CHARLES UNIVERSITY IN PRAGUE

PHARMACY FACULTY IN HRADEC KRALOVE

DEPARTMENT OF BIOLOGICAL AND MEDICAL SCIENCES

DIPLOMA THESIS

Resting Energy Expenditure During Lactation

Head of diploma thesis: PharmDr. Miloslav Hronek, Ph.D.

HRADEC KRÁLOVÉ, 2010 MARTINA PLACHA

I would like to thank Pharm Dr. Miloslav Hronek, Ph.D. for his boundless patience and continuous help with writing this diploma thesis.

Aim of study

The focus of the entire study was to derive a new equation for predicting resting energy expenditure (REE) during pregnancy based on the Harris-Benedict equation, since the basis of Harris-Benedict equation does not take into account pregnant women. This thesis is a fragment of the study focusing on finding correlations of measured REE in lactating women to anthropometrical parameters by analyzing maternal and lactation changes in the body and milk production. In the past, in Czech Republic, Resting Energy Expenditure (REE) was not known in pregnant or lactating women. The measurements of resting energy expenditure were conducted in 5 different time periods via indirect calorimetry to determine correlations between Resting Energy Expenditure and measured anthropometrical values. The results obtained from our study are intended for clinical use since up until now resting energy expenditure was obtained from internationally published results, and were not a direct measure for Czech women due to differences in terms of nutrition and calories. As well, by comparing our results to similar studies conducted internationally we can determined whether our results are similar or prove to be entirely different from international results.

Theoretical Component

1 Human Energy Requirements

The energy requirements of humans is estimated from measures of energy expenditure along with additional energy needs for growth, pregnancy and lactation. The recommendations for dietary energy intake from food must satisfy the energy requirements to attain and maintain optimal health, physiological function and well-being (FAO/WHO/UNU, 2004). The daily energy requirements of the individual are highly variable. They are dependent on numerous factors, for example, age, sex, amount of physical activity, stage of ovarian cycle in females, and environmental temperature (www. gpnotebook.com).

2 Energy Requirement

The general assumption is that requirements for energy will be fulfilled through the consumption of a diet that satisfies all nutrient needs. Energy requirement is the amount of food energy needed to balance energy expenditure in order to maintain body size, body composition and a level of necessary and desirable physical activity consistent with long-term good health. Also included is, the energy needed for optimal growth and for the deposition of tissues during pregnancy, and for the secretion of milk during lactation consistent with the good health of the mother and child. The recommended level of dietary energy intake for a population group is the mean energy requirement of the healthy, well-nourished individuals who represent that group (FAO/WHO/UNU, 2004).

2.1 Daily Energy Requirements and Daily Energy Intakes

Daily requirements or recommended daily intakes are energy requirements and recommended levels of intake, expressing the requirement of average energy needed over a certain number of days. As well, the recommended energy intake is the amount of energy that should be ingested as a daily average over a certain period of time. The fact that physical activity and eating habits may vary on some days of the week, periods of seven days are often used when estimating the average daily energy expenditure and recommended daily intake (FAO/WHO/UNU, 2004).

2.2 Average Requirement and Inter-individual Differences

The energy requirement estimates are derived from measurements of a collection of individuals of the same gender and similar age, body size and physical activity. These measurements give the average energy requirement – or recommended level of dietary intake – for a class or population group of people. These requirements are then used to predict the requirements and recommended levels of energy intake for other individuals with similar characteristics that did not undergo these measurements. Although there remain unknown factors that produce variations among individuals of the same class or population group (FAO/WHO/UNU, 2004).

2.3 Energy Requirements of Pregnancy

Energy requirements and recommendations for energy intake of pregnant women should be population-specific, because of differences in body size, lifestyle and underlying nutritional status. Even within a particular society, high variability is seen in the rates of gestational weight gain and energy expenditure of pregnant women, and therefore in their energy requirements (FAO/WHO/UNU, 2004).

2.4 Energy Requirements of Lactation

Energy requirement of lactation is the energy needed to produce an appropriate volume of milk that must be added to the woman's habitual energy requirement (FAO/WHO/UNU, 2004).

2.5 Determinants of Energy Cost of Pregnancy

The energy cost of pregnancy is determined by the energy needed for maternal gestational weight gain, which is associated with protein and fat accumulation in maternal, fetal and placental tissues, and by the increase in energy expenditure associated with basal metabolism and physical activity (FAO/WHO/UNU, 2004).

2.6 Determinants of Energy Cost of Lactation

The main factors that influence the energy needs of lactating women are the duration of breastfeeding and the extent of exclusive breastfeeding, these vary significantly in different societies therefore dietary energy recommendations for lactating women should be population specific (FAO/WHO/UNU, 2004).

3 *Introduction to Metabolism*

Metabolism is a set of chemical reactions that occur in living organisms to maintain life processes via the organism taking in various substances from the external environment and integrating, transforming, and eliminating the breakdown products. Metabolism can be divided into two categories, catabolic and anabolic. Catabolic reactions result in breaking down organic matter to produce energy, for example, proteins to amino acids, while anabolic reactions use energy to construct components of cells, for example, carbohydrates to triglycerides. Three basic classes of molecules are vital for life, amino acids, carbohydrates, and lipids. Metabolic reactions use these molecules during construction of cells and tissues or break them down and use them as a source of energy (Holecek, 2010).

3.1 Metabolic Response to Food

Eating requires energy for the ingestion and digestion of food, and for the absorption, transport, interconversion, oxidation and deposition of nutrients. Metabolic processes increase heat production and oxygen consumption, known as dietary-induced thermogenesis, specific dynamic action of food and thermic effect of feeding. The metabolic response to food increases total energy expenditure by about 10% of the basal metabolic rate over a 24-hour period in individuals eating a mixed diet (FAO/WHO/UNU, 2004).

3.2 Pregnancy

During pregnancy, extra energy is needed for the growth of the fetus, placenta and various maternal tissues, such as in the uterus, breasts and fat stores, as well as for changes in maternal metabolism and the increase in maternal effort at rest and during physical activity (FAO/WHO/UNU, 2004).

3.3 Lactation

The energy cost of lactation has two components: a) the energy content of the milk secreted; and b) the energy required to produce that milk. Well-nourished lactating women can derive part of this additional requirement from body fat stores accumulated during pregnancy (FAO/WHO/UNU, 2004).

4 Energy Metabolism

The *metabolism* of the body simply means all the chemical reactions in all the cells of the body (Guyton, 2006). Humans oxidize carbohydrates, proteins, and fats producing primarily CO_2, H_2O, and the energy necessary for life processes. CO_2, H_2O, and energy are also produced when food is burned outside the body. The amount of energy liberated by the catabolism of food in the body is the same as the amount liberated when food is burned outside the body. The energy liberated by catabolic processes in the body is used for maintaining body functions, digesting and metabolizing food, thermoregulation, and physical activity. It appears as external work, heat, and energy storage. Energy output is equal to external work + energy storage + heat. The amount of energy liberated per unit of time is the *metabolic rate*. Essentially all of the energy of contractions appear as heat, because little or not external work is done. Energy is stored by forming energy rich compounds. The amount of energy storage varies, but in fasting individuals it is zero or negative. In an adult individual who has not eaten recently and who is not moving or growing or reproducing or lactating, all of the energy output appears as heat (Ganong, 2005).

4.1 Basal Metabolism

Comprises a range of functions that are essential for life; cell function, replacement, synthesis, secretion and metabolism of enzymes and hormones to transport proteins and other substances and molecules. As well as, maintaining of body temperature, uninterrupted work of cardiac and respiratory muscles, and brain function. Basal metabolism increases in pregnancy as a result of accelerated tissue synthesis, increased active tissue mass, and increased cardiovascular and respiratory work (FAO/WHO/UNU, 2004).

4.2 Basal Metabolic Rate

The speed of metabolism is the basal metabolic rate and is a measure of basal metabolism. It is standardly expressed as heat production per unit of body surface area per day. Uniform criteria are taken for its measurement, so minimizing the effects of variation with (www.gpnotebook.com):

- age
- sex
- body weight
- height
- time of day
- hormonal variations e.g. individual thyroid function, stage of ovarian cycle in females
- recent food
- recent exercise
- ambient temperature variation

For the average man, **basal** metabolic rate (BMR) is about 1671.92 kilocalories per day. In practice, the **resting** metabolic rate (RMR) is a more practical index used in the assessment of energy requirement (www.gpnotebook.com).

BMR is measured under standard conditions that include being awake in the supine position after 10 to 12 hours of fasting and eight hours of physical rest, and being in a state of mental relaxation in an ambient environmental temperature that does not elicit heat-generating or heat-dissipating processes (FAO/WHO/UNU, 2004). Additionally, BMR requires measurement in the early hours of the morning when metabolism is minimal(www.gpnotebook.com). It is important to note that in clinical practice the terms BMR and Resting Energy Expenditure (REE) are used interchangeably (Cuerda, 2007).

The Harris-Benedict equations can be used to provide approximations of REE based on data taking into account sex, age, weight and height (Roza, 1984):

For women:

$$\mathbf{REE_{HB} = 655.096 + 9.563 \times W + 1.850 \times H - 4.676 \times A}$$

For men:

$$\mathbf{REE_{HB} = 66.473 + 13.752 \times W + 5.003 \times H - 6.755 \times A}$$

Where **W** is the weight in kg; **H** is height in cm; **A** is age in years.

Harris-Benedict equations tend to overestimate energy expenditure by 10-15%. Nevertheless, they can be applied to work out a practically-relevant daily energy expenditure in combination with the energies of activity and diet-induced thermogenesis (www.gpnotebook.com).

As well, Schofield's equation can be used to estimate BMR. The units are weight in kg and BMR in kcal/24hr (www.gpnotebook.com).

AGE/ MALE /FEMALE

15-18 years 17.6 x weight + 656 13.3 x weight + 690

18-30 years 15.0 x weight + 690 14.8 x weight + 485

30-60 years 11.4 x weight + 870 8.1 x weight + 842

over 60 years 11.7 x weight + 585 9.0 x weight + 656

More accurately, BMR can be measured clinically in one of two ways (www.gpnotebook.com):

1) direct calorimetry where the subject sits in a respiratory chamber and the amount of heat produced is measured

2) indirect calorimetry is based on the respiratory quotient; a more practical means of assessing energy requirements.

4.3 Resting Metabolic Rate

The *resting* metabolic rate is an index closely related to the basal metabolic rate. It is the background energy required for basic metabolic functions when lying quietly awake. It is more practical than BMR because it does not have to be measured at the time of minimal expenditure in the early hours of the morning. Typically, it represents 60-70% of the total daily energy expenditure - approximately 20-24 kcal/kg/day - after subtraction of the energies of activity and dietary thermogenesis (www.gpnotebook.com).

4.4 Factors affecting Metabolic Rate

Factors affecting metabolic rate are many, the most important is muscular exertion because O_2 consumption is elevated not only during exertion but also long afterward and it is necessary to repay the O_2 deficit. Recently ingested foods also increase the metabolic rate due to their specific dynamic action which is the obligatory energy expenditure that occurs during the food's assimilation into the body. As environmental temperature decreases below body temperature, heat producing mechanisms like shivering are activated and metabolic rate rises. If environmental temperature is high enough to raise the body temperature, the metabolic processes accelerate and the metabolic rate rises around 14 % for each degree Celsius of elevation. Other factors include; height, weight, surface area, sex, age, growth, reproduction, lactation, emotional state, body temperature, circulating levels of thyroid hormones and epinephrine and nor epinephrine levels (Ganong, 2005).

4.5 Respiratory Quotient

The respiratory quotient is the ratio in the steady state of the volume of CO_2 (diffusing from blood into alveoli of the lung) produced to the volume of O_2 (diffusing in the opposite direction) consumed per unit of time (Ganong, 2005). The normal value is approximately 0.8, and may be used to calculate the basal metabolic rate via indirect calorimetry (www.gpnotebook.com) The respiratory quotient can be calculated through the measured VO_2 and VCO_2. RQ is the ratio of VCO_2 to VO_2.

RQ= $VCO_2 \div VO_2$

RQ= respiratory quotient; VCO_2= carbon dioxide production; VO_2= oxygen consumption

RQ reflects the substrates, i.e. proteins, lipids, carbohydrates used during metabolism, each substrate has its own RQ value. If the values are less than 0.65 and more than 1.25, a non-steady state condition is usually implied (Indirect Calorimetry Handbook).

Table 1: Respiratory quotients for Various Substrates - RQ= VCO2 ÷ VO2

(Indirect Calorimetry Handbook).

Substrate Oxidation	RQ
Protein	0.8
Lipids (fats)	0.7
Carbohydrates (CHO)	1.0

RQ of CHO is 1.00 and that of fat is about 0.7 because H and O are present in CHO in the same proportions as in water, whereas in various fats, extra O_2 is necessary for the formation of H_20 (Ganong, 2005). RQ of protein in the body has an average value of 0.82 (Ganong, 2005). The approximate amounts of CHO, protein, lipids being oxidized in the body at any given time can be calculated from the RQ and the urinary nitrogen excretion. 24 hour urinary urea nitrogen excretion is used to calculate the non-protein RQ from which the amount of metabolized fats and carbohydrates can be determined (Holecek, 2010). 24 hour urinary creatinine excretion also gives an estimate of skeletal muscle mass depletion; however, is influenced by meat content of diet and renal function (www.gpnotebook.com).

4.6 Importance of Conditions in Relation to RER and RQ

The important difference between respiratory exchange ratio (RER) and respiratory quotient (RQ) is that RER is the ratio of CO_2 to O_2 at any given time whether equilibrium has been reached or not. It is affected by factors other than metabolism, and can be calculated for reactions outside the body, for individual organs and tissues, and for the whole body (Ganong, 2005). The airway measurement of VO_2 and VCO_2 in fact correspond to RER, while the RQ reflects the exchange of these gases at the cellular level. The steady state condition occurs when the measured RER and RQ are similar in value. That is, the exchange of oxygen and carbon dioxide are similar at the cellular level as those measured at the airway (Indirect Calorimetry Handbook).

5 Measuring Energy Expenditure

Measurement of resting energy expenditure can be measured in two ways by direct and indirect calorimetry. Indirect calorimetry is based on the principle that gas volumes and concentrations exchanged at the mouth reflect cellular metabolic activity. The patient's metabolic rate is calculated through measuring the difference between inspired and expired levels of oxygen consumption and carbon dioxide production. From this measurement we obtain oxygen consumption, VO_2, and carbon dioxide production, VCO_2 (Indirect Calorimetry Handbook).

Once these values are obtained, conversion to REE is possible through a metabolic cart by applying the Weir equation. In order for the Weir equation to be clinically acceptable, daily (24 hour) urinary nitrogen measurements have to be obtained as well to determine the protein metabolism not revealed in the exhaled gas analysis (Indirect Calorimetry Handbook).

Weir Formula (Holecek, 2010):

$$EE = [(3.941 \times VO_2) + (1.106 \times VCO_2)] \times 1.44 - 2.17 \times UN$$

EE = energy expenditure (kcal/day)

$VO_2 = O_2$ consumption (ml/min)

$VCO_2 = CO_2$ production (ml/min)

UN = total urinary nitrogen (g/day)

5.1 Accurate Monitoring

Indirect calorimetry studies can be performed in a variety of ways. They can be carried out on a continuous basis over several hours, days, or over alternating shorter periods of time. The most ideal situation would be to have data collected over a 24 hour period to give a more overall picture of the patient's energy expenditure. Indirect calorimetry studies measured over alternating shorter periods of time can give an accurate picture as well, provided the monitoring times are carefully selected under controlled conditions. It is imperative that the patient remains in steady-state conditions, yet most patients will often slip in and out of the steady-state. These changes must be taken into consideration and accounted for when analyzing the data. To provide an accurate picture when using alternating shorter periods of time, current protocol suggests a minimum time of 30 minutes resting conditions prior to beginning data collection. Prior to beginning measurement, patient should not have undergone any strenuous activity, be at rest but not asleep during the measurement, the room should be quiet and at a temperature ranging from 20 to 25˚C The actual time of collecting data can last from 5 to 15 minutes. Upon collection of the data, it is stored in the metabolic computer and placed into interpretation format for analysis and application (Indirect Calorimetry Handbook).

