

**NUTRITIONAL STATUS OF PREGNANT WOMEN  
(UNDER 20 YEARS OF AGE) WITH SPECIAL  
EMPHASIS ON IRON AND FOLIC ACID STATUS**

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of Master of Nutrition at the University of Stellenbosch**



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**“Declaration**

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

Signature:

Date:

## **ABSTRACT**

Pregnancy and growth have been found to have a detrimental effect on the micronutrient status of adolescent girls. Dietary studies in adolescents have shown serious shortfalls in their dietary iron and folate intake. The competition for nutrients between the fetus and a pregnant adolescent may carry the risk of complications such as intrauterine growth retardation, pre-eclampsia, both maternal and fetal intrapartum mortality, the increased risk of birth injuries and low birth weight.

The aim of the study was to assess the nutritional status of rural black, pregnant teenagers attending the antenatal clinic at Siloam Hospital in the Limpopo Province, with special emphasis on iron and folic acid intake, and evaluation of the newborn babies in terms of weight status and neural tube defects. The nutritional status was determined in 40 pregnant and 40 non-pregnant adolescent girls. The pregnant girls were selected during their first visit to the antenatal clinic, and the non-pregnant girls were selected from nearby schools.

The demographic and dietary history questionnaires were used to collect information from the subjects. The dietary intake of the subjects was collected by the completion of a pre-tested quantified food frequency questionnaire. The anthropometric questionnaire was used to get information from the pregnant adolescents and the control group. The infant anthropometric measurements questionnaire provided information on the infant and the outcome of birth. Blood was collected from the pregnant adolescent girls and the control subjects.

Anaemia was observed in 57.5% of the pregnant and 27.5% of the non-pregnant adolescents (haemoglobin <11 g/dl and <12 g/dl, respectively). The prevalence of low serum ferritin (<12 ug/L) and low transferrin saturation (<16%) was high in both the pregnant (30% and 60% respectively) and the non-pregnant adolescents (17% and 72.5% respectively). Iron deficiency was observed in 45% of the

pregnant girls and 35% of the control subjects. The prevalence of iron deficiency anaemia in the pregnant girls (30%) and the control subjects (22.5%) was high. Low red blood cell folate in pregnant and non-pregnant girls was uncommon. Low serum vitamin B12 was common in most of the pregnant girls.

Analysis of the quantitative food frequency questionnaire (QFFQ) that was completed for 80 of the pregnant and non-pregnant girls showed that 55% had low dietary iron intakes (<67% of the RDA) ( $p = 0.7307$ ). Forty-five percent of the pregnant girls reported taking iron supplements. The QFFQ showed 20% of pregnant girls with low intake of folate ( $p = 0.0577$ ). Forty-five percent of pregnant girls also reported taking folate supplements. Anthropometric measurements of pregnant girls and their dietary intake could not be correlated to the birth outcome. The social profile of the subjects did not seem to influence their iron and folate status and the outcome of pregnancy.

The findings in this study showed that pregnant adolescents appeared to be similar to the non-pregnant adolescent girls socio-economically, anthropometric and nutritionally. We recommend that educational programmes targeted at adolescents and teenagers in the Siloam area should reach girls that could potentially fall pregnant. An educational programme must include: reproductive needs (avoidance of sex or safe sex); nutritional needs, especially targeted at improving nutritional status to meet future reproductive needs; early booking at the antenatal clinic and effective supplementation during pregnancy. The need to improve the dietary and nutrient intake of the adolescent girls should be addressed within the current framework of the Integrated Nutrition Programme (INP). A food fortification programme with essential micronutrients such as iron, folic acid, zinc and Vitamin A to improve the micronutrient status should be promoted among the adolescent girls.

## UITREKSEL

Daar is bevind dat swangerskap en groei 'n nadelige effek het op die mikronutriëntstatus van vroulike adolessente. Dieetstudies in adolessente het ernstige tekortkominge in dieetyster- en foliaatinnames getoon. Die kompetisie vir nutriente tussen die fetus en die swanger adolessent kan verantwoordelik wees vir komplikasies soos intra-uterine groeivertraging, preeklampsie, verhoogde mortaliteit van beide moeder en baba tydens kraam, 'n verhoogde risiko vir geboortebeserings en lae geboortegewig.

Die doel van die studie was om die effek van voedingstatus by swart, swanger tieners by die voorgeboortekliniek in Siloam Hospital in die Limpopo-provinsie te bepaal, met spesifieke verwysing na die yster- en foliensuurinname, asook die evaluering van die pasgebore babas in terme van gewig en neurale buis defekte. Die voedingstatus van 40 swanger en 40 nie-swanger adolessente meisies is bepaal. Die swanger meisies is ewekansig geselekteer gedurende die eerste besoek aan die voorgeboortekliniek, en die nie-swanger meisies is geselekteer by nabygeleë skole.

Die demografiese en dieetgeskiedenisvraelyste is gebruik om inligting van die proefpersone in te samel. Voorafgetoetste gekwantifiseerde voedselrekwensie vraelyste is gebruik om die voedselinname van proefpersone te bepaal. Antropometriese vraelyste is gebruik om antropometriese inligting van die swanger adolessente en die kontrole groep. Die antropometriese vraelys vir babas is gebruik om inligting ten opsigte van die baba aan te teken asook die verloop van die swangerskap. Bloedmonsters is van die swanger tieners en die kontrole groep ingesamel.

Anemie is waargeneem by 57.5% van die swanger en 27.5% van die nie-swanger adolessente (hemoglobien  $<11$  g/dl en  $<12$  g/dl onderskeidelik). Die voorkoms van lae serum ferritien ( $<12$  ug/L) en lae transferriënersadiging ( $<16\%$ ) was

hoog by beide die swanger (30% en 60%) en die nie-swanger adolessente (17.5% en 72.5% onderskeidelik). Ystergebrek is in 45% van die swanger meisies en in 35% van die kontrole groep waargeneem. Die voorkoms van ystergebrekanemie in die swanger meisies (30%) en die kontrole groep (22.5%) was hoog. Lae rooibloedsel-folaat by swanger en nie-swanger meisies is nie algemeen waargeneem nie. Lae serum vitamien B12 was algemeen by die meeste swanger meisies.

Ontleding van die gekwantifiseerde voedsel-frekwensievraelys (KVFV) wat vir 80 van die swanger en nie-swanger meisies voltooi is, het getoon dat 55% 'n lae dieet-ysterinnome gehad het (<67% van die ADT) ( $p=0.7307$ ). Vyf-en-veertig persent van die swanger meisies het bevestig dat hulle ystersupplemente gebruik het. Die gekwantifiseerde voedsel-frekwensievraelys (KVFV) het getoon dat 20% van die swanger meisies 'n lae folaatinnome het (0.0577). Vyf-en-veertig persent van die swanger meisies het ook genoem dat hulle folaatsupplemente gebruik het. Die antropometriese metings van swanger meisies en dieetinname kon nie gekorreleer word met die verloop van die geboorte nie. Dit blyk dat die sosiale profiel van die meisies nie 'n effek op die yster- en folaatstatus en op die verloop van swangerskap gehad het nie.

Die bevindinge van hierdie studie toon dat swanger en nie-swanger adolessente meisies sosio-ekonomies, antropometriese en nutrisioneel dieselfde voorkom. Dit word aanbeveel dat voorligtingsprogramme vir adolessente en tieners in die Siloam-area meisies moet bereik wat moontlik swanger sal word. 'n Voorligtingprogram moet die volgende insluit: reprodktiewe behoeftes (vermyding van seks of veilige seks); voedingbehoefte, veral geteiken om voedingstatus te verbeter om ten einde toekomstige voorplantingsbehoefte te vervul; vroeë besoeke aan die voorgeboortekliniek en effektiewe supplementering gedurende swangerskap. Die behoefte om die dieet en nutriëntinnome van adolessente meisies te verbeter moet binne die huidige raamwerk van die Geïntegreerde Voedingsprogram aangespreek word. 'n

Voedselversterkingsprogram met essensiële mikronutriënte soos yster, foliensuur, sink en vitamien A om die mikronutriëntstatus van adolessente meisies te verbeter, moet bevorder word.

## **Dedication**

This thesis is dedicated to the following people for their encouragement and support throughout my studies: my lovely wife, Lusani; my dearest mom and dad, and the rest of the family.

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**ABBREVIATIONS**

BMI	Body Mass Index
CED	Chronic-Energy-Deficient
DHF	Dihydrofolic Acid
DNA	Deoxyribonucleic Acid
EC	European Commission
EU	European Union
FAO	Food and Agriculture Organization
INP	Integrated Nutrition Programme
IOM	Institute of Medicine
IU	International Units
IUGR	Intra-uterine Growth Retardation
MCHC	Mean Corpuscular Haemoglobin Concentration
MCH	Mean Corpuscular Haemoglobin
MCV	Mean Corpuscular Volume
MG	Milligram
MJ	Millijoule
MRC	Medical Research Council
MUAC	Mid-Upper Arm Circumference
NE	Niacin Equivalent
NRIND	National Research Institute for Nutritional Diseases
NTD	Neural Tube Defects
PAHO	Pan American Health Organization
PRI	Population Reference Intakes
QFFQ	Quantitative Food Frequency Questionnaire
RDA	Recommended Dietary Allowance
RE	Retinol Equivalents
RNA	Ribonucleic Acid
RNI	Recommend Nutrient Intake
SAS	Statistics Analysis System
SAVACG	South African Vitamin A Consultative Group
SCN	Sub-committee of Nutrition
SD	Standard Deviation
SGA	Small for Gestational Age
TFR	Transferrin Receptor
THF	Tetrahydrofolic Acid
TIBC	Total Iron Binding Capacity
µg	Microgram
UK	United Kingdom
UNU	United Nations University
USA	United States of America
USAID	United States Agency for International Development
WHO	World Health Organization

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# **INTRODUCTION AND LITERATURE REVIEW**

## **1. INTRODUCTION**

### **1.1 The importance of nutrition during pregnancy**

The nutritional status of a woman before and during pregnancy has a great influence on the outcome and development of the fetus, and maternal health (1). Maternal nutrition plays an important role because the demand for nutrients during pregnancy is higher and the consequences of poor nutrition are severe (2). Maternal nutrition affects both the successful completion of pregnancy and the development of the fetus, and most of the studies conducted in the past have linked poor maternal nutrition to poor pregnancy outcome (3,4).

