requirements[2-5]. There is also evidence of energy saving during physical activity in pregnancy[6-8], which is one of the major components of energy expenditure over a day. This saving in energy expenditure, however, cannot account for the discrepancy between the measured increment in energy intake and theoretical estimate of the energy cost of pregnancy. It is possible that the enhancement of efficiency in other physiological processes associated with energy expenditure may result in an energy saving during pregnancy. Basal metabolism and diet induced thermogenesis account for approximately 80 percent of the 24 hour energy expenditure[9], in sedentary individuals and any energy saving associated with these processes may, possibly, help explain how women meet the energy cost of pregnancy, without a substantial increase in their energy intake.

There is evidence that the basal metabolic rate (BMR) is reduced during early and mid-pregnancy[4,10-14], enabling the mother to save energy that may be channelled towards meeting the cost of pregnancy. However, reports on Asian women[15], and well nourished Western women[3,6,16-18] have not been able to demonstrate these changes. The information available on the thermic effect of a meal (TEM), from longitudinal studies[18,19], and cross-sectional studies[6,20] is also contradictory, with some authors subscribing to an energy

saving associated with the TEM[19,20]; while others finding no saving associated with this process[6,20]. Studies on African women have demonstrated a very low energy cost of pregnancy, and an even lower increment in energy intake during this period[10,14]. Whereas, studies on well nourished European women from the Netherlands[4], Scotland[3] and England[17] show that the energy cost of pregnancy is substantial and very near the energy intake recommended by the joint FAO/WHO/UNU expert consultation[1]. However, the measured increments in energy intake in the same women were very much lower than the measured expenditure[3-5,17]. The available data suggest that the energy requirements in pregnancy are varied and depend on the population being examined[21].

It is thus becoming increasingly important to determine if the discrepancy observed between the energy costs of pregnancy and the increment in energy intake exist in all population groups, as it could have serious implications when arriving at recommendations for energy requirements during pregnancy. This study has attempted to uncover changes in maternal energy metabolism associated with the BMR and the TEM by comparison with a non-pregnant, non-lactating control group. The energy cost of pregnancy in Indian women has been estimated from an analysis of the weight gained during pregnancy, and serial measurements of the BMR during this period.

SUBJECTS AND METHODS

Study design (Table 1)

Two groups of twenty-two women each, were recruited for the study. The first group consisted of non-pregnant, non-lactating subjects (Control Group), in the reproductive age group, while the second consisted of women who were less than 11 weeks pregnant (Pregnant Group). The basal metabolic rate (BMR) and thermic effect of a meal (TEM) were measured between the 6th and 10th day, during the pre-ovulatory phase, of the menstrual cycle of the Control Group; and during the 12th, 24th and 34th week of gestation in the Pregnant Group. Urine samples were collected following the BMR and TEM for estimation of total urinary nitrogen (TUN) excretion, in order to calculate non-

protein respiratory quotients and substrate oxidation rates during these measurements. Fasting (basal) blood samples were obtained from 6 subjects of each group on the day that the metabolic measurements were carried out, for the estimation of haemoglobin, glucose, insulin, tri-iodothyronine (T_3), free T_3 (FT_3), thyroxine (T_4), estradiol and progesterone. Anthropometric measurements (height, weight, mid-arm circumference and skinfold thicknesses) were made on the same day, following the metabolic measurements. In the Pregnant Group body weight was also measured at term; and again at 4 weeks postpartum together with mid-arm circumference and skinfold thicknesses. A written record of the measured volume of all food and drinks consumed was obtained for 5 days prior to these metabolic and anthropometric measurements. These data were used to calculate energy and macro-nutrient intakes.

Table 1. Study Design

	Control Group	**Pregnant Group**				
		Gestation			Term	Postpartum
Weeks		**12**	**24**	**34**		**4**
Height	x	x	-	-	-	-
Weight	x	x	x	x	x	x
Mid-arm circumference	x	x	x	x	-	x
Skinfold thicknesses	x	x	x	x	-	x
Basal metabolic rate	x	x	x	x	-	-
Thermic effect of a meal	x	x	x	x	-	-
Substrate oxidation rate	x	x	x	x	-	-
Fasting blood sample[a]	x	x	x	x	-	-
Urine samples	x	x	x	x	-	-
Dietary intake[b]	x	x	x	x	-	-
Birth weight of baby					x	-
Weight of placenta					x	-

[a]In a sub-group of 6 subjects from each group; [b]recorded for 5 days prior to metabolic measurements

Subjects

Subjects were either from the middle or upper socio-economic class as assessed by Kuppuswamy's[22] urban socio-economic assessment scale, were non-vegetarians and had *ad libitum* access to food. The Control Group subjects were recruited from amongst the staff and students of St. John's Medical College and Hospital in Bangalore. They were non-smokers, in good health, weight stable, and not on any kind of chronic medication or oral contraceptives. Pregnant Group subjects were recruited for participation either through the Obstetric departments of St. John's Medical College Hospital, St. Martha's Hospital or by personal approach. Pregnant subjects were in good health and had no impairment of appetite due to 'morning sickness' prior to the initial metabolic measurement at 12 weeks of gestation. Subjects from both groups were well educated and were interested in nutritional issues. They were familiarized with the ventilated hood system prior to the actual metabolic measurements.

Methods

Energy expenditure was measured by respiratory gas exchange measurements using a 'ventilated hood'. The ventilated hood measurement system consisted of a plastic hood which surrounded the subject's head and a soft plastic collar surrounding the neck and shoulders. A fixed flow (50 l/min) of room air was maintained through the hood by connecting the outlet of the hood through a calibrated rotameter (Fischer Controls, UK) to a suction pump. A small sample of air (1 l/min) was drawn off from the outlet of the rotameter for minute to minute estimation of oxygen (O_2) and carbon-dioxide (CO_2) concentrations using a paramagnetic oxygen analyzer (Servomex 540 A, Taylor Instruments, UK) and an infra-red carbon-dioxide analyzer (Type SSI, Analytical Development Co., U.K.) respectively. O_2 and CO_2 concentrations of the room (reference) air were monitored every hour for 10 minutes, prior to the start of a metabolic measurement. Moisture content and temperature were measured by means of a dew-point hygrometer of the optical condensing type (General Eastern Systems, 1100 DP, USA) and a mercury thermometer placed in the air stream emerging from the rotameter, respectively. Atmospheric pressure was measured by means of a mercury barometer at the start of each measurement protocol. Oxygen consumption (VO_2) and carbon-dioxide production (VCO_2) rates (calculated to STPD conditions; standardized for temperature,

pressure and dryness), Respiratory Quotient (RQ = VCO_2/VO_2), and energy expenditure[23] were calculated from this data and recorded every minute using a computerized data acquisition and storage system[24]. The gas analyzers were calibrated before the start of each measurement using pure nitrogen (100%) (Indian Oxygen Ltd., Bangalore) for 'zero', and a mixture of carbon-dioxide (1%), oxygen (21%) and nitrogen (78%) (Bhoruka Gases Ltd., Bangalore), for the 'span' setting. These calibration gases were passed through the analyzers at the end of the measurement protocol, 6 hours after the initial calibration, to check for instrument drift. All data was subsequently corrected for instrument drift during this period. This measurement system had been validated against other methods of measurement of oxygen consumption[25]. Calibration using the N_2 infusion technique[26] yielded a net discrepancy of less than 0.4 percent in the estimation of oxygen consumption. Ethanol burns conducted at regular intervals over the duration of the study produced energy expenditures within ± 2 percent of expected values. Room temperatures were maintained between 24 and 29 °C on all days of the study , while barometric pressures ranged from 681 to 692 mm Hg.

The measurement protocol employed was as follows (Figure 1). Subjects were instructed to complete their evening meal by 19:30 hours, and to be in bed by 22:00 hours on the night prior to the metabolic measurements. On the morning of the metabolic measurement they were instructed to wake between 6:00 and 6:30 hours, complete their toilet and empty their bladder while noting the exact time. They were then transported to the laboratory by the department vehicle. On entering the laboratory all subjects were instructed to rest in bed for 30 min. prior to the start of the BMR measurement. At the end of this mandatory rest period the basal metabolic rate was measured for 30 min, after an initial 10 min period to allow O_2 consumption to stabilize while within the ventilated hood. Basal metabolic rate was calculated by obtaining the mean energy expenditure over this 30 min period.

Following the measurement of the BMR the hood was removed. A fasting blood sample (10 ml) was collected from the antecubital vein using aseptic precautions for the estimation of haemoglobin, glucose using the orthotoluidine method, insulin, triiodothyronine (T_3), thyroxine (T_4), thyroid stimulating hormone (TSH) using locally

available RIA kits (Bhabha Atomic Research Centre, Bombay, India), while free T_3 (FT_3), estradiol and progesterone were measured using DPC RIA kits (Diagnostic Products Corporation, Los Angeles, USA) in 6 subjects from each group.

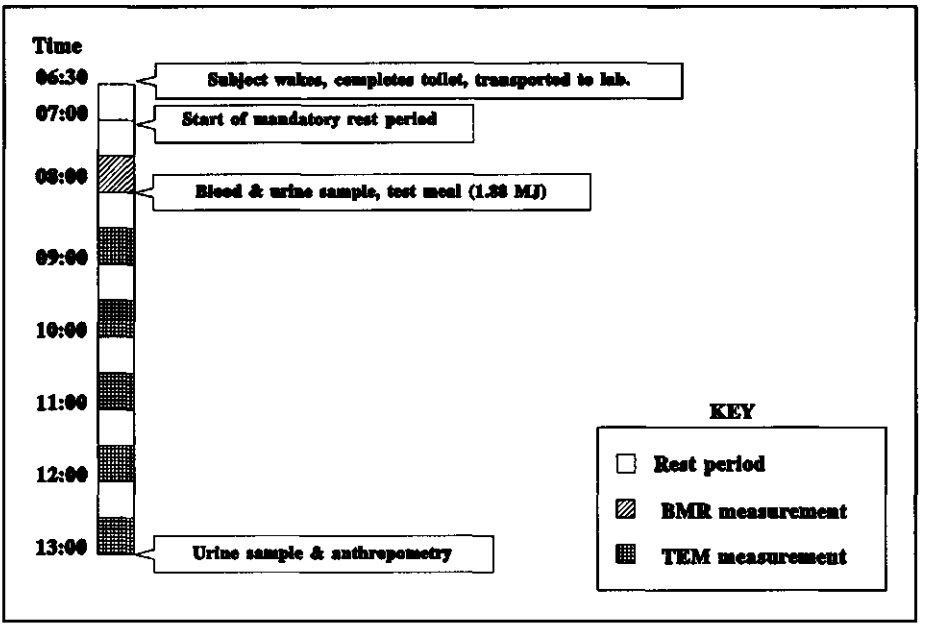

Figure 1. Measurement protocol

The subjects were then instructed to void. The voided urine volume was noted and then acidified with concentrated hydrochloric acid (1 ml acid per 100 ml urine). An aliquot was stored for estimation of total urinary nitrogen (TUN) excretion, over the preprandial period, by the micro-kjeldhal method[27]. (All urine voided during the postprandial period together with the final urine sample obtained at the end of the metabolic measurements, was collected in the same manner, for estimation of TUN excretion, over the postprandial period).

Following the collection of the basal urine sample the subjects were served a standard test meal which consisted of tinned milk powder, rice cereal and sugar, together with a

glass of lemon juice. The cereal was mixed with the powdered milk and part of the sugar and made up to 200 ml with water. The remaining sugar was mixed with 200 ml of water to which lemon juice was added for flavour. Together this provided 1.88 MJ (450 kcal) of energy, with 10 percent of the energy being derived from protein (11.2 g/100 g), 15 percent from fat (7.6 g/100 g) and 75 percent from carbohydrate (84.2 g/100 g). Energy and nutrient composition was derived from the manufacturers' product information. All tinned products were used well before their date of expiry, and produced by the same manufacturer.

After the ingestion of the meal, the ventilated hood was replaced and the O_2 consumption and CO_2 production were measured intermittently for the next 5 hours. The second thirty minutes in each hour were considered to be a 'measurement period' and representative of the energy expenditure for the entire hour; the initial 30 min being designated as a 'rest period'. The subject was instructed to lie awake and motionless in the recumbent position during the 'measurement periods'. Between the 'measurement periods', i.e. during the 'rest periods', they were also asked to lie quietly in bed though some movement and reading was permitted. Ten minutes prior to the start of each 'measurement period' they were instructed to rest motionless to allow their O_2 consumption to stabilize, while within the ventilated hood, in preparation for the next 'measurement period'.

The thermic effect of the standard test meal (TEM) was calculated by obtaining the mean increment in energy expenditure during the 'measurement period' (30 min) in each hour (which was considered representative for the entire hour) above pre-meal basal values. The cumulative increment in energy expenditure for the 5 hours following the ingestion of the test meal was expressed as a percentage of the energy content of the meal. The post-meal total energy output (PMTEO) was calculated by obtaining the total energy expenditure during the 5 hours after the ingestion of the test meal[28]. Substrate oxidation rates were calculated during the pre- and postprandial period in each subject, using total oxygen consumption (VO_2), carbon-dioxide (VCO_2) production rates and total urinary nitrogen (TUN) excretion during the pre and postprandial periods[29]. No correction was made to the protein oxidation rate to take into account any change in the

blood urea pool between the fasted and fed states. Non-Protein Respiratory Quotients (NPRQ) were calculated after subtracting the oxygen consumed (PVO_2) and carbon dioxide produced ($PVCO_2$) in protein oxidation, using the formulas of Jequier et. al.[30], from the measured VO_2 and VCO_2 of the subject $[(VCO_2\text{-}PVCO_2)/(VO_2\text{-}PVO_2)]$.

On completion of all metabolic measurements for the day, anthropometric measurements were made for assessment of nutritional status. These included height, weight, skinfold thicknesses (biceps, triceps, subscapular, supra-iliac) and mid-arm circumference (MAC). Height was measured using a moveable anthropometer (Nivotoise, France) and recorded to the nearest 0.1 cm. Body weight was measured on a digital weighing scale (Soehnle, Germany) and recorded to the nearest 100 g. Subjects stood upright with the head in the horizontal plane, in light indoor clothing. For the measurement of the skinfold thicknesses (biceps, triceps, subscapular, supra-iliac) a Holtain skinfold calliper (Crymmych, U.K.) was used. All the skinfolds were measured on the right side of the body and recorded to the nearest 0.2 mm. Every skinfold was measured in triplicate and the mean of these 3 values was used for calculation. Fat mass (FM) was calculated after estimating percent body fat from the sum of four skinfolds and applying the formula of Durnin & Womersley[31]. Fat free mass (FFM) was estimated from the difference of weight and FM.

A week prior to the metabolic measurements the subjects were visited at home and provided with two separate household measures of known volume, together with a tape measure, to measure the volume of all solid and liquid food consumed. In addition a booklet was provided on each occasion to record the time of the meal, a general description of the food/drink consumed, details of any unconventional food item eaten, the exact volume consumed as measured in the household measures provided and dimensions of solid food that could not be measured in the household measures provided. All subjects were instructed by a trained dietitian as to the proper use of the household measures and the type of detail required in the description of the food items or beverages being consumed. The method of recording this information in the booklet provided was demonstrated in a simple example on the initial page of each booklet. No special advice as to the type or quantity of food to be consumed was given, except to

encourage all subjects to eat to the dictates of their appetite and not change their eating pattern during this period. This record was maintained for 5 consecutive days prior to the metabolic measurement. On the day of the metabolic and anthropometric measurements the dietitian checked the dietary record maintained by the subject and clarified any ambiguities, following the completion of the metabolic and anthropometric measurements. The volume of food consumed was converted to weight by weighing a similar food item, prepared in the laboratory using a standardized recipe, in the standard household measure used by the subject. Fruits, sweets, snacks etc were purchased based on the dimensions provided by the subjects and weighed in the laboratory. These data were then used for estimation of energy and macro-nutrient content of the diet by the use of standard food composition tables[32-34].