Table 2: Overview of Indirect Calorimetry terms, Symbols and Normal Values (Indirect Calorimetry Handbook).

Variable	Symbol	Normal value*
Oxygen Consumption	VO_2	250 mL/min (3.6 mL/min/kg IBW)
Carbon Dioxide	VCO_2	200 mL/min (2.9 mL/min/kg IBW)
Respiratory Quotient	RQ	0.65-1.25
Respiratory Exchange Ratio	RER	0.65-1.25 (assume steady state conditions)*
Energy Expenditure	EE	Dependent on measurement conditions
Basal Energy Expenditure	BEE	Not applicable
Resting Energy Expenditure	REE	1800-2200 kcal/24hr (25-30 kcal/kg)

IBW= ideal body weight

* A steady-state condition exists when the exchange of gases (oxygen and carbon dioxide) at the cellular level and those measured at the airway are similar. This requires that both perfusion and ventilation are relatively stable during the monitoring period. If rapid changes in either occur, gas measurements at the airway (RER) will not accurately reflect cellular levels (RQ) (Indirect Calorimetry Handbook).

5.2 Collection of Data

Upon attaching the patient to the metabolic cart, that data is collected for 5 minutes to ascertain a baseline reading. Once the data is collected, it should be reviewed for errors and for possible indications that the patient is not in a steady state (**Table 3**) (Indirect Calorimetry Handbook).

Table 3: Acceptable Ranges for Indirect Calorimetry Data.

Variable	**Acceptable Range**
RQ	0.65-1.25
VO_2	+/- 5% from baseline value
VCO_2	+/- 5% from baseline value
Minute ventilation	+/- 10% from baseline value

Many metabolic carts automatically alert the operator if the patient is not in the steady state, and as well indicate deviations from the steady state in the data record. After about 15 minutes of collecting data, the test can be stopped (Indirect Calorimetry Handbook).

5.3 Interpreting the Data

The measured REE is a representation of the number of calories burned by a patient and is expressed in the units kcal per 24 hours. The patient's overall diet can be adjusted to provide the right amount of calories because the REE is an actual expression of the metabolic rate. RQ is used to establish the mixture of substrates used in generating REE (**Table4**) (Indirect Calorimetry Handbook).

Table 4: Using RQ Ratio to Determine Substrate Utilization

RQ	Condition
1.0	Carbohydrate metabolism
0.71	Lipid metabolism
0.80	Protein metabolism
0.85	Mixed Substrate metabolism
< 0.65	Non-steady state condition—hypoventilation/ketosis
> 1.25	Non-steady state condition—hyperventilation/ isocapnic buffering

Upon successfully measuring the RQ and urinary nitrogen along with REE, caloric intake should match, sometimes even exceed, the REE with a balanced mixture of substrate and nutrients (Indirect Calorimetry Handbook).

5.4 Purpose for Indirect Calorimetry

1) To accurately measure the REE and RQ to guide nutritional support

2) To determine substrate utilization in conjunction with urinary nitrogen

3) To determine VO_2 as a guide for monitoring the work of breathing and targeting adequate oxygen delivery

4) To assess the contribution of metabolism to ventilation

(Indirect Calorimetry Handbook)

5.5 Types of Energy Expenditures

When assessing energy expenditure (EE), a distinction has to be made in regards to which caloric expenditure is being measured, whether it is basal energy expenditure (BEE), resting energy expenditure (REE) , activity energy expenditure (AEE), or total energy expenditure (TEE) (**Table 5**) (Indirect Calorimetry Handbook).

Table 5: Definitions of Various Energy Expenditures.

EE	Energy Expenditure	# of calories consumed in a given period of time.
BEE	Basal Energy Expenditure	EE measured at basal level of metabolism ; patient is at rest; fasting; non-REM sleep; in a healthy state.
REE	Resting Energy Expenditure	EE measured at resting conditions; patient is awake; resting quietly
AEE	Activity Energy Expenditure	EE during activity.
TEE	Total Energy Expenditure	REE + AEE = TEE for a 24 hour period.

6 Findings in International Studies

Studies performed internationally recognize that metabolic adjustments occur during pregnancy and lactation to support fetal growth and milk synthesis. Energy expenditure increases during pregnancy because of the metabolic contribution of the uterus and fetus and the increased work of the heart and lungs (Butte, 1999). Extra dietary energy is required during pregnancy to make up for the energy deposited in maternal and fetal tissues and the rise in energy expenditure attributable to increased basal metabolism and to changes in the energy cost of physical activity (Butte, 2004). Milk synthesis is assumed to be a continuous process, and the costs should be reflected in the basal metabolic rate. Therefore it is expected that the BMR should be somewhat higher in the lactating women than in the non-pregnant, non-lactating women. If this is not the case, there could be an indication of energy-sparing adaptation (Dewey, 1997).

6.1 BMR & REE in Pregnant Women and Lactating Women

In Butte's study consisting of 76 women (40 lactating and 36 non-lactating) at the 37th week of gestation and 3 and 6 months postpartum were measured with room calorimetry. The study found that BMR was 18 to 20% higher during pregnancy than postpartum- 1661 kcal/day, 1396 kcal/day, and 1410 kcal/day - at 37th week of gestation, 3 months postpartum, and 6 month postpartum respectively. Butte concludes in her study that increased rates of energy expenditure were evident in both late pregnancy and lactation (Butte, 1999). In another study carried out by Butte, BMR was obtained via oxygen and carbon dioxide consumption continuously measured in a room calorimeter for 24 hours. The study was performed on 63 women ;17 with a low body mass index (BMI; in kg/m^2), 34 with a normal BMI, and 12 with a high BMI, estimated at 0, 9, 22, and 36 wk of pregnancy and at27 weeks postpartum. BMR in the low BMI group at the 36th week of pregnancy was found to be 1573 ± 210 kcal/day, in the normal BMI at the 36th week of pregnancy BMR was 1673 ± 172 kcal/day, in the high BMI, BMR was found to be 2016 ± 254 kcal/day. At the 27th week of postpartum BMR in the low BMI group was 1254 ± 169 kcal/day, in the normal BMI it was 1323 ± 136 kcal/day, and in the high BMI 1505 ± 171 kcal/day .BMR increased gradually throughout pregnancy at a mean rate of 10.7 ± 5.4 kcal/gestational week. BMR in pregnant women differed between BMI groups, low BMI group was lower than normal BMI group which was lower than the high BMI group. When adjusted for weight or fat free mass and fat mass BMR did not vary significantly between BMI groups. Postpartum BMR did not differ significantly from pre-gravid BMR, with or without adjustment for weight or fat free mass and fat mass in all BMI groups (Butte,2004).

Prentice conducted a study consisting of 8 healthy, well nourished women studied at pre-pregnancy and at 6,12, 18,24 ,30, and 36 weeks of gestation via 24 hour whole body calorimetry. Prentice found in his study that BMR increased over a four-fold range from 8.6 to 35.4 % in the 36th week of pregnancy in comparison to non-pregnant non-lactating women (Prentice, 1989).

van Raaij[1] conducted a study on 57 healthy pregnant Dutch women before, during, and after pregnancy and obtained data on basal metabolic rate measured via indirect calorimetry. In 23 women, BMR was measured before and throughout pregnancy. At 6 weeks gestation BMR was 78 ± 111 kcal/day higher than before pregnancy. No significant differences in BMR occurred between the 6, 12^{th} , and 24^{th} week but at the 36^{th} week BMR was 290 ± 166 kcal/day above pre-pregnancy level. Overall, BMR was 20% higher at the 36^{th} week of gestation than before pregnancy (van Raaij, 1989).

Forsum measured resting energy expenditure on roughly 20 healthy Swedish women via doubly labeled water before, during and after pregnancy. Forsum compared BMR pre-pregnancy and 2 and 6 months postpartum. The following results were obtained from the study- 1337.54 ± 143.3 kcal/day, 1409.19 ± 167.2 kcal/day, 1433.08 ± 143.3 kcal/day- respectively (Forsum, 1992), showing a higher BMR in postpartum than pre-pregnancy.

Spaaij conducted a study on 24 women before pregnancy and 2 months postpartum on lactating women via indirect calorimetry. The study found that REE was 58.46 ± 127.3 kcal/day higher postpartum than during pregnancy – before pregnancy REE was 1306.94 ± 120.4 kcal/day, and during lactation 1368.87 ± 137.6 kcal/day, therefore showing that REE increased during lactation (Spaaij, 1994).

Sadurskis studied 23 healthy lactating Swedish women, BMR was studied pre-pregnancy and three times postpartum. The results were the following- 1340 ± 53 kcal/day, 1520 ± 246 kcal/day, 1410 ± 172 kcal/day, 1440 ± 150 kcal/day- before pregnancy, 5 -10 days postpartum, 2 months postpartum and 6 months postpartum respectively, again showing that BMR increased for lactating women (Sadurskis, 1988).

Motil's study measured BMR via indirect calorimetry on 12 lactating women at 1, 5, and 13 month postpartum and on non-lactating women. Motil's study found that BMR remained relatively unchanged between lactating and non-lactating women. The results for the lactating women were 18.5 ± 1.1 kcal/kg, 17.0 ± 3.1 kcal/kg, 18.9 ± 1.1 kcal/kg at 1, 5, and 13 months respectively. For the non-lactating women the results were 17.3 ± 2.5 kcal/kg (Motil, 1990).

6.2 RQ

Butte's study found that the respiratory quotient was significantly higher during pregnancy than postpartum- 0.828 ± 0.032, 0.802 ± 0.042, 0.789 ± 0.042 at 37^{th} week of gestation, 3 months postpartum, and 6 month postpartum respectively. Butte concludes that elevated respiratory quotient during pregnancy continue during lactation. Butte's study found that the lactating group's adjusted RQ did not change significantly with time- 0.874, 0.876- at 3 and 6 months postpartum respectively (Butte, 1999).

van Raaij's results for RQ were higher throughout pregnancy than before pregnancy. At 12, 24, and 36weeks gestation RQ's were 0.024 ± 0.052, 0.051 ± 0.064, and 0.043 ± 0.051 respectively (van Raaij, 1989).

Denne and Knuttgen found an in increase in respiratory quotients in pregnant subjects, again indicating higher rates of net carbohydrate utilization, yet Blackburn found no changes in respiratory quotients. For lactating women Spaaij found that the respiratory quotient was lower and van Raaij and Frigerio found no changes in respiratory quotient (Butte, 1999).

6.3 Carbohydrate

In Butte's study, carbohydrate oxidation as a percentage of TEE, was highest in pregnancy- 54 ± 6 %, 49 ± 8%, 50 ±8 %- at 37th week of gestation, 3 and 6 months postpartum respectively. Butte's study concluded that higher carbohydrate utilization at 37th week of gestation was maintained through to the 6th month postpartum in lactating women, which is consistent with the preferential use of glucose by the fetus and mammary gland (Butte, 1999).

6.4 Protein

In terms of substrate utilization Butte's study found that protein oxidation as a percentage of TEE was significantly lower during pregnancy than postpartum- 16 ± 3%, 20 ± 3%, and 21 ± 3% at 37th week of gestation, 3 and 6 months postpartum respectively. In Butte study protein oxidation as a percentage of TEE was significantly higher postpartum than during pre-pregnancy (Butte , 1999).

6.5 Lipids

Butte's results conclude that lipid oxidation did not change very much over time 30 ± 7, 31 ± 9, 29 ± 8 at 37th week of gestation, 3 months postpartum, and 6 month postpartum respectively (Butte, 1999).

6.6 Energy expenditure

The only difference in energy output between pregnant and lactating women is that lactating women have extra energy output due to milk production, otherwise, other energy expenditure parameters are the same i.e. basal metabolism, physical activity and thermic effect of food (Lederman, 2004). Suggestions have been made to indicate that lactating women may compensate for energy demand by reducing energy expenditure. This could occur through a decrease in basal metabolic rate, dietary- induced thermogenesis, or physical activity (Dewey, 1997).

6.7 Weight

During pregnancy, energy expenditure generally rises because of an increase of maternal and fetal weight(Butte, 1999). Vinoy's study evaluated 15 lactating Bangladesh mothers who showed evidence of chronic malnutrition (BMI 14.9- 18.1 kg/m^2), the measurements were conducted in 5 different time periods- 3.5, 5.5, 7.5, 10, and 13 months post partum and found that on average the mother's weight fell by 1.3kg during the 5 periods of lactation (Vinoy, 2000). The following results were obtained form Butte's study .Weight (kg) showed a decrease from the 37th week of gestation to the 3rd and 6th month postpartum. The weight ranged from 75.7 ± 9.7 kg to 65.5 ± 11.0 kg to 62.7 ± 10.0 kg respectively (Butte, 1999). In another study carried out by Butte, performed on 63 women ;17 with a low body mass index (BMI; in kg/m^2), 34 with a normal BMI, and 12 with a high BMI, estimated at 0, 9, 22, and 36 wk of pregnancy and at27 wk postpartum, the following weights were observed. Weight in the low BMI at the 36th week of pregnancy was found to be 63.0 ± 4.7 kg , in the normal BMI it was 72.2 ± 8.4 kg, and in the high BMI it was 84.6 ± 10.4 kg (Butte,2004).

7 Method used to Measure the Subjects

Many of the anthropometrical values were measured using an electronic balance, the Bioimpedance Inner Scan Body Composition Monitor, model BC 532, Tanita Corporation, Tokyo, Japan. It is important to note that this balance does not take into account pregnant women, therefore the reading should only be used as a reference rather than using absolute values. The monitor works by using Advanced Dual Frequency Technology where safe, low-level electrical signals are passed through the body via patented Tanita footpads on the monitor platform. The signal flows through the fluids in the muscle and other body tissues but it does meet resistance when it passes through body fat since it contains little fluid, this resistance is known as impedance. These impedance readings are entered into medically researched mathematical formulas to calculate body composition (Body Composition Monitor Manual). Body weight was measured with the electronic balance InnerScan Body Composition Monitor. Total Body Water Percentage is the total amount of fluid in a patient's body expressed as a percentage of their total weight. Maintaining a healthy total body water percentage ensures efficient body function and reduces the risk of developing health problems. The general guide for average total body water percentage for healthy female is 45 to 60%. It is important to note that total body water percentage will tend to decrease as the percentage of body fat increases, therefore a patient with a high percentage of body fat may be below the average body water percentage. Body Fat Percentage is the amount of body fat in proportion to body weight. Healthy body fat range standard for females aged 18-36 years old is 21 to 33%. Anything below that is classified as underfat and any value above is either overfat or obese. Basal Metabolic Rate (BMR) is the number of calories required for basal metabolism, it is the minimum level of energy the body needs when at rest to function effectively, including the respiratory and circulatory organs, neural system, liver, kidneys, and other organs. About 70% of daily calories consumed every day are used for the basal metabolism. The customary way of calculating BMR is using a standard equation using weight and age (Body Composition Monitor Manual). Tanita Body Composition Monitor has developed a more in depth method to calculate and measure BMR based on the impedance measurement, this method has been validated via indirect calorimetry.

Visceral fat is the fat located in the internal abdominal cavity, surrounding the vital organs. The Tanita Body Composition Monitor measures visceral fat rating from 1 to 59. The range from 1 to 12 is an indication of healthy level of visceral fat. From 13 to 59 is an indication of excess level of visceral fat. Muscle Mass is the weight of muscle in body, this includes the skeletal muscles, smooth muscles, and the water contained in these muscles. Physique rating is used to assess the patient's physique based on the ratio of body fat and muscle mass in body (Body Composition Monitor Manual).

Table 6: Physique Rating Results.

Result	**Physique Rating**
1	Hidden Obese
2	Obese
3	Solidly-built
4	Under exercised
5	Standard
6	Standard Muscular
7	Thin
8	Thin & Muscular
9	Very Muscular

.