The competition for nutrients between the fetus and a pregnant adolescent may carry the risk of complications such as intra-uterine growth retardation, pre-eclampsia, intrapartum mortality both maternal and fetal, the increased risk of birth injuries and low birth weight (4,5,6). This may be explained by the fact that pregnant adolescents are still growing and their bodies need more nutrients. Most of the important nutrients are likely to be inadequate in the diets of the adolescents. Their premature body status will not have had the time to store the much-needed nutrients for their own development because their needs are high (7).

Adolescents gain up to 50% of their weight, more than 20% of their adult height, and 50% of their adult skeletal mass during this period resulting in increased nutritional needs. Increased physical activity, combined with poor eating habits and other considerations, such as menstruation and pregnancy, contribute to the potential risk for poorly nourished adolescents. The main nutritional problems affecting adolescent populations include iron deficiency and anaemia; vitamin A deficiency; poor intake of calcium; zinc; folate; intra-uterine growth retardation in pregnant adolescent girls and obesity. In the Ontario Health status survey, adolescents' diet tended to be higher in fat and lower in carbohydrates (8).

Nutrition is one of the most important factors determining the quality of the environment of the developing fetus and the overall outcome of pregnancy. Nutrition services such as nutrition education and antenatal clinics may improve the outcome of a first pregnancy, lay the groundwork for better care and timing of future pregnancies, and improve the nutrition of families of adolescent parents (9).

Every year, one out of ten young women between the age of 15 and 19 falls pregnant in the United States of America (USA) (10). A proportion of these infants born to these women are of low birth weight, preterm and need specialized care (9). In South Africa, adolescent child bearing is significant with teenage pregnancies representing one third of all births (330 per 1000 live births) (11). The low socio-economic status associated with most of the pregnant teenagers may be the reason for the large number of infants with a low birth weight and for the other complications and unfavourable outcomes of their pregnancies (12). Some of the problems that are found among pregnant adolescents under the age of 16 years are: preterm births, pregnancy-induced hypertension, anaemia, and sexually transmitted diseases (13). These conditions leave pregnant adolescents at a greater risk of complications.

## **2. Physiological changes during pregnancy**

Pregnancy is a normal physiological process associated with major changes affecting all the maternal organs and metabolic pathways. Changes in haematological values such as haemoglobin may be significant as pregnancy progresses from first to third trimester and to delivery, and then returns normal after delivery (14). Plasma volume and red cell mass are controlled by different mechanisms in pregnancy. Plasma volume begins to rise in the first trimester and reaches a peak at about the 32<sup>nd</sup> week (15). The average plasma volume expansion is about 50%, so that enough oxygen, which is transported bound to haemoglobin in the red cells, can be available to support the growing fetal and maternal tissue

(16,17). There is a greater increase of plasma volume in multigravidas than primigravidas because of the increase in the weight borne by multigravidas compared to primis (16).

There is an increase in the need for those nutrients most important for making blood: protein, iron, folacin, and vitamin B12. The red cell mass begins to rise in the late first trimester. The red cell mass is usually increased up to 20%. This disproportionate increase in plasma volume results in haemodilution and is responsible for the physiological anaemia of pregnancy (15). The concentration of haemoglobin and the packed cell volume usually fall despite the absolute increase in total haemoglobin. The purpose of these many changes in the composition and volume of maternal blood is to facilitate the transport of nutrients to and removal of waste products from the fetus (17).

The kidney tubules are unable to adjust completely and a percentage of nutrients that would have been reabsorbed in the non-pregnant woman are excreted in the urine of a pregnant woman (18). More fluid is needed by the woman's body because of increased blood volume for the mother, the development of blood for the fetus, the amniotic fluid (which surrounds the fetus in the uterus and protects it from trauma or shock) and the increased urine output because the pregnant woman is now getting rid of waste products for both herself and her baby (17). Cardiac output rises with 30% to 50% of pre-pregnancy levels by the early third trimester. Hyperventilation during pregnancy produces a 40% to 50% increase in tidal volume.

There is a high glomerular filtration rate because of increased blood volume. Serum creatinine and urea nitrogen decrease from pre-pregnancy values. Cell-mediated immunity may be depressed. There are progressive decreases in the haemoglobin concentration, erythrocyte count, packed-cell volume, total serum protein and certain fractions thereof (including albumin), and vitamin A and ascorbic acid, and decreased urinary excretion of thiamin and riboflavin.

Changes in the plasma concentration of vitamin B12 during pregnancy have been reported to affect intestinal absorption and placental nutrient transport (19). There is also an increase in the serum concentration of carotene, tocopherols, cholesterol and N-methyl nicotinamide (17).

There is an increase in cardiac hypertrophy and increased pulse rate. Blood pressure usually decreases and then returns to normal in the third trimester. Maternal oxygen requirements increase and there is a lowered threshold for carbon dioxide, making the pregnant woman feel dyspnoeic with an increased need to breathe. The growing uterus pushes the diaphragm upward, making breathing difficult (18).

Gastrointestinal tract changes affect nutritional status in several ways. Early in pregnancy nausea and vomiting may occur, followed by a return of appetite which can be ravenous. Cravings for and aversions to food may be accompanied by the decreased ability to taste saltiness. An increased progesterone level relaxes the uterine muscle so that it can expand with fetal growth. This also causes diminished gastrointestinal motility so that constipation is often a problem. A relaxed lower oesophageal sphincter can result in regurgitation and heartburn. Heartburn and constipation are common and there is evidence of reduced gastric tone, motility and secretion.

### **3. Assessment of nutritional status in pregnant adolescents**

The usefulness of anthropometric measurements in assessing nutritional status was first realized by Richer, who used skinfold thickness as an index of fatness (20). Anthropometry provides a simple, reliable and low-cost method of assessing maternal nutrition status. Maternal nutrition has long been known as a fundamental determinant of fetal growth, birth weight, and infant morbidity, as well as women's health, productivity and caring capacity (21). Assessment of maternal status during pregnancy is commonly based on height, weight, mid-upper arm circumference, and various measures of skinfold thickness, and body mass index before pregnancy or during the first trimester.

In 1990, the World Health Organization (WHO), Pan American Health Organization (PAHO), the United States Agency for International Development (USAID) and Mother Care International convened an international meeting to examine the relationship between maternal anthropometry and pregnancy outcome and its application to primary health care (22,23). Measurements made during pregnancy are expected to reflect both the nutritional status of the women and, indirectly, growth of the fetus. Measurements taken early in pregnancy should also be used to predict how well a pregnant woman can cope with the physiological demands of pregnancy.

The most widespread use of anthropometric measurements during pregnancy has been in evaluating the risk of fetal growth retardation and selecting women or populations for nutritional interventions aimed at improving fetal growth or prolonging gestation. However, some of the changes that occur during normal human pregnancy can change the biological meaning of anthropometric measurements. Many of these changes relate to the growth of the fetus and of maternal tissue such as the breasts and uterus, whereas others include the increases in body hydration and blood volume that occur quite early in pregnancy (24).

## Height

Height is a reflection of the interaction of the genetic potential for growth and environmental factors (25). Biological changes may also affect the interpretation of maternal height in pregnancy. The normal lordosis has been found to reduce the maternal height as pregnancy progresses (26). Maternal height is an index which indicates the past nutritional state. Measuring a woman's height provides a good indicator of childhood growth and skeletal pelvic structure is a good predictor of the risk of cephalopelvic disproportion and obstructed labour, which is a major cause of maternal death in developing countries (27).

The use of maternal height as an indicator of health and nutritional status must take account of the environmental context in which growth occurred. The environmental conditions that lead to poor maternal linear growth may also result in poor growth and sub-optimal development of the anatomical and physiological systems that sustain optimal fetal growth or maximize maternal health (25). The biological changes that occur during pregnancy may affect the interpretation of maternal height relative to the non-pregnant state. In very young adolescents, in whom significant linear growth potential remains, some increase in height may be observed during pregnancy, but is likely to be very small (26).

Adolescents may be misclassified as being at risk for poor pregnancy outcomes because of short stature relative to adults, when in fact the greater risk is related to other factors such as inadequate dietary intake and poor weight gain associated with adolescent pregnancy. Short maternal height has also been shown to be associated with an increased risk of having babies which are small for gestational age (SGA) in several populations, and cut-off points of maternal height that is between 140 cm and 150 cm have been proposed for screening (28).

Maternal stunting reflects conditions that prevailed during the women's early childhood and may have little relevance to their current nutritional status. For instance, supplementary feeding for women of short stature will not increase their height but may well improve their weight gain. The WHO has shown that those women who were shorter than the median height for their population are particularly prone to having small babies if their weight is also low and if their weight gain during pregnancy is small. Short stature has been associated with the increased risk of low birth weight and obstructed labor during childbirth (28).