Statistical analysis

Data are presented as Mean ± SD. A two-tailed unpaired t-test was used for comparison between the Control Group and Pregnant Group at 12 weeks gestation. An analysis of variance for repeated measurements designs (ANOVA-RMD), was used to analyze longitudinal data in the Pregnant Group. A paired t-test, with the Bonferroni correction, was used to identify where the differences, detected by the ANOVA-RMD, existed. Correlations coefficients were used to establish relationships between variables. Statistical significance accepted at the 5 percent level. All data was analyzed using SPSS/PC+ (Version 4.0, SPSS Inc. 444 N.Michigan Avenue, Chicago, Illinois, USA) statistical package on an IBM-PC 386 compatible computer.

Ethical approval

Ethical approval was obtained for the study from the Human Investigation Committee of the Medical School. Informed consent in writing was obtained from all subjects prior to the start of the measurements.

RESULTS

Of the 22 pregnant subjects recruited only 18 completed all planned measurements. Hence, what follows is data on 18 non-pregnant, non-lactating subjects and 18 pregnant subjects at 12, 24 and 34 weeks of pregnancy. The anthropometric characteristics of both groups studied are given in Table 2. There were no significant differences in age, parity, height, weight, body mass index (kg/m^2; BMI), fat free mass (FFM) and mid-arm circumference (MAC) between the Control Group and Pregnant Group at 12 weeks of gestation. The sum of 4 skinfold thicknesses, and percent body fat were significantly higher ($p<0.05$) in the Pregnant Group at 12 weeks of gestation, as compared to the Control Group, however, the difference in fat mass (FM) was of borderline significance ($p=0.06$). The supra-iliac skinfold could not be measured in many subjects at 24 and 34 weeks of gestation, therefore this data has been omitted. There was a significant increase ($p<0.05$) on an ANOVA-RMD, in body weight with the progression of pregnancy in the Pregnant Group. The body weight at 24 weeks of gestation was 59.6 ± 8.4 kg, 64.2 ± 8.7 kg at 34 weeks gestation and 66.2 ± 8.7 kg at term. There was also a significant ($p<0.05$) increase in the BMI of the Pregnant Group at 24 weeks (23.5 ± 2.9 kg/m^2), and 34 weeks (25.3 ± 3.0 kg/m^2) of gestation; however, there were no significant changes in the MAC with the progression of pregnancy (27.2 ± 2.5; and 27.4 ± 2.4 cm, at 24 and 34 weeks of gestation, respectively). The average length of gestation calculated from the first day of the last menstrual cycle was 38.7 ± 1.1 weeks (271 ± 7 days). Of the 18 births 14 were normal vaginal deliveries, while 4 were delivered by caesarian section. 9 male and 9 female babies were born.

The basal metabolic rate (BMR) in the Pregnant Group at 12 weeks of gestation was significantly higher ($p<0.05$) in absolute terms (Figure 2), and when expressed per kg FFM, as compared to the Control Group. There was a significant increase ($p<0.05$) in the absolute BMR within the Pregnant Group with the progression of pregnancy, however, when expressed per unit body weight there were no significant differences between the three trimesters (Table 3). 15 of the 18 Pregnant Group subjects received test meals of 1.88 MJ on all occasions, while 3 subjects could not finish the entire test

meal on the first occasion and, therefore, these subjects received the amount ingested on the first occasion, at subsequent measurements. There were no significant differences in the thermic effect of the test meal (TEM), expressed as a percentage of the energy content of the test meal, between the Control Group and the Pregnant Group at 12 weeks gestation, nor was there any significant difference in the TEM between the different time points in pregnancy within the Pregnant Group.

Table 2. Subject characteristics and changes in anthropometry[a]

Groups	**Control Group** (n=18)		**Pregnant Group** (n=18)	
Time of measurement	**Pre-ovulatory**		**12 weeks gestation**	
Age (years)	26.2 ±	6.0	29.6 ±	5.2
Parity	0.3 ±	0.7	0.6 ±	0.6
Height (m)	1.61 ±	0.06	1.59 ±	0.07
Weight (kg)	53.2 ±	8.3	54.8 ±	8.3
BMI (kg/m^2)	20.4 ±	2.4	21.7 ±	3.0
Mid-arm circumference (cm)	25.3 ±	2.4	26.6 ±	2.6
Sum of 4 skinfolds (mm)	56.9 ±	13.8	75.2 ±	26.2[b]
Fat[c] (%)	28.5 ±	3.6	32.4 ±	5.7[b]
Fat mass (kg)	15.2 ±	3.4	18.1 ±	5.1
Fat free mass (kg)	37.9 ±	5.0	36.8 ±	4.2

[a]Mean ± SD; [b]Control vs Pregnant Group, significantly ($p<0.05$) different on an unpaired 't-test'

[c]Calculated from the sum of 4 skinfold thicknesses and applying the formulas of Durnin & Womersley (1974).

The absolute post meal total energy output (PMTEO) was significantly ($p<0.05$) higher in the Pregnant Group at 12 weeks of gestation, as compared to the Control Group. This difference persisted when the PMTEO was expressed per kg FFM, however, it was not observed when expressed per kg body weight. There was a progressive increase in the absolute PMTEO with the progression of pregnancy, however, when expressed per kg body weight there was no significant difference between trimesters within the Pregnant Group (Table 3).

Table 3. Basal metabolic rate, thermic effect of a meal, and post meal total energy output in the Control and Pregnant Groups[a]

Groups	Control Group (n=18)		Pregnant Group (n=18)	
Time of measurement	**Pre-ovulatory**	**12 weeks gestation**	**24 weeks gestation**	**34 weeks gestation**
Basal Metabolic Rate				
(kJ/min)	3.21± 0.22	3.56± 0.38[b]	3.91± 0.47[c]	4.29± 0.55[dc]
(MJ/d)	4.62± 0.32	5.13± 0.55[b]	5.63± 0.68[c]	6.17± 0.79[de]
(kJ/kg wt/d)	88.3 ± 11.4	94.4 ± 7.5	95.0 ± 7.7	96.5 ± 6.2
(kJ/kg FFM/d)	123.5 ± 15.7	139.9 ± 6.8[b]		
Thermic Effect of the Meal				
(% of energy in the meal)	7.0 ± 1.7	7.2 ± 2.1	7.2 ± 2.4	7.3 ± 2.2
Post Meal Total Energy Output				
(kJ/min over 5h)	3.65± 0.24	4.00± 0.39[b]	4.33± 0.47[c]	4.72± 0.59[de]
(kJ/kg wt/5h)	20.9 ± 2.6	22.1 ± 1.8	21.9 ± 1.3	22.2 ± 1.4
(kJ/kg FFM/5h)	29.3 ± 3.6	32.7 ± 2.0[b]		

[a]Mean ± SD; [b]Control vs Pregnant Group at 12 weeks of gestation, significantly ($p<0.05$) different on an unpaired 't-test';
[c]12 vs 24 weeks, [d]12 vs 34 weeks, [e]24 vs 34 weeks gestation, significantly different on ANOVA-RMD ($p<0.05$) and a paired t-test using the Bonferroni correction

Table 4. Substrate oxidation rates and non-protein respiratory quotient (NPRQ) in the Control and Pregnant Groups[a]

Groups	Control Group (n=18)		Pregnant Group (n=18)	
Time of measurement	**Pre-ovulatory**	**12 weeks gestation**	**24 weeks gestation**	**34 weeks gestation**
NPRQ - Basal	0.84 ± 0.08	0.84 ± 0.04	0.88 ± 0.04[c]	0.85 ± 0.05
NPRQ - Postprandial	0.95 ± 0.04	0.95 ± 0.04	0.94 ± 0.04	0.92 ± 0.04[d]
Protein oxidation rates (mg/min)				
Basal	39.0 ± 22.1	33.5 ± 17.8	29.4 ± 10.4	26.3 ± 9.8
(% of total energy)	(21.2)	(16.2)	(12.7)	(10.5)
Postprandial	37.6 ± 9.8	33.0 ± 14.2	32.3 ± 5.4	32.0 ± 6.7
(% of total energy)	(17.2)	(13.7)	(12.4)	(11.3)
Fat oxidation rates (mg/min)				
Basal	35.3 ± 19.2	38.1 ± 10.3	33.9 ± 13.8	45.9 ± 15.6[e]
(% of total energy)	(43.4)	(41.5)	(33.0)	(41.1)
Postprandial	12.1 ± 10.6	15.3 ± 10.6	18.4 ± 14.9[d]	29.3 ±16.6[e]
(% of total energy)	(12.4)	(14.3)	(15.8)	(23.3)
Carbohydrate oxidation rates (mg/min)				
Basal	65.1 ± 33.9	87.6 ± 28.8[b]	125.4 ± 29.0[c]	121.9 ± 34.9[d]
(% of total energy)	(35.6)	(42.4)	(54.3)	(48.5)
Postprandial	154.1 ± 22.9	173.2 ± 25.7[b]	187.6 ± 26.6	185.3 ± 29.4
(% of total energy)	(70.4)	(72.0)	(71.8)	(65.4)

[a]Mean ± SD; [b]Control vs Pregnant Group at 12 weeks of gestation, significantly ($p<0.05$) different on an unpaired 't-test';
[c]12 vs 24 weeks, [d]12 vs 34 weeks and [e]24 vs 34 weeks gestation, significantly different on an ANOVA-RMD ($p<0.05$) and a paired t-test using the Bonferroni correction

Basal and postprandial non-protein respiratory quotients (NPRQ) were comparable in the Control and Pregnant Group at 12 weeks of gestation (Table 4). However, the postprandial NPRQ within the Pregnant Group fell significantly ($p<0.05$), with the progression of pregnancy, while basal NPRQs rose significantly from 12 weeks to 24 weeks of gestation and then appeared to fall again, though this was not significant. Basal and postprandial carbohydrate oxidation rates at 12 weeks of gestation in the Pregnant Group were significantly ($p<0.05$) higher than the Control Group. There was a significant ($p<0.05$) increase in basal carbohydrate, and in basal and postprandial fat oxidation rates, with the progression of pregnancy. There was also an apparent reduction in the basal protein oxidation rate with the progression of pregnancy, however, this did not reach statistical significance; postprandial protein oxidation rates appeared to remain constant through out pregnancy.

Plasma hormone and substrate levels in a sub-group of 6 Control Group and 6 Pregnant Group subjects are given in Table 5. There were no significant differences in the haemoglobin, fasting glucose and insulin between the Control and Pregnant Group at 12 weeks gestation, or between trimesters within the Pregnant Group. Total T_3 and T_4 levels in the Pregnant Group were significantly higher than the Control Group at 12 weeks of gestation, however, there were no significant differences between trimesters within the Pregnant Group. Free T_3 levels were -16.5, +59.1 and +46.5 percent at 12, 24 and 34 weeks of gestation respectively, of the Control Group values. These differences, however, did not reach statistical significance. Plasma estradiol and progesterone levels were significantly higher in the Pregnant Group at 12 weeks of gestation, as compared to the Control Group, and rose significantly ($p<0.05$) with the progression of pregnancy.

An analysis of the weight gain during pregnancy is given in Table 6. The weight gain from 12 weeks to term was 11.4 ± 3.7, which was 21.3 ± 7.8 percent of the body weight at 12 weeks gestation. There was a significant ($p<0.05$) inverse correlation ($r=-0.52$) between the percent weight gain and weight measured at 12 weeks of gestation.

Table 5. Plasma haemoglobin (Hb), glucose, insulin, tri-iodothyronine (T_3), free tri-iodothyronine (FT_3), thyroxine (T_4), thyroid stimulating hormone (TSH), estradiol, progesterone in the Control and Pregnant Groups[a]

Groups	Control Group (n=6)		Pregnant Group (n=6)	
Time of measurement	Pre-ovulatory	12 weeks gestation	24 weeks gestation	34 weeks gestation
Hb (g/dl)	12.5 ± 1.3	13.7 ± 1.4	11.9 ± 1.9	11.4 ± 1.1
Glucose (mg/dl)	65.0 ± 6.5	63.8 ± 5.4	58.3 ± 9.0	60.4 ± 10.1
Insulin (μU/ml)	12.9 ± 5.24	12.6 ± 5.1	13.2 ± 5.06	16.5 ± 11.44
T_3 (ng/ml)	1.5 ± 0.26	2.1 ± 0.34[b]	2.3 ± 0.19	2.1 ± 0.41
FT_3 (pg/ml)	1.3 ± 1.06	1.1 ± 0.57	2.0 ± 1.09	1.9 ± 0.86
T_4 (μg/dl)	9.2 ± 1.45	12.5 ± 0.53[b]	13.3 ± 2.13	10.8 ± 2.25
TSH (μIU/ml)	1.0 ± 0.75	0.65 ± 0.54	1.0 ± 0.55	1.2 ± 0.61
Progesterone (ng/ml)	0.2 ± 0.16	29.5 ± 9.90[b]	62.2 ± 36.4[c]	169.5 ± 88.8[de]
Estradiol (ng/ml)	0.1 ± 0.03	1.1 ± 0.42[b]	6.6 ± 3.34[c]	11.5 ± 4.32[de]

[a]Mean ± SD; [b]Control vs Pregnant Group at 12 weeks of gestation, significantly ($p<0.05$) different on an unpaired 't-test';
[c]12 vs 24 weeks, [d]12 vs 34 weeks, [e]24 vs 34 weeks gestation, significantly different on ANOVA-RMD ($p<0.05$) and a paired t-test using the Bonferroni correction

Table 6. Analysis of weight gain in pregnancy[a]

Weight gain (kg) (12-40 weeks)	11.4 ± 3.7
Weight gain (% of weight at 12 weeks)	21.3 ± 7.8
Weight of fetus (kg)	3.06 ± 0.41
Weight of placenta (kg)	0.63 ± 0.15
Weight of uterus etc.[b](kg)	4.6 ± 0.6
Weight of adipose tissue gained (kg)	3.1 ± 3.6
Gain in fat mass[c](kg) (12-40 weeks)	2.2 ± 2.9

[a]Mean ± SD

[b]based on the assumption that an infant with a 3.4 kg birth weight would cause an increase in maternal organs (uterus, amniotic fluid, mammary glands, blood, and extravascular fluid) of 5.1 kg (Hytten, 1991)

[c]Fat content of adipose tissue assumed to be 80 percent (Keys & Brozek, 1953), and that 0.4 kg of weight gained is accounted for by an increase in breast mass (Hytten, 1991).

The mean estimated gain in adipose tissue from 12 weeks to term, using the factorial approach (Table 6), was 3.1 ± 3.60. Assuming an increase of 0.4 kg in breast tissue[35] and that 80 percent of adipose tissue is fat mass[36] then fat mass gained from 12-40 weeks of gestation was 2.2 ± 2.9 kg.

In a subgroup of 14 subjects body weight and skinfold measurements were also made at 4 weeks postpartum. The body weight, sum of 4 skinfold thicknesses, percent body fat was 51.9 ± 6.3, 71.4 ± 28.0 mm and 31.5 ± 6.1 percent respectively, at 12 weeks gestation; as compared to, 55.5 ± 6.9 kg, 60.3 ± 22.1 mm and 29.3 ± 5.6 percent respectively at 4 weeks postpartum. The gain in fat mass estimated from measures of skinfold thickness obtained at 12 weeks of gestation and 4 weeks postpartum, in these 14 subjects, was not significant, and only of the order of 0.1 ± 2.8 kg. There was, however, a significant increase in body weight of 3.6 ± 3.06 kg ($p<0.01$), which would imply a gain of 2.6 ± 2.4 kg fat mass. The gain in FM calculated by the factorial

approach, used in Table 6, in these 14 subjects was 2.4 ± 2.4 kg (adipose tissue 3.4 ± 3.0 kg), a value very similar to that arrived at from the analysis of the difference in weight measured at 12 weeks gestation and 4 weeks postpartum.