Experimental Part

1 Study Design and Subjects

There were a total of 24 subjects in the study examined in 5 different time periods. The P0 being at the end of pregnancy, the L1 from birth up to 7 weeks post partum, L2 from the 10^{th} to the 16^{th} week post partum, L3 from the 20^{th} to the 26^{th} week post partum, and L4 from the 34^{th} to the 47^{th} week post partum. The pregnant subjects P0 were used as the comparison with L1 to L4 lactation subjects to observe changes in REE and other anthropometrical measures.

Table7: Anthropometrical Parameters of Subjects in the Study.

	Time Period	# of Subjects in Study	Age	Height	BMI	C.W	W.B.P	W.B.B	W.G.P	M.M	(C.W) - (I.B.W)	I.B.W
P0	End of Pregnancy	19	28.41	167.71	26.75	75.17	62.84	75.17	12.33	24.17	14.32	60.85
L1	Up to 7 weeks post partum	23	28.76	166.72	23.35	64.8	61.42	74.78	13.36	22.54	4.53	60.27
L2	10 to 16 weeks post partum	23	28.92	166.96	22.51	62.64	61.18	73.61	12.42	22.37	2.23	60.41
L3	20 to 26 weeks post partum	22	29.3	167.3	22.54	62.89	62.81	77.03	14.22	22.9	2.28	60.61
L4	34 to 47 weeks post partum	24	29.79	167.2	22.16	61.73	63.17	76.81	13.64	25.05	1.18	60.55

Age is in years; **Height** is in cm; **BMI** is in kg/m^2; **C.W**= current weight in kg; **W.B.P**= weight before pregnancy in kg; **W.B.B** is weight before birth in kg; **W.G.P** is weight gain in pregnancy in kg; **M.M** is muscle mass in kg; (**C.W-I.B.W)** is current weight subtracted from ideal body weight in kg; **I.B.W** is ideal body weight in kg.

2 Statistical Evaluation of Results

Statistical analysis was conducted via the programs GraphPad Prism5 (GraphPad Software, La Jolla, CA, USA) and Excel 2007 (Microsoft, Redmond, WA, USA). From these programs, descriptive statistics, the D'Agostino test of normality and Pearson's coefficient for determining correlation between REE and anthropometrical parameters were used. As well the ANOVA test was conducted to determine if the results from P0, L1, L2, L3, and L4 differ from each other.

3 Results of this study

3.1 Anthropometrical Parameters

3.1.1 Anthropometrical Parameters for Pregnant Women P0

Table 8: Anthropometrical Parameters*

Statistical Results	Week of Pregnancy	Age	Height	BMI	C.W	W.B.P	W.B.B	W.G.P	M.M	(C.W) – (I.B.W)	IBW
Median	37	29	167.3	26.08	76.2	62	76.2	11.8	47.1	13.75	60.61
Mean	37.11	28.41	167.7	26.75	75.17	62.84	75.17	12.33	47.53	14.32	60.85
Std. Deviation	0.8753	3.104	7.181	2.949	8.714	6.962	8.714	4.887	4.024	7.827	4.258
Std. Error	0.2008	0.7527	1.647	0.6765	1.999	1.597	1.999	1.121	0.9231	1.796	0.977
Lower 95% CI of Mean	36.68	26.82	164.2	25.33	70.97	59.49	70.97	9.971	45.59	10.55	58.8
Upper 95% CI of Mean	37.53	30.01	171.2	28.18	79.37	66.2	79.37	14.68	49.47	18.09	62.9
Coefficient of Variation	2.36%	10.92%	4.28%	11.02%	11.59%	11.08%	11.59%	39.64%	8.47%	54.66%	7.00%

***Std. Deviation** is Standard Deviation; **Std. Error** is Standard Error; **CI** is Confidence Interval; **Age** is in years; **Height** is in cm; **BMI** is in kg/m^2; **C.W**= current weight in kg; **W.B.P**= weight before pregnancy in kg; **W.B.B** is weight before birth in kg; **W.G.P** is weight gain in pregnancy in kg; **M.M** is muscle mass in kg; (**C.W-I.B.W)** is current weight subtracted from ideal body weight in kg; **I.B.W** is ideal body weight in kg.

3.1.2 Anthropometrical Parameters for Lactating Women L1

Table 9: Anthropometrical Parameters

Statistical Results	Week After Birth	Age	Height	BMI	C.W	W.B.P	W.B.B	W.G.P	M.M	I.B.W	(C.W) - (W.P.P)
Median	4	29	166.8	23	64.9	62	77	13	42.3	60.31	2.75
Mean	4.2	28.76	166.7	23.35	64.8	61.42	74.78	13.36	42.38	60.27	3.886
Std. Deviation	1.19	3.059	6.608	2.814	7.875	7.118	9.17	4.909	2.582	3.918	3.838
Std. Error	0.238	0.6118	1.322	0.5627	1.575	1.424	1.834	0.9817	0.5164	0.7837	0.8182
Lower 95% CI of Mean	3.709	27.5	164	22.19	61.55	58.48	70.99	11.33	41.31	58.65	2.185
Upper 95% CI of Mean	4.691	30.02	169.5	24.51	68.05	64.36	78.57	15.39	43.45	61.88	5.588
Coefficient of Variation	28.34%	10.64%	3.96%	12.05%	12.15%	11.59%	12.26%	36.74%	6.09%	6.50%	98.74%

(C.W.) – (W.P.P) is current weight subtracted from weight pre-pregnancy in kg.

3.1.3 Anthropometrical Parameters for Lactating Women L2

Table 10: Anthropometrical Parameters

Statistical Results	Week After Birth	Age	Height	BMI	C.W	W.B.P	W.B.B	W.G.P	M.M	I.B.W	(C.W) - (W.P.P)
Median	12	29	166.7	21.94	62.9	60	74	13	41.9	60.25	2.9
Mean	12.36	28.92	167	22.51	62.64	61.18	73.61	12.42	41.79	60.41	3.045
Std. Deviation	1.655	3.174	6.701	3.194	8.678	7.848	8.738	3.368	2.717	3.974	1.586
Std. Error	0.3311	0.6349	1.34	0.6387	1.736	1.8	2.005	0.7726	0.5434	0.7947	0.4783
Lower 95% CI of Mean	11.68	27.61	164.2	21.2	59.06	57.4	69.39	10.8	40.67	58.77	1.98
Upper 95% CI of Mean	13.04	30.23	169.7	23.83	66.23	64.97	77.82	14.04	42.91	62.05	4.111
Coefficient of Variation	13.39%	10.98%	4.01%	14.19%	13.85%	12.83%	11.87%	27.11%	6.50%	6.58%	52.09%

3.1.4 Anthropometrical Parameters for Lactating Women L3

Table 11: Anthropometrical Parameters

Statistical Results	Week After Birth	Age	Height	BMI	C.W	W.B.P	W.B.B	W.G.P	M.M	I.B.W	(C.W) - (W.P.P)
Median	24	29	166.7	21.6	63	62.5	78	13.5	41.9	60.25	2.3
Mean	23.87	29.3	167.3	22.54	62.89	62.81	77.03	14.22	41.97	60.61	3.463
Std. Deviation	1.1	3.183	6.721	3.408	8.703	7.432	9.166	5.37	2.501	3.986	3.223
Std. Error	0.2293	0.6637	1.402	0.7107	1.815	1.858	2.292	1.342	0.5215	0.8311	1.14
Lower 95% CI of mean	23.39	27.93	164.4	21.06	59.12	58.85	72.15	11.36	40.89	58.89	0.7679
Upper 95% CI of mean	24.35	30.68	170.2	24.01	66.65	66.77	81.92	17.08	43.05	62.33	6.157
Coefficient of variation	4.61%	10.86%	4.02%	15.12%	13.84%	11.83%	11.90%	37.76%	5.96%	6.58%	93.09%

3.1.5 Anthropometrical Parameters for Lactating Women L4

Table 12: Anthropometrical Parameters

Statistical Results	Week After Birth	Age	Height	BMI	C.W	W.B.P	W.B.B	W.G.P	M.M	I.B.W	(C.W) - (W.P.P)
Median	36	30.5	166.6	21.56	62.3	64	78	13	41.6	60.19	2.95
Mean	38.29	29.79	167.2	22.16	61.73	63.17	76.81	13.64	41.96	60.55	3.66
Std. Deviation	3.793	3.107	6.694	3.731	9.275	7.18	9.17	5.385	2.944	3.969	2.835
Std. Error	0.7743	0.6341	1.366	0.7616	1.893	1.692	2.161	1.269	0.6138	0.8103	0.8964
Lower 95% CI of Mean	36.69	28.48	164.4	20.59	57.82	59.6	72.25	10.96	40.69	58.87	1.632
Upper 95% CI of Mean	39.89	31.1	170	23.74	65.65	66.74	81.37	16.32	43.23	62.22	5.688
Coefficient of Variation	9.91%	10.43%	4.00%	16.84%	15.02%	11.37%	11.94%	39.48%	7.01%	6.56%	77.45%

3.2 Body Composition

Table 13: Body Composition*

	Time Period	# of Subjects in Study	A.W.B	B.F	V.F	M.M	BMR	B.S.A	E.M.A	P.R
P0	End of Pregnancy	19	47.9	33.01	4.42	47.53	1532.84	1.85	40.42	3.53
L1	Up to 7 weeks post partum	23	48.87	30.54	3.08	42.38	1370.52	1.72	32.56	4.16
L2	10 to 16 weeks post partum	23	49.84	28.94	2.72	41.79	1360.72	1.7	29.24	4.36
L3	20 to 26 weeks post partum	22	49.88	28.88	2.74	41.97	1404.44	1.71	29.74	4.61
L4	34 to 47 weeks post partum	24	51.13	27.42	2.54	41.96	1346.13	1.69	27.67	4.71

***A.W.B** is amount of water in body in %; **B.F** is body fat in %; **V.F** is visceral fat in %; **M.M** is muscle mass in kg; **BMR** is basal metabolic rate in kcal; **B.S.A** body surface area in m^2; **E.M.A** is estimated metabolic age in years; **P.R** is physique rating.

3.2.1 Body Composition for Pregnant Women P0

Table 14: Body Composition

Statistical Results	A.W.B	B.F	V.F	BMR	B.S.A	E.M.A	P.R
Median	47.1	33.6	5	1531	1.85	43	3
Mean	47.9	33.01	4.421	1533	1.845	40.42	3.526
Std. Deviation	3.892	5.36	1.121	129	0.1271	8.94	1.577
Std. Error	0.8929	1.23	0.2572	29.6	0.02916	2.051	0.3617
Lower 95% CI of Mean	46.02	30.42	3.881	1471	1.784	36.11	2.766
Upper 95% CI of Mean	49.78	35.59	4.962	1595	1.906	44.73	4.286
Coefficient of Variation	8.13%	16.24%	25.36%	8.42%	6.89%	22.12%	44.71%

3.2.2 Body Composition for Lactating Women L1

Table15: Body Composition

Statistical Results	A.W.B	B.F	V.F	BMR	B.S.A	E.M.A	P.R
Median	48.6	31	3	1369	1.732	31	5
Mean	48.87	30.54	3.08	1371	1.724	32.56	4.16
Std. Deviation	3.388	5.464	1.152	91.11	0.1156	11.58	1.491
Std. Error	0.6776	1.093	0.2304	18.22	0.02311	2.317	0.2982
Lower 95% CI of Mean	47.47	28.29	2.605	1333	1.677	27.78	3.545
Upper 95% CI of Mean	50.27	32.8	3.555	1408	1.772	37.34	4.775
Coefficient of Variation	6.93%	17.89%	37.40%	6.65%	6.70%	35.58%	35.84%

3.2.3 Body Composition for Lactating Women L2

Table 16: Body Composition

Statistical Results	A.W.B	B.F	V.F	BMR	B.S.A	E.M.A	P.R
Median	49.4	29.6	2	1361	1.715	29	5
Mean	49.84	28.94	2.72	1361	1.701	29.24	4.36
Std. Deviation	3.805	6.292	1.37	102	0.1232	13.09	1.604
Std. Error	0.7611	1.284	0.274	20.4	0.02463	2.617	0.3208
Lower 95% CI of Mean	48.27	26.28	2.155	1319	1.65	23.84	3.698
Upper 95% CI of Mean	51.41	31.59	3.285	1403	1.751	34.64	5.022
Coefficient of Variation	7.63%	21.74%	50.36%	7.50%	7.24%	44.76%	36.79%

3.2.4 Body Composition for Lactating Women L3

Table 17: Body Composition

Statistical Results	A.W.B	B.F	V.F	BMR	B.S.A	E.M.A	P.R
Median	50	28.7	3	1354	1.713	29	5
Mean	49.88	28.88	2.739	1404	1.706	29.74	4.609
Std. Deviation	4.195	6.738	1.421	244.9	0.1188	12.97	1.971
Std. Error	0.8746	1.405	0.2963	51.07	0.02478	2.704	0.411
Lower 95% CI of Mean	48.07	25.96	2.125	1299	1.654	24.13	3.756
Upper 95% CI of Mean	51.7	31.79	3.354	1510	1.757	35.35	5.461
Coefficient of Variation	8.41%	23.33%	51.88%	17.44%	6.97%	43.60%	42.77%

3.2.5 Body Composition for Lactating Women L4

Table 18: Body Composition

Statistical Results	A.W.B	B.F	V.F	BMR	B.S.A	E.M.A	P.R
Median	50.6	27.85	2.5	1337	1.702	26	5
Mean	51.13	27.42	2.542	1346	1.691	27.67	4.708
Std. Deviation	4.574	7.123	1.474	106.7	0.1229	13.11	2.053
Std. Error	0.9338	1.454	0.3008	22.25	0.02509	2.676	0.4191
Lower 95% CI of Mean	49.19	24.41	1.919	1300	1.639	22.13	3.841
Upper 95% CI of Mean	53.06	30.43	3.164	1392	1.743	33.2	5.575
Coefficient of Variation	8.95%	25.98%	57.99%	7.93%	7.27%	47.39%	43.61%

3.3 Evaluation of Urine

Before the assessment, urine was collected over a 24-hour period to measure daily urinary nitrogen (UN, in grams per day), characterizing protein metabolism. Urinary nitrogen content was determined by a standard kinetic ultraviolet assay (Roche/Hitachi 917 Analyzer, Roche Diagnostics, Indianapolis, IN, USA) at the University Hospital (Hronek, 2009). The norm for volume of urine in 24 hours is 1 to 2 Litres. For urea in urine (mmol/L) the norm is 220- 440 mmol/L and urea in urine in 24 hrs. (mmol/d) the norm is 330-580 mmol/L.

Table 19: Evaluation of Urine

	Time Period	# of Subjects in Study	Creatinine (mmol/L)	Creatinine (mmol/d)	Volume of Urine in 24 hrs.	Urea in Urine (mmol/L)	Urea in Urine in 24 hrs.(mmol/d)	Urea in Urine in 24 hrs.(g/d)
P0	End of Pregnancy	19	4.53	10.69	2551.58	145.26	342.09	20.55
L1	Up to 7 weeks post partum	23	4.77	9.97	2479	162.2	338.58	20.34
L2	10 to 16 weeks post partum	23	4.77	9.9	2340.8	170.72	356.4	21.41
L3	20 to 26 weeks post partum	22	5.24	10.13	2289.35	194.96	389.25	23.38
L4	34 to 47 weeks post partum	24	5.58	11.52	2383.04	179.35	384.57	22.14

The norm for **Volume of Urine in 24hrs.** is 1-2L; for **Urea in Urine (mmol/L)** the norm is 220-400 mmol/L; for **Urea in Urine in 24 hrs. (mmol/d)** the norm is 330-580 mmol/L.