### **Weight**

Body weight includes all compartments at each level of body composition. Body weight represents the sum of protein, fat, water, and bone mineral mass, and does not provide any information on relative changes in these four chemical components (29) Body weight serves as a reflection of past growth performance of the mother. It also reflects the recent and concurrent health and nutritional status. The purpose of measuring body weight is to ensure that adequate nutrients are available to the fetus and to add to mother's reserves for lactation. Body weight is also used to evaluate the long-term response to nutrition interventions. There is a strong correlation between preterm delivery and inadequate maternal weight gain, but the fetal contribution to total maternal weight gain cannot be separately determined during pregnancy. Many studies have indicated that both underweight and overweight affect maternal and newborn outcomes (27).

Body weight measured no more than two months before conception is an acceptable approximation of pre-pregnancy weight. If this value is unavailable, the nearest value for measured pre-pregnancy weight may be based on maternal recall or on a measurement made during the first trimester of pregnancy (30). Most studies consider that use of body weight measured in early pregnancy introduces recall bias, and that use of body weight measured in early pregnancy introduces first trimester weight-gain bias. In a study of a group of adolescents in

the USA, recalled pre-pregnancy weight correlated closely with measured weight (31). The use of recalled weight may be a problem in a population with low education status. Body weight measured at various times during pregnancy has been widely used to assess the maternal health status. Because body weight changes rapidly during pregnancy, gestational weight changes are routinely monitored as part of the prenatal care in many parts of the world (22,30). Results from the WHO collaborative study showed that there is a strong correlation between a single measure of maternal weight late in pregnancy and babies that are small for gestational age and low birth weight. Rosso (32) also reported a positive association between maternal size (height and weight) and newborn birth weight.

Given that maternal diet and weight gain during pregnancy are determinants of birth weight, it might be expected that improved diet and weight gain would increase birth weight and decrease infant mortality correspondingly. Weight becomes of limited value when assessing the degree of malnutrition in the presence of disease. Interpretation may be difficult in the presence of oedema, ascites, massive tumor growth or organomegaly. Heavy roundworm infestation and enlarged malarial spleen may contribute to weight increase.

### **3.2.1 Changes in weight during pregnancy**

Maternal weight increases significantly during the course of normal pregnancy (33). Maternal characteristics influence weight gain during pregnancy. These include factors such as age and race which cannot be modified; body habits and socio-economic and marital status, which are not easily modified during the prenatal period; cigarette smoking, drug and alcohol abuse, depression, and social support; which may be more amenable to intervention (21,34). No specific amount of weight gain during pregnancy is right for everyone.

The report of the Institute of Medicine (IOM) (Table 1) recommended that weight gain for normal weight women should be 0.36-0.53 kg per week in the last two trimesters of pregnancy. It has been recommended that adolescents weight gain during pregnancy must be higher than that of adults. It has been demonstrated by Rees et al that restriction of the actual weight gain of pregnant adolescents to the rate recommended for adults may lead to the birth of smaller than optimal infants (35).

**Table 1: Recommendations for adult pregnancy weight gain by body mass index at conception (21)**

BMI Category	Recommended total gain (kg)
Low (BMI<19.8)	12.8 – 18.0
Normal (BMI 19.8 – 26.0)	11.5 – 16.0
High (BMI 26.0 – 29.0)	7.0 – 11.5
Obese (BMI>29.0)	>6.0

The influence of socio-economic status on weight gain during pregnancy cannot be underestimated (36). The nutritional status before pregnancy is linked to poor weight gain in adolescents. Weight gain during adolescent pregnancy has been the focus of recent controversy because of the potential of improving pregnancy outcome by modifying weight gain (37,38). Weight gain during adolescent pregnancy is generally encouraged clinically through monitoring weight gain during the course of prenatal care. However, the course of weight gain among adolescent gravidas is poorly described, and the amount of weight gain, rates of weight gain, and pattern of gain that should be recommended for adolescent gravidas are still debated (39). Hediger et al, in the study amongst adolescent pregnant women found that the birth weight did not appear to improve for the infants of overweight adolescents except when weight gain is low (40). For black adolescents, birth weight is not further improved at any maternal pre-pregnancy BMI with excessive weight gain.

In another study conducted among pregnant adolescents in the USA, it was found that they gained weight equal to the highest range recommended by the IOM (Table 1). Maternal thinness is always associated with the risk of pre-term delivery, but had no other relation to the course of labour. It was also associated with the number of small for gestational age and low birth weight babies (41). Obesity was associated with an increased risk of hypertensive and diabetic disturbances and complications in the course of labour. It was also associated with more macrosomic, large-for-date babies and marginally, with the number of babies who were transferred to the neonatal intensive care unit after birth, pre-eclampsia and urinary tract infections (42,43). Caesarean section rate increased only in morbidly obese women. Even moderate overweight is a significant risk factor for obstetric complications (44).

The difference in morbidity of the low birth weight and small for gestational age babies of the thin women compared with the similar babies of the obese women was found to be minor. This may indicate that, to some extent, low birth weight is

hereditary and is not always a bad prognostic sign of the baby's condition (45). However, small for gestational age and low birth weight babies are major contributors to perinatal death and childhood morbidity (46).

Stevens – Simons et al found that the rate of small for gestational age babies decreased significantly as BMI increased (47). It has been shown in this study that patients with a low BMI and poor weight gain are at the highest risk for small for gestational age (SGA) babies and that those patients with a higher BMI and poor weight gain do not share the risk. The average total weight gain in pregnancy is as low as 6kg in rural parts of developing countries such as Tanzania, but ranges from 10 to 16 kg in developed countries. During pregnancy, the average woman should gain about ten kilograms. Maternal weight gain, one of the most important determinants of infant birth weight, is particularly interesting to health care providers because it can be modified during the prenatal period (48).

Below average weight gain is associated with low birth weight or smallness for gestational age. Total weight gain is of epidemiological interest but of little or no predictive value. The researchers concluded that maternal weight should no longer be measured routinely in pregnancy, after the booking visit (42,43). This might create unnecessary anxiety amongst pregnant women, and the correlation between routine weighing and SGA babies was found to be small. Women with low initial weights often gain more during pregnancy than women with high initial weight. Low weight gains or weight losses are most likely to occur in obese women (49). Many studies have shown that increasing pre-pregnancy weight raises mean birth weight and reduces the percent low birth weight, often by half. Women who have low weights also show higher incidences of early delivery and of perinatal mortality if their weight gain is not above average (49). Limited gestational weight gain has been continuously advocated for a variety of reasons. These include facilitating delivery, preventing pre-eclampsia, improving the maternal condition, or preventing later obesity.

Relatively little of the total weight gain during pregnancy occurs during the first trimester, while gain in the last two trimesters is linear. Birth weight is an important determinant of child health and survival. Low birth weight (<2.5kg) infants are 40 times more likely to die in the neonatal period. Low birth weight-for-length at birth may be a risk factor for chronic disease in later life.

Barker concluded that undernutrition in utero may lead to changes in blood pressure, cholesterol metabolism, insulin response to glucose, and a range of other metabolic, endocrine, and immune functions known to be important in human disease (50,51). In one study conducted in 1092 pregnant women, it was found that maternal weight gain was not effective at detecting women who give birth to small for gestational age infants. The effectiveness of routine measurements of maternal weight gain to detect women at risk of having a growth retarded fetus remains unclear.

It was found that the correlation between weight gain and infant birth weight was low. Weight changes occurring between 28 and 32, 32 and 36, and 28 and 36 weeks were not significantly associated with infant weight after taking into account gestation, maternal age, smoking and parity. Booking weight, booking BMI, and the average weekly gain did have a significant effect on infant birth weight. The study also confirms that the increased prevalence of small for gestational age infants is associated with weight loss or static weight in the third trimester but that this has no practical predictive value. The evidence provided does not support the theory that maternal weight gain might predict infant weight. (52).

Different views have been expressed on weight gain during adolescent pregnancy. Whether maternal weight gain can be used to predict the outcome of pregnancy is still debatable. As younger girls are still growing it may be necessary for them to achieve a greater pregnancy weight gain in order to achieve a satisfactory birth weight. If the weight gain of pregnant adolescents can be recommended above the

cut-off points of the Institute of Medicine, there could be a problem of overweight. Pregnant adolescent girls should be encouraged to have as varied a diet as possible during pregnancy instead of placing undue emphasis on weight gain.

### **3.3. Mid-upper arm circumference**

Mid-upper arm circumference (MUAC) is used to reflect the past and current nutritional status of an individual, and is less responsive than weight to short term changes in health and nutritional condition. MUAC is almost stable throughout pregnancy even if measured at a later stage of pregnancy (22). MUAC may be used to identify women who may be at risk for intra-uterine growth retardation, as a marker for possible maternal malnutrition and to identify women that may benefit from nutritional intervention to prevent poor outcomes. Since the arm contains subcutaneous fat, muscle and bones, a decrease in mid-upper arm circumference may therefore reflect either a reduction in muscle mass or a reduction in subcutaneous tissue, or both (29). MUAC is largely independent of gestational age and regarded as a proxy indicator of maternal pre-pregnancy weight, although the association has been found to be too weak to permit MUAC to be a substitute for weight (53).

### **3.4 Body mass index**

The exact importance of BMI is often difficult to determine. It may be used as an overweight index, on the assumption that excess weight for height reflects excess adiposity. The ability of measures such as pre-pregnancy BMI and gestational weight to predict risk of low birth weight and pregnancy complications has led to their widespread acceptance as clinical tools. The importance of nutritional status as a factor in reproductive outcomes as well as maternal BMI may also be used as an indicator of risk for small for gestational age babies and maternal malnutrition.