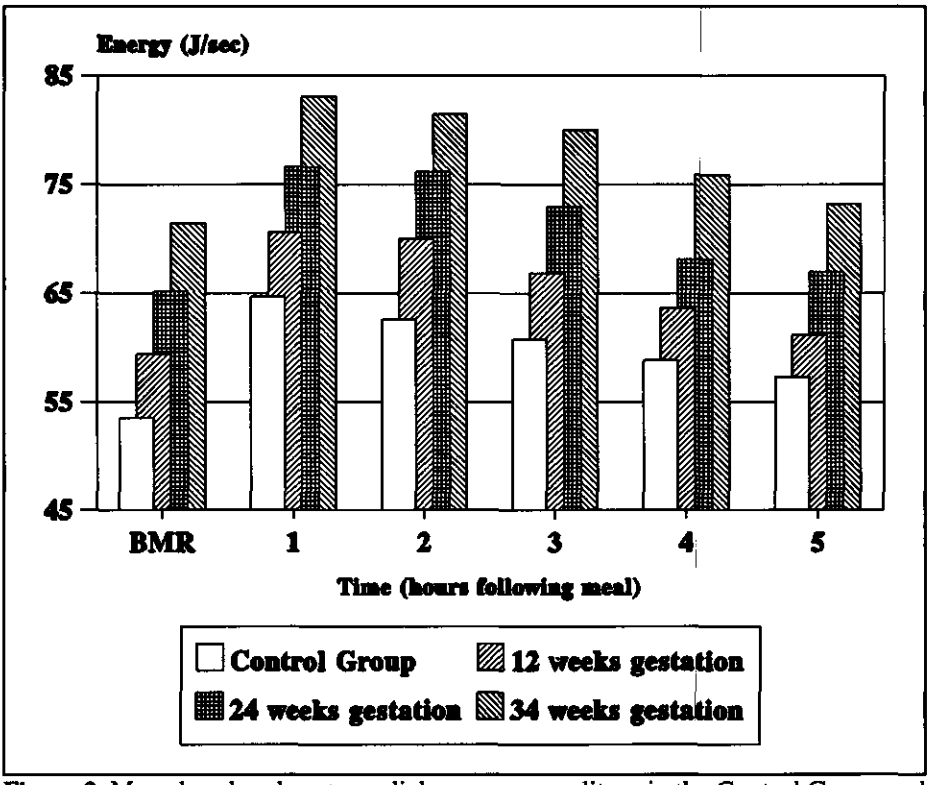

Figure 2. Mean basal and postprandial energy expenditure in the Control Group and Pregnant Group at 12, 24 and 34 weeks of gestation

The energy cost of pregnancy was estimated at 288 ± 171 MJ. This estimate was based on a gain in fat stores of 2.18 kg (100 ± 133 MJ), 0.44 kg fat (20 MJ) and 0.84 kg protein (25 MJ) deposited in the foetus and increased maternal tissues, and a cumulative increase in basal metabolism of 143 ± 65 MJ (Table 7).

Table 7. Energy cost of pregnancy[a]

	g	MJ
Tissue deposition[b]		
Gain in fat stores	2180	100 ± 133
Fat deposition[c]	436	20 ± 7
Protein deposition[d]	841	25 ± 8
Cumulative increase in basal metabolism[e]	-	143 ± 65
TOTAL	-	288 ± 171
Cumulative increase in energy intake[f]	-	290 ± 280

[a]Mean ± SD

[b]values of 29.3 kJ (7 kcal) and 46 kJ (11 kcal) were applied as the energy costs of depositing each gram of protein and fat respectively (allowing for both the energy content of the tissue and the energy cost of deposition) (Durnin et. al., 1987)

[c]Fat deposition in foetus, placenta, and increased maternal tissues assumed to be 461 g at 40 weeks of gestation, based on a deposition of 480 g in subjects gaining 12.5 kg body weight (Hytten, 1991).

[d]Protein deposition in foetus, placenta and increased maternal tissues assumed to be 888 g at 40 weeks gestation, based on a deposition of 925 g in subjects gaining 12.5 kg body weight (Hytten, 1991).

[e]Calculated, using mean values obtained at 12 weeks gestation as baseline, as follows: [(BMR at 24 weeks gestation - BMR at 12 weeks gestation) x 93.1 days of gestation] + [(BMR at 34 weeks gestation - BMR at 12 weeks gestation) x 93.1 days of gestation].

[f]Calculated, using mean values obtained at 12 weeks gestation as baseline, as follows: [(Mean intake at 24 weeks - mean intake at 12 weeks) x 93.1 days of gestation] + [(mean intake at 34 weeks - mean intake at 12 weeks) x 93.1 days of gestation].

An estimate of the protein, fat, carbohydrate and energy intake based on 5 day measured records of dietary intake in the pre-ovulatory phase of the menstrual cycle of the Control Group and during the 12th, 24th and 34th week of gestation in the Pregnant Group are given in Table 8. There was no significant difference between the Control Group and the Pregnant Group at 12 weeks of gestation. However, the Pregnant Group displayed a steady increase in both energy and macro-nutrient intake, with progression of pregnancy, the energy intakes being significantly higher ($p<0.05$) at the 34 week of gestation as compared to 12 weeks of gestation. The mean increment in energy intake per day, using energy intake at 12 weeks of gestation as baseline, was 1.55 MJ/d (371 kcal/d), which would result in a cumulative increment of 290 ± 280 MJ (69288 ± 67017 kcal) over the second and third trimesters. The increment in the protein intake over that measured at 12 weeks of gestation amounted to 7.5 ± 16.6 g at 24 weeks and 8.7 ± 18.0 g at 43 weeks gestation providing an average increment of 8 g/d over the final two trimesters.

Energy intake at 12, 24 and 34 weeks gestation were correlated to the corresponding BMR at 12 ($p<0.05$, $r=0.57$), 24 ($p=0.06$, $r=0.46$) and 34 ($p<0.05$, $r=0.57$) weeks of gestation. There was, however, no significant correlation between the total increment in energy intake and the total cost of pregnancy. The cumulative increase in energy intake over the final two trimesters was also not significantly correlated with the cumulative increase in basal energy expenditure. However, the correlation coefficient ($r=0.44$) between the increment in energy intake from 12 to 34 weeks with the increment in BMR over the same period was just short of statistical significance ($p=0.07$).

DISCUSSION

During pregnancy extra energy is needed for the growth of the fetus, placenta, and associated maternal tissues. It is, however, difficult to recommend an average energy intake during pregnancy, as women of small stature tend to have small babies[1,35], and gain less weight and hence require less additional energy than the average.

Table 8. Energy (kJ/d) and macro-nutrient (g/d) intakes in Control Group and Pregnant Group at the 12th, 24th and 34th week of gestation[a]

Groups	**Control Group** (n=18)		**Pregnant Group** (n=18)	
Time of measurement	**Pre-ovulatory**	**12 weeks gestation**	**24 weeks gestation**	**34 weeks gestation**
Energy	7.58 ± 1.30	7.61 ± 2.38	9.01 ± 3.02[b]	9.33 ± 3.34[c]
Protein (% of total energy)	52 ± 15 (11.4)	53 ± 17 (11.7)	60 ± 19 (11.2)	62 ± 21 (11.1)
Fat (% of total energy)	75 ± 20 (37.4)	69 ± 24 (34.3)	84 ± 30[b] (35.0)	86 ± 36 (34.8)
Carbohydrate (% of total energy)	232 ± 33 (51.3)	249 ± 95 (54.8)	295 ± 118[b] (54.8)	305 ± 128 (54.7)

[a]Mean ± SD; [b]12 vs 24 weeks gestation, [c]12 vs 34 weeks gestation, significantly ($p<0.05$) different on ANOVA-RMD and a paired t-test using the Bonferroni correction

Mean increment in energy intake per day, using the energy intake at 12 weeks of gestation as baseline, was 1.55 MJ/d (371 kcal/d) over the last two trimesters of pregnancy, providing in total 290 ± 280 MJ (69295 ± 67021 kcal).

The additional energy needs during pregnancy would also depend on the degree of reduction of physical activity. It is, however, clearly desirable to increase dietary intake to spare maternal tissue, allow for satisfactory growth of the fetus, adnexa, and breast tissue, and to sustain a desirable pattern of physical activity. The deposition of some fat is also associated with a more satisfactory infant birth weight. However, there is now considerable evidence to show that energy intake is not substantially altered from pre-pregnant levels[3-5]. It is, therefore, reasonable to expect that during pregnancy maternal metabolism is adapted to reduce energy expenditure to below pre-pregnancy levels. Therefore, the basal metabolic rate (BMR) and thermic effect of a meal (TEM) were measured longitudinally during pregnancy, and compared to a non-pregnant non-lactating group of women (Control Group), with the objective of determining if any energy saving was associated with these physiological processes.

The Control Group was anthropometrically comparable to the Pregnant Group at the initial measurement (12 weeks of gestation), except for the significant difference in the percent body fat. The values of percent fatness, fat mass (FM) and fat free mass (FFM), presented in Table 2 should, however, be viewed in the light of the fact that the equations of Durnin & Womersley[31] were not derived for use in pregnant women. In the 14 subjects whose skinfold thicknesses were also measured at 4 weeks postpartum there was a non-significant reduction in the sum of 4 skinfold thicknesses and percent body fat, as compared to 12 weeks of gestation, though there was a significant ($p<0.05$) weight gain of 3.6 ± 3.06 kg. This would imply that the skinfold thicknesses measured at 12 weeks gestation did not reflect the true fat mass, or that the weight gained was not over the upper body where these skinfold thicknesses were measured. This is in keeping with current knowledge, that the weight gain during pregnancy is predominantly over the lower body[35]. The reduction in skinfold thickness postpartum is thought to be due to the loss of accumulated water, and/or due to the regression of the increased skin vascularity characteristic of pregnancy[35]. Therefore the inability to demonstrate a gain in FM based on these 4 skinfold thicknesses is not surprising. In fact this approach has been found to be the least accurate by Spaaij et. al.[5]. It is, therefore, also likely that FFM was underestimated as a consequence of the overestimation of FM from the sum of four skinfold thicknesses. The weight gain from 12 weeks of gestation to term was 11.4 ± 3.7

kg, similar values have been reported by Thomson & Billewicz[37] of 11.4 kg and by Humphreys[38] of 11.7 kg in primigravid women. The significant inverse correlation between the percentage (of weight at 12 weeks of gestation) weight gained and weight measured at 12 weeks of gestation would indicate that percent weight gained over pregnancy was greater in women with initially low body weights at 12 weeks of gestation.

The BMR was significantly ($p<0.05$) higher in the Pregnant Group, as compared to the Control Group in absolute terms at 12 weeks of gestation. On expressing the BMR per kg FFM it remained significantly higher. However, as the FFM was probably underestimated, the true difference in the BMR expressewd per kg FFM may not be as large as reported. The BMR, expressed per kg body weight, in the Pregnant Group at 12 weeks of gestation was 7 percent higher than the Control Group, however, this did not reach statistical significance. There was no significant change in BMR expressed per kg body weight with the progression of pregnancy. The increase in BMR, as compared to the Control Group, is in contrast to some reports of reduced BMR during early and mid-pregnancy[10,13,14], but similar to another report on Asian women[15]. The increase in the BMR can be accounted for by the metabolic contribution of the uterus and its contents and by the increased work of maternal heart and lungs[39]. Other possible explanations include evidence of greater protein turnover during pregnancy[40]. At a conservative estimate the energy cost of protein synthesis accounts for 15-20 percent of O_2 consumption[41-43]. The concomitant significant increase in the total circulating thyroid hormones (Table 5) could possibly provide an alternative explanation for this rise in BMR. The effect of the thyroid hormones on metabolic rate is well documented[9]. The rise in total plasma tri-iodothyronine (T_3) and thyroxine (T_4) during pregnancy has been reported earlier[44,45], and was attributed to the high levels of estradiol stimulating the liver to produce increased amounts of thyroid binding globulin (TBG). This in turn would be responsible for providing increased binding sites for circulating thyroid hormones, similar changes have been reported in women taking exogenous estradiol, such as the contraceptive pill[46], the consensus of opinion being that net thyroxine turnover is unchanged in normal pregnancy[45]. However, the free thyroxine index[47] which is usually within the normal range in pregnancy may have some values that fall above the upper limit of normal[48]. A more recent report[49] has demonstrated a significant rise of between

5 and 30 percent in the plasma levels of the free T_4 and between 6 and 15 percent in free T_3 during the three trimesters of pregnancy, above pre-pregnant levels, while still remaining within normal limits of the euthyroid range. This rise in the free thyroid hormone is thought to reflect a possible increased maternal need for energy or heat generation[50]. The free T_3 (FT_3) measured in 6 subjects were 60 and 45 percent higher at 24 and 34 weeks of gestation respectively, as compared to the Control Group, though these values did not reach statistical significance. There is also evidence of human chorionic gonadotropin (hCG) having thyrotropic activity mediated, at least in part, by TSH receptors[51,52], suggesting that in early pregnancy, when hCG concentrations are highest, thyroid hormones may indeed have a role in stimulating metabolism. However, no definite conclusions can be reached based on the data available in this study. Clearly, it is an area that deserves closer attention in future studies.

There does not appear to be any reduction in the thermic effect of a meal (TEM) during pregnancy, and its role as a possible adaptive mechanism to conserve energy during pregnancy does not find much support from the data in this study, as is the case in other recent reports[12,18]. Given an within-individual variability of 7.6 and 22.7 percent in the BMR and TEM[53] we would have a 90 percent chance to detect differences ≥ 9 percent in the BMR and 26 percent in the TEM, when making between group comparisons, or differences of ≥ 6.5 and 19 percent in the BMR and TEM respectively, when making within group comparisons[54]. Illingworth et. al.[19] were the first to suggest a possible energy saving role for the TEM during the second trimester of pregnancy. Similarly, Contaldo et. al.[20] also found a significant reduction in the TEM during late pregnancy, as compared to non-pregnant, non-lactating controls. Both these studies had small sample sizes. However, another cross-sectional study with 4 subjects in late-pregnancy, 6 in early pregnancy and 6 non-pregnant, non-lactating controls, did not find any significant differences[6]. Recently Robinson et. al.[55] have demonstrated a 53 percent reduction in postprandial thermogenesis in the third trimester, as compared to a Control Group control group. This reduction was attributed to a progressively decreasing insulin sensitivity. Postprandial thermogenesis was, however, only measured for two hours following the mixed meal. The significantly increased post meal total energy output (PMTEO) in the Pregnant Group ($p<0.05$), as compared to the Control Group, even

when corrected for changes in FFM at 12 weeks of gestation, appears to be a reflection of the significantly higher BMR observed in them.

Basal and postprandial non-protein respiratory quotients (NPRQ) were similar in both groups at 12 weeks of gestation. Both the basal and postprandial carbohydrate oxidation rates were significantly higher in the Pregnant Group at 12 weeks of gestation, as compared to the Control Group; however fat oxidation rates appeared to be comparable. When compared to the Control Group, basal and postprandial protein oxidation rates in the Pregnant Group at 12 weeks of gestation, appeared to be lower, though this did not reach statistical significance. Basal protein oxidation within the Pregnant Group seemed to decline with the progress of pregnancy, although this too was not statistically significant, while the postprandial oxidation rates appeared to remain unchanged. Protein sparing during pregnancy has been described earlier by De Benoist et. al.[56], and also by Fitch & King[57]. There appeared to be a greater oxidation in absolute terms of carbohydrate and fat, both in the basal and postprandial periods in the group, with the progression of pregnancy. The proportion of fat being oxidized becoming progressively larger in the postprandial period, as indicated by the falling NPRQ (Table 4). This pattern of a gradually reducing NPRQ with the progression of pregnancy is also seen in the study on Dutch women[18], though it was less pronounced.