3.3.1 Evaluation of Urine for Pregnant Women P0

Table 20: Evaluation of Urine

Statistical Results	Creatinine (mmol/L)	Creatinine (mmol/d)	Volume of Urine in 24 hrs.	Urea in Urine (mmol/L)	Urea in Urine in 24 hrs. (mmol/d)	Urea in Urine in 24 hrs. (g/d)
Median	4.1	10.3	2560	128	345.6	20.76
Mean	4.532	10.69	2552	145.3	342.1	20.55
Std. Deviation	1.706	2.433	742.4	57.27	93.06	5.589
Std. Error	0.3913	0.5582	170.3	13.14	21.35	1.282
Lower 95% CI of Mean	3.71	9.522	2194	117.7	297.2	17.85
Upper 95% CI of Mean	5.354	11.87	2909	172.9	386.9	23.24
Coefficient of Variation	37.64%	22.75%	29.09%	39.42%	27.20%	27.20%

3.3.2 Evaluation of Urine for Lactating Women L1

Table 21: Evaluation of Urine

Statistical Results	Creatinine (mmol/L)	Creatinine (mmol/d)	Volume of Urine in 24 hrs.	Urea in Urine (mmol/L)	Urea in Urine in 24hrs. (mmol/d)	Urea in Urine in 24 hrs. (g/d)
Median	4.9	10	2080	164	340.8	20.47
Mean	4.768	9.973	2479	162.2	338.6	20.34
Std. Deviation	1.906	2.46	1305	71.15	100.2	6.018
Std. Error	0.3812	0.4919	260.9	14.23	20.04	1.204
Lower 95% CI of Mean	3.981	8.958	1941	132.8	297.2	17.85
Upper 95% CI of Mean	5.555	10.99	3017	191.6	379.9	22.82
Coefficient of Variation	39.97%	24.66%	52.62%	43.86%	29.59%	29.59%

3.3.3 Evaluation of Urine for Lactating Women L2

Table 22: Evaluation of Urine

Statistical Results	Creatinine (mmol/L)	Creatinine (mmol/d)	Volume of Urine in 24 hrs.	Urea in Urine (mmol/L)	Urea in Urine in 24hrs. (mmol/d)	Urea in Urine in 24 hrs. (g/d)
Median	4.2	9.8	2420	163	369.6	22.2
Mean	4.768	9.9	2341	170.7	356.4	21.41
Std. Deviation	2.323	2.178	727.6	88.45	108.4	6.509
Std. Error	0.4646	0.4355	145.5	17.69	21.67	1.302
Lower 95% CI of Mean	3.809	9.001	2040	134.2	311.7	18.72
Upper 95% CI of Mean	5.727	10.8	2641	207.2	401.1	24.09
Coefficient of Variation	48.72%	22.00%	31.08%	51.81%	30.41%	30.41%

3.3.4 Evaluation of Urine for Lactating Women L3

Table 23: Evaluation of Urine

Statistical Results	Creatinine (mmol/L)	Creatinine (mmol/d)	Volume of Urine in 24 hrs.	Urea in Urine (mmol/L)	Urea in Urine in 24 hrs. (mmol/d)	Urea in Urine in 24 hrs. (g/d)
Median	4.3	10.2	2100	181	374	22.46
Mean	5.243	10.13	2289	195	389.3	23.38
Std. Deviation	2.902	2.534	905.3	97.46	122.1	7.332
Std. Error	0.6051	0.5283	188.8	20.32	25.46	1.529
Lower 95% CI of Mean	3.989	9.039	1898	152.8	336.5	20.21
Upper 95% CI of Mean	6.498	11.23	2681	237.1	442	26.55
Coefficient of Variation	55.34%	25.00%	39.55%	49.99%	31.36%	31.36%

3.3.5 Evaluation of Urine for Lactating Women L4

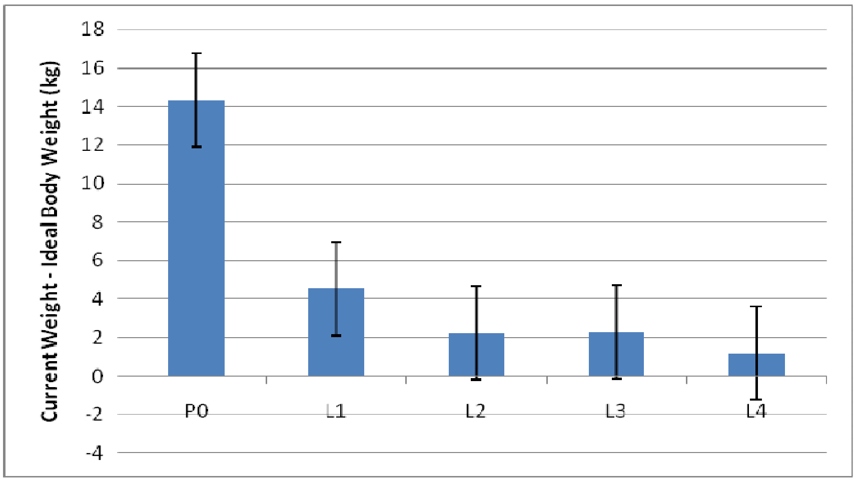
Table 24: Evaluation of Urine

Statistical Results	Creatinine (mmol/L)	Creatinine (mmol/d)	Volume of Urine in 24 hrs.	Urea in Urine (mmol/L)	Urea in Urine in 24 hrs. (mmol/d)	Urea in Urine in 24 hrs. (g/d)
Median	5.5	11.1	2340	165	391.3	23.16
Mean	5.583	11.52	2383	179.3	384.6	22.14
Std. Deviation	2.457	2.941	1038	64.49	110.9	8.034
Std. Error	0.5123	0.6132	216.3	13.45	23.12	1.64
Lower 95% CI of Mean	4.52	10.25	1934	151.5	336.6	18.75
Upper 95% CI of Mean	6.645	12.79	2832	207.2	432.5	25.53
Coefficient of Variation	44.01%	25.52%	43.54%	35.96%	28.84%	36.29%

3.3.6 Graphical Evaluation of Results

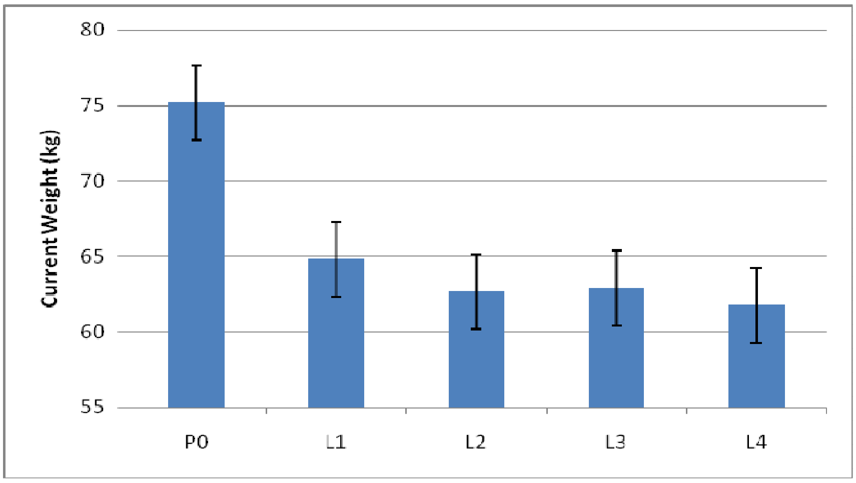
From this graph we can see that the difference in current weight subtracted from ideal body weight significantly decreased from P0 to L1 and continued to decrease up to L4.

Graph 1. Average of Current Weight (kg) minus Ideal Body Weight (kg) values in the 5 Different Time Periods



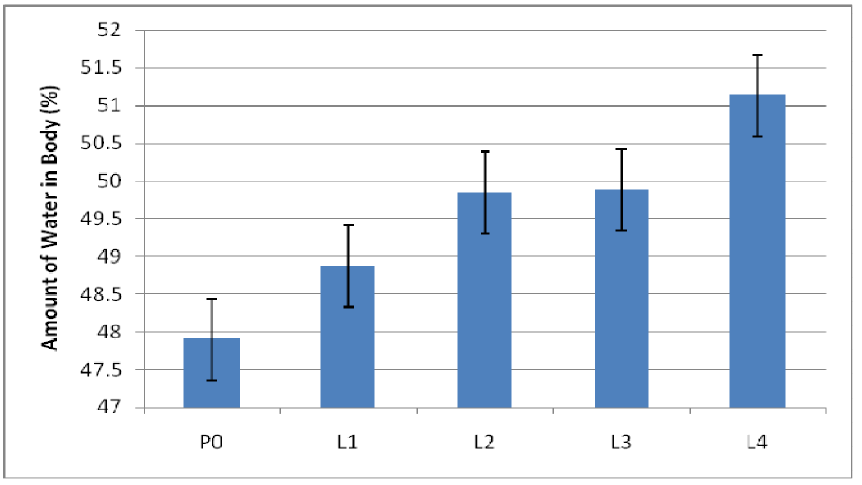
The weight of the women in the study decreased significantly from P0 to L1 and from L1 to L4 remained relatively stable.

Graph 2. Average of Current Weight (kg) values in the 5 Different Time Periods



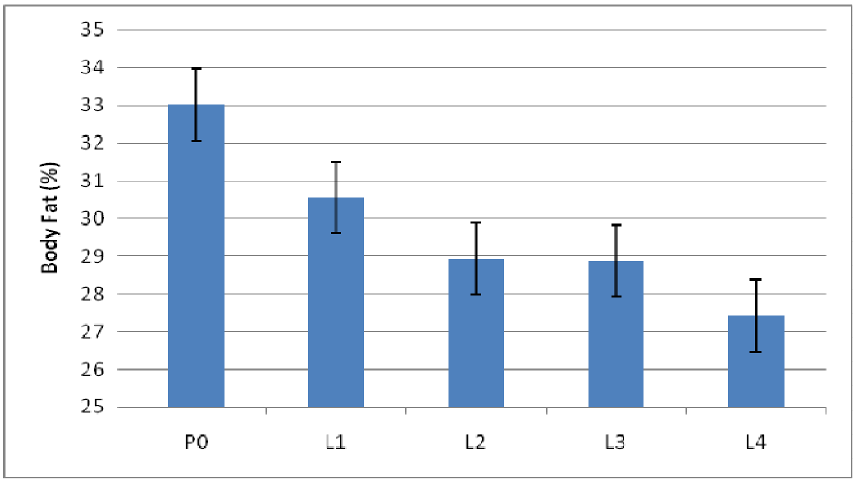
The amount of water in the body increased greatly from P0 to L4.

Graph 3. Average Amount of Water in Body (%) values in the 5 Different Time Periods



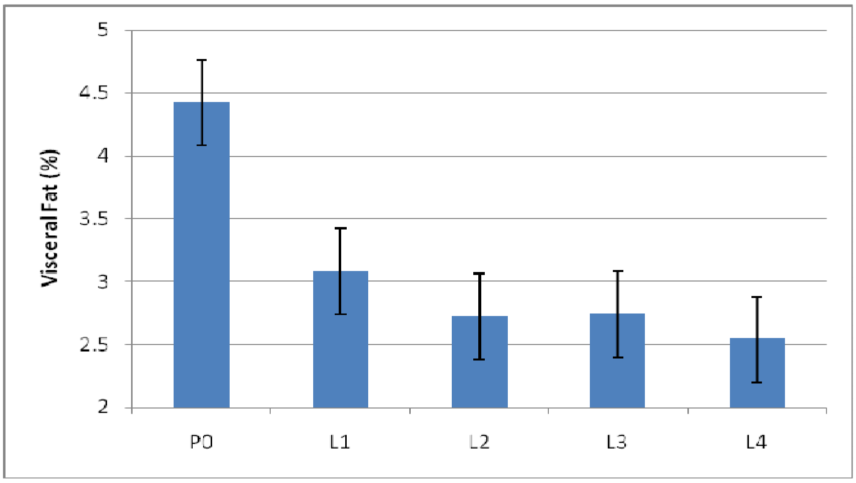
The amount of body fat showed a steady decrease from P0 to L4.

Graph 4. Average Amount of Body Fat (%) values in the 5 Different Time Periods



Visceral fat was highest in P0 and substantially decreased in L1 and fluctuated mildly between L1 to L4.

Graph 5. Average Visceral Fat (%) values in the 5 Different Time Periods

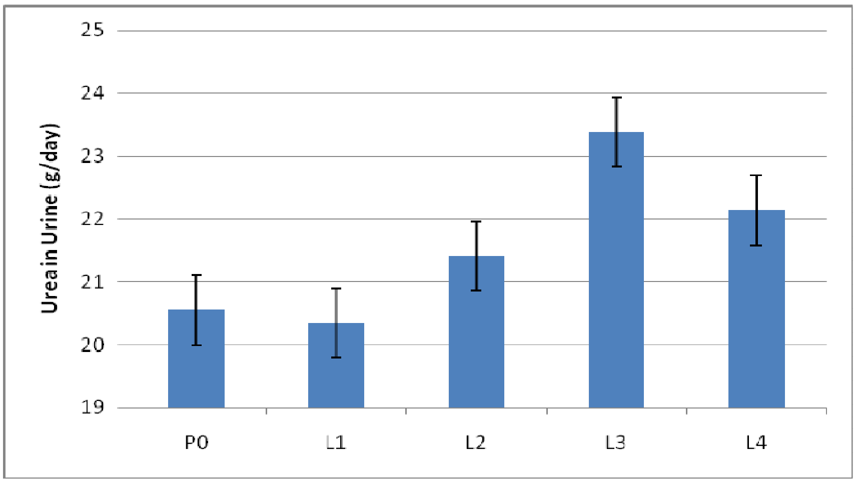


Urine Samples

Urine samples were collected at the time of measurement from the women over a 24 hour period.

The urea in urine over a period of 24 hours fluctuated from P0 to L4.

Graph 6. Average of Urea in Urine in 24 hours (g/day) values in the 5 Different Time Periods

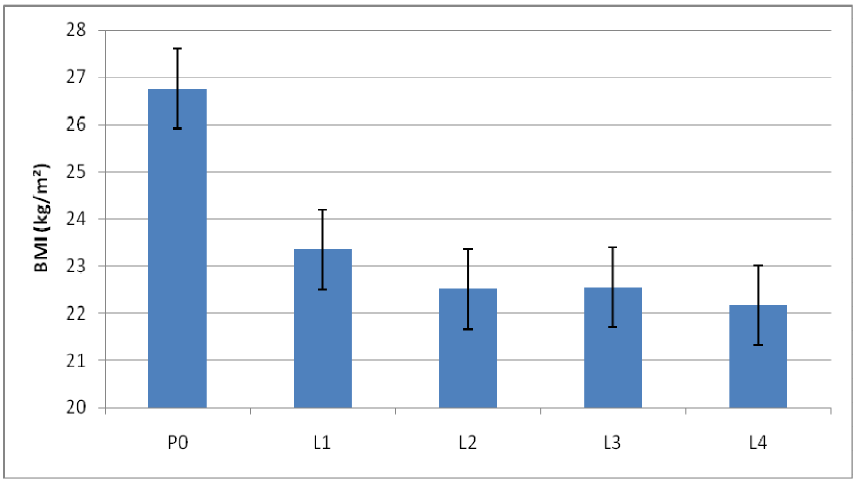


BMI

Body Mass Index (BMI) is an indicator of weight adequacy in relation to height. It is calculated as weight in kilograms divided by height in meters squared. The acceptable range for adults is 18.5 to 24.9. BMI was highest in P0 and decreased significantly in L1 to L4.

BMI = kg / m²

Graph 7. Average of BMI (kg/m²) values in the 5 Different Time Periods



3.4 Measured Resting Energy Expenditure

The measurement of Resting Energy Expenditure was carried out via indirect calorimetry. The women arrived from their homes after 12 hours of fasting and were at rest 30 minutes before assessment. The indirect calorimeter was calibrated before each examination according to the standard procedures for this machine. A ventilated hood system (Vmax Series, V6200 Autobox; SensorMedics, Yorba Linda, California, USA) was used to measure the difference between inspired and expired levels of oxygen and carbon dioxide; the determinants of VO2 and VCO2 were obtained over a 30-minute period while the women were lying in a supine position (Hronek, 2009).

These values were then converted to an REE expressed in kilocalories per day (REE) using the Weir equation (Hronek, 2009).