The Institute of Medicine recommends a weekly gain of 0.4 kg during the second and third trimesters for women of normal pre-pregnant BMI, 0.5 kg for those who are underweight, and 0.3 kg for overweight women. Body mass index, which is a derived index, was found to be less predictive of SGA risk than indices such as pre-pregnancy weight or attained weight, although a clear relationship does exist (54). In an Indian study, birth weights were related to the nutritional status of the mothers, as measured by BMI. Low birth weight prevalence ranges from 28% for women with adequate BMI to 36% for mild chronic-energy-deficient (CED) mothers, 41% for moderate CED and 53% for severe CED (55). In East Java, Kusin et al. found that the lower a woman's pre-pregnant BMI, the more weight she was likely to gain during pregnancy (56) as indicated in Table 1.

### **3.5 Dietary assessment**

Dietary assessment and dietary status are important parts in describing the relationships between diet, health and disease. It is only in the twentieth century that dietary assessment has been linked to nutrient intake (57,58). Basic questions such as the following have to be asked when selecting a dietary assessment method:

- What is being assessed?
- Who is the target group?
- What is the focus of analysis?
- How accurate is the tool used?

Some of the methods that are used in dietary assessment include 24-hour recall, food frequency recalls, semi-quantitative food frequency recalls, and dietary histories. The problems encountered in the accurate assessment of habitual diet are well documented (59,60).

Food frequency questionnaires have been used increasingly in epidemiological research and have been reported to give reliable estimate of nutrient intake in a wide variety of settings (61). The main objective of the food frequency

questionnaire is to rank individuals appropriately in the distribution of nutrient intake. An advantage of the food frequency questionnaire over short-term records is that it covers a longer period and may therefore, give a better approximation of the usual diet (60).

In one study conducted among pregnant women (in Southampton in England), the food frequency questionnaire appeared to give meaningful estimates of nutrient intake in early pregnancy, which can be used to rank individuals within the distribution (62). However, the food frequency questionnaire has limitations like any other dietary assessment method. The food frequency questionnaire is a shortcut method and does not capture all the information required to calculate nutrient intake. Total consumption is difficult to obtain because not all foods can be included in questionnaire and underestimation can occur. Respondent burden rises as the number of food items queried increases.

#### **4. Nutrient intake during pregnancy**

Pregnancy represents a period of high nutrient needs, and the diet of a pregnant woman should provide for her nutrient needs. Dietary recommendations for pregnant women differ in the amounts and the specifics of the recommended nutrients. Most studies have reported that a large number of pregnant women consume amounts of major nutrients well below the recommended intakes (63). In particular, many teenagers tend to consume low quality diets, regardless of their family backgrounds and knowledge of nutrition (64,65,66) many teenage women will restrict the amount of food they eat in an attempt to control their weight.

Adolescents also commonly skip meals and give low priority to healthy dietary habits. Adequate nutrition during adolescence means adequate dietary intake and/or body stores of both macronutrients and micronutrients, with respect to the activity level of the individual. However, dietary studies in adolescents have shown serious shortfalls in intake. Among 13 to 15 year old girls in Haryana,

India, for example, the mean intake of energy, protein, iron, riboflavin, niacin, and vitamin A were all found to be below recommended allowances (67). Few studies have attempted to relate adolescent micronutrient status to low birth weight risk. There is increased need for energy during pregnancy for the growth of the fetus, placenta, and associated maternal tissues. Energy metabolism differs greatly among women during pregnancy, depending on their situation during pregnancy and energy status before pregnancy (68). It is not easy to recommend an average energy intake during pregnancy because women of small stature tend to gain less weight and have small babies, and hence require less additional energy than an adult woman of normal stature.

## **4.1 Energy and macronutrient requirements during pregnancy**

### **4.1.1 Energy**

An increase in energy and protein intake is associated with an increase in maternal weight gain and fetal growth; however, in a review of several studies, it was found that the supplementation with increased energy and protein intake in pregnant women had no beneficial impact on the health of mothers or that of fetuses (69). Many researchers have different views on this matter. The total energy requirements for pregnant women can be divided into three parts: the obligatory need for energy by the fetus; uterine and other tissues; maternal fat storage and the additional energy expended in association with basal metabolism of the newly formed tissues (70). In some of the studies conducted on energy expenditure, it was found that maternal fat storage and basal metabolism are not fixed energy expenditure but vary widely among similar women in the same population who give birth to healthy infants. The data suggest that the incremental energy expenditure associated with pregnancy is flexible, some women expend far less than the recommended standard and others expend far more energy (71,72).

The energy recommendation by the United Nations agencies such as the Food and Agriculture Organization (FAO), the World Health Organization (WHO) and United Nations University (UNU), for the entire pregnancy is 1200 kilojoules (285 kcal) per day in addition to the normal recommendation. The total energy needed for pregnancy as recommended by FAO/WHO/UNU is compared with those of the United Kingdom, the USA, and The Netherlands recommendations (Table 2). The estimated total amount of energy needed for pregnancy due to extra tissue and increased metabolism amounts to about 80 000 kcal (72). Metabolism of energy in pregnancy seems to be influenced by energy intake during pregnancy, maternal pre-pregnancy energy status or the size of the fetus. Women with low fat stores may have a limited energy supply at conception, and adaptations may occur to save energy for fetal growth (73). Many women in developing countries have to maintain pregnancy on dietary intakes lower than those recommended by the FAO/WHO/UNU. This may result in infants with a low birth weight.

Studies among Gambian women have indicated that they were able to complete their pregnancy successfully despite being undernourished. This has been attributed to an energy-sparing adaptation such as decreased basal metabolic rate (74,75). In some women, the basal metabolic rate actually decreases or increases only slightly in the early stages of pregnancy and does not increase substantially until late gestation. The reduction in physical activity has been reported in some women to save energy. This saving of energy largely meets the cost of pregnancy (76). Many women are likely to reduce their activity because of heavy farming duties as well as manual housework. It is not easy to prescribe energy intakes for the individual woman since it cannot be predicted how she will respond metabolically or behaviourally to pregnancy. It is important to give attention to each individual woman and the circumstances such as weight gain that might have a negative impact on the newborn.

**Table 2: Current recommendations for energy intakes during pregnancy (adapted from Prentice et al (77))***Existing FAO/WHO/UNU recommendations during pregnancy and comparison with UK, USA and Dutch values*

<b>Recommending body</b>	<b>Trimester (s)</b>	<b>Increment MJ/day (kcal day)</b>	<b>Total for pregnancy MJ (kcal)</b>	<b>Qualifying comments</b>
FAO/WHO/UNU (1985)	1 – 3	1.20 (300)	336 (80 300)	For healthy women who reduce activity
	1 – 3	0.84 (200)	235 (56 150)	
UK (1991)	3 <sup>rd</sup>	0.80 (190)	74 (17 000)	Underweight women and those not reducing activity may need more
USA (1989)	2 <sup>nd</sup> and 3 <sup>rd</sup>	1.25 (300)	233 (55 700)	
The Netherlands (1981)	1 - 3	0.60 (140)	168 (40 150)	Reduction in physical activity assumed

#### **4.1.2 Protein**

Proteins are the main structural constituents of the cells and tissues of the body, and they make up the greater portion of the substance of the muscles and organs.

Proteins are necessary for:

- Growth and development of the body,
- Body maintenance; repair and replacement of damaged tissues,
- The production of metabolic and digestive enzymes, as an essential constituent of certain hormones, such as thyroxine and insulin (78).

The recommendation of protein intake during pregnancy differs among the Canadian Recommended Nutrient Intake, the European Union, the Population Reference Intakes (PRI), United Kingdom Recommended Nutrient Intake (UK RNI), the USA Recommended Dietary Allowance (RDA), and World Health Organization (WHO) panels (79,80,81,82,83,84). The UK panel recommends the lowest total protein intake of 51g per day. The recommendations of European Community (EC) panel and the WHO committee are 55g per day. The USA recommends 60g per day and the Canadian panel recommended that four times more protein be added to the diet of a pregnant woman than that recommended by the UK panel. It is unlikely that many pregnant women will consume as large amounts of protein as proposed by the Canadian panel.

#### **1.1.1 Fat**

Fat is very energy dense and therefore changes in body fat stores have a large impact on the energy cost of pregnancy. The essential fatty acids are linoleic acid and alpha-linoleic acid. The UK and EC panels have established dietary standards for linoleic acid of 1% and 2% of the total energy intake, respectively. The Canadian panel was the only group to recommend an incremental intake of linoleic acid in pregnancy of 0.3g per day in the first trimester and 0.9g per day in the second and third trimester.

No recommended intakes were established by the WHO and US panels for essential fatty acids (79,80,81). Polyunsaturated fatty acids such as omega-6 and omega-3 have important roles in fat transport and metabolism, immune function, and in maintaining the function and integrity of cellular membranes.

## **1.1 Micronutrient requirements in pregnancy**

### **4.2.1 Fat soluble vitamins**

#### ***Vitamin A***

Vitamin A is an important nutrient because of its importance in reproduction, the immune system, cellular differentiation, vision and development of the fetus. Vitamin A can be fatal if the fetus is exposed to either too little or too much vitamin A in its early development. Vitamin A intake must be closely monitored because of its teratogenic consequences with high dietary or supplemental intake (85). Vitamin A deficiency may not be clinically detectable in the mother, but the developing fetus may be more vulnerable to sub-clinical deficiency (86,87). Too little vitamin A during the critical period when organ differentiation is occurring is linked to resorptions, abortions, and congenital anomalies in human beings. Animal studies reveal that excessive exposure in early pregnancy, when organogenesis is occurring rapidly and the need is greatest, predisposes a newborn to teratogenesis ( 88). In communities where night blindness is reported by pregnant women, the WHO (89) and the International Vitamin A Consultative Group (90) recommend that no more than a 3000 µg RE (10 000 IU) Vitamin A supplement be given daily.