It has been observed that mothers who gain most weight during pregnancy will deliver heavier babies[58-60]. Thus the risk associated with a low birth weight is reduced if weight gain in pregnancy is adequate. The weight gain during pregnancy in this study (Table 6) is comparable to well nourished Western populations[4,5], being 21.3 percent of the weight at 12 weeks of gestation. The weight of the babies born, however, were lower when compared to Western babies, perhaps a reflection of the generally shorter and lighter mothers in this study, as compared to Western women, but higher than the birth weight of babies (2.70 ± 0.39 kg) born to rural Indian women[61], and to high income urban Indian women (2.74 kg)[62]. The fact that maternal size affects birth weight has been observed in different populations[59,63,64]. It has also been shown that women of Indian origin, whether living in India[64,65], or in Europe[66] have smaller babies than European women of the same height; the difference being as much as 300-400g.

The subjects in our study were well nourished and had *ad libitum* access to food and were, therefore, comparable to the subjects in studies on Western populations. The energy cost of pregnancy was estimated to be 288 MJ. A figure that is close to the FAO/WHO/UNU[1] recommendation of 335 MJ. This value is also similar to the energy cost of pregnancy obtained in well nourished Western populations[3-5,17]. A constant feature in all these studies is the large between subject variability. The energy cost of pregnancy, however, was in contrast to the reported low costs of pregnancy in the Gambia[10,14]. The cumulative increase in energy intake was almost 290 MJ, once again with a large between subject variability. This would suggest that the extra requirements of pregnancy were met entirely by increasing energy intakes (Table 8). Though the increments in energy intake were calculated over those measured at 12 weeks of gestation, the average increment in energy intake per day expressed over the whole 257 days of gestation (271 days - 14 days, as the length of gestation was calculated from the first day of menstruation of the last reported menstrual period) amounted to approximately 1.13 MJ/day (270 kcal/day). This is very close to the 1.26 MJ/day recommended by the ICMR[67] and the 1.2 MJ/d recommended by the FAO/WHO/UNU joint expert consultation[1]. The increment of protein intake observed was also very close to the FAO/UNU/WHO[1] recommendations of 6 g/d, but far short of the ICMR values of an extra 15 g/d, through out pregnancy. The relationship between the increment in the BMR and energy intake between 12 and 34 weeks of gestation was close to statistical significance ($p=0.07$) and would suggest that most of the extra intake was a consequence of the progressively higher BMR observed during pregnancy, if the food consumption data provides a true reflection of the energy intake during this period. The inability to demonstrate a relationship between the total cost of pregnancy and the total increment in energy intake may have been due to differing levels of physical activity existing between the pregnant subjects studied.

A reduction in physical activity may be one of the mechanisms by which energy is saved during pregnancy[7,8,15]. In this study, however, the large concomitant increase in energy intake over pregnancy in this study, accounts for almost all the extra costs of pregnancy, in contrast to the studies in Western populations[3-5,17]. This may be due to various factors. The methodology employed for the estimation of energy intake was not as precise as the

weighed intake method used in most Western studies. However, it is was less cumbersome and only 3 measurements were made through out pregnancy. Subjects had no idea, in terms of weight, of how much they were eating and therefore, less likely to change their eating patterns. Another possible contributory factor is that there is little cultural pressure to maintain a slim profile during pregnancy and motherhood. Hence, mothers are more likely to eat to the dictates of their appetite than their counterparts in the West.

In summary, there does not appear to be any energy saving associated with the BMR or TEM during pregnancy. In fact, there seems to be an increase in the BMR during pregnancy, over the non-pregnant, non-lactating state, even after correcting for changes in body weight and composition. Well nourished Indian women have weight and fat gains similar to well nourished Western women. Birth weight weights of infants born appeared to be lower, but placental weights appeared to be comparable to those reported in well nourished populations. The energy cost of pregnancy was estimated to be 288 MJ, close to the 335 MJ recommended by the FAO/WHO/UNU expert consultation[1]. The extra energy intake during pregnancy was very close to the estimated cost of pregnancy, and similar to the FAO/WHO/UNU[1] recommendations, though the increment in intake was only observed in the second and third trimesters of pregnancy.

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Chapter 5

THE EFFECT OF LACTATION ON ENERGY EXPENDITURE, ANTHROPOMETRY, AND ENERGY INTAKE DURING THE FIRST SIX MONTHS POSTPARTUM IN WELL NOURISHED INDIAN WOMEN

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ABSTRACT

The basal metabolic rate (BMR), thermic effect of a meal (TEM), anthropometry and energy intake were compared in a group of 18 non-pregnant, non-lactating controls and 17 Pregnant-Lactating women at 12 weeks of gestation, 12 and 24 weeks postpartum; in order to uncover any enhancement in metabolic efficiency during lactation. An adequate lactational performance was inferred from the weight gained (2.06 ± 0.41) by the infants from birth to 12 weeks of age, while being exclusively breast fed. The BMR was significantly higher in the Pregnant-Lactating Group all time points, as compared to the Control Group, while the TEM was comparable between groups, and at all time points, within the Pregnant-Lactating Group. This would indicate that there was no energy saving associated with these processes during lactation. The mean weight gain from 12 weeks of gestation to term was 11.2 ± 3.7 kg; the mean birth weight was 3.05 ± 0.45 kg while placental weight was 0.63 ± 0.15 kg. The loss in body weight, in a sub-group of 13 subjects, from 4 to 12 weeks postpartum was 0.36 ± 1.69 kg. This would provide at most 242 kJ/d assuming that the entire weight lost was fat mass. Energy intake was not significantly different between the Control Group and the Pregnant-Lactating Group at 12 weeks of gestation, however, energy intakes at 12 and 24 weeks postpartum were significantly higher than that measured at 12 weeks of gestation by 2.0 and 1.5 MJ/d, close to FAO/UNU/WHO recommendation of 2.1 MJ/d. In the light of an adequate lactational performance, low rate of body fat mobilization during the period of exclusive breast feeding, and energy intakes close to that recommended by the FAO/WHO/UNU, it would appear that the total energy cost of lactation (3.0 MJ/d) may be an overestimate.

INTRODUCTION

Lactation imposes considerable physiological stress on the metabolism of the mother. The Food and Agricultural Organization /World Health Organization /United Nations University (FAO/WHO/UNU) joint expert consultation[1] recommended an extra energy intake of 2.1 MJ/d (500 kcal/d) to meet the cost of lactation. This was based on the assumption that the efficiency of conversion of food energy to milk is 80 percent and that approximately 835 kJ/d (200 kcal/d) are mobilized from maternal fat stores built up during pregnancy. Studies on food intake in well nourished women from industrialized countries have indicated that increments in food intake rarely meet this recommendation[2-4]. This may be due to several reasons. It is possible that the volume of breast milk produced is being overestimated, or possibly that the energy content of breast milk is lower than suggested[5]; or that there is a greater efficiency in the conversion of dietary energy to milk energy[5-7]. Alternatively body fat, accumulated during pregnancy, may be mobilized to meet this extra energy requirement[8-13]. Another possibility may be that there is an enhanced metabolic efficiency[2,5,11,12] and a lower physical activity level[2,5], which would result in a reduction in energy expenditure during this period.

Changes in requirements in individuals at different time points and whether these requirements have wide validity or are influenced by factors such as ethnicity, climate, or adaptation were listed as priority areas of research in the report of the FAO/WHO/UNU joint expert consultation[1]. Most of the studies on energy expenditure and intake during lactation have been carried out in well nourished Western populations. More evidence from different parts of the world is required before an extrapolation can be made to all populations. This study was designed to examine how well nourished Indian women cope with the energy stress imposed by lactation, by studying changes in the basal metabolic rate, thermic effect of a meal (which together would account for almost 80 percent of the total energy expenditure over 24-hours in sedentary individuals[14]), energy intake and anthropometry that may occur during this period and comparing them to non-pregnant non-lactating women in the reproductive age group.

SUBJECTS AND METHODS

Study design (Table 1)

Two groups, of twenty-two women each, were recruited for the study. The first group consisted of non-pregnant, non-lactating controls (Control Group) in the reproductive age group, while the second consisted of women who were less than 11 weeks pregnant (Pregnant-Lactating Group). The BMR and TEM were measured, between the 6th and 10th day, during the pre-ovulatory phase of the menstrual cycle, in the Control Group; and in the Pregnant-Lactating Group, at 12 weeks of gestation, as well as, 12 and 24 weeks postpartum. Urine samples were collected following the BMR and TEM for estimation of total urinary nitrogen (TUN) excretion, in order to calculate non-protein respiratory quotients and substrate oxidation rates during these measurements. Fasting blood samples were obtained from 6 subjects of each group on the day that the metabolic measurements were carried out, for the estimation of haemoglobin, glucose, insulin, tri-iodothyronine (T_3), free T_3 (FT_3), thyroxine (T_4), estradiol and progesterone. Anthropometric measurements (height, weight, mid-arm circumference and skinfold thicknesses) were made on the same day, following the metabolic measurements, body weight and skinfold thicknesses were also measured at 4 weeks postpartum. A written record of the measured volume of all food and drinks consumed was obtained for 5 days prior to the metabolic measurements. These data were used to calculate energy and macro-nutrient intakes. The birth weight and weight at 12 weeks of age of the infants were also measured.

Subjects

Subjects were either from the middle or upper socio-economic class as assessed by Kuppuswamy's[15] urban socio-economic assessment scale, were non-vegetarians and had *ad libitum* access to food. The Control Group were recruited from amongst the staff and students of St. John's Medical College and Hospital in Bangalore. They were non-smokers, in good health, weight stable, and were not on any kind of chronic medication or oral contraceptives. Pregnant-Lactating Group subjects were recruited for participation either through the Obstetric departments of St. John's Medical College Hospital, St.

Martha's Hospital or by personal approach. All subjects were in good health and had no impairment of appetite due to 'morning sickness' prior to the initial metabolic measurement at 12 weeks of gestation. All Pregnant-Lactating Group subjects were committed to breast feeding their infants following delivery. Subjects from both groups were well educated and were interested in nutritional issues. They were familiarized with the ventilated hood system prior to the actual metabolic measurements.

Methods

Energy expenditure was measured by respiratory gas exchange measurements using a 'ventilated hood'. The ventilated hood measurement system consisted of a plastic hood which surrounded the subject's head and a soft plastic collar surrounding the neck and shoulders. A fixed flow (50 l/min) of room air was maintained through the hood by connecting the outlet of the hood through a calibrated rotameter (Fischer Controls, UK) to a suction pump. A small sample of air (1 l/min) was drawn off from the outlet of the rotameter for minute to minute estimation of oxygen (O_2) and carbon-dioxide (CO_2) concentrations using a paramagnetic oxygen analyzer (Servomex 540 A, Taylor Instruments, UK) and an infra-red carbon-dioxide analyzer (Type SSI, Analytical Development Co., U.K.) respectively. O_2 and CO_2 concentrations of the room (reference) air were monitored every hour for 10 minutes, prior to the start of a metabolic measurement. Moisture content and temperature were measured by means of a dew-point hygrometer of the optical condensing type (General Eastern Systems, 1100 DP, USA) and a mercury thermometer placed in the air stream emerging from the rotameter, respectively. Atmospheric pressure was measured by means of a mercury barometer at the start of each measurement protocol. Oxygen consumption (VO_2) and carbon-dioxide production (VCO_2) rates (calculated to STPD conditions; standardized for temperature, pressure and dryness), Respiratory Quotient (RQ = VCO_2/VO_2), and energy expenditure[16] were calculated from this data and recorded every minute using a computerized data acquisition and storage system[17].

The gas analyzers were calibrated before the start each measurement using pure nitrogen (100%) (Indian Oxygen Ltd., Bangalore) for 'zero', and a mixture of carbon-dioxide (1%), oxygen (21%) and nitrogen (78%) (Bhoruka Gases Ltd., Bangalore), for the 'span'

Table 1. Study Design

Groups	Control Group (n=18)		Pregnant-Lactating Group (n=17)	
Time of measurement	**Pre-ovulatory**	**12 weeks gestation**	**12 weeks postpartum**	**24 weeks postpartum**
Height	x	-	-	-
Weight[a]	x	x	x	x
Mid-arm circumference[a]	x	x	x	x
Skinfold thicknesses[a]	x	x	x	x
Basal Metabolic Rate	x	x	x	x
Thermic effect a Meal	x	x	x	x
Substrate oxidation rates	x	x	x	x
Fasting blood sample[b]	x	x	x	x
Urine samples	x	x	x	x
Dietary Intakes[c]	x	x	x	x
Baby's weight[d]			x	-

[a]Also measured at 4 weeks postpartum in 13 women from the Pregnant-Lactating Group

[b]Measured in 6 subjects from each group

[c]Recorded for 5 days prior to metabolic measurements.

[d]Birth-weight also obtained

setting. These calibration gases were passed through the analyzers at the end of the measurement protocol, 6 hours after the initial calibration, to check for instrument drift. All data was subsequently corrected for instrument drift during this period. This measurement system had been validated against other methods of measurement of oxygen consumption[18]. Calibration using the N_2 infusion technique[19] yielded a net discrepancy of less than 0.4 percent in the estimation of oxygen consumption. Ethanol burns conducted at regular intervals over the duration of the study produced energy expenditures within ± 2 percent of expected values. Room temperatures were maintained between 24 and 29 °C on all days of the study , while barometric pressures ranged from 681 to 692 mm Hg.

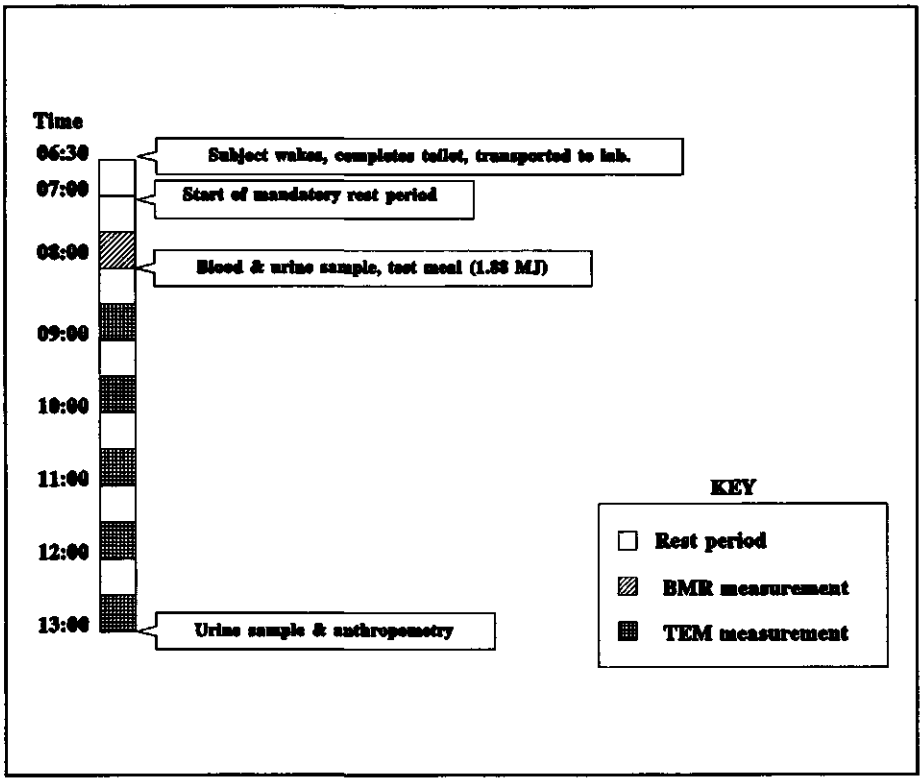

Figure 1. Measurement protocol

The measurement protocol employed was as follows (Figure 1). Subjects were instructed to complete their evening meal by 19:30 hours, and to be in bed by 22:00 hours on the night prior to the metabolic measurements. On the morning of the metabolic measurement they were instructed to wake between 6:00 and 6:30 hours, complete their toilet and empty their bladder while noting the exact time. They were then transported to the laboratory by the department vehicle. On entering the laboratory all subjects were instructed to rest in bed for 30 min. prior to the start of the BMR measurement. At the end of this mandatory rest period the basal metabolic rate was measured for 30 min, after an initial 10 min period to allow O_2 consumption to stabilize, while within the ventilated hood. Basal metabolic rate (BMR) was calculated by obtaining the mean energy expenditure over this 30 min period.