REE = ([3.941 x VO_2] + [1106 x VCO_2] x 1.44 – 2.17 x UN

Predictive Equations for Estimation of REE

In non-pregnant women, REE (in kilocalories per day) can be predicted from body weight (W, in kilograms), and age (A, in years) with the Schofield equations (REE_s) (Schofield), which were used in the 1985 World Health Organization/Food and Agriculture Organization of the United Nations/United Nations University report (Hronek, 2009).

$$\mathbf{REE_S = 14.8 \times W + 487 \text{ (for A from 18 to 29 y)}}$$

$$\mathbf{REE_S = 8.3 \times W + 846 \text{ (for A from 30 to 59 y)}}$$

In clinical practice, the Harris Benedict (REE_{HB}) equation is a widely used alternative equation that calculates REE in kilocalories per day according to weight (**W**, in kilograms), height (**H**, in centimeters), and age (**A**, in years) for women (Roza, 1984):

$$\mathbf{REE_{HB} = 655.0955 + 9.5634 \times W + 1.8496 \times H - 4.6756 \times A}$$

As well, the equation of Kleiber for women (REE_k) can be used to calculate REE in kilojoules per day (Kleiber, 1947), although this is not widely used in clinical practice owing to its complicated mathematic expression (Hronek, 2009):

$$\mathbf{REE_K = 275.3 \times W^{0.75} \times (1 + 0.004 [30 - A] + 0.018 [H/W^{1/3} - 42.1])}$$

However, no predictive equations are available to calculate REE at different time periods in lactating women (Hronek, 2009).

Table 25: Parameters from Indirect Calorimetry.

	Time Period	# of Subjects in Study	VO2 (L/min)	VCO2 (L/min)	RQ	MREE (kcal/day)	PBMR (kcal/day)	REE (%)	REE/kg (kcal/kg)	REE/BSA (kcal/m²)
P0	End of Pregnancy	19	0.25	0.2	0.84	1674.4	1542.84	107.79	22.26	906.05
L1	Up to 7 weeks post partum	23	0.2	0.16	0.77	1345.06	1448.71	92.86	20.91	781.12
L2	10 to 16 weeks post partum	23	0.2	0.16	0.83	1349.861	1427.78	95.02	21.96	799.02
L3	20 to 26 weeks post partum	22	0.2	0.16	0.84	1317.32	1428.93	92.23	21.16	774.07
L4	34 to 47 weeks post partum	24	0.19	0.16	0.94	1297.93	1415.43	91.77	21.3	768.11

RQ is respiratory quotient; **MREE** is measured resting energy expenditure in kcal/day, **PBMR** is predicted basal metabolic rate in kcal/day

3.4.1 REE for Pregnant Women P0

Table 26: Parameters from Indirect Calorimetry

Statistical Results	**VO2 (L/min)**	**VCO2 (L/min)**	**RQ**	**MREE (kcal/day)**	**PBMR (kcal/day)**	**REE (%)**	**REE/kg (kcal/kg)**	**REE/BSA (kcal/m²)**
Median	0.242	0.203	0.853	1656	1560	105	22	882
Mean	0.2457	0.2041	0.8382	1674	1543	107.8	22.26	906.1
Std. Deviation	0.03781	0.03013	0.1167	255.4	97.13	11.79	1.955	100.9
Std. Error	0.008674	0.006913	0.02677	58.58	22.28	2.705	0.4485	23.15
Lower 95% CI of Mean	0.2275	0.1896	0.782	1551	1496	102.1	21.32	857.4
Upper 95% CI of Mean	0.2639	0.2186	0.8944	1797	1590	113.5	23.2	954.7
Coefficient of Variation	15.39%	14.76%	13.92%	15.25%	6.30%	10.94%	8.78%	11.14%

3.4.2 REE for Lactating Women L1

Table 27: Parameters from Indirect Calorimetry

Statistical Results	VO2 (L/min)	VCO2 (L/min)	RQ	MREE (kcal/day)	PBMR (kcal/day)	REE (%)	REE/kg (kcal/kg)	REE/BSA (kcal/m²)
Median	0.199	0.158	0.7565	1325	1449	91.83	21.21	779.1
Mean	0.2006	0.1573	0.7722	1345	1449	92.86	20.91	781.1
Std. Deviation	0.02398	0.01657	0.1038	153.1	88.55	6.304	1.513	52.05
Std. Error	0.005001	0.003455	0.02076	31.91	17.71	1.314	0.3155	10.85
Lower 95% CI of Mean	0.1902	0.1502	0.7294	1279	1412	90.13	20.25	758.6
Upper 95% CI of Mean	0.211	0.1645	0.815	1411	1485	95.58	21.56	803.6
Coefficient of Variation	11.95%	10.53%	13.44%	11.38%	6.11%	6.79%	7.24%	6.66%

3.4.3 REE for Lactating Women L2

Table 28: Parameters from Indirect Calorimetry

Statistical Results	VO2 (L/min)	VCO2 (L/min)	RQ	MREE (kcal/day)	PBMR (kcal/day)	REE (%)	REE/kg (kcal/kg)	REE/BSA (kcal/m²)
Median	0.203	0.165	0.7881	1351	1430	94.93	22.45	803
Mean	0.2009	0.162	0.825	1350	1428	95.02	21.96	799
Std. Deviation	0.02207	0.01874	0.141	147.8	94.48	9.742	3.052	78.09
Std. Error	0.004602	0.003907	0.02819	30.82	18.9	2.031	0.6364	16.28
Lower 95% CI of Mean	0.1913	0.1539	0.7668	1286	1389	90.8	20.64	765.2
Upper 95% CI of Mean	0.2104	0.1701	0.8832	1414	1467	99.23	23.28	832.8
Coefficient of Variation	10.99%	11.56%	17.09%	10.95%	6.62%	10.25%	13.90%	9.77%

3.4.4 REE for Lactating Women L3

Table 29: Parameters from Indirect Calorimetry

Statistical Results	VO2 (L/min)	VCO2 (L/min)	RQ	MREE (kcal/day)	PBMR (kcal/day)	REE (%)	REE/kg (kcal/kg)	REE/BSA (kcal/m²)
Median	0.1955	0.155	0.7642	1296	1435	91.38	20.74	764
Mean	0.1963	0.16	0.8356	1317	1429	92.23	21.16	774.1
Std. Deviation	0.02427	0.02266	0.1754	159.8	95.15	8.716	2.432	70
Std. Error	0.005175	0.00483	0.0374	34.06	19.84	1.858	0.5185	14.93
Lower 95% CI of Mean	0.1855	0.15	0.7579	1246	1388	88.36	20.09	743
Upper 95% CI of Mean	0.207	0.17	0.9134	1388	1470	96.09	22.24	805.1
Coefficient of Variation	12.37%	14.16%	20.99%	12.13%	6.66%	9.45%	11.49%	9.04%

3.4.5 REE for Lactating Women L4

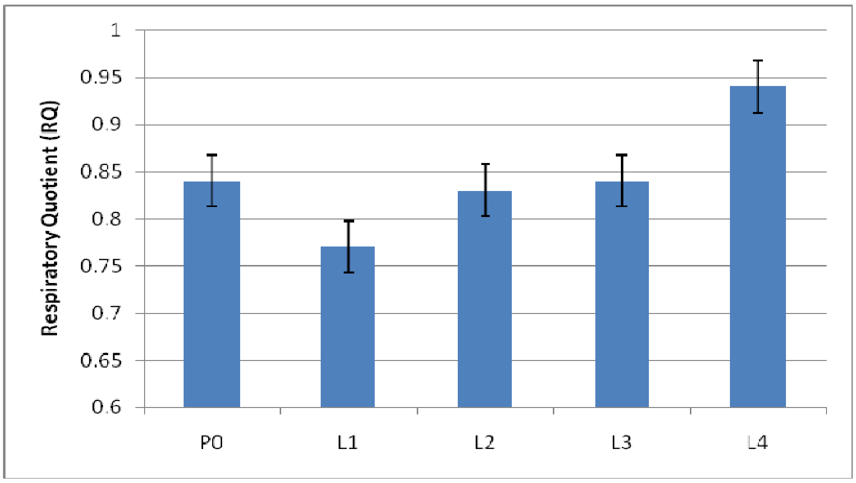
Table 30: Parameters from Indirect Calorimetry

Statistical Results	VO2 (L/min)	VCO2 (L/min)	RQ	MREE (kcal/day)	PBMR (kcal/day)	REE (%)	REE/kg (kcal/kg)	REE/BSA (kcal/m²)
Median	0.1865	0.162	0.8201	1248	1422	90.56	20.94	752.8
Mean	0.1916	0.1626	0.9443	1298	1415	91.77	21.3	768.1
Std. Deviation	0.02173	0.02174	0.3104	145	99.8	8.792	2.86	69.86
Std. Error	0.004435	0.004438	0.06337	29.6	20.37	1.795	0.5839	14.26
Lower 95% CI of Mean	0.1824	0.1534	0.8132	1237	1373	88.05	20.1	738.6
Upper 95% CI of Mean	0.2008	0.1718	1.075	1359	1458	95.48	22.51	797.6
Coefficient of Variation	11.34%	13.37%	32.87%	11.17%	7.05%	9.58%	13.43%	9.10%

3.4.6 Graphical Results of Measured REE

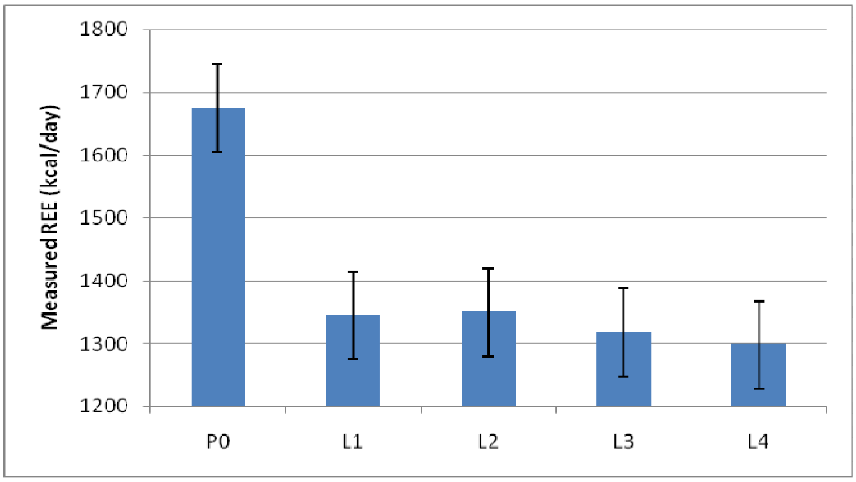
The RQ values were similar in P0, L2 and L3, lowest in L1 and highest in L4.

Graph 8. Average of Respiratory Quotient (RQ) values in the 5 Different Time Periods



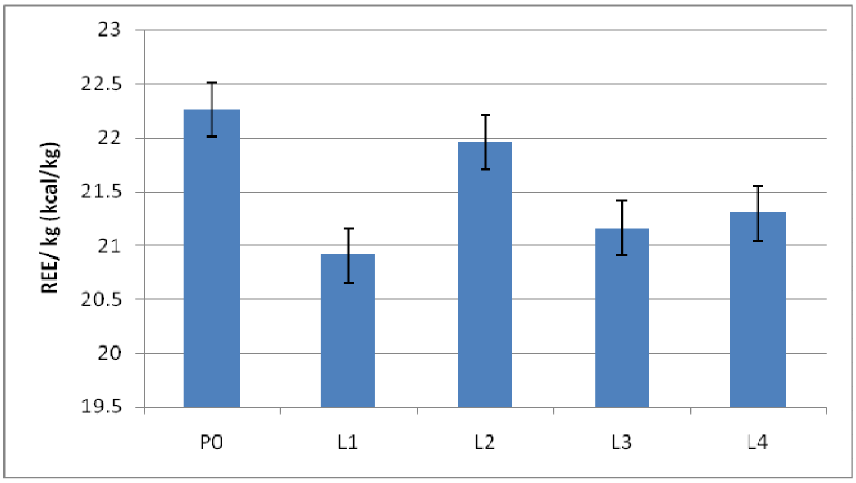
The measured REE was highest in P0 and showed little variation from L1 to L4.

Graph 9. Average of Measured Resting Energy Expenditure (kcal/day) values in the 5 Different Time Periods



Resting energy expenditure per kilogram was highest in P0 and L2, and relatively similar in L3 and L4, and lowest in L1.

Graph 10. Average of REE/kg (kcal/kg) values in the 5 Different Time Periods



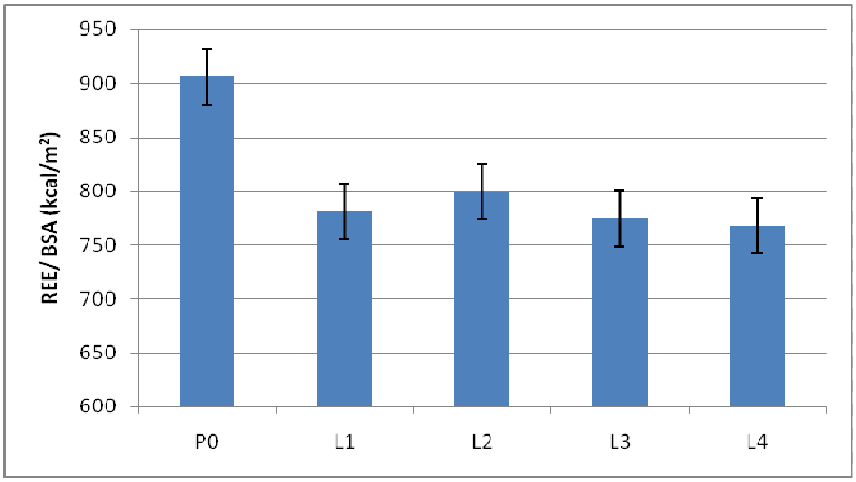
Body surface area (BSA, in meters squared) was calculated by the DuBois equation from height (H, in meters) and weight (W, in kilograms) (DuBois, 1916).

$$\mathbf{BSA = H^{0.725} \times W^{0.425} \times 71.84}$$

One variable that correlates well with metabolic rate is the body surface area, this is expected since heat exchange occurs at the body surface. BMR's relation to weight would be $BMR = 3.52W^{0.75}$.

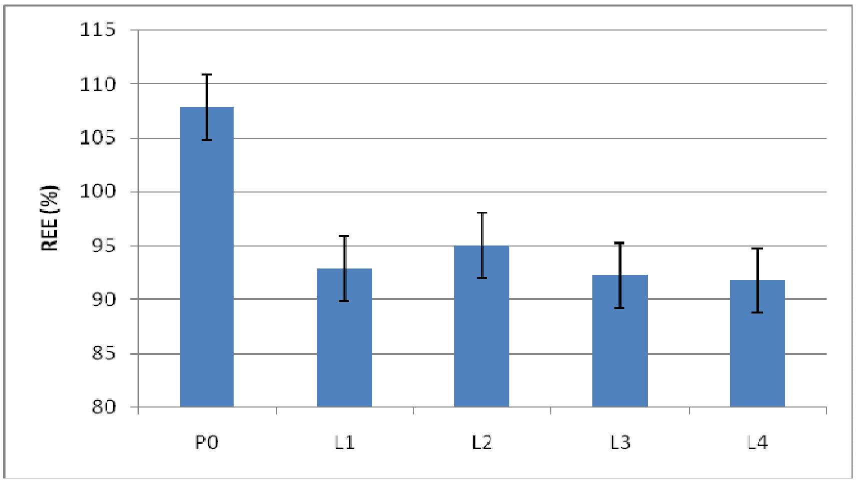
The highest REE/BSA was found in P0, while in L1 to L4 REE/BSA remained similar.

Graph 11. Average of REE/BSA (kcal/m²) values in the 5 Different Time Periods



The highest REE % was found in P0 and was relatively similar in L1 to L4.