A total daily intake of 700 or 800 retinol equivalents is recommended for pregnancy by the five panels. The US and Canadian panels recommend 800 RE from the first to the third trimester (Table 3) (79,82). They do not recommend an additional increment for pregnancy over the standard for non-pregnant women. The WHO recommended 600 RE throughout pregnancy (84). The UK and the EC

panel recommended 700 RE from the first to the last trimester (80,83). The panel from the US and the Canadian conclude that most women in those countries could provide the additional increment of vitamin A from hepatic reserves. Intakes above 10 000 IU per day have been associated with birth defects (91). The UK panel advises pregnant women not to take supplements containing vitamin A unless so prescribed by a health worker and not to eat liver or products made from it since animal livers can contain large amount of vitamin A. Vitamin A is most likely to be inadequately supplied by the diets of female adolescents. The South African Vitamin A Consultative Group (SAVACG) indicates that one in three children in South Africa have marginal vitamin A status (92). This may give an indication that adolescent pregnant women in rural areas are no exception.

### ***Vitamin D***

The additional amount of 2,5 µg/day of vitamin D has been recommended by the Canadian panel, bringing the total to 5,0 µg/day (Table 3) (82). The USA and the UK recommend a total intake of 10µg/day or 400IU (79,80). Since vitamin D ensures an adequate absorption of calcium, maternal vitamin D status may improve fetal calcium accretion. Deficiency of vitamin D may result in hypocalcaemia, hyperparathyroidism and a defect of dental enamel.

### ***Vitamin E***

Vitamin E is an antioxidant that prevents oxidation of unsaturated fatty acid by trapping free radicals. The Canadian panel recommends intake of 9.2 mg, whereas the USA recommends 10 mg (79,82). The increased rate of metabolism during pregnancy may enhance the production of free radicals and increase vitamin needs. Maternal circulating tocopherol rise during pregnancy, in conjunction with rising plasma lipid levels, making more tocopherol available to the fetus.

### ***Vitamin K***

There is limited data on vitamin K intake during pregnancy. The UK panel recommends the intake of 1 µg/kg/day for all adults. The USA recommends 64ug/day or about 1ug/kg/day for, non-pregnant, pregnant and lactating women.

**Table 3: Recommended daily intake of vitamins during pregnancy (79-84)****Panel Nutrients**

	Vitamin A (RE <sup>a</sup> )	Vitamin D (µg)	Vitamin E (mg α- TE <sup>b</sup> )	Vitamin K (µg)	Ascorbic acid (mg)	Thiamin (mg)	Riboflavin (mg)	Niacin (NE)	Vitamin B6 (mg/day)	Folate (µg/day)	Vitamin B12 (µg/ day)
Canada (RNI <sup>c</sup> )	800	4.5	7.2 + 2	No PRI	40	0.9	1.2	17	15µg/g protein	380	1.2
EC (PRI <sup>d</sup> )	700	No PRI	No PRI	No PRI	55	100µg MJ	1.6	1.6µg MJ	15µg/g protein	400	1.6
UK (RNI)	700	10	No PRI	No PRI	50	0.8	1.4	13	1.2	300	1.5
USA (RDA <sup>e</sup> )	800	10	10	65	70	1.5	1.6	17	1.6+0.6	400	2.2
WHO <sup>f</sup>	600									370- 470	1.4

<sup>a</sup> Retinol Equivalents<sup>b</sup> Alpha-tocopherol<sup>c</sup> (1991) Reference Nutrient Intake (RNI)<sup>d</sup> European Community (1993) Population Reference Intake (PRI)<sup>e</sup> (1989) Recommended Dietary Allowance<sup>f</sup> World Health Organization (1985)

**Table 4: Recommended daily intake of minerals and electrolytes during pregnancy (79-84)**

Panel	Nutrients									
	Calcium (mg)	Phosphorus (mg)	Magnesium (mg)	Sodium (mg)	Potassium (mg)	Iron (mg)	Zinc (mg)	Iodine (µg)	Selenium (µg)	Copper (mg)
Canada	1300	1050	215	NA	NA	23	15	185	NA	NA
EC	700	550	150-300	NA	3100	20 + supplement	7	130	55	1.1
UK	700	550	270	1600	3500	14.5	7	140	60	1.2
USA	1200	1200	320	NA	NA	30	15	175	65	1.5-3.0
WHO						60	13	200	39	1.15

#### **4.2.2 Water soluble vitamins**

##### ***Vitamin C***

All four panels recommend an additional 10 mg ascorbic acid during pregnancy. Actual requirements are assumed to be higher, because fetal turnover rates of ascorbic acid are probably greater than those of adults (Table 3).

##### ***Thiamin***

The panel in the USA recommends an additional 0.4 mg/day to accommodate fetal growth, changes in maternal thiamin utilization and increased energy intakes. Other panels recommend modest increases with either an additional 0.1 mg/day or suggest no change in requirements (Table 3). All the panels agree that thiamin requirements are related to energy metabolism.

##### ***Riboflavin***

All the panels agreed about the extra demand for riboflavin placed on the pregnant mother by the fetus is approximately 0.3 mg per day. As pregnancy progresses, urinary riboflavin excretion falls (Table 3).

##### ***Niacin***

Opinion on the dietary need for niacin varied widely among the five panels (79,84). The UK and the EC panels conclude that the additional need for niacin will be met by the increased conversion of tryptophan to niacin during pregnancy, and that an increased intake of niacin is unnecessary. The panel from the USA recommends additional 2 niacin equivalents because of the increased energy requirements. The EC recommends 17 NE. In the UK the recommended intake of niacin are either 13 or 17 NE per day (Table 3).

##### ***Vitamin B6***

All panels conclude that the dietary requirement for vitamin B6 is related to the protein intake. The Canadian, the EC and the UK panels use conversion factors of

15µg vitamin B6 per gram protein and the USA uses 16 µg. Based on the recommended increase in dietary protein during pregnancy, the Canadian panel advises an additional daily intake of 0.36 mg of vitamin B6 and the EC panel of 0.15 mg vitamin B6. The USA recommends an additional 0.6 mg/day. The UK concludes that no increase is necessary (Table 3).

### ***Vitamin B12***

The maternal vitamin B12 status is important in pregnancy. A low maternal intake or poor absorption of the vitamin in pregnancy may reduce fetal stores as well as breastmilk concentrations, and may later adversely affect the child's mental and physical development (93,94). All the panels, except the UK group, recommend an additional daily intake of vitamin B12 (Table 3). The WHO panel recommends an additional 0.4 µg per day; the other panels advise 0.2 µg per day.

### **4.2.3 Minerals**

#### ***Calcium***

Guidelines on the need for calcium during pregnancy differ from one country to another. There is lack of knowledge about the requirements of calcium during pregnancy and lactation (95). Most of the recommendations by different panels were derived by adding an increment to the figure for non-pregnant, non-lactating women to cover the calcium costs of fetal growth and breast-milk production. Very little data is available on the influence of calcium intake on the outcome of pregnancy. Maternal bone loss during pregnancy might lead to osteoporosis and fracture in later life by reducing peak bone mass (97). Calcium deficiency has been associated with a high incidence of eclampsia in pregnant women (97). Bucher et al (98) have found that calcium supplementation in pregnant women in the range of 1.5 to 2 grams per day appears to result in a modest reduction in systolic and diastolic blood pressure during pregnancy, and substantially reduces the odds of pre-eclampsia. The Canadian and USA panels increase intake of calcium for pregnant women by 400 or 500 mg/day, over that recommended for

non-pregnant women (79,82). The UK and the EC panels conclude that mobilization of maternal calcium depots would meet the needs for fetal growth. In pregnant adolescents there is increased need for both fetal and maternal growth (Table 4) (80,83).

### ***Phosphorus***

The requirements for phosphorus are unknown. The panels from the EC, the UK and the USA recommend equal amounts of dietary calcium and phosphorus and conclude that neither inadequate nor excessive intakes of phosphorus are a problem. The Canadian panel states that calcium and phosphorus should be parallel. They recommend a total of 1300mg of calcium and of 1050mg phosphorus during gestation (Table 4) (82).

### ***Magnesium***

The panel in the USA panel estimates an incremental need of 20mg per day for magnesium to cover fetal and maternal needs. The panel recommends a total intake of 320mg. The UK concludes that the physiological adaptations during pregnancy will meet additional fetal and maternal needs. The Canadian panel recommends an additional amount of 15mg/day. The EC recommends an intake range from 150 to 300mg per day (Table 4).

## **4.2.4 Trace elements**

### ***Zinc***

Zinc is essential for the normal growth of human beings. Zinc deficiency has been associated with infertility, abortions, malformations, fetal intra-uterine growth retardation, prematurity, perinatal death, and intrapartum and postpartum haemorrhage and placental abruption (99,100,101). The relationship between zinc deficiency, pre-term delivery and low birth weight is becoming clearer, despite difficulties in measuring actual deficiency. A significant positive correlation has been found between plasma zinc concentrations at entry into prenatal care and

duration of gestation, while low dietary zinc intake has been found to double the risk of low birth weight and treble the risk of pre-term delivery (102,103). The US and Canadian panels recommend 15 mg per day of zinc in pregnant women. The EC and UK recommend 7mg (Table 4). The WHO says that a pregnant woman in the third trimester should consume 13,3mg/day of zinc. The other groups conclude that an increase in dietary zinc might be necessary since there is no evidence of physiological adaptations in zinc utilization among pregnant women except in the UK and the EC.