Following the measurement of the BMR the hood was removed. A fasting blood sample (10 ml) was collected from the antecubital vein using aseptic precautions for the estimation of haemoglobin, glucose using the orthotoluidine method, insulin, triiodothyronine (T_3), thyroxine (T_4), thyroid stimulating hormone (TSH) using locally available RIA kits (Bhabha Atomic Research Centre, Bombay, India), while free T_3 (FT_3), estradiol and progesterone were measured using DPC RIA kits (Diagnostic Products Corporation, Los Angeles, USA) in 6 subjects from each group.

Subjects were then instructed to void. The voided urine volume was noted and then acidified with concentrated hydrochloric acid (1 ml acid per 100 ml urine). An aliquot was stored for estimation of total urinary nitrogen (TUN) excretion, over the preprandial period, by the micro-kjeldhal method[20]. (All urine voided during the postprandial period together with the final urine sample obtained at the end of the metabolic measurements, was collected in the same manner, for estimation of TUN excretion, over the postprandial period).

Following the collection of the basal urine sample the subjects were served a standard test meal which consisted of tinned milk powder, rice cereal and sugar, together with a glass of lemon juice. The cereal was mixed with the powdered milk and part of the sugar and made up to 200 ml with water. The remaining sugar was mixed with 200 ml of water

to which lemon juice was added for flavour. Together this provided 1.88 MJ (450 kcal) of energy, with 10 percent of the energy being derived from protein (11.2 g/100 g), 15 perecnt from fat (7.6 g/100 g) and 75 percent from carbohydrate (84.2 g/100 g). Energy and nutrient composition was derived from the manufacturers' product information. All tinned products were used well before their date of expiry, and produced by the same manufacturer.

After the ingestion of the meal, the ventilated hood was replaced and the O_2 consumption and CO_2 production were measured intermittently for the next 5 hours. The second thirty minutes in each hour were considered to be a 'measurement period' and representative of the energy expenditure for the entire hour; the initial 30 min being designated as a 'rest period'. The subject was instructed to lie awake and motionless in the recumbent position during the 'measurement periods'. Between the 'measurement periods', i.e. during the 'rest periods', they were also asked to lie quietly in bed though some movement and reading was permitted. Pregnant-Lactating Group subjects were allowed to breast feed their infants during these 'rest periods'. Ten minutes prior to the start of each 'measurement period' they were instructed to rest motionless to allow their O_2 consumption to stabilize, while within the ventilated hood, in preparation for the next 'measurement period'.

The thermic effect of the meal (TEM) was calculated by obtaining the mean increment in energy expenditure during the 'measurement period' (30 min) in each hour (which was considered representative for the entire hour) above pre-meal basal values. The cumulative increment in energy expenditure for the 5 hours following the ingestion of the test meal was expressed as a percentage of the energy content of the meal. The post-meal total energy output (PMTEO) was calculated by obtaining the total energy expenditure during the 5 hours after the ingestion of the test meal[21]. Substrate oxidation rates were calculated during the pre- and postprandial period in each subject, using total oxygen consumption (VO_2), carbon-dioxide (VCO_2) production rates and total urinary nitrogen (TUN) excretion during the pre and postprandial periods[22]. No correction was made to the protein oxidation rate to take into account any change in the blood urea pool between the fasted and fed states. Non-Protein Respiratory Quotients (NPRQ) were

calculated after subtracting the oxygen consumed (PVO_2) and carbon dioxide produced ($PVCO_2$) in protein oxidation, using the formulas of Jequier et. al.[23], from the measured VO_2 and VCO_2 of the subject [$(VCO_2-PVCO_2)/(VO_2-PVO_2)$].

On completion of all metabolic measurements for the day, anthropometric measurements were made for assessment of nutritional status. These included height, weight, skinfold thicknesses (biceps, triceps, subscapular, supra-iliac) and mid-arm circumference (MAC). Height was measured using a moveable anthropometer (Nivotoise, France) and recorded to the nearest 0.1 cm. Body weight was measured on a digital weighing scale (Soehnle, Germany) and recorded to the nearest 100 g. Subjects stood upright with the head in the horizontal plane, in light indoor clothing. For the measurement of the skinfold thicknesses (biceps, triceps, subscapular, supra-iliac) a Holtain skinfold calliper (Crymmych, U.K.) was used. All the skinfolds were measured on the right side of the body and recorded to the nearest 0.2 mm. Every skinfold was measured in triplicate and the mean of these 3 values was used for calculation. Fat mass (FM) was calculated after estimating percent body fat from the sum of four skinfolds and applying the formula of Durnin & Womersley[24]. Fat free mass (FFM) was estimated from the difference of weight and FM.

A week prior to the metabolic measurements the subjects were visited at home and provided with two separate household measures of known volume, together with a tape measure, to measure the volume of all solid and liquid food consumed. In addition a booklet was provided on each occasion to record the time of the meal, a general description of the food/drink consumed, details of any unconventional food item eaten, the exact volume consumed as measured in the household measures provided and dimensions of solid food that could not be measured in the household measures provided. All subjects were instructed by a trained dietitian as to the proper use of the household measures and the type of detail required in the description of the food items or beverages being consumed. The method of recording this information in the booklet provided was demonstrated in a simple example on the initial page of each booklet. No special advice as to the type or quantity of food to be consumed was given, except to encourage all subjects to eat to the dictates of their appetite and not change their eating

pattern during this period. This record was maintained for 5 consecutive days prior to the metabolic measurement. On the day of the metabolic and anthropometric measurements the dietitian checked the dietary record maintained by the subject and clarified any ambiguities, following the completion of the metabolic and anthropometric measurements. The volume of food consumed was converted to weight by weighing a similar food item, prepared in the laboratory using a standardized recipe, in the standard household measure used by the subject. Fruits, sweets, snacks etc were purchased based on the dimensions provided by the subjects and weighed in the laboratory. These data were then used for estimation of energy and macro-nutrient content of the diet by the use of standard food composition tables[25-27].

Statistical analysis

Data are presented as Mean ± SD. An unpaired t-test was used for comparisons between the Control Group and Pregnant-Lactating Group. An analysis of variance for repeated measurements designs (ANOVA-RMD), was used to analyze longitudinal data in the Pregnant-Lactating Group. A paired t-test, with the Bonferroni correction, was used to identify where the differences, detected by the ANOVA-RMD, existed. Statistical significance was accepted at the 5 percent level. All data was analyzed using SPSS/PC+ statistical package (Version 4.0, SPSS Inc. 444 N.Michigan Avenue, Chicago, Illinois, USA) on an IBM-PC 386 compatible computer.

Ethical Approval

Ethical approval was obtained for the study from the Human Investigation Committee of the Medical School. Informed consent in writing was obtained from all subjects prior to the start of the measurements.

RESULTS

Of the 22 subjects recruited in each group 18 Control and 17 Pregnant-Lactating Group subjects completed all planned measurements. Subject characteristics are given in Table

2. The sum of 4 skinfold thicknesses, percent body fat, and fat mass (FM) were significantly ($p < 0.05$) higher, while fat free mass (FFM) was significantly ($p < 0.05$) lower at 12 weeks of gestation in the Pregnant-Lactating Group, as compared to the Control Group. Body mass index (kg/m^2, BMI), mid-arm circumference (MAC) and percent body fat were significantly ($p < 0.05$) higher at 12 weeks postpartum in the Pregnant-Lactating Group, as compared to the Control Group. In the Pregnant-Lactating Group at 24 weeks postpartum BMI and MAC remained significantly higher than that of the Control Group. Body weight, BMI and FFM were significantly ($p < 0.05$) higher at 12 weeks postpartum, as compared to, 12 weeks of gestation in the Pregnant-Lactating Group. In the Pregnant-Lactating Group at 24 weeks postpartum FFM continued to remain significantly ($p < 0.05$) higher than that estimated at 12 weeks of gestation.

All 17 Pregnant-Lactating Group subjects had normal pregnancies. The average length of gestation was 38.7 ± 1.1 weeks. The subjects gained 11.2 ± 3.7 kg of weight from 12 weeks of gestation to term, which was 20.9 ± 7.8 per cent of their weight at 12 weeks of gestation. 13 of the subjects had normal vaginal deliveries, while 4 subjects had their babies delivered by caesarian section. There were 8 male and 9 female babies born. The mean birth weight of the 17 babies was 3.05 ± 0.45 kg, while mean placental weight was 0.63 ± 0.15 kg. 14 of the 17 subjects from the Pregnant-Lactating Group exclusively breast fed their infants till after the 12 weeks postpartum measurement, the other 3 had started supplementary formula feeds in addition to breast feeding prior to the 12 weeks postpartum measurement. All mothers continued to breast feed their infants till after the 24 weeks postpartum metabolic measurement, though supplementary feeding had also been started.

The body weight at 4 weeks postpartum could only be measured in 13 of the 17 subjects studied. There was a significant ($p < 0.05$) weight gain from 12 weeks of gestation (51.8 ± 6.8 kg) to 4 weeks postpartum (55.0 ± 7.0) of 3.2 ± 2.8 kg, while the percent body fat was significantly ($p < 0.05$) reduced from 31.7 ± 6.3 percent at 12 weeks of gestation to 29.2 ± 5.8 percent at 4 weeks postpartum. In the same 13 Pregnant-Lactating Group subjects at 12 weeks postpartum, body weight (54.7 ± 7.1 kg) and percent body fat (29.6 ± 5.5 %) remained relatively unchanged (0.36 ± 1.69 kg), as compared to measurements

Table 2. Subject characteristics[a]

Groups	Control Group (n=18)		Pregnant-Lactating Group (n=17)	
Time of measurement	**Pre-ovulatory**	**12 weeks gestation**	**12 weeks postpartum**	**24 weeks postpartum**
Age (years)	26.2 ± 6.0	29.6 ± 5.4		
Height (m)	1.61± 0.06	1.58± 0.06		
Weight (kg)	53.2 ± 8.3	55.0 ± 8.5	56.9 ± 7.6[c]	56.6 ± 7.6
BMI (kg/m^2)	20.4 ± 2.4	22.0 ± 2.9	22.8 ± 2.7[bc]	22.7 ± 2.8[b]
Mid-arm Circumference (cm)	25.2 ± 2.5	26.7 ± 2.7	27.2 ± 2.3[b]	27.3 ± 2.2[b]
Sum of 4 skinfolds (mm)	56.9 ± 13.8	76.4 ± 26.5[b]	68.9 ± 24.7	65.0 ± 21.2
Fat[e](%)	28.5 ± 3.6	32.6 ± 5.8[b]	31.1 ± 5.7[b]	30.5 ± 5.2
Fat mass (kg)	15.2 ± 3.4	18.2 ± 5.2[b]	18.0 ± 5.0	17.5 ± 4.6
Fat free mass (kg)	37.9 ± 5.0	36.7 ± 4.3[b]	38.9 ± 3.9[c]	39.1 ±4.0[d]

[a]Mean ± SD; [b]significantly ($p<0.05$) different vs Control Group on an 'unpaired t-test'; [c]12 weeks gestation vs 12 weeks postpartum, [d]12 weeks gestation vs 24 weeks postpartum, significantly different on ANOVA-RMD ($p<0.05$) and a paired t-test using the Bonferroni correction.

[e]Calculated from the sum of 4 skinfold thicknesses and applying the formulas of Durnin & Womersley (1974)

made at 4 weeks postpartum. There was no significant difference (0.29 ± 2.00 kg) in the body weight measured at 12 and 24 weeks postpartum in the whole group of 17 Pregnant-Lactating Group subjects.

14 of the 17 babies born, who were exclusively breast fed till 12 weeks of age, were also weighed at 12 weeks postpartum. There was a significant ($p<0.05$) increase in body weight of 2.06 ± 0.41 kg, from a birth-weight of 3.01 ± 0.45 kg, to that measured at 12 weeks of age (5.08 ± 0.63 kg).

The metabolic data on the Control and Pregnant-Lactating Groups are presented in Table 3. The BMR was significantly higher at 12 weeks of gestation and at 12 and 24 weeks postpartum in absolute terms in the Pregnant-Lactating Group, as compared to the Control Group. When expressed per kg body weight there was no significant difference, though the values tend to remain higher. However, on expressing the BMR per kg FFM values at 12 weeks of gestation were significantly ($p<0.05$) higher, while 12 and 24 weeks postpartum values though tending to be higher, were not significantly different from the Control Group. There were no significant differences in the TEM between the Control Group and Pregnant-Lactating Group at any time point (Figure 2).

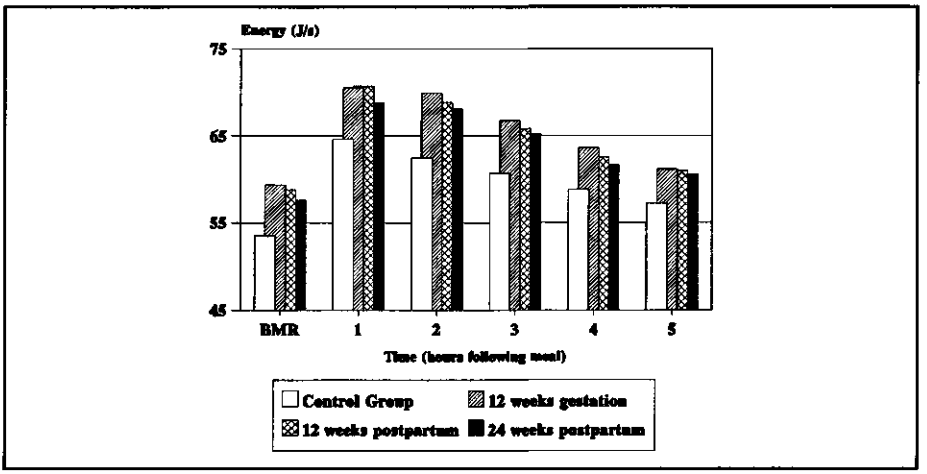

Figure 2. Mean basal and postprandial energy expenditures in the Control and Pregnant-Lactating Groups

Table 3. Basal metabolic rate, thermic effect of a meal, post meal total energy output in Control and Pregnant-Lactating Groups[a]

Groups	Control Group (n=18)		Pregnant-Lactating Group (n=17)	
Time of measurement	**Pre-ovulatory**	**12 weeks gestation**	**12 weeks postpartum**	**24 weeks postpartum**
Basal Metabolic Rate				
(kJ/min)	3.21± 0.22	3.58± 0.38[b]	3.54± 0.33[b]	3.46± 0.41[b]
(MJ/d)	4.62± 0.32	5.15± 0.55[b]	5.10± 0.48[b]	4.98± 0.60[b]
(kJ/kg wt/d)	88.3 ± 11.4	94.4 ± 7.5	90.7 ± 10.4	89.0 ± 10.4[d]
(kJ/kg FFM/d)	123.5 ± 15.7	140.8 ± 6.0[b]	131.3 ± 10.4[c]	127.9 ± 13.8[d]
Thermic Effect of a Meal				
(% of energy in the meal)	7.0 ± 1.7	6.9 ± 1.7	6.9 ± 1.5	7.3 ± 1.9
Post Meal Total Energy Output				
(kJ/min over 5h)	3.65± 0.24	4.00± 0.39[b]	3.96± 0.36[b]	3.86± 0.35[b]
(kJ/kg wt/5h)	22.0 ± 1.8	21.1 ± 2.2	20.9 ± 2.6	20.6 ± 2.0
(kJ/kg FFM/5h)	29.3 ± 3.6	32.7 ± 2.0[b]	30.6 ± 2.2[c]	29.9 ± 2.5[d]

[a]Mean ± SD; [b]significantly ($p<0.05$) different vs Control Group; [c]12 weeks gestation vs 12 weeks postpartum, [d]12 weeks gestation vs 24 weeks postpartum, significantly different on ANOVA-RMD ($p<0.05$) and a paired t-test using the Bonferroni correction.