Graph 12. Average of REE (%) values in the 5 Different Time Periods



3.5 Utilization of Substrates

Table 31: Utilization of Substrates

	Time Period	# of Subjects in Study	U of C (g/day)	U of C (kcal/day)	U of C (%)	U of L (g/day)	U of L (kcal/day)	U of L (%)	U of P (g/day)	U of P (kcal/day)	U of P (%)
P0	End of Pregnancy	19	125.53	521.37	31.42	70.41	599.84	38.76	128.32	554.11	33.89
L1	up to 7 weeks post partum	23	51.55	207.18	16.95	66.71	630.86	46	123.82	534.68	39.73
L2	10 to 16 weeks post partum	23	62.55	255.82	19.57	61.15	572.8	41.9	134.59	581.18	43.05
L3	20 to 26 weeks post partum	22	70.3	289.75	22.89	62.46	550.07	45.23	148.4	639.95	48.45
L4	34 to 47 weeks post partum	24	79.18	328.09	25.33	46.84	443.32	34.42	139.45	602.09	45.64

U of C (g/day) is Utilization of Carbohydrates (g/day); **U of C** (kcal/day) is Utilization of Carbohydrates (kcal/day); **U of C (%)** is Utilization of Carbohydrates (%); **U of L (g/day)** is Utilization of Lipids (g/day); **U of L (g/day)** is Utilization of Lipids (kcal/day); **U of L (%)** is Utilization of Lipids (%); **U of P (g/day)** is Utilization of Proteins (g/day); **U of P (kcal/day)** is Utilization of Proteins (kcal/day); **U of P (%)** is Utilization of Proteins (%).

3.5.1 Utilization of Substrates for Pregnant Women P0

Table 32: Utilization of Substrates

Statistical Results	U of C (g/day)	U of C (kcal/day)	U of C (%)	U of L (g/day)	U of L (kcal/day)	U of L (%)	U of P (g/day)	U of P (kcal/day)	U of P (%)
Median	134	560	39	61	575	35	130	562	34
Mean	125.5	521.4	31.42	70.41	599.8	38.76	128.3	554.1	33.89
Std. Deviation	98.7	416.8	24.06	40.14	390.4	17.61	34.45	148.6	10.32
Std. Error	22.64	95.61	5.52	9.736	94.69	4.271	7.903	34.09	2.368
Lower 95% CI of Mean	77.96	320.5	19.82	49.77	399.1	29.71	111.7	482.5	28.92
Upper 95% CI of Mean	173.1	722.2	43.02	91.05	800.6	47.82	144.9	625.7	38.87
Coefficient of Variation	78.63%	79.94%	76.58%	57.01%	65.09%	45.42%	26.85%	26.82%	30.45%

3.5.2 Utilization of Substrates for Lactating Women L1

Table 33: Utilization of Substrates

Statistical Results	U of C (g/day)	U of C (kcal/day)	U of C (%)	U of L (g/day)	U of L (kcal/day)	U of L (%)	U of P (g/day)	U of P (kcal/day)	U of P (%)
Median	39.5	164	11.5	80	754	55	124.5	539	39
Mean	51.55	207.2	16.18	66.71	630.9	46	123.8	534.7	39.73
Std. Deviation	54.49	233.2	18.59	29.57	279.8	17.88	37.75	163.1	11.9
Std. Error	11.62	49.71	3.963	6.453	61.06	3.902	8.049	34.76	2.537
Lower 95% CI of Mean	27.39	103.8	7.941	53.25	503.5	37.86	107.1	462.4	34.45
Upper 95% CI of Mean	75.7	310.6	24.42	80.17	758.2	54.14	140.6	607	45
Coefficient of Variation	105.71%	112.55%	114.87%	44.32%	44.35%	38.88%	30.49%	30.50%	29.96%

3.5.3 Utilization of Substrates for Lactating Women L2

Table 34: Utilization of Substrates

Statistical Results	U of C (g/day)	U of C (kcal/day)	U of C (%)	U of L (g/day)	U of L (kcal/day)	U of L (%)	U of P (g/day)	U of P (kcal/day)	U of P (%)
Median	44	184.5	15	62.5	591	45	142.5	614	45.5
Mean	62.55	255.8	19.57	61.15	572.8	41.9	134.6	581.2	43.05
Std. Deviation	57.81	246.6	16.98	32.09	305.1	21.04	36.38	156.9	12.16
Std. Error	12.33	52.56	3.706	7.175	68.23	4.704	7.757	33.44	2.592
Lower 95% CI of Mean	36.91	146.5	11.84	46.13	430	32.05	118.5	511.6	37.65
Upper 95% CI of Mean	88.18	365.1	27.3	76.17	715.6	51.75	150.7	650.7	48.44
Coefficient of Variation	92.44%	96.38%	86.76%	52.48%	53.27%	50.21%	27.03%	26.99%	28.25%

3.5.4 Utilization of Substrates for Lactating Women L3

Table 35: Utilization of Substrates

Statistical Results	U of C (g/day)	U of C (kcal/day)	U of C (%)	U of L (g/day)	U of L (kcal/day)	U of L (%)	U of P (g/day)	U of P (kcal/day)	U of P (%)
Median	64.5	270	21.5	66	601	44	152	656.5	47.5
Mean	70.3	289.8	21.75	62.46	550.1	45.23	148.4	640	48.45
Std. Deviation	56.53	240.8	17.16	21.58	251.6	13.78	45.28	193.9	14.04
Std. Error	12.64	53.84	3.837	5.985	67.24	3.822	10.13	43.36	3.138
Lower 95% CI of Mean	43.84	177.1	13.72	49.42	404.8	36.9	127.2	549.2	41.88
Upper 95% CI of Mean	96.76	402.4	29.78	75.5	695.3	53.56	169.6	730.7	55.02
Coefficient of Variation	80.41%	83.09%	78.90%	34.55%	45.73%	30.46%	30.51%	30.30%	28.97%

3.5.5 Utilization of Substrates for Lactating Women L4

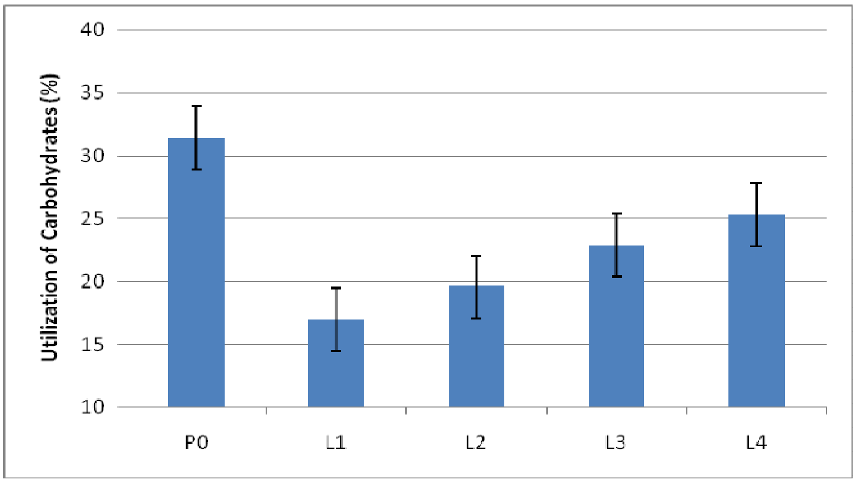
Table 36: Utilization of Substrates

Statistical Results	U of C (g/day)	U of C (kcal/day)	U of C (%)	U of L (g/day)	U of L (kcal/day)	U of L (%)	U of P (g/day)	U of P (kcal/day)	U of P (%)
Median	56	233	20	42	396	28	143	616	45.5
Mean	79.18	328.1	25.33	46.84	443.3	34.42	139.5	602.1	45.64
Std. Deviation	62.4	263.7	18.18	25.79	244.4	19.49	39.13	169.4	11.49
Std. Error	13.3	56.22	3.967	5.918	56.07	4.47	8.343	36.11	2.449
Lower 95% CI of Mean	51.51	211.2	17.06	34.41	325.5	25.03	122.1	527	40.54
Upper 95% CI of Mean	106.9	445	33.61	59.27	561.1	43.81	156.8	677.2	50.73
Coefficient of Variation	78.81%	80.37%	71.75%	55.07%	55.13%	56.61%	28.06%	28.13%	25.17%

3.5.6 Graphical Evaluation of Utilization of Substrates

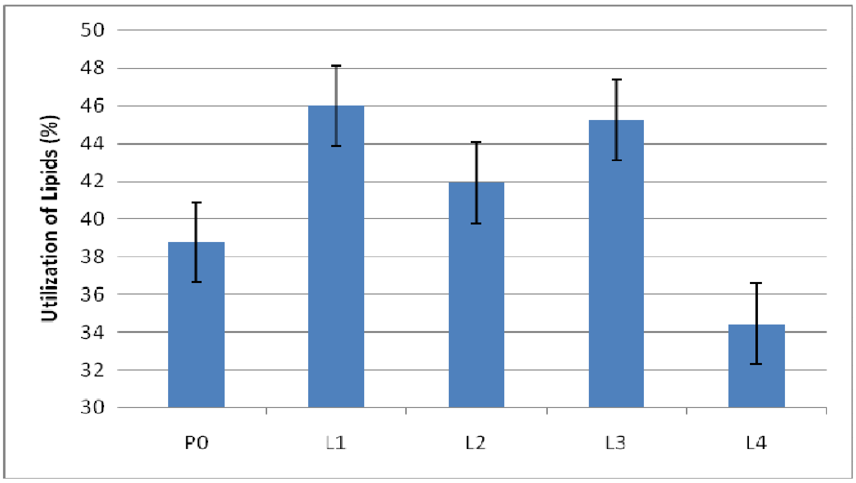
Utilization of carbohydrates (%) was highest in P0, it was the lowest in L1 but slowly showed an increase up to L4.

Graph 13. Average of Utilization of Carbohydrates (%) values in the 5 Different Time Periods



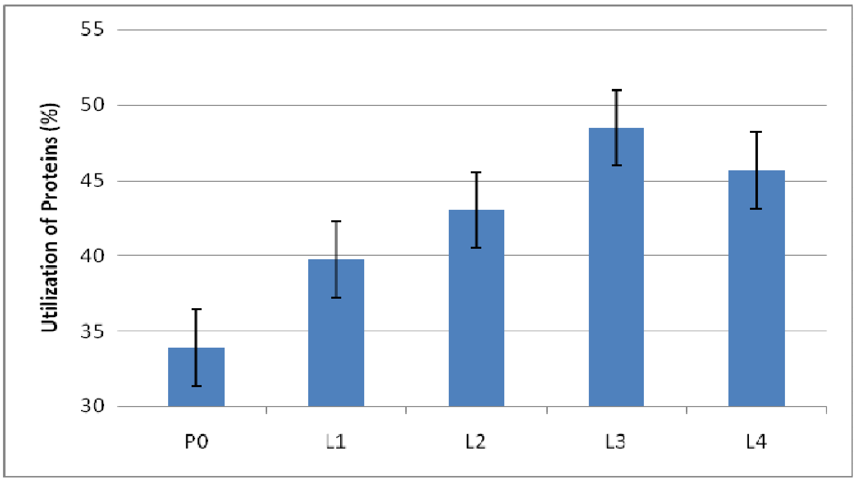
The utilization of lipids (%) fluctuated from P0 to L4, the highest utilization being in L1 and L3, the lowest in L4.

Graph 14. Average of Utilization of Lipids (%) values in the 5 Different Time Periods



Utilization of proteins was highest in L3 and lowest in P0 with increases relative to P0 in L1, L2 and L4**.**

Graph 15. Average of Utilization of Proteins (%) values in the 5 Different Time Periods

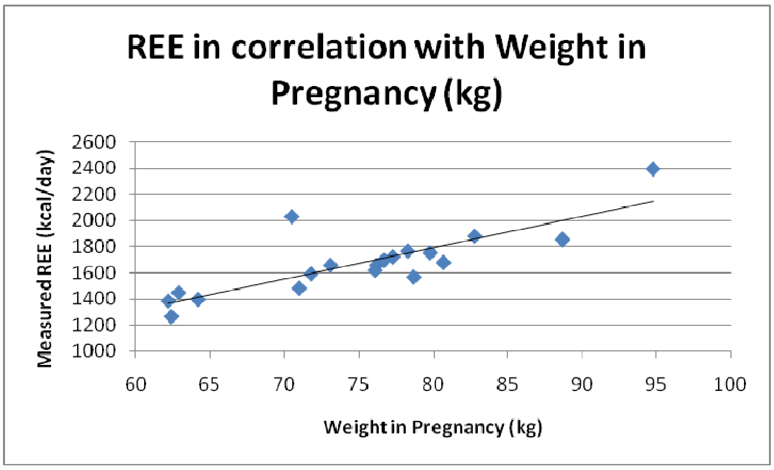


3.6 Correlation of Measurements

3.6.1 Results for P0- Pregnant Women

During pregnancy the increase in body weight strongly correlated to the increase in measured REE.

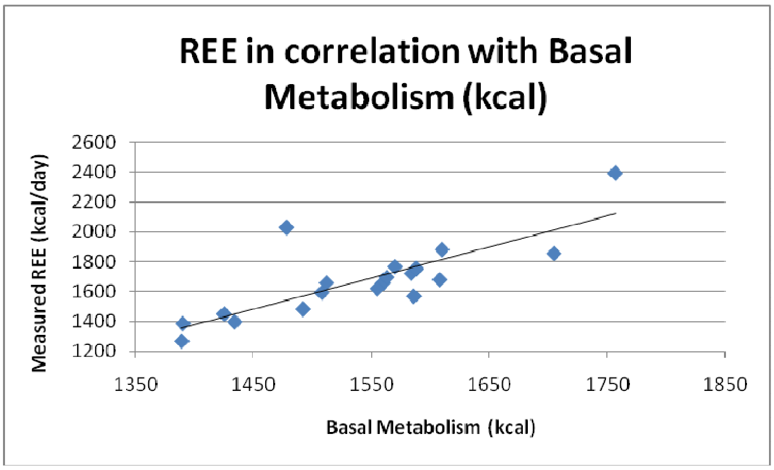
Graph 16 . P0 - Measured REE in correlation with Weight in Pregnancy (kg)



P = <0.0001   r = 0.8174

Basal metabolism, measured with electronic balance, the Bioimpedance Inner Scan Body Composition Monitor, increases along with the measured REE.

Graph 17. P0 - Measured REE in correlation with Basal Metabolism (kcal)

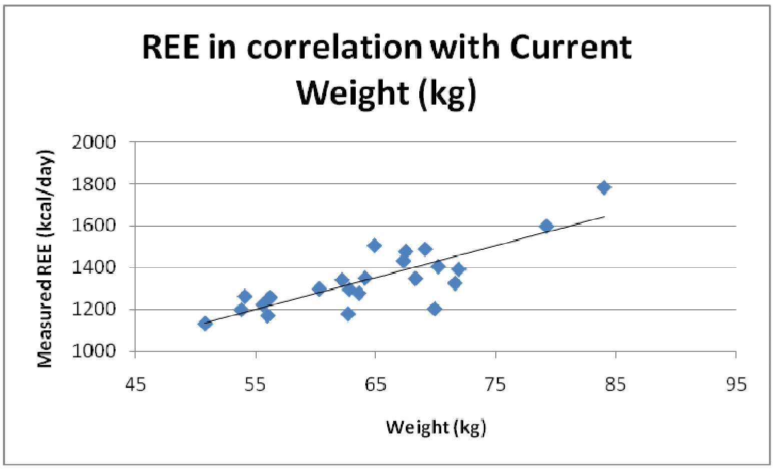


P = <0.0001 r = 0.7935

3.6.2 Results for L1- Lactating Women

Increase in current weight in correlation to increase in measured REE also results in an increase in basal metabolism.

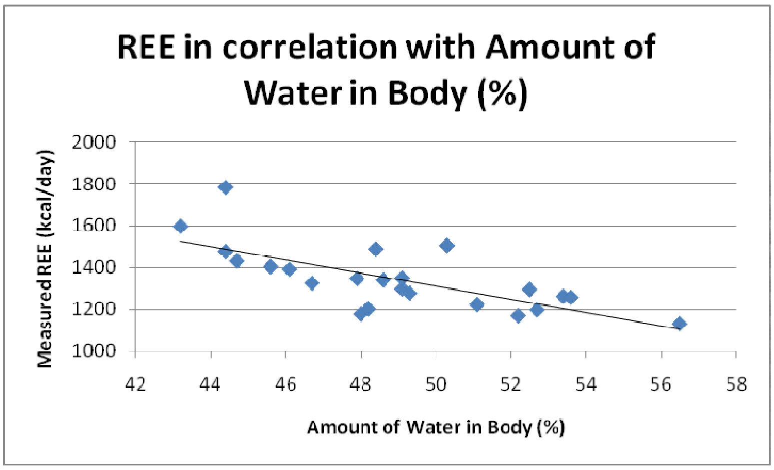
Graph 18. L1 - Measured REE in correlation with Current Weight (kg).