### ***Iodine***

Iodine is an essential component of the thyroid hormone molecule. Its deficiency during fetal development can cause hypothyroidism and irreversible mental retardation (104). Iodine deficiency disorders are of particular concern to women due to their adverse effects on their reproductive functions. Iodine-deficient mothers have higher rates of miscarriage and stillbirth as well as higher mortality rates from cephalopelvic disproportion (105). Recommendations by Canada and the USA for iodine are an increment of 25µg/day for a total intake of 175 and 185µg per day, respectively (79,82). The WHO recommends 200µg/day. The EC and the UK felt that no increment was necessary for pregnancy (Table 4).

### ***Selenium***

The USA recommends an increment of 10µg/day based on the assumption that a total of 1,25mg selenium is retained during pregnancy (79). The WHO recommends that an additional 9µg/day be consumed (84). The UK and the EC agree that since adaptive changes in the metabolism of selenium occur during pregnancy, no advantage is seen in recommending extra selenium at this time (80,832). The Canadian feels that there is insufficient evidence to recommend a dietary allowance for selenium (Table 4).

### *Copper*

None of the panels recommend additional copper in pregnancy. The required amount is estimated to be 21mg. Recommended total daily intake ranges from 1,1 to 3,0 mg.

## **5. Iron**

Iron is one of the most important trace metals needed in very small amounts in the body (106). Human beings do not easily acquire iron. Iron is one of the micronutrients that function as a co-factor for many enzymes, (107,108,109) and plays an essential role in many metabolic processes including the transport of oxygen, oxidative metabolism and cellular growth (110). Its role in haemoglobin formation and the aetiology of anaemia has received intense attention especially, in pregnancy.

### **5.1 Prevalence of iron deficiency**

Anaemia in pregnancy is usually caused by iron and folate deficiency. There are other forms of anaemia that may result from aplasia or bone marrow suppression by chronic systemic disease (111). Malabsorption of iron in celiac disease and gastrectomy may positively contribute to anaemia. Pulmonary haemosiderosis, bleeding into the joints in rheumatoid arthritis, urinary loss from chronic microangiopathic haemolysis or paroxysmal nocturnal haemoglobinuria and angiodysplasia of the gastrointestinal tract have been positively linked to iron deficiency (112). Blood losses due to threatening abortion, infection and acute pyelonephritis have also been linked to anaemia in pregnancy.

Iron deficiency and iron deficiency anaemia together with folic acid are the most prevalent nutrient deficiencies affecting pregnant women (113). The production of small erythrocytes and a diminished level of circulating haemoglobin, i.e. microcytic hypochromic anaemia, characterize iron deficiency. The prevalence of iron deficiency is estimated to affect 51% of the world's population (114). In developing countries, the prevalence of anaemia in pregnancy averages 56%, ranging between 35% and 75% among different regions of the world (112). It is estimated that 6% to 11% of reproductive women, 14% of young women aged 15 – 19 years, and 25% of pregnant women are iron deficient in the United States

and Canada (115,116). Data from studies conducted in South Africa (Table 5) among pregnant women of different ethnic origin, reflect that there is a high prevalence of iron deficiency and iron deficiency anaemia.

**Table 5: Reported prevalence of iron deficiency among South African women (117-122)**

<b>Study</b>	<b>Ethnic origin</b>	<b>Total number of subjects</b>	<b>Prevalence of iron deficiency</b>
Patel et al (1992)(117)	Black	236	40 – 51%
Baynes et al (1986)(118)	Black	229	17%
Lamparelli et al (1988)(119)	Indian	100	20%
Lamparelli et al (1988)(120)	Coloured	224	19%
Macfarlane et al (121)	Indian	774	25%
Kruger et al (1994) (122)	Coloured	105	25%

## **5.2 Iron absorption**

Iron absorption can be divided into three different stages: iron uptake, intraenterocyte transport and storage and extraenterocyte transfer. No absorption of iron occurs in the mouth, oesophagus or stomach. Iron is converted from the ferric form in the stomach by hydrochloric acid, to the ferrous form which is absorbable. The majority of iron absorption takes place in the duodenum (123,124). The total amount of iron absorbed by the body depends on the total amount in the diet, bioavailability, and the regulation of its absorption by the body (115,125,126). Absorption is also enhanced in the presence of erythroid hyperplasia, rapid iron turnover and a high concentration of unsaturated transferrin (124).

The presence of haem iron sources such as meat in the diet increases the absorption of iron from other non-haem sources such as vegetables. Substances such as ascorbic acid, amino acid and sugars increase absorption of non-haem iron by keeping iron in its ferrous form (127). Other substances like phytates, eggs, coconut milk and desferioxamine decrease iron absorption (128). Haem iron which comes from haemoglobin and myoglobin is more easily absorbed by humans than non-haem iron. The major sources of haem iron are beef, fish and poultry. The non-haem iron consists of iron from other foods such as vegetables, grains, fruits, eggs, and dietary products as well as from the non-haem iron of meats, poultry, and fish and from soluble iron supplements (1298). Haem iron has 20% to 40% absorption while non-haem iron has only 5% to 10% absorption (130).

## **5.3 Iron requirements in pregnancy**

There is increased need for iron in pregnancy because of the expansion of the red cell volume and the requirements of the developing fetus and placenta (103). The average iron requirements in pregnancy are between 800mg to 1000 mg (Table 6)

(131,132). The net cost of pregnancy is less than the total iron needed because the iron used in the expansion of the red cell mass is not lost from the body and can be returned to the stores after pregnancy (115,133).

**Table 6: The iron cost of a normal pregnancy**

	1 <sup>st</sup> half of pregnancy (mg)	2 <sup>nd</sup> half of pregnancy (mg)	Total (mg)	Net cost of pregnancy (mg)
Expansion of red cell mass	-	500	500	-
Blood loss at and after delivery	-	-	-	250
Fetal iron	-	290	290	290
Fetal iron in placenta	-	25	25	25
Basal losses (skin, etc)	110	110	220	
Total	110	925	1035	565

During the first trimester of pregnancy, iron requirements are lower owing to the cessation of menses, and iron stores may even increase. At around 16 weeks of gestation the maternal blood volume and the red cell mass expand so that iron requirements increase substantially (134). The iron need is greatest in the third trimester (135). The intake of iron by the fetus occurs at week 30, during a time when maternal serum ferritin is constant. Total circulating serum transferrin increases by about 250% between conception and term, probably in response to oestrogenic hormones.

When fetal iron demand is high, such as for the increased synthesis of haemoglobin by the fetus in diabetic pregnancy, the fetus may be able to mobilize its own iron stores to support erythropoiesis (108). It is estimated that about 5.6 mg (range 3.5 – 8.8 mg per day) is needed daily during the second and third trimesters (136). There are discrepancies in studies in the estimated amount of iron absorbed during pregnancy. Further data are clearly needed on this question. Maternal haemoglobin tends to recover spontaneously to approach pre-pregnancy values during the first months postpartum. In pregnancy the external loss in urine, faeces, and sweat amounts to about 220 mg for the gestational period. The fetus uses about 290 mg, and another 25 mg are contained in the placenta and cord. The amount of iron lost in haemorrhage at delivery is estimated at 250 mg (Table 6).

#### **6.4. Aetiology of anaemia in pregnancy**

Iron deficiency may occur because of inadequate diet, impaired absorption, blood loss, or repeated pregnancies. Menstrual bleeding and haemorrhage from the alimentary tract may contribute significantly to the deficiency. Geophagia interferes with the absorption of iron, probably because the ingested clay either strongly chelates or precipitates iron as insoluble compounds in the lumen of the gut. Inadequate uptake of iron occurs in malabsorption syndromes and in chronic diarrhoea from any cause. Iron deficiency may also occur as a result of blood loss due to haemorrhage from wounds, the nose or mouth, genitourinary tract,

haemorrhoids and occult gastrointestinal bleeding. Hereditary haemorrhagic telangiectasia is a rare cause of chronic bleeding and iron-deficiency anaemia. Aspirin may cause diffuse haemorrhage gastritis (118,125,128).

Young women have additional iron deficits because of blood during menstruation (128,131). Dietary intake also plays a role (116). It is well established that there is a marked variation in menstrual blood loss among different women, and that loss in a single woman is very constant from menarche and throughout her fertile life (137). Iron can also be lost through parasites such as *trichuris trichiura* (126). Recently, Aids has been included in the differential diagnosis of patient with anaemia (138).

### **5.5 Consequences of iron deficiency**

Iron deficiency anaemia during pregnancy is said to have negative consequences such as the poor outcome of pregnancy, low birth weight, urinary tract infections, pyelonephritis and pre-eclampsia (139). However, there is a need for more data on these issues (140). Iron deficiency also affects other metabolic processes, including mitochondrial electron transport, catecholamine metabolism, DNA synthesis, several enzyme systems, neurotransmitter synthesis, protein synthesis and organogenesis (129). Impairments of work performance, neurologic function, the immune response, and epithelial tissues have been attributed to iron deficiency (99,116,130). The tissues of the gastrointestinal tract have been reported to be extremely sensitive to iron deficiency. Glossitis, stomatitis, oesophageal webs, and chronic gastritis may partly result from other coincidental deficiencies (102,131).