The PMTEO, however, was significantly higher at 12 weeks of gestation and at 12 and 24 weeks postpartum in the Pregnant-Lactating Group, as compared to the Control Group, reflecting the significant differences in the BMR. There were no significant differences in the BMR (absolute or expressed per kg body weight or FFM), TEM, or PMTEO, in the Pregnant-Lactating Group between 12 and 24 weeks postpartum.

Non-protein respiratory quotients and substrate oxidation rates are given in Table 4. NPRQs in the basal and postprandial period were comparable between the Control Group and the Pregnant-Lactating Group at 12 weeks of gestation and 12 and 24 weeks postpartum. Basal and postprandial protein oxidation rates at 12 weeks of gestation and 12 weeks postpartum appeared to be lower than Control Group, but this was not statistically significant. Basal fat oxidation rates was similar in the Control Group and the Pregnant-Lactating Group at 12 weeks of gestation and 12 and 24 weeks postpartum. Postprandial fat oxidation rates in the Pregnant-Lactating Group, however, were significantly ($p<0.05$) higher at 12 and 24 weeks postpartum, as compared to the control group. Basal and postprandial carbohydrate oxidation rates were significantly ($p<0.05$) higher at 12 weeks of gestation, as compared to the Control Group. After delivery basal carbohydrate oxidation rates remained significantly ($p<0.05$) higher than the Control Group, but postprandial rates were comparable. There were no significant differences in basal and postprandial carbohydrate oxidation rates at 24 weeks postpartum in the Pregnant-Lactating Group, as compared to the Control Group.

The results of the analyses of the fasting blood samples are presented in Table 5. There were no significant differences between the two groups in the haemoglobin, glucose and insulin concentrations at any time point. T_3 and T_4 values were significantly ($p<0.05$) higher at 12 weeks of gestation, but were not significantly different at the 12 and 24 weeks postpartum measurements in the Pregnant-Lactating Group, as compared to the Control Group. TSH and FT_3 values were comparable in both groups at all time points. Estradiol and progesterone values were significantly higher at 12 weeks of gestation.

Table 4. Substrate oxidation rates and non-protein respiratory quotient (NPRQ) in the Control and Pregnant-Lactating Groups[a]

Groups	Control Group (n=18)		Pregnant-Lactating Group (n=17)	
Time of measurement	**Pre-ovulatory**	**12 weeks of gestation**	**12 weeks postpartum**	**24 weeks postpartum**
NPRQ				
Basal	0.84 ± 0.08	0.84 ± 0.04	0.85 ± 0.05a	0.84 ± 0.10
Postprandial	0.95 ± 0.04	0.94 ± 0.04	0.93 ± 0.04	0.91 ± 0.07
Protein oxidation rates mg/min				
Basal	39.0 ± 22.1	34.0 ± 18.2	31.0 ± 12.8	32.8 ± 14.0
(% of total energy)	(21.2)	(16.4)	(15.0)	(16.3)
Postprandial	37.6 ± 9.8	32.7 ± 14.6	34.5 ± 9.6	36.8 ± 13.0
(% of total energy)	(17.2)	(13.6)	(14.6)	(15.8)
Fat oxidation rates mg/min				
Basal	35.3 ± 19.2	38.1 ± 10.6	37.8 ± 13.6	37.2 ± 22.0
(% of total energy)	(43.4)	(41.3)	(41.2)	(41.7)
Postprandial	12.1 ± 10.6	16.1 ± 10.3	20.3 ± 12.9[b]	22.1 ± 16.0[b]
(% of total energy)	(12.4)	(15.0)	(19.4)	(21.3)
Carbohydrate oxidation rates mg/min				
Basal	65.1 ± 33.9	87.7 ± 29.7[b]	90.2 ± 26.4[b]	84.2 ± 63.0
(% of total energy)	(35.6)	(42.3)	(43.7)	(42.0)
Postprandial	154.1 ± 22.9	171.0 ± 24.8[b]	155.6 ± 23.8	146.8 ± 51.0
(% of total energy)	(70.4)	(71.3)	(66.0)	(63.0)

[a]Mean ± SD; [b]significantly ($p<0.05$) different vs Control Group on an 'unpaired t-test'

Table 5. Plasma haemoglobin (Hb), glucose, insulin, tri-iodothyronine (T_3), free tri-iodothyronine (FT_3), thyroxine (T_4), thyroid stimulating hormone (TSH), estradiol, progesterone in the Control and Pregnant-Lactating Groups[a]

Groups	**Control Group** (n=6)		**Pregnant-Lactating Group** (n=6)	
Time of measurement	**Pre-ovulatory**	**12 weeks gestation**	**12 weeks postpartum**	**24 weeks postpartum**
Hb (g/dl)	12.5 ± 1.3	13.7 ± 1.4	11.9 ± 0.6	11.8 ± 1.2
Glucose (mg/dl)	65.0 ± 6.5	63.8 ± 5.4	68.1 ± 15.2	67.6 ± 8.1
Insulin (μU/ml)	12.9 ± 5.2	12.6 ± 5.3	9.5 ± 5.0	14.1 ± 9.2
T_3 (ng/ml)	1.5 ± 0.3	2.1 ± 0.3[b]	1.3 ± 0.4	1.2 ± 0.5
FT_3 (pg/ml)	1.3 ± 1.1	1.1 ± 0.6	1.4 ± 1.0	1.3 ± 0.7
T_4 (μg/dl)	9.2 ± 1.5	12.5 ± 0.5[b]	7.9 ± 1.4	7.3 ± 1.6
TSH (μIU/ml)	1.0 ± 0.8	0.7 ± 0.5	1.0 ± 0.8	1.4 ± 1.0
Estradiol (ng/ml)	0.07 ± 0.03	1.11 ± 0.42[b]		
Progesterone (ng/ml)	0.2 ± 0.2	29.5 ± 9.9[b]		

[a]Mean ± SD; [b]significantly ($p<0.05$) different vs Control Group on an 'unpaired t-test'.

Table 6. Energy and macro-nutrient intakes in the Control and Pregnant-Lactating Groups[a]

Groups	**Control Group** (n=18)		**Pregnant-Lactating Group** (n=17)	
Time of measurement	**Pre-ovulatory**	**12 weeks gestation**	**12 weeks postpartum**	**24 weeks postpartum**
Energy (MJ/d)	7.58 ± 1.30	7.58 ± 2.45	9.60 ± 2.77[bc]	9.09 ± 2.53[b]
Protein (g/d)	52 ± 15	52 ± 17	65 ± 19[bc]	62 ± 16
(% of energy)	(11.4)	(11.5)	(11.2)	(11.4)
Fat (g/d)	75 ± 20	69 ± 25	88 ± 31[c]	86 ± 33
(% of energy)	(37.4)	(34.0)	(34.3)	(35.6)
Carbohydrate (g/d)	232 ± 33	249 ± 98	314 ± 101[bc]	288 ± 92[b]
(% of energy)	(51.3)	(54.0)	(54.5)	(53.0)

[a]Mean ± SD; [b]significantly ($p<0.05$) different vs Control Group on an 'unpaired t-test'; [c]12 weeks gestation vs 12 weeks postpartum, significantly different on an ANOVA-RMD ($p<0.05$) and a paired t-test using the Bonferroni correction.

There were no significant differences either in the energy or macro-nutrient intakes at 12 weeks of gestation in the Pregnant-Lactating Group, as compared to the Control Group (Table 6). However, in the Pregnant-Lactating Group at 12 weeks postpartum there were significant ($p < 0.05$) increases in the energy, protein and carbohydrate intakes, as compared to the Control Group, as well as, values at 12 weeks of gestation.

The mean increase in energy intake at 12 weeks postpartum, as compared to the 12 weeks of gestation, was 2016 ± 2390 kJ (482 ± 571 kcal)/d. The increment in protein, fat and carbohydrate intake between the same time points were 12.8 ± 19.5, 19.5 ± 27.2 and 64.7 ± 81.1 g/d respectively. The energy and carbohydrate intake at 24 weeks postpartum in the Pregnant-Lactating Group were significantly ($p < 0.05$) higher than the Control Group. There were no significant differences in the energy or macronutrient intakes in the Pregnant-Lactating Group measured at 12 and 24 weeks postpartum, though there was trend towards lower intakes at 24 weeks postpartum. Total energy intake was still 1508 ± 2564 kJ (361 ± 613 kcal)/d higher than that measured at 12 weeks of gestation, while protein fat and carbohydrate intakes were higher by 10 ± 18, 18 ± 30 and 39 ± 102 g/d respectively.

DISCUSSION

All the women in the present study were well nourished and healthy. Their body weight gain during pregnancy, birth weight of their infants and placental weights are comparable to well nourished Western women[28]. This study was designed to examine if during lactation, in well nourished Indian women, there was any energy saving associated with the basal metabolic rate (BMR) and thermic effect of a meal (TEM) which could help subsidize its energy cost. As breast milk production was not measured, a proxy indicator of adequate lactational performance is provided by the weight gain of the baby from birth to 12 weeks postpartum, in the subgroup of 14 women. This weight gain (2.06 kg) amounted to almost 70 percent of the birth weight (3.01 kg) and suggests that breast milk production, and consumption by the baby was more than adequate for optimal growth,

as these 14 infants were exclusively breast fed till after the 12 weeks postpartum measurement.

Maternal body weight at 12 weeks postpartum was significantly ($p < 0.05$) higher than at 12 weeks of gestation by 1.96 kg, but the reduction in the skinfold thickness by 7.5 mm would suggest that fat mass (FM) was lost (0.2 kg) over the same period. This would indicate that there was no gain in FM and that increment in weight was due to an increment in fat free mass (FFM). This conclusion can be misleading as skinfold measurements during pregnancy are unreliable because water retention and increased skin vascularity during pregnancy may result in erroneously thicker skinfolds[28]. It is generally accepted that the entire weight gain, calculated from the difference of body weight measured prior to pregnancy and 4 weeks postpartum, is attributed to a gain in adipose tissue and breast mass[28]. The use of skinfold thicknesses to determine fat gain during pregnancy was also found to be the least accurate of 5 different approaches used by Spaaij et. al.[4]. This inaccuracy in the estimation of FM from skin fold thicknesses was attributed to site specific changes[29-31]. It is, therefore, likely that at 12 weeks of gestation, in the Pregnant-Lactating Group, percent body fat and FM were overestimated and that FFM was underestimated. The skinfold method for the estimation of percent body fat and FM was employed in the current study for lack of alternative measures.

The observation, in a subgroup of 13 subjects, that the difference in body weight at 4 and 12 weeks postpartum was approximately 0.4 kg, while the increment in weight from 12 weeks of gestation to 4 and 12 weeks postpartum was 3.2 ± 2.8 and 2.8 ± 2.2 kg respectively, would suggest that hardly any fat was being mobilized (6.4 g/d). This would provide at most 242 kJ/d (57.9 kcal/d), assuming that the entire weight lost was fat mass. Similar, small losses of body weight (0.26 kg), were observed by Goldberg et. al.[3], between the 4 and 12 weeks postpartum. The weight loss between 12 and 24 weeks postpartum in the current study were also small (0.3 kg) providing 130 kJ/d (31.1 kcal/d), again assuming that all the weight lost was fat mass. This suggests that only small amounts of fat are mobilized during the initial 24 weeks postpartum, in well nourished Indian women.

The significant rise in the BMR, even when corrected for changes in body weight in the Pregnant-Lactating Group, as compared to the Control Group, would suggest that there is no energy saving associated with this process during lactation. The BMR expressed per kg body weight at 12 weeks postpartum was approximately 2.7 percent higher than Control Group values, but about 4 percent lower than values at 12 weeks of gestation. As the FFM was probably underestimated at 12 weeks of gestation in the Pregnant-Lactating Group, therefore, the BMR expressed per kg FFM must be an overestimate. The BMR at 24 weeks postpartum was not significantly different from that measured at 12 weeks postpartum, which can be attributed to the fact that the Pregnancy-Lactation Group subjects continued to breast feed till after 24 weeks postpartum. However, the BMRs measured during lactation in other longitudinal studies were similar to those measured after the cessation of lactation[3,12,32]. In a study on undernourished Indian women the BMR, measured every month for the first 6 months postpartum, was found to be similar to a group of non-pregnant, non-lactating controls[33].

The TEM measured at 12 weeks of gestation and at 12 and 24 weeks postpartum in the Pregnant-Lactating Group was comparable to the Control Group value. The postprandial fat oxidation rates in the Pregnant-Lactating Group subjects at the 12 and 24 weeks postpartum, were significantly higher, while the postprandial NPRQs tended to be lower, than the Control Group, suggesting a greater utilization of fat in the fed state. This pattern of fat utilization was not present during the fasted state.

Given an within-individual variability of 7.6 and 22.7 percent in the BMR and TEM respectively[34] we would have a 90 percent chance (power = 0.90) to detect differences $\geq$ 9 percent in the BMR and 26 percent in the TEM, when making between group comparisons, or differences of $\geq$ 6.5 and 19 percent in the BMR and TEM respectively, when making within group comparisons[35]. In other words, we can conclude that there was no significant energy saving associated with either the BMR or the TEM in the present study.

Thyroid hormones levels in the Pregnant-Lactating Group, at 12 and 24 weeks postpartum, appeared to have returned close to Control Group values, suggesting that

the reduction in BMR observed during the postpartum measurements may indeed be linked to the reduction in the total thyroid hormone levels. As hormone levels were only measured in a subgroup of 6 women a possible link between the raised BMR and thyroid hormone levels during pregnancy[34] can only be a matter of speculation requiring further detailed investigation, though the consensus of opinion is that net thyroxine turnover is not changed in normal pregnancy[36]. A more recent study by Harada et. al.[37] measured absolute free hormone levels in 339 pregnant women and 40 female controls and found a slight but significant rise in absolute free T_4 levels from 11 weeks of gestation onwards, and a significant rise in absolute free T_3 levels at 13-20 weeks of gestation in the pregnant subjects. These elevated levels were not above the normal range of the female controls. This rise in the absolute free hormone levels were attributed to a possible increased secretion of thyroid hormones, changes in peripheral metabolism, and or shifts in intracellular or extracelluar compartments[37].

The mean increment in energy (2.0 MJ/d) and protein (12.8 g/d) intakes in the Pregnant-Lactating Group at 12 weeks postpartum, as compared to 12 weeks of gestation, were similar to the FAO/WHO/UNU[1] recommendations of an extra energy intake of 2.1 MJ/d, but considerably lower than the extra 17.5 g/d of protein suggested for the first 6 months of lactation. The energy intakes at 24 weeks postpartum tended to be lower than at 12 weeks postpartum, but still significantly higher than the Control Group. The FAO/WHO/UNU[1] estimate of the energy cost of lactation assume an additional 835 kJ/d (200 kcal/d) being mobilized from fat stores, which would result in approximately 22 g of fat loss per day. This assumption does not find much support in the current study as changes in body weight and FM were small from 4 to 24 weeks postpartum. Studies on poor rural Philippino women have demonstrated that even with much lower increments in energy intake of 1.02 and 0.77 MJ/d (244 and 185 kcal/d), at 6 and 30 weeks postpartum respectively[32] lactational performance is adequate, using growth patterns of the breast-fed infants as proxy indicators for the adequacy of lactational performance. These authors, therefore, suggest that the current recommendations for energy intake during lactation are too high. Studies in well nourished lactating Dutch women[2] have demonstrated that the cost of lactation, between 5-13 weeks, was 2.64 MJ/d (630 kcal/d) with an average breast milk production of 720

± 124 g/d. This energy cost was met by eating 1.74/d MJ (415 kcal/d) more; mobilizing fat to produce 0.15 MJ/d (35 kcal); and by reducing energy expenditure by 0.75 MJ/d (180 kcal/d). These authors also reach the same conclusion with regard to the current recommendations of energy intakes during lactation.