P = <0.0001 r = 0.8218

The correlation of amount of water in body (%) to measured REE is that as water increases REE decreases.

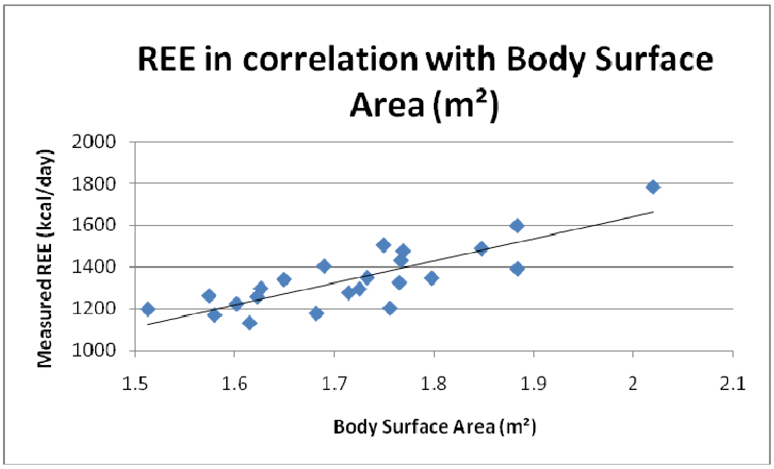
Graph 19. L1 - Measured REE in correlation with Amount of Water in Body (%).



P = 0.0001 r = -0.7109

The body surface are (m^2) increases with measured REE.

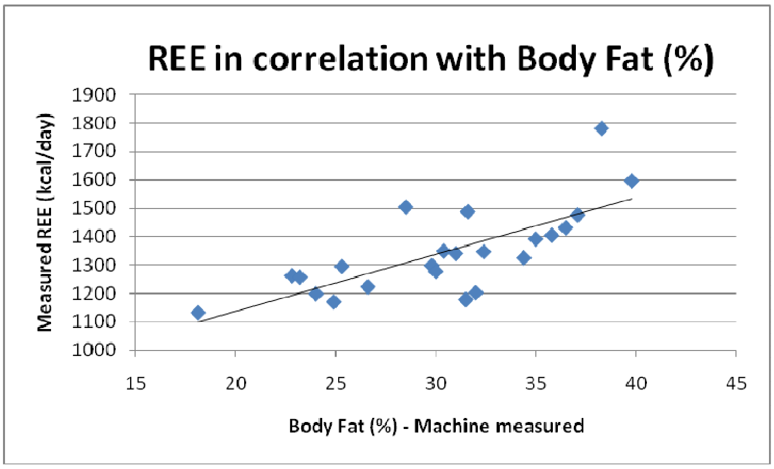
Graph 20. L1 - Measured REE in correlation with Body Surface Area (m²).



P = < 0.0001 r = 0.8246

The body fat (%) increased along with measured REE.

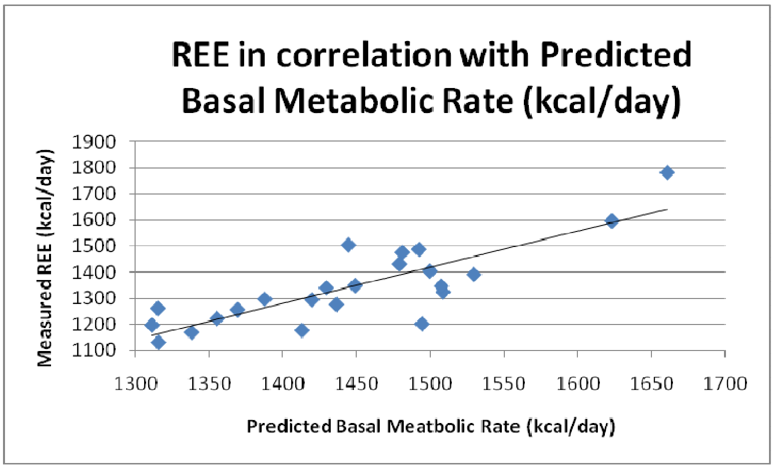
Graph 21. L1 Measured REE in correlation with Body Fat (%)



P = <0.0001 r = 0.7290

The predicted BMR increases along with the measured REE.

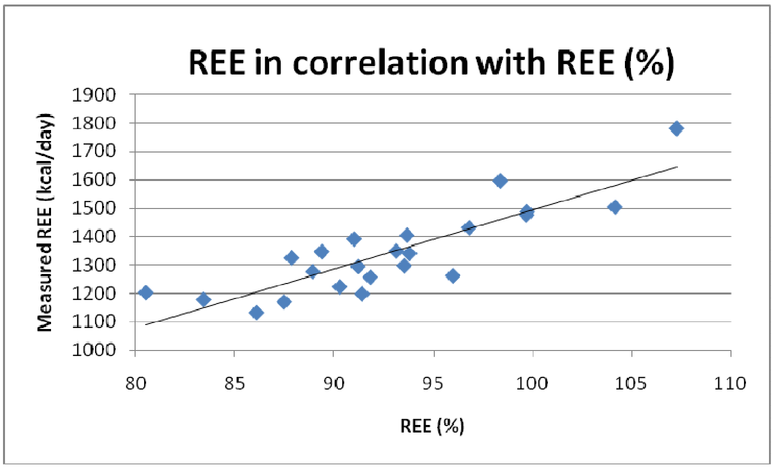
Graph 24 . L1 Measured REE in correlation with Predicted Basal Metabolic Rate (kcal/day)



P = <0.0001 r = 0.8305

The REE (%) increases as does measured REE.

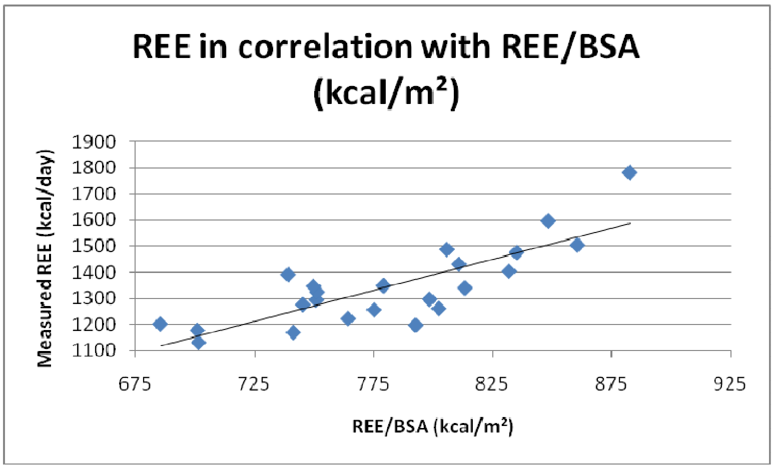
Graph 25 . L1 Measured REE in correlation with REE (%)



P = <0.0001 r = 0.8561

The REE/BSA increased as did REE.

Graph 26. L1 Measured REE in correlation with REE/BSA (kcal/m²)

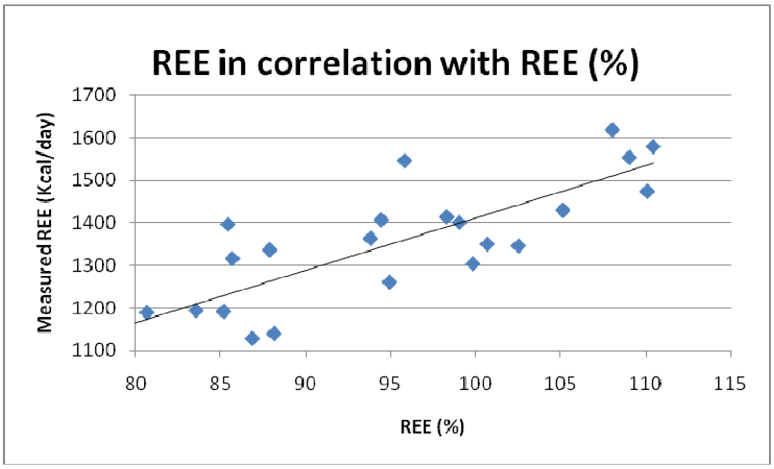


P = <0.0001 r = 0.8077

3.6.3 Results for L2- Lactating Women

The REE (%) increased as did measured REE.

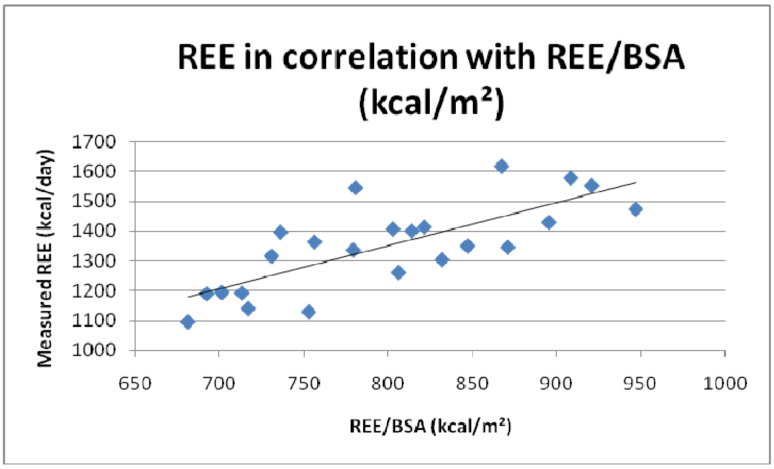
Graph 27. L2 Measured REE in correlation with REE (%).



P = < 0.0001 r = 0.8061

The REE/BSA increased as did measured REE.

Graph 28. L2 Measured REE in correlation with REE/BSA(kcal/m²).

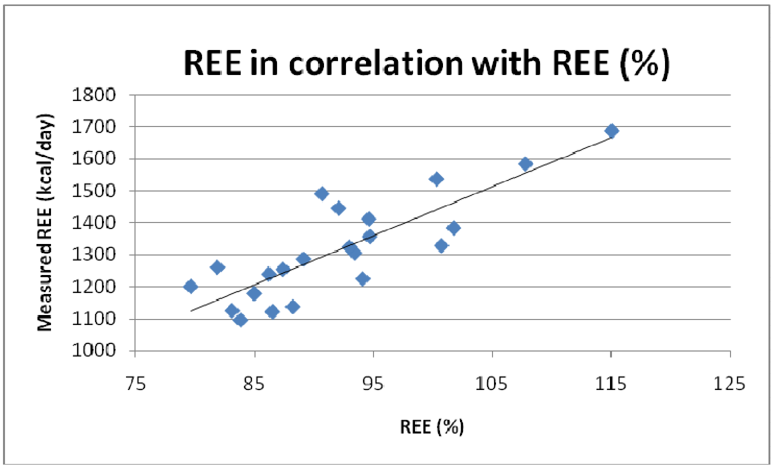


P = <0.0001 r = 0.7583

3.6.4 Results for L3- Lactating Women

The REE (%) increased as did measured REE.

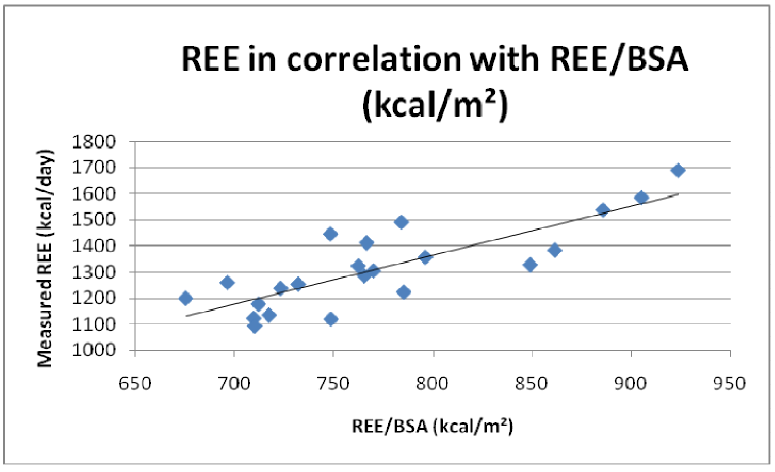
Graph 29. L3 Measured REE in correlation with REE (%).



P = < 0.0001 r = 0.8352

The REE/BSA increased with measured REE.

Graph 30. L3 Measured REE in correlation with REE/BSA (kcal/m²).

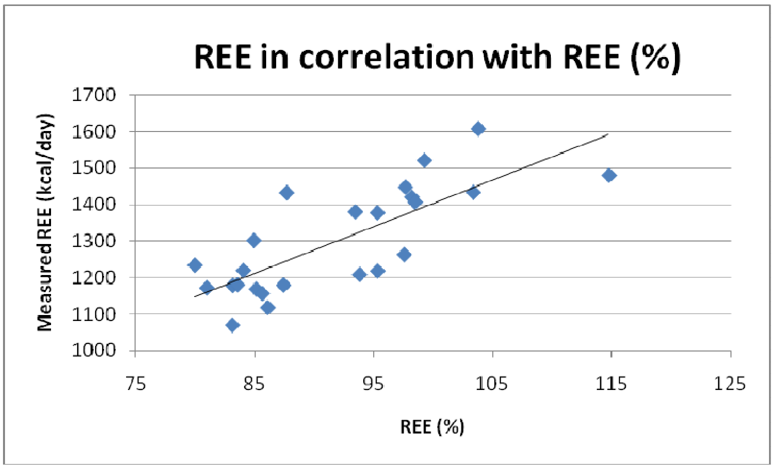


P = < 0.0001 r = 0.8219

3.6.5 Results for L4- Lactating Women

The REE (%) increased as did measured REE.

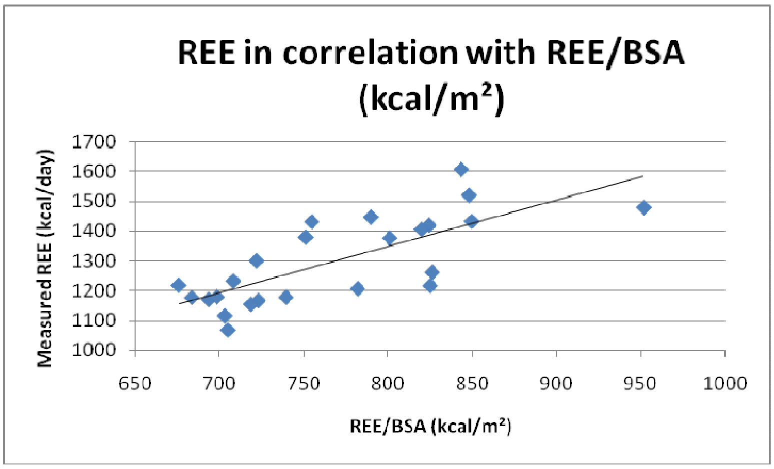
Graph 31. L4 Measured REE in correlation with REE (%).



P = < 0.0001 r = 0.774

The REE/BSA increased as did measured REE.

Graph 32. L4 Measured REE in correlation with REE/BSA (kcal/m²).



P = < 0.0001 r = 0.7467

10 Discussion

Our study focuses on measuring resting energy expenditure (REE) in kcal/day in lactating women as it is unknown in the Czech women via indirect calorimetry, as well measuring anthropometrical parameters with Inner Scan Body Composition Monitor balance to determine if there were any correlations between measured REE and the obtained anthropometrical parameters. Other studies performed internationally recognize that metabolic adjustments occur during pregnancy and lactation to support fetal growth and milk synthesis yet the effect of body composition on these changes is poorly understood Energy expenditure increases during pregnancy because of the metabolic contribution of the uterus and fetus and the increased work of the heart and lungs. Variation in energy expenditure among subjects in international studies was largely due to differences in fat free mass, which in pregnancy is composed of the expanded plasma, high-energy requiring fetal and uterine tissues, and moderate-energy requiring skeletal muscle mass. Fat mass, a tissue with low energy requirements, contributed to the variation in energy expenditure, but to a much lesser extent (Butte, 1999). Results from our study can be compared to similar studies undergone internationally which studied the same parameters as our study, however in a different context for example TEE instead of REE.