Abnormalities, such as lower oestrogen levels and an increased risk of placental hypertrophy are common in pregnancy (143). The placentas of anaemic women may become large in response to hypoxia (144). Increased placental weight and a high ratio of placental weight to birth weight, which are associated with an

increased risks of high blood pressure in the infant's later life, have been linked to maternal anaemia during pregnancy and specifically to maternal-iron deficiency anaemia (144).

Iron deficiency anaemia in the first trimester is a significant risk factor for prematurity and infants with a low birth weight (115,143,145). The deficiency in the second and third trimester has very little impact on fetal outcome, although relationships may have been obscured by the difficulty of correctly diagnosing the condition later in pregnancy. Maternal anaemia and/or iron deficiency can be harmful to both the mother and the infant. Most of the adverse effects are not well understood and require further documentation and quantification (107). The severity of anaemia has always been closely associated to perinatal mortality. However, the mechanism associated with the higher mortality of anaemic women is not well understood. Cardiac failure during labour may be a result of severe anaemia. Anaemic mothers may less be able to tolerate haemorrhagic blood loss during childbirth, have a greater risk of infections and experience the slow healing of wounds (109).

Anaemia impairs the transport by haemoglobin of oxygen to the uterus, placenta and also the developing fetus, thus compromising the outcome of pregnancy. Maternal haemoglobin is negatively correlated with both human chorionic gonadotropine and placental weight across the normal haemoglobin range. Both low and high maternal haemoglobin may be associated with low birth weight (145,146). Because maternal haemoglobin and haematocrit tend to increase during the last trimester of pregnancy, lower infant's birth weight associated with preterm delivery is usually associated with lower maternal haemoglobin or haematocrit (147). Low birth weight as a result of prematurity is a risk for infant morbidity, poor growth, and the early depletion of iron stores in infancy.

Pregnant adolescents are at high risk for iron deficiency anaemia because of the high iron requirement for their own growth and the increased demands of pregnancy, coupled with a low dietary intake (148,149). While national prevalence data on iron deficiency anaemia in pregnant adolescents are not available, one Ontario study found 22% of 58 pregnant adolescents had iron deficiency anaemia and 78% had depleted iron stores (162). Another study also reported a two to three fold higher risk of premature birth and low birth weight was associated with iron deficiency anaemia (haemoglobin <11 g/dl) in the first trimester (148). It was found that 70% of pregnant adolescents had depleted their iron stores (ferritin <12 – 15 µg/l) at 11 – 16 weeks gestation, while only 7.4% had iron deficiency anaemia, and 3.6% were anaemic but not iron deficient (150).

In infants, iron deficiency anaemia results in developmental delays and behavioural disturbances such as decreased motor activity, poor social interaction and inattention to tasks (151,152). Poor performance on developmental scales in infants and toddlers is associated with iron deficiency anaemia (153). Iron in the brain is involved in the synthesis of neurotransmitters that affect human behaviour (154). Whether maternal iron deficiency causes a lowering of the availability of iron to the young infant, with subsequent adverse effects on brain biochemistry and cognition is still a question. Iron deficiency anaemia is likely to affect the mother's attention and motivation. These effects could, in turn, affect the frequency and duration of interaction with her infant (113). Increased fatigue, resistance to cold and poor tolerance to blood loss in pregnant women has been associated with anaemia due to iron deficiency in pregnant women.

## **5.6 Assessment of iron deficiency**

The assessment of the iron status in pregnancy is rather difficult and complicated because of physiological changes in the body during pregnancy. Many different methods are used to assess the iron status including dietary intake, haematocrit, haemoglobin, mean erythrocyte protoporphyrin, mean cell volume, bone marrow iron stain, serum iron, total iron binding capacity, serum transferrin saturation, serum ferritin, and serum transferrin receptor (155,156). They vary considerably in their sensitivity and specificity. The choice of tests to detect iron-deficiency anaemia also depends on the population to be studied. For example, the underlying prevalence of iron deficiency and whether other factors such as infection are common in the area must be considered.

### ***5.6.1 Haemoglobin***

In 1991 a WHO Technical Working Group defined anaemia in pregnancy as a haemoglobin level below 11g/dl, with a cut-off value of 10.5g/dl for the mid-trimester in pregnancy (157). The use of haemoglobin alone to diagnose iron deficiency anaemia can give a false indication. This is because haemoglobin concentration can be altered by/in chronic inflammation, chronic infection, cigarette smoking, polycythaemia, dehydration, haemorrhage, protein-energy malnutrition, vitamin B12 deficiency, folic acid deficiency and haemoglobinopathies (158). Haemoglobin decline to its lowest peak at week 16 of gestation and starts to recover during the third trimester from 24 weeks. This decline in haemoglobin is a result of haemodilution, wherein plasma volume expansion exceeds the expansion of the red cell mass (159). Because of haemodilution during the second trimester, proposed cut-off values for anaemia for pregnant women in trimesters 1, 2, and 3, respectively, are: haemoglobin, 11.0, 10.0, and 11.0; and haematocrit, 33, 32, and 33 (160).

### ***5.6.2 Serum ferritin***

A long-term negative iron balance leads to the depletion of the storage iron pool, leading to declines in plasma ferritin concentrations. Assessment of the size of the storage pool is done by the measurement of serum or plasma ferritin concentrations (161). Serum ferritin is considered a valid estimate of a woman's iron stores. Ferritin levels fall during pregnancy, but gradually rise towards the end of pregnancy in women with an adequate iron status. Ferritin is also an acute phase reactant, and its concentration is increased with inflammation, infections, liver diseases, vitamin B12 deficiency, folic acid deficiency, leukaemia, Hodgkin's disease, excessive alcohol intake and hyperthyroidism (161,162,163).

In a study conducted among non-pregnant Zairean women, it was found that serum ferritin is not a reliable indicator of iron status unless inflammation is taken into consideration because the presence of inflammation may mask depleted iron stores, and hence make its validity questionable (164). Plasma volume expansion and haemodilution will also cause ferritin concentrations to decrease during the second and third trimester. Ferritin is stable, is not affected by the recent ingestion of iron and appears to reflect the iron stores accurately and quantitatively, particularly in the lower range associated with iron deficiency (115). A normal serum ferritin value in the first trimester does not necessarily guarantee adequate iron status in the second or third trimester (113). A low serum ferritin concentration of less than 12µg/l is used as a cut-off value for iron deficiency (131,141). Ferritin levels in early pregnancy can be used to predict the need for iron supplements (165).

### ***5.6.3 Serum iron***

A low serum iron is not diagnostic of iron deficiency (130). Serum iron levels fluctuate widely and are affected by the recent ingestion of iron (115). Serum iron is low in pregnancy because of placental transfer (109). The serum iron of adult

non-pregnant women ranges between 13 and 27 mol/l (60-150µg/dl). The serum iron is low in iron deficiency anaemia and can be raised by the administration of iron to treat anaemia. Serum iron is not a reliable method to diagnose iron deficiency in pregnancy due to the increased plasma volume.

#### ***5.6.4 Total iron binding capacity***

The plasma concentration of transferrin may be measured by the amount of iron that it will bind. This determination is called the total iron binding capacity (TIBC). The normal serum TIBC is about 45 to 80 µmol/L (250-450 µg/dl). In the presence of iron deficiency anaemia, total iron binding protein is usually increased. In the presence of chronic disorders such as chronic infections, rheumatoid arthritis, or malignancies, the TIBC may be normal or diminished. In acute diseases, such as acute infections or myocardial infarction, and after immunizations, total iron binding protein is usually normal. In acute diseases, such as acute infections or myocardial infarction, and after immunizations, the total iron binding protein is usually normal. In pregnancy TIBC is usually raised and this may affect the measured transferrin saturation as well. TIBC is not a reliable method to use in pregnancy because it is increased and may give false indication of iron status (166).

#### ***5.6.5 Transferrin saturation***

Transferrin saturation declines in the presence of iron deficiency (161). Inadequate iron is available for essential body iron proteins. People in this stage of iron depletion have transferrin saturation below 15-16% and an inadequate supply of iron from the bone marrow to support erythropoiesis. Transferrin saturation is calculated as 100 multiplied by serum iron divided by total iron binding capacity. Values are about 30% lower in mid-afternoon, and substantially lower in the evening, with a nadir near midnight. In chronic disorders such as chronic infections, rheumatoid arthritis, or malignancies, transferrin saturation

may be normal or low. In acute diseases, such as acute infections or myocardial infarction, and after immunizations, transferrin saturation is diminished. Transferrin saturation may not be a good method to use during pregnancy because it is derived from a formula that uses the serum iron and total iron binding capacity.

#### ***5.6.6 Serum transferrin receptor***

Transferrin receptor is a transmembrane glycoprotein that controls the uptake of circulating iron into cells (167). Transferrin receptors can be detected in human serum, and the amount in circulation has been shown to vary with individual's iron status (168). Serum concentration of transferrin receptor has been suggested as a reliable index of iron depletion especially in pregnancy and in the anaemia of chronic disease or in the presence of infections. In the presence of iron deficiency, transferrin receptor (TfR) concentrations are higher. This has been reported as one of the parameters that can be used to distinguish between iron deficiency anaemia and other anaemia's, including the anaemia of chronic disease (156). A low or normal serum TfR concentration distinguishes patients with acute inflammation and anaemic patients with chronic inflammatory diseases from those with iron deficiency anaemia (169).