The energy cost of lactation may possibly be met, despite low energy intakes, by reducing physical activity and, possibly, a greater efficiency in the conversion of dietary energy to milk energy. Goldberg et. al.[3] suggested that the cost of lactation is met by an increase in energy intake and reduction in physical activity. The study on undernourished Indian women have also reported the loss of body weight (body fat stores) and reduced physical activity as possible mechanisms by which the energy cost of lactation is met[33]. The other possibility is that the efficiency of conversion of food energy into breast milk is higher than the 80 percent assumed by the FAO/WHO/UNU joint expert consultation[1]. Frigerio et. al.[6] have proposed the figure of 95 percent as being more appropriate to calculate the energy cost of lactation. They argue that the use of the value of 94 percent for the efficiency of milk production would reduce the energy requirements for milk synthesis in lactation by 400 kJ/d (96 kcal/d). Other studies[3,5,7] have also indicated that the net metabolic efficiency of human milk synthesis is nearer the value suggested by Frigerio et. al[6].

In conclusion, the extra energy required during lactation appears to be met largely by increases in energy intake and to a lesser extent by fat mobilization, rather than any enhancement in the efficiency of maternal metabolism, in well nourished Indian women. However, the possibility of an enhanced efficiency in energy metabolism during lactation in chronically energy deficient women cannot be ruled out. In the light of an adequate lactational performance, low rate of body fat mobilization during the period of exclusive breast feeding, and energy intakes close to that recommended by the FAO/WHO/UNU joint expert consultation[1], it would appear that the total energy cost of lactation (3.0 MJ/d) may be an overestimate or the efficiency of conversion of food energy to milk energy may be an underestimate.

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Chapter 6

GENERAL DISCUSSION

The studies presented in this thesis were aimed at investigating energy expenditure and by extension, requirements, in well nourished Indian women in the reproductive age group under various physiological conditions. There has been a considerable amount of work carried out in developed countries with regard to energy expenditure following the recognition by the Food and Agriculture Organization /World Health Organization Ad Hoc expert committee[1] that energy requirements should be based on measurements of energy expenditure. However, the influence of ethnicity, climate, and differing social, cultural, and dietary habits require further investigation in population groups from all parts of the world for a truly global perspective.

All subjects recruited were either from the upper or middle socio-economic group, as assessed by Kuppuswamy's[2] urban socio-economic scale, had ad libitum access to food and were in good health. As studies on energy expenditure and intake require a considerable amount of subject compliance most women recruited tended to have a keen interest in nutrition and often a tertiary education.

The basal metabolic rate of Indian women

The basal metabolic rate (BMR) which accounts for almost 70 percent of the total 24-hour energy expenditure (TEE) in the sedentary individual[3] was measured in 60 well nourished Indian women aged 18-30 years, in the mid-follicular phase of their menstrual cycle (Chapter 2). The BMR of these subjects, adjusted for differences in weight (4.9 MJ/d), were found comparable to the BMR measured in 52 Indian women 50 years earlier (4.8 MJ/d).

Schofield et. al.[4] had compiled BMR data from all over the world, measured over the last

60 years, and developed a simple predictive equations for BMR from body weight. It was observed that the BMR of Indians (which comprised part of the database) was 12.8 percent lower in males and 12.9 percent lower in females of all ages, when compared to their age-, sex-, or weight matched European/American counterparts. It was implied that this was an ethnic feature of this population. Henry & Rees[5] reviewed the BMR measurements made in the tropics and using data not previously used by Schofield et. al.[4]. They demonstrated that the BMR of people living in the tropics was significantly lower than that predicted by Schofield's equations but the deviation seen in tropical women (-3.8%, n=350) was not as large as that seen in tropical men (-10.8%, n=350). However, the phase of the menstrual cycle was not taken into account when making these BMR measurements and the possible influence this may have had on producing the observed differences was questioned.

We, therefore, compared our data, which was collected in the mid-follicular phase of the menstrual cycle in all subjects, with data published in the literature on current European/American BMRs. This comparison indicated that present day Indian women have BMRs that are approximately 7 percent lower than their European/American counterparts. This can be explained possibly by differences in body composition and climate. This difference was further reduced when only the American data was used for the comparison. Thus the possibility of an ethnic feature being responsible for the entire observed difference seems unlikely.

A comparison of the measured BMR with that predicted by the equation of Schofield et. al.[4] showed that the equation was over-predicting the BMR in current European/American women by about 4 percent, while those of current Indian women by about 9 percent. This would suggest that the predictive equation of Schofield et. al.[4] for women in the 18-30 age group would significantly over-predict the energy requirements of well-nourished populations all over the world. International agencies that deliberate on these matters should review recommendations based on these equations. Further studies on the BMR together with accurate measures of body composition would go a long way in establishing if true differences in the BMR exist between young Indian women and their Western counterparts.

Changes in energy expenditure over the course of a menstrual cycle.

Of the main components of the total 24-hour energy expenditure, the BMR and the TEM together account for almost 80 percent of the TEE in sedentary individuals[3]. The hormones associated with the cyclical changes in the menstrual cycle are known to be thermogenic and may interact with other thermogenic hormones in the body, which may alter the energy expenditure in the pre- and post-ovulatory phases of the menstrual cycle. An increase in energy expenditure during the post-ovulatory phase the menstrual cycle have been reported by several workers[6-9]. However, other reports have failed to demonstrate such an increase in the BMR or TEM[10]. Reported increases of between 7-16 percent of the TEE[7,11] during the post-ovulatory period would require a significant increase in energy intake during this period. Changes in nutritional requirements in individuals at different time points, and whether these requirements have wide validity or are influenced by factors such as ethnicity, climate, or adaptation were listed as priority areas of research in the report of the joint Food and Agricultural Organization /World Health Organization /United Nations University (FAO/WHO/UNU) expert consultation[12].

The study presented in this thesis (Chapter 3) was designed to examine the effect of the different phases of the menstrual cycle on the BMR and TEM in well-nourished Indian women. In addition the change in BMR and the TEM between two consecutive menstrual cycles was also examined. The study was designed to have a 90 percent chance to be able to demonstrate a true difference of 5 percent in the BMR and 15 percent in the TEM measured in the pre (follicular) and post (luteal) ovulatory phases of a single menstrual cycle, and a true difference of 7.5 and 20 percent in the BMR and TEM respectively, between the pre- ovulatory phase of two consecutive menstrual cycles.

There were no significant differences in the BMR between the pre and post-ovulatory phases of the same menstrual cycle. The TEM, however, was significantly elevated during the post- ovulatory or luteal phase of the menstrual cycle, as compared to the

pre-ovulatory or follicular phase, by 18.5 percent. There are several possible explanations for this. Progesterone is know to have a thermogenic effect[13-15], however, it should have had an equal effect both on the basal and postprandial energy expenditures. This was not observed in the present study. Insulin is another hormone thought to be thermogenic[16-19]. Insulin levels rise markedly in response to a meal, it is possible that the tissue responsiveness to insulin is enhanced by estradiol[20], which is also present in higher concentrations during the post-ovulatory phase of the menstrual cycle. This would enhance glucose entry into the cell and result in an increased TEM. In addition estradiol is thought to elevate catecholamine levels in the plasma by competitive inhibition of catechol-O-methyl transferase[21] (COMT) and inhibiting biological inactivation of the neurotransmitter by COMT in the liver[22]. This adrenergic activation may further stimulate ovarian progesterone production[23]. The elevated TEM observed during the post-ovulatory (luteal) phase of the menstrual cycle may, therefore, be a consequence of the activation of the sympathetic nervous system, which is thought to account for between 30-70 percent of the response[23,24]. The elevation of the TEM in the post-ovulatory phase of the menstrual cycle may be of academic significance, however, in nutritional terms it amounts to less than 20 kJ per meal. This is unlikely to result in any substantial increase in energy requirements. Fasting plasma glucose, insulin, thyroid stimulating hormone (TSH), tri- iodothyronine (T_3), thyroxine (T_4) measured in 5 of the subjects in both phases of the same menstrual cycle were found to be comparable. There was no observed difference in the dietary energy or macro-nutrient intake between the pre- and post-ovulatory phases of the menstrual cycle in contrast to the observations of Dalvit[26], but in keeping with the observations of Fong and Kretsch[27].

The differences in the BMR and the post meal total energy output (PMTEO), between the same phase of two consecutive menstrual cycles were small, and not statistically significant, however, the difference in the TEM was approximately 10 percent. This would suggest that the calculation of the TEM is influenced by small changes in the BMR.

We conclude that the changes in energy expenditure in relation to the BMR and TEM over the course of a single menstrual cycle, and between two consecutive cycles, are

small, in contrast to other reports. In nutritional terms the significant rise in post-ovulatory TEM is small and is unlikely to alter energy requirements in well-nourished women from the reproductive age group.

Energy expenditure during pregnancy

Enhanced metabolic efficiency was postulated as a possible mechanism by which well nourished women were able to meet the high energy cost of pregnancy, while only increasing their dietary intakes marginally. The study presented in this thesis (Chapter 4) was carried out to determine if any enhancement in metabolic efficiency could be detected in the BMR and TEM during pregnancy, as almost 80 percent of the total 24-hour energy expenditure is accounted for by these processes[3]. Also to determine if the cost of pregnancy in well nourished Indian women was comparable to other well nourished women and establish to what extent increments in dietary intake met the additional energy costs of pregnancy.

Results indicated no enhancement of metabolic efficiency in relation to the basal metabolic rate (BMR) and the thermic effect of a meal (TEM). In fact the BMR expressed per kg body weight or FFM appeared to be higher than the non-pregnant, non-lactating controls. The increase in the BMR has been attributed to the metabolic contribution of the uterus and its contents and by the increased work of maternal heart and lungs[28]. Other possible explanations include evidence of greater protein turnover during pregnancy[29], as even at a conservative estimate the energy cost of protein synthesis accounts for 15-20 percent of O_2 consumption[30-32]. The concomitant significant increase in the total circulating thyroid hormones could possibly provide an alternative explanation for this rise in BMR. However, this association of the increase in BMR with the circulating levels of absolute free T_3 needs further detailed examination.

Estimates of body composition from skinfold thicknesses even at 12 weeks of gestation appeared to be unreliable. The estimated gain in fat mass (FM) (calculated from body

weight and percent body fat estimated from the sum of 4 skinfold thicknesses, using the equations of Durnin and Womersley[33]), from 12 weeks of gestation to 4 weeks postpartum, was only about 0.1 kg, despite an increase of almost 3.6 kg in body weight. This would imply that the skinfold thicknesses measured at 12 weeks gestation did not reflect the true fat mass, or that the weight gained was not over the upper body where these skinfold thicknesses were measured. This is in keeping with current knowledge, that the weight gain during pregnancy is predominantly over the lower body[34]. The reduction in postpartum skinfold thickness is thought to be due to the loss of accumulated water, and/or due to the regression of the increased skin vascularity characteristic of pregnancy[34]. Therefore the inability to demonstrate a gain in fat mass based on these 4 skin-fold thicknesses is not surprising. This approach has been found to be the least accurate of five different approaches used to estimate fat gain during pregnancy by Spaaij et. al.[35] and was attributed to site specific changes[36-38]. This method was employed to measure body fatness for lack of alternative measures. It is, therefore, likely that the differences in BMR and PMTEO when expressed per kg FFM, were smaller than reported, as percent body fat and FM were probably overestimated at 12 weeks of gestation.

The cumulative increase in BMR over the final two trimesters was 143 MJ, while the gain in maternal fat stores (estimated by the factorial approach) amounted to 100 MJ, leaving 45 MJ as the cost incurred for the growth of the fetus, placenta, uterus, breasts and formation of blood and extracellular and amniotic fluid. This resulted in an energy cost of pregnancy of 288 MJ. This value is comparable to that obtained for well nourished Western women[35,39-41], but considerably more than that observed in African women[42].

In contrast to the studies in the West on European women, a significant increase in energy intake over the last two trimesters of pregnancy, which amounted to 290 MJ, was observed. This would indicate that all the extra cost of pregnancy was met by an increase in maternal food intake. This is perhaps the first time that increments in food intake have been shown to be adequate to meet the energy cost of pregnancy. Though a clear relationship between the total energy cost of pregnancy and total increment in food

intake could not be established, there was a correlation (r=0.44), of borderline significance (p=0.07), between the increment in the BMR and that of energy intake, between 12 and 34 weeks of gestation. The methodology employed to estimate energy intake was less accurate than that employed by studies in the West. However, this may have been to our advantage as it was less likely to influence the pattern of eating, subjects not having any idea as to how much they were eating in terms of weight. Besides, there was probably little cultural pressure on women to maintain slim figures during pregnancy and motherhood. A combination of these factors may have been instrumental in obtaining these data.

In conclusion there appears to be no enhancement in the efficiency of energy metabolism associated with the BMR and TEM during pregnancy. The gain in fat mass (estimated by the factorial approach) compares well with values reported for other well nourished women. The energy cost of pregnancy was found to be similar to that obtained in well nourished populations, however, the birth weight of infants were lower than those observed in European women, possibly related to the smaller stature of the mother. Extra energy intakes over pregnancy were large and apparently accounted for all the extra energy expenditure incurred during this period. The FAO/WHO/UNU[12] recommendations for extra energy intakes during pregnancy appear to be justified.

Energy expenditure during lactation

Lactation imposes a considerable stress on the mother. The FAO/WHO/UNU joint expert consultation[12] recommended a extra 2.1 MJ/d based on the assumptions that the efficiency of conversion of food energy to breast milk energy was 80 percent, and that about 835 kJ (200 kcal)/d of energy was mobilized from fat stores. However, studies in developed countries, where mothers have ad libitum access to food have demonstrated that the energy intake during lactation while being higher than the pre-pregnant state is frequently less than 2.1 MJ/d [43-45]. This presents several possibilities. The volume of breast milk being produced may be overestimated, or the efficiency of conversion of food

energy to breast milk energy may be higher than 80 percent[44-47]. There may be a mobilization of fat stores built up during pregnancy, to meet the extra requirements of lactation[48-53]. The degree of physical activity may possibly be reduced, thus saving energy[46]. Finally there is a possibility of an enhanced metabolic efficiency which may help subsidize the cost of pregnancy [43,46,51,52].

The study presented in the thesis (Chapter 5) attempts to address the final possibility. As breast milk production was not measured directly or indirectly, a proxy indicator of adequacy of lactational performance was provided by the increment in weight of the infant from birth to 12 weeks of age. There was no reduction in the BMR or TEM in absolute terms or when expressed per kg body weight or FFM, indicating the absence of any enhancement of metabolic efficiency. The increments in energy and protein intake at 12 weeks postpartum were 2.0 MJ (482 kcal)/d and 13 g/d respectively, over those measured at 12 weeks of gestation. The FAO/WHO/UNU joint expert consultation[12] recommendation for energy intake during lactation was based on the assumptions that the total energy cost of lactation was around 3.1 MJ (750 kcal)/d and that approximately 835 kJ (200 kcal)/d of energy would be provided by the mobilization of body fat, the remainder by an increase in food intake and a reduction of physical activity. However, the 0.36 kg reduction in weight indicated that the mobilization of body fat was considerably below that envisaged by the FAO/WHO/UNU joint expert consultation[12]. The adequate lactational performance observed in this study would suggest that the cost of lactation was met largely by an increment in food intake (and perhaps a reduction in physical activity), mobilized fat appeared to contribute only a small part of the total energy requirement. As the increment in energy intake was close to that recommended by the FAO/WHO/UNU joint expert consultation[12] and lactational performance was adequate (despite the low quantities of fat being mobilized) either the total energy cost of lactation or the volume of breast milk required for optimal infant growth may have been overestimated by the FAO/WHO/UNU joint expert consultation[12]. The other possibility being that the efficiency of conversion of food energy to milk energy may have been underestimated.