10.1 REE

Extra dietary energy is required during pregnancy to make up for the energy deposited in maternal and fetal tissues and the rise in energy expenditure attributable to increased basal metabolism and to changes in the energy cost of physical activity (Butte, 2004). During pregnancy, energy expenditure generally rises because of an increase of maternal and fetal weight(Butte, 1999).

In our study REE was measured via indirect calorimetry. The results were P0 1543 ± 97.1 kcal/day, L1 1449 ± 88.6 kcal/day, L2 1428 ± 94.5 kcal/day, L3 1429 ± 95.2 kcal/day, L4 1415 ± 99.8 kcal/day , the REE in the end of pregnancy was 6.48 to 9.05% higher than in the lactation periods, during the lactation periods REE remained in a similar high range .

In international studies similar results were obtained. In Butte's study, BMR was 18 to 20% higher in the pregnant subjects than in the post-partum subjects, overall increased rate of REE was evident in both late pregnancy and lactation. In anther study conducted by Butte, where the normal BMI pregnant group REE was compared to normal BMI post-partum REE group, showed that the pregnant REE was 26.5% higher than post- partum REE. A similar study done by Prentice found BMR to increase from 8.6 to 35.4% in the 36th week of pregnancy, however this study compared non-pregnant and non-lactating women to pregnant women. Forsum, Spaaij, and Sadurski all conducted similar studies on pre-pregnant and post partum lactating women and all three studies concluded that the post-partum lactating women had a higher REE than the pre-pregnant women. Motil's study is interesting to note as he compared lactating women to non-lactating women and found that BMR remained relatively unchanged. Overall, we see that the highest REE is found in pregnant women towards the end of pregnancy. Varying results are obtained on lactating women, however since Butte's 1999 study is the most similar to ours, it is in agreement with our results that REE is highest in pregnant women and remains in a similar high range in the lactating women.

Denne and Knuttgen found an in increase in respiratory quotients in pregnant subjects, which would indicate higher rates of net carbohydrate utilization (Butte,1999), yet Blackburn found no changes in respiratory quotients. For lactating women Spaaij found that the respiratory quotient was lower and van Raaij and Frigerio found no changes in respiratory quotient.

Butte concludes that elevated respiratory quotient during pregnancy continue during lactation, this being consistent with preferential use of glucose by fetus and mammary gland (Butte,1999). Butte's study found that the respiratory quotient was significantly higher during pregnancy than postpartum- 0.828 ± 0.032, 0.802 ± 0.042, 0.789 ± 0.042 at 37th week of gestation, 3 months postpartum, and 6 month postpartum respectively. In our study the respiratory quotients were P0 0.8382 ± 0.1167, L1 0.7722 ± 0.1038, L2 0.8250 ± 0.1410, L3 0.8356 ± 0.1754, L4 0.9443 ± 0.3104, in our study we find that the respiratory quotient was highest in L4, and P0 was only higher that L1 and L2 but not L3 and L4.

In terms of substrate utilization Butte's study found that protein oxidation as a percentage of TEE was significantly lower during pregnancy than postpartum (P = 0.004). In our study we found that utilization of the protein substrate as a percentage of REE in pregnancy was lower as well; P0 =33.89 ± 10.32, L1 = 39.73 ± 11.9, L2 = 43.05 ± 12.16, L3 = 48.45 ± 14.04 L4 = 45.64 ± 11.49. Butte's study concluded that higher carbohydrate utilization at 37th week of gestation was maintained through to the 6th month postpartum in lactating women, which is consistent with the preferential use of glucose by the fetus and mammary gland. Our study found that the highest utilization of carbohydrates (% of REE) was highest during the pregnant period and decreased in the lactating period relative to the pregnant period; P0 = 31.42 ± 24.06, L1 = 16.18 ± 18.59, L2 = 19.57 ± 16.98, L3 = 21.75 ±

17.16 L4 = 25.33 ± 18.18. As for lipid oxidation (% of TEE) Butte's results conclude that lipid oxidation did not change very much over time 30 ± 7, 31 ± 9, 29 ±8 at 37th week of gestation, 3 months postpartum, and 6 month postpartum respectively. Our study found that utilization of lipids (% of REE) were P0 = 38.76 ± 17.61, L1 = 46 ± 17.88, L2 = 41.90 ± 21.04, L3 = 45.23 ± 13.78, L4 = 34.42 ± 19.49. Our study found that utilization of lipids was highest at L1 followed by L3, then L2, then P0 and finally L4, showing quite some variation.

Weight gain during pregnancy results from products of conception (fetus, placenta, and amniotic fluid), increases in various maternal tissues (uterus, breasts, blood, and extracellular extra vascular fluid), and increases in maternal fat stores (Butte). Vinoy's study evaluated 15 lactating Bangladesh mothers who showed evidence of chronic malnutrition (BMI 14.9- 18.1 kg/m^2), the measurements were conducted in 5 different time periods- 3.5, 5.5, 7.5, 10, and 13 months post partum and found that on average the mother's weight fell by 1.3kg during the 5 periods of lactation. In our study of healthy Czech women we had 4 periods of lactation and when comparing weight from L1 to L4 we found on average a 2.38 kg decrease in weight, despite the difference between malnourished and healthy women, we still see a trend of a decrease in weight as lactation continues. The following results were obtained form Butte's study . Weight (kg) showed a decrease from the 37th week of gestation to the 3rd and 6th month postpartum. The weight ranged from 75.7 ± 9.7 kg to 65.5 ± 11.0 kg to 62.7 ± 10.0 kg respectively. In our study, the current weight (kg) of P0 75.2 ± 8.7 kg, L1 64.8 ± 7.9 kg, L2 62.6 ± 8.7 kg , L3 62.9 ± 8.7 kg, L4 61.7 ± 9.3 kg also showed a general decrease in weight.

From our study we found that we are in accordance with the international studies in terms of predicting a higher REE in pregnant women. As for lactating women, it is difficult to compare and find a difference in REE between lactating women and pregnant women as most studies compare either non pregnant non lactating women REE to pregnant women REE, or non pregnant women REE to lactating women REE.

Our results of Respiratory Quotient (RQ) do not agree with the international findings, some studies found RQ to be highest in pregnant women, some found no changes at all and other found RQ to be lower in lactating women. Our findings show RQ to be highest in the lactating period of L4, followed by L3, and then the next highest value for RQ being in the pregnant period P0.

As for substrate utilization our results are in accordance with protein utilization, being lowest in the pregnant group and increasing in the lactating groups. In terms of carbohydrate utilization, our results agree with international findings that carbohydrate utilization was highest in the pregnant period. Lipid oxidation however varied widely from international findings, in Butte's study, lipid oxidation does not vary widely, but in our study we found a range of lipid oxidation from 34.42 to 46% where as Butte's results only varied from 29 to 31%.

Our results of weight are also in agreement with international findings that weight decreased from the end of pregnancy throughout lactation.

In terms of correlation findings, very high correlations were found in relation to measured resting energy expenditure in the following parameters.

In the time period P0:

• Weight in pregnancy (kg) ($P< 0.0001$)

•Predicted basal metabolism ($P< 0.0001$)

In the time period L1:

•Current weight (kg) ($P< 0.0001$)

• Amount of water in body (%) ($P< 0.0001$)

• Body surface area (m^2) ($P< 0.0001$)

• Body fat (%) ($P< 0.0001$)

• Predicted basal metabolic rate (kcal/day) ($P< 0.0001$)

• REE (%) ($P< 0.0001$)

• REE/BSA ($kcal/m^2$) ($P< 0.0001$)

In the time period L2:

• REE (%) ($P< 0.0001$)

• REE/BSA($kcal/m^2$) ($P< 0.0001$)

In the time period L3:

• REE (%) ($P< 0.0001$)

• REE/BSA ($kcal/m^2$) ($P< 0.0001$)

In the time period L4:

- REE (%) ($P < 0.0001$)
- REE/BSA ($kcal/m^2$) ($P < 0.0001$)

Abstract

Aim: In the past, in Czech Republic, Resting Energy Expenditure (REE) was not known in pregnant or lactating women. The results obtained from our study are intended for clinical use since up until now Resting Energy Expenditure was obtained from internationally published results, and were not a direct measure for Czech women due to differences in terms of nutrition and calories.

Objective: The measurements of Resting Energy Expenditure were conducted in 5 different time periods via indirect claorimetry to determine correlations between Resting Energy Expenditure and measured anthropometrical values.

Design: There were a total of 24 subjects in the study examined in 5 different time periods. The P0 being at the end of pregnancy, the L1 from birth up to 7 weeks post partum, L2 from the 10^{th} to the 16^{th} week post partum, L3 from the 20^{th} to the 26^{th} week post partum, and L4 from the 34^{th} to the 47^{th} week post partum. The pregnant subjects P0 were used as the comparison with L1 to L4 lactation subjects to observe changes in REE and other anthropometrical measures. The measurement of Resting Energy Expenditure was carried out via indirect calorimetry. The women arrived from their homes after 12 hours of fasting and were at rest 30 minutes before assessment.

Results: In our study resting energy expenditure was measured via indirect calorimetry. The results were P0 1543 ± 97.1 kcal/day, L1 1449 ± 88.6 kcal/day, L2 1428 ± 94.5 kcal/day, L3 1429 ± 95.2 kcal/day, L4 1415 ± 99.8 kcal/day , the REE in the end of pregnancy was 6.48 to 9.05% higher than in the lactation periods, during the lactation periods REE remained in a similar high range .

Conclusion: From our study we found that we are in accordance with the international studies in terms of predicting a higher REE in pregnant women. Maximum REE in pregnancy, lower and balanced was determined. As for lactating women, it is difficult to compare and find a difference in REE between lactating women and pregnant women as most studies compare either non pregnant non lactating women REE to pregnant women REE, or non pregnant women REE to lactating women REE.

Sources

Blackburn MW, Calloway DH. Basal Metabolic Rate and Work Energy Expenditure of Mature, Pregnant Women. J Am Diet Assoc 1976;69:24–8.

Bronstein MN, Mak RP, King JC. Unexpected Relationship between Fat Mass and Basal Metabolic Rate in Pregnant Women. Br J Nutr 1996;75:659–68.

Butte NF, Hopkinson JM, Mehta N, Moon JK, Smith EB. Adjustments in Energy Expenditure and Substrate Utilization during Late Pregnancy and Lactation. Am J Clin Nutr 1999; 69:299-307

Butte NF, Wong WW, Treuth MS, Ellis KJ, Smith EB. Energy Requirements during Pregnancy based on Total Energy Expenditure and Energy Deposition. Am J Clin Nutr 2004;79:1078–87.

Cuerda C, Ruiz A, Velasco C, Breto´n I, Camblor M, Garcı´a-Peris P.How accurate are Predictive Formulas calculating Energy Expenditure in Adolescent Patients with Anorexia Nervosa? Clin Nutr 2007; 26:100–106.

Denne SC, Patel D, Kalhan SC. Leucine Kinetics and Fuel Utilization During a Brief Fast in Human Pregnancy. Metabolism 1991;40:1249–56.

DuBois D, DuBois EF. A Formula to Estimate the Approximate Surface Area if Height and Weight be Known. Arch Intern Med 1916;17:863–871.

Forsum E, Kabir N, Sadurskis A, Westerterp K. Total Energy Expenditure of Healthy Swedish Women During Pregnancy and Lactation. Am J Clin Nutr 1992;56:334–42.

Frigerio C, Schutz Y, Whitehead R, Jequier E. A New Procedure to Assess the Energy Requirements of Lactation in Gambian women. Am J Clin Nutr 1991;54:526– 33.

Fuller NJ, Jebb SA, Laskey MA, Coward WA, Elia M. Four-component model for the Assessment of Body Composition in Humans: Comparison with Alternative Methods, and Evaluation of the Density and Hydration of Fat-Free Mass. Clin Sci 1992;82:687–93.

Ganong WF. Review of Medical Physiology. 22nd ed. New York: Lange Medical, 2005: 279-282.

Goldberg GR, Prentice AM, Coward WA, et al. Longitudinal Assessment of Energy Expenditure in Pregnancy by the Doubly Labeled Water Method. Am J Clin Nutr 1993;57:494–505.

de Groot LCPGM, Boekholt HA, Spaaij CK, et al. Energy Balances of Healthy Dutch Women Before and During Pregnancy: Limited Scope for Metabolic Adaptations in Pregnancy. Am J Clin Nutr 1994;59:827–32.

Guyton, Hall Medical Physiology 11th ed. Philadelphia. Elsevier Inc.; 2006: 883-887

Hronek M., Zadak Z. New Equation for the Prediction of Resting Energy Expenditure During Pregnancy. Nutrition 2009; 25: 947- 953

Kleiber M. Body Size and Metabolic Rate. Physiol Rev 1947;27:511.

Knuttgen HG, Emerson K. Physiological Response to Pregnancy at Rest and During Exercise. J Appl Physiol 1974;36:549–53.

Lederman, SA. Influence of Lactation on Body Weight Regulation. Nutrition Reviews 2004; 62: 112-119

Motil KJ, Montandon CM, Garza C. Basal and Postprandial Metabolic Rates in Lactating and Non-lactating women. Am J Clin Nutr 1990;52:610–5.

Prentice AM, Goldberg GR, Davies HL, Murgatroyd PR, Scott W. Energy-Sparing Adaptations in Human Pregnancy assessed by Wholebody Calorimetry. Br J Nutr 1989;62:5–22.

Roza AM, Shizgal HM. The Harris Benedict Equation Reevaluated: Resting Energy Requirements and the Body Cell Mass. Am J Clin Nutr 1984; 40:168–182

Sadurskis A, Kabir N,Wager J, Forsum E. Energy Metabolism, Body Composition, and Milk Production in Healthy Swedish Women during Lactation. Am J Clin Nutr 1988;48:44–9.

Schofield WN, Schofield C, James WPT. Basal Metabolic Rate—review and Prediction, together with an annotated bibliography of source material. Hum Nutr Clin Nutr 1985;39C:1–96.

Spaaij CJK, van Raaij JMA, de Groot LCPGM, van der Heijden LJM, Boekholt HA, Hautvast JGAJ. Effect of Lactation on Resting Metabolic Rate and on Diet- and Work-induced Thermogenesis. Am J Clin Nutr 1994;59:42–7.

van Raaij JMA, Schonk CM, Vermaat-Miedema SH, Peek MEM, Hautvast JGAJ.Body Fat Mass and Basal Metabolic Rate in Dutch Women Before, During, and After Pregnancy: a Reappraisal of Energy Cost of Pregnancy. Am J Clin Nutr 1989;49:765–72.

van Raaij JMA, Schonk CM, Vermaat-Miedema SH, Peek MEM, Hautvast JGAJ. Energy Cost of Lactation, and Energy Balances of Well-Nourished Dutch Lactating Women: Reappraisal of the Extra Energy Requirements of Lactation. Am J Clin Nutr 1991;53:612–9.

Vinoy S, Rosetta L,Mascie- Taylor CGN. Repeated Measurements of Energy intake, Energy Expenditure and Energy Balance in Lactating Bangladeshi Mothers. European Journal of Clinical Nutrition (2000) 54, 579±585

Weir J. New Method for Calculating Metabolic Rate with Special Reference to Protein Metabolism. J Physiol 1949;109:1–9.

Instruction Manuals:

Tanita Body Composition Monitor Manual; no author given, date not give on manual

Indirect Calorimetry Handbook; no author given, date not given on manual

Internet sources:

FAO/WHO/UNU. Report on Human Energy Requirements. Expert Consultation. Rome; 2004. http://www.fao.org. Viewed at 01/30/2010.

Anonymous author. Indirect Calorimetry. 2007. www.gpnotebook.com. Viewed at 09/25/2009.