Conclusion

In conclusion, the differences in the basal metabolic rate of present day non-pregnant, non- lactating Indian and European/American women in the reproductive age group can possibly be explained by differences in body composition and climate, rather than the effect of a postulated ethnic feature alone. Changes in the BMR and TEM over the course of a single menstrual cycle are small and unlikely to affect energy requirements in women of the reproductive age group. There is no evidence of an enhancement of the efficiency of energy metabolism with regard to the BMR and TEM, either during pregnancy or lactation in well nourished Indian women. Therefore, no energy saving can be expected that may possibly help subsidize the energy cost of pregnancy or lactation. Estimates of energy intakes suggest that almost the entire cost of pregnancy and lactation is met by concomitant increments in energy intake. Well nourished Indian women apparently cope with the stress of pregnancy and lactation by eating more. The estimated cost of pregnancy of well nourished Indian women is similar to that observed in well nourished populations in developed countries, justifying FAO/UNU/WHO[12] recommendations for energy intake during pregnancy. However, in the light of an adequate lactational performance, increments in energy intake close to values recommended by the FAO/WHO/UNU[12], and mobilization of only very small amounts of fat from stores, it would appear that the FAO/WHO/UNU[12] joint expert consultation[12] may have overestimated either the total energy cost of lactation or the volume of breast milk required for optimal infant growth. The other possibility being that the efficiency of conversion of food energy to milk energy may have been underestimated.

Future studies on the BMR in non-pregnant non-lactating Indian women with accurate estimates of body composition, perhaps using stable isotopes to estimate total body water, would go a long way to resolving the presence or absence of an ethnic feature which may be responsible for the lower BMRs seen in women from the tropics. The possibility of an enhanced efficiency of energy metabolism in chronically energy deficient (CED) women during pregnancy and lactation cannot be ruled out. Future studies on

CED women examining the BMR and the total energy expenditure, possibly by a combination of indirect calorimetry and the doubly labelled water (DLW) technique would provide invaluable information on energy metabolism and body composition in these individuals.

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SUMMARY

This thesis has examined the energy expenditure, and hence energy requirements, of young Indian women in the reproductive age group. The measured basal metabolic rates (BMR) of present day Indian women (4.9 MJ/d, n=60) were found to be similar to published BMRs, measured in Indian women over 50 years ago (4.8 MJ/d, n=52), when adjusted for differences in body weight. Published BMRs of present European/American women were found to be approximately 7 percent higher than present Indian women, after adjusting for differences in body weight. Factors such as differences in body composition and climate may be partly responsible for this difference, rather than an *ethnic* factor alone. Schofield's equation, used to predict the BMR of women in the 18-30 age group, from simple anthropometric measures such as body weight, was found to over-estimate the BMR of young Indian women by approximately 9 percent. This equation also overestimated the BMR of young American women in the reproductive age group by almost 7 percent. This would suggest that this commonly used equation may be overestimating the energy requirements of all populations (Chapter 2).

The effect of the menstrual cycle on the BMR and the thermic effect of a meal (TEM) was measured in the pre-ovulatory and post-ovulatory phases of a single menstrual cycle in young Indian women. Studies on well nourished women had suggested that there was a significant increase in energy expenditure (basal metabolic rate, sleeping metabolic rate, and total energy expenditure) during the post-ovulatory phase of the menstrual cycle. Such differences, should they exist, may influence the energy requirements in women from the reproductive age group. In the current study (n=13) the BMR was not significantly different between the pre- and post-ovulatory phases of the menstrual cycle, however, the TEM was approximately 18 percent higher ($p<0.05$) during the post-ovulatory phase. This increment in the TEM was small in absolute terms and is unlikely to affect the energy requirements of these individuals. The differences in BMR and the post meal total energy output (PMTEO) were also found to be small between the same phase of consecutive menstrual cycles, however, the difference in TEM amounted to about 10 percent suggesting that small changes in the BMR affect its calculation (Chapter 3).

Pregnancy is an energetically expensive phase during the life of a woman and would appear to require substantial increments in energy intake. However, the measured increment in energy intake in well nourished women rarely appear to be adequate to meet all the costs of pregnancy. Therefore, saving of energy by an enhancement of the efficiency of maternal energy metabolism was postulated as a possible mechanism by which the costs of pregnancy and lactation are met. The BMR and the TEM were, therefore, measured at 12, 24, and 34 weeks of gestation, in a group of 18 well nourished Indian women and compared to a group of 18 non-pregnant, non-lactating controls. The BMR was found to be higher throughout pregnancy, as compared to the non-pregnant, non-lactating controls, while the TEM was not different at any time point studied. This would suggest that no energy saving was associated with either basal metabolism or diet induced thermogenesis during pregnancy. The mean weight gain from 12-40 weeks of gestation was 11.4 kg, mean birth weight of the infant was 3.06 kg and the mean placental weight was 0.63 kg. The estimated gain in adipose tissue and fat mass from an analysis of the weight gain during pregnancy was 3.1 and 2.2 kg, respectively. These figures suggest that well nourished Indian women have a pregnancy performance comparable to well nourished women from developed countries. The total energy cost of pregnancy was estimated to be 288 MJ, from the cumulative in crease in basal metabolism (143 MJ), estimates of fat and protein deposited in the foetus (45 MJ) and the estimated gain in fat stores (100 MJ). This figure is close to the Food and Agriculture /World Health Organization /United Nations University (FAO/WHO/UNU) estimate of the cost of pregnancy of 335 MJ. The dietary energy intake, estimated at the same time as the metabolic and anthropometric measurements during pregnancy, appeared to be substantially increased during the second and third trimesters of pregnancy, in contrast to the small increases seen in women from developed countries. This increase in energy intake (290 MJ) was apparently adequate to meet all the extra energy expenditure associated with pregnancy (Chapter 4).

Lactation is thought to impose a considerable stress on the mother and the FAO/WHO/UNU joint expert consultation estimated its energy cost to be about 3.1 MJ (750 kcal)/d. The FAO/WHO/UNU joint expert consultation recommended an extra energy intake of 2.1 MJ/d based on the assumption that the average woman would start

lactation with 150 MJ (36000 kcal) of fat reserves, and that a normal body composition would be re-established by 6 months postpartum. This reserve of fat was expected to provide approximately 835 kJ (200 kcal)/d. However, recent studies have not been able to demonstrate a large mobilization of fat during lactation, nor have large increments in energy intake been observed. The suggestion was, therefore, made that this discrepancy between the theoretical estimate of the energy cost of lactation and the energy provided by increments in intake and mobilization of fat may partly be explained by an enhancement of the efficiency of energy metabolism. The BMR and TEM was, therefore, compared in 18 non-pregnant, non-lactating controls and 17 Pregnant-Lactating women at 12 weeks of gestation, 12 and 24 weeks postpartum. The BMR was found to be significantly ($p<0.05$) higher in the Pregnant-Lactating group at all time points studied. The TEM was not significantly different between the groups studied. This would suggest that there was no energy saving associated with basal metabolism and diet induced thermogenesis during lactation. During this period (0-24 weeks postpartum) only small amounts of fat were mobilized. In the light of an adequate lactational performance (as assessed the gain in weight from birth to 12 weeks postpartum in the infants while exclusively breast feeding), energy intakes close to that recommended by the FAO/WHO/UNU joint expert consultation, and small amounts of fat being mobilized, it would appear that either the total energy cost of lactation or the volume of milk required for optimal growth of the infant is currently being overestimated. The other possibility is that the efficieny of conversion of food energy to milk energy is being underestimated (Chapter 5).

SAMENVATTING

In dit proefschrift is onderzoek beschreven naar het energieverbruik en, daarmee samenhangend, de energiebehoefte van jonge Indiase vrouwen in de vruchtbare leeftijd. Indien gecorrigeerd wordt voor het lichaamsgewicht, is het bij deze vrouwen gemeten basaalmetabolisme (BMR, basal matabolic rate) (4,9 MJ/dag, n=60) vergelijkbaar met de waarde zoals deze bij Indiase vrouwen meer dan 50 jaar geleden gemeten is (4,8 MJ/dag, n=52). In de literatuur gerapporteerde BMRs van hedendaagse Europese en Amerikaanse vrouwen zijn, na corrigeren voor lichaamsgewicht, ongeveer 7% hoger zijn dan die van de Indiase vrouwen in ons onderzoek. Dit is waarschijnlijk niet alleen toe te schrijven aan een ethnisch verschil, maar verschillen in lichaamssamenstelling en klimaat zijn wellicht ook ten dele verantwoordelijk. Het gebruik van de vergelijking van Schofield om de BMR van vrouwen in de leeftijdsgroep van 18-30 jaar te voorspellen op basis van eenvoudige anthropometrische grootheden als lichaamsgewicht, leidt tot een overschatting van de BMR van de jonge Indiase vrouwen in ons onderzoek met ongeveer 9%. Deze vergelijking zou echter ook de BMR van jonge Amerikaanse vrouwen met bijna 7% overschatten. Dit doet vermoeden dat deze veelgebruikte voorspellingsvergelijking in zijn algemeenheid het basaalmetabolisme, en dus de energiebehoefte doet overschatten (Chapter 2).

Onderzoek uitgevoerd bij goed gevoede vrouwen doet vermoeden dat er een significante toeneming is in energieverbruik (o.a. in basaalmetabolisme, metabolisme tijdens slaap, en dagelijks energieverbruik) in de post-ovulaire fase van de menstruele cyclus. Indien dit daadwerkelijk het geval is, dan kan dat wellicht de dagelijkse energiebehoefte beïnvloeden. In onze studie (n=13) was de BMR in de pre- en postovulaire fase van dezelfde menstruele cyclus niet significant van elkaar verschillend, maar het thermisch effect van een maaltijd (TEM, thermic effect of meal) was in de post-ovulaire fase ongeveer 18% hoger ($p<0.05$). Deze toeneming in de TEM is echter in absolute zin gering en zal de energiebehoefte van deze vrouwen waarschijnlijk niet beïnvloeden. Ook tussen dezelfde fasen van opeenvolgende cycli waren de verschillen in BMR en absolute energieverbruik na een maaltijd (PMTEO, post meal total energy output) klein, maar de verschillen in TEM liepen op tot ongeveer 10 procent, hetgeen nog eens duidelijk

maakt dat kleine veranderingen in BMR de berekening van de TEM flink beïnvloeden (Chapter 3).

Energetisch gezien is de zwangerschap een kostbare fase in het leven van een vrouw. Men zou dus substantiële toenemingen in energie-inneming mogen verwachten. In goed gevoede vrouwen is deze toeneming in energie-inneming echter zelden toereikend om in de totale kosten van de zwangerschap te kunnen voorzien. Daarom is wel verondersteld dat een verhoging van de efficiëntie van het energiemetabolisme van de moeder een goed mechanisme zou zijn om de kosten van de zwangerschap, althans voor een deel, te dekken. Teneinde deze hypothese te onderzoeken werden de BMR en de TEM gemeten in een groep van 18 goed gevoede Indiase vrouwen bij 12, 24, en 34 weken in de zwangerschap, en werden de waarden vergeleken met die van een niet-zwangere niet-lacterende controle groep (n=18). De BMR was tijdens zwangerschap hoger dan in de controle group, maar de TEM bleef onveranderd. Dit suggereert dat tijdens de zwangerschap geen energie gespaard wordt door aanpassing van basaalmetabolisme of van 'door voeding geïnduceerde thermogenese'. De gemiddelde gewichtstoeneming van 12 tot 40 weken zwangerschap was 11,4 kg, het gemiddelde geboortegewicht was 3,06 kg en het gemiddelde placenta-gewicht 0,63 kg. De geschatte toeneming in vetweefsel en vetmassa berekend uit de gewichtstoeneming over de zwangerschap bedroeg respectievelijk 3,1 en 2,2 kg. Deze cijfers doen vermoeden dat goed gevoede Indiase vrouwen een 'pregnancy performance' hebben welke vergelijkbaar is met die van goed gevoede vrouwen uit westerse landen. De totale kosten van de zwangerschap werden geschat op 288 MJ, bestaande uit een cumulatieve stijging in basaalmetabolisme (143 MJ), een geschatte afzetting van eiwit en vet in foetus (45 MJ) en de geschatte toeneming in vetopslag (100 MJ). Dit cijfer wijkt niet veel af van de FAO/WHO/UNU-schatting van de kosten van de zwangerschap (335 MJ). De energie-inneming met de voeding nam substantieel toe in het tweede en derde trimester van de zwangerschap, in tegenstelling tot de geringe toenemingen die bij vrouwen in de westerse landen gevonden worden. De toeneming in energie-inneming (290 MJ) was blijkbaar voldoende om de extra kosten van het zwanger zijn te compenseren (Chapter 4).

Men neemt aan dat de lactatie een aanzienlijke energetische stress betekent voor de

moeder en de FAO/WHO/UNU schat de extra kosten op 3,1 MJ (750 kcal) /dag. De FAO/WHO/UNU beveelt een extra energie-inneming van 2,1 MJ/dag aan gebaseerd op de aanname dat de gemiddelde vrouw de lactatie ingaat met 150 MJ (36000 kcal) aan vetreserves, en dat haar lichaamsamenstelling rond 6 maanden postpartum weer genormaliseerd is. Deze vetreserve wordt geacht gemiddeld voor 835 kJ (200 kcal) /dag te leveren. In recente studies kon tijdens de lactatie echter geen grote vetmobilisatie aangetoond worden, maar ook geen grote toenemingen in energie-inneming. Daarom is wel gesuggereerd dat de discrepantie tussen de theoretische schatting van de energiekosten van de lactatie enerzijds, en de energie uit voedsel en uit vetmobilisatie anderzijds, misschien ten dele verklaard kon worden door een verhoging van de efficiëntie van het energiemetabolisme. De BMR en de TEM van 18 niet-zwangere niet-lacterende vrouwen werden daarom vergeleken met die van 17 zwangere/lacterende vrouwen bij 12 weken zwangerschap en bij 12 en 24 weken postpartum. De BMR bij de zwangere/lacterende groep was op alle tijdsmomenten significant hoger ($p<0.05$). De TEM was niet verschillend tussen beide groepen. Dit suggereert dat er tijdens de lactatie geen energie bespaard wordt middels aanpassing van het basaalmetabolisme of van 'door voeding geinduceerde thermogenese'. Gedurende deze periode (0-24 weken postpartum) werden slechts kleine hoeveelheden vet gemobiliseerd. Daar de 'lactational performance' adequaat lijkt (gebaseerd op de gewichtstoeneming van geboorte tot 12 weken postpartum in zuigelingen die uitsluitend borstvoeding krijgen), de energie-innemingen dicht bij de aanbevelingen liggen, en slechts kleine hoeveelheden vet gemobiliseerd worden, lijkt het erop dat òf de totale energiekosten van de lactatie òf het melkvolume dat nodig is voor de optimale groei van het kind momenteel overschat worden. Een andere mogelijkheid is dat de efficiëntie waarmee voedselenergie omgezet wordt in melkenergie onderschat wordt (Chapter 5).

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CURRICULUM VITAE

Leonard Sunil Piers was born on 26 October 1961 in Bangalore, India. He completed his secondary education in 1977, at St. Joseph's Boys High School, and pre-university education in 1979, at St. Joseph's College. In 1980 he joined St. John's Medical College, Bangalore. He successfully completed his medical studies in 1985 and rotating internship, at St. John's Medical College Hospital, in 1986, following which he was awarded a 'Bachelors degree in Medicine and Surgery' (M.B., B.S.) from the Bangalore University, India. He then joined the Nutrition Research Centre and Department of Physiology in May 1986, as a 'Research Fellow'. In January 1988 he started the postgraduate program in 'Human Physiology'. On successfully completing the program he was awarded the 'Doctor of Medicine' (M.D.) degree in Human Physiology in 1990, from the Bangalore University, India. He was also awarded a 'Senior Research Fellowship' by the Indian Council of Medical Research (ICMR) in 1989, for a period of 3 years. In 1989 he spent 3 months at the Department of Human Nutrition, Wageningen Agricultural University, The Netherlands, and successfully completed the preparatory phase of the Ph.D. program. Since then he has been engaged in carrying out the work described in this thesis at the Nutrition Research Centre, Bangalore, India. He returned in October 1993 to complete the final phase of the Ph.D. program at the Department of Human Nutrition, Wageningen Agricultural University, The Netherlands.

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