Serum concentration may be low in patients with inflammatory diseases irrespective of their iron status. Plasma TfR concentrations increase even in mild iron deficiency of recent onset. The plasma concentration of TfR is increased in B-thalassaemia, autoimmune haemolytic anaemia, sickle cell anaemia, hereditary spherocytosis, polycythaemia vera, secondary polycythaemia, myelofibrosis, and chronic lymphocytic leukaemia (170,171). The plasma TfR concentration is decreased in haemochromatosis, aplastic anaemia-bone marrow ablation, post transplantation anaemia, and chronic renal failure (156,170,172). Since TfRs are not affected by infection or inflammation, they can be useful in areas where the

prevalence of infection is high (173). Serum transferrin receptor has been widely used in detecting iron deficiency anaemia in pregnancy (174,175,176).

## **5.7 Treatment of iron deficiency**

Many solutions have been suggested over the years on the treatment of iron deficiency. Dietary modification forms the basis of the education given to pregnant women on how they can increase their iron intake. Dietary intake alone has been found not to meet the requirements of pregnant women (126).

### ***5.7.1 Iron supplementation***

Iron supplementation during pregnancy has been shown not to affect the outcome of pregnancy, but to improve maternal iron status parameters (177,178). Iron supplementation is still controversial in developed countries (132). Some prefer iron to be supplemented in the second half of pregnancy, whereas others prefer supplementation after an assessment of iron status has established an iron deficit. Routine supplementation of iron seems to be favoured by many researchers as they argue that it is difficult and expensive to determine the iron status of all pregnant women, since iron overload is unlikely to cause problems in childbearing women (179). One of the reasons why researchers do not prefer routine supplementation is that there is poor compliance among pregnant women, with gastrointestinal complaints, nausea and vomiting being the common problems (180,181). The incidence of side-effects increases as the dose of iron increases. Although some of the side effects may be associated with pregnancy itself, if they are attributed to the supplement, compliance decreases.

The World Health Organization recommends 60 mg per day of elemental iron for pregnant and lactating women (182). They also recommend that a blood transfusion should be considered when there is evidence of decompensation, cardiac failure, a haemoglobin of <4g/dl, or when haemoglobin is <8g/dl near

delivery (150). In 1990 the Institute of Medicine recommended that 30 mg of iron per day should be given to pregnant women. The recommendation was to supplement all pregnant women, regardless of iron status (183). The United Nations Administrative committee on Coordination/SCN recommends that 60 mg of ferrous iron and 250 µg folate should be supplemented in regions where there is a low prevalence of iron deficiency anaemia. Folic acid is included in the supplementation programme because folate deficiency often accompanies iron deficiency and may lead to anaemia. In areas where the prevalence of iron deficiency anaemia is high, the dose should be 120 mg of elemental iron and 500 µg of folate (183).

The effectiveness of iron supplementation during pregnancy has always been a difficult task to accomplish. The effectiveness of a supplementation programme depends on:

- The supply of iron tablets,
- The ability of the primary health care system to provide supplementation through service delivery,
- The quality of counseling about the need for iron supplementation and,
- The benefits and side effects and willingness of the pregnant women to consume the iron supplements (184).

The type of diet that women consume and whether iron pills should be given daily or on a weekly basis adds to the controversy (185,186). The effect of iron supplementation on the zinc status of pregnant women should also be taken into consideration (187). Iron decreases the absorption of zinc (188). It is only the inorganic, non-haem form of dietary iron which competes with the absorption of dietary zinc, as opposed to iron in the form of haemoglobin, from red muscle meats, which has no effect (189,190).

Iron fortification is slow to be implemented in developing countries because of the cost, decentralized food production and staple foods of low iron bioavailability that do not lend themselves for fortification. In the South African population previous studies have indicated that a considerable proportion of the black male population has excessive body iron from the consumption of beer prepared in iron containers. This should be taken into account when food fortification programs are embarked upon (191).

### ***5.7.2 Benefits of maternal iron supplementation during pregnancy***

Iron supplementation in pregnancy improves the maternal iron status in both industrialized and poor countries (192). In a study conducted among pregnant women in Niger, it was found that the prevalence of anaemia and iron deficiency decreased during the last trimester after they have been given iron supplements. Serum ferritin concentrations were significantly higher in infants with mothers in the iron-supplemented group than those with mothers in the placebo group (193). Other studies did not find the benefit of iron supplementation in haemoglobin concentration (194,195). Increases in haemoglobin, haematocrit, mean corpuscular volume, serum ferritin, serum iron, and transferrin saturation, compared with unsupplemented controls are usually apparent within 3 months. Iron supplementation during pregnancy also improves maternal iron status postpartum. A study of Finish women also reported that supplementation increased ferritin (but not haemoglobin) concentrations postpartum (196). In South Africa the third most common cause of maternal deaths is haemorrhage (197). It is therefore of utmost importance to increase the haemoglobin level during the antenatal period in anaemic women to reduce the impact of this complication.

### **5.7.3 Infants benefits**

An iron deficient mother can still provide enough iron to the fetus, because of the increase in placental transferrin receptors in iron deficient women. In the Nigerian women no association was found between maternal and cord blood haemoglobin or ferritin concentrations after supplementation (199). Only three studies extended to older infants and suggest that maternal iron status during pregnancy is, in fact, a strong predictor of iron status in infant later in life. In most studies, infants of anaemic mothers were found to be iron deficient. (200). Supplementation of iron is more likely to benefit the mother than her child (201). Excessive iron intake has been associated with lower rate of weight gain (182). Iron deposits may lead to increased catabolism of vitamin C resulting in the reduced release of iron into circulation from the reticuloendothelial cells and inappropriately low serum ferritin concentrations (202). Haemochromatosis due to iron overload has been observed in the United States (203,204).

### **5.8 Pica**

Pica is the compulsive ingestion of non-food substances, described as craving for substances such as clay (geophagia), starch (amylophagia) and ice or freezer frost (pagophagia). In a study conducted among African-American women, their serum ferritin concentration and haemoglobin were lower during the second and third trimester of pregnancy because of pica. The head circumferences of infants born to women with pica who consumed freezer frost/ice were smaller than those of women without pica. It was concluded that pagophagia might be the result of iron deficiency because the diets of women with pica contained less iron (205). Stress has also been attributed to pica. Infants born to women with pica had higher autonomic ratings than those of women without pica. These infants were more irritable and cried more frequently (206).

## **6. Folic acid**

Over the years, folate has been known as the most common vitamin deficiency in North Africa and Western Europe (207). This vitamin is well known as a major cause of megaloblastic anaemia. At a cellular level, folic acid is reduced first to dihydrofolic acid (DHF) and then to tetrahydrofolic acid (THF) which forms the cornerstone of cellular folate metabolism (208). Folate is a generic descriptor for the many different forms of the vitamin which exhibit the biological activity of the parent molecule folic acid (pteroylmonoglutamic acid), consisting of a pteridine nucleus linked through a methylene bridge to a para-aminobenzoic acid and L-glutamic acid residue (209). Folic acid is used in vitamin supplements, and a number of other folate compounds occur naturally in food such as green leafy vegetables. It is estimated that 25-50% of folate is available in the average diet with 10% available in brewer's yeast (210).

The main function of folic acid is in the synthesis of nucleic acid such as deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and protein synthesis (211,212). Folate enzymes are used mainly in the transfer and use of one-carbon units in a variety of essential reactions in mammalian tissues involving amino acid metabolism, and the synthesis of purine and pyrimidines. Folate co-enzymes are responsible for the synthesis of thymidylate that is an essential precursor of de novo biosynthesis of DNA (213).

### **6.1 Causes of folate deficiency**

Several studies have indicated that pregnant women and women from low socio-economic status may be at risk for developing folate deficiency (214). The primary causes of folic acid deficiency are the inadequate intake of slightly cooked fresh foods. Food preparation is important in the preservation of folate. Boiling, exposure to heat, air and ultraviolet light may result in substantial losses (213).

Secondary causes are inadequate absorption due to the malabsorption syndrome. The deficiency may also appear because of inadequate utilization due to folic acid antagonists, enzyme deficiency, scurvy and vitamin B12 deficiency. There are increased requirements of folic acid due to prematurity, infancy and increased metabolism because of pregnancy and growth (210,215). Gastro-intestinal haemorrhage may also be associated with folic acid deficiency (210). Low folate levels are attributed to smoking and alcohol (213).

## **6.2 Diagnosis of folate deficiency**

Folate deficiency can be assessed by dietary and biochemical measurements. With dietary intake, caution should be exercised when interpreting folate intake data from literature. Clinical signs may not be reliable in the early stages of deficiency. When assessing folic acid intake by biochemical methods, Herbert developed the following stages in the development of folate deficiency (216):

1. The first stage involves the greater loss of folate than the individual can absorb. This can be assessed by serum folate, which decreases prior to tissue depletion, usually after only two to three weeks of negative folate balance. Serum levels of less than 3ng/ml indicate negative balance. There are doubts as to whether this method is reliable, because serum folate can be influenced by the recent ingestion of food and the plasma volume expansion.
2. The second stage is tissue depletion that can be diagnosed by erythrocyte folate level below 140ng/ml. It is a reliable index because it indicates a long-standing deficiency.
3. The third stage is characterized by severe depletion of the vitamin. Intracellular folate becomes inadequate for normal folate-dependent metabolism and biochemical function is impaired. It can be diagnosed by abnormal deoxyuridine suppression test.
4. Final stage is manifested by anaemia. Mean corpuscular volume (MCV) is increased (>94fl), and haemoglobin becomes <12g/dl in women.

Red cell folate is known as the best indicator of folate status (215). Red cell folate reflects neither daily nor other short-term variations in plasma folate levels. It is thought to give a better indication of overall tissue levels, but the turnover of red blood cells is slow and there will be a delay before significant reductions are evident in the folate concentrations of the red cells, due to folate deficiency. Plasma folate reflects recent dietary intake. The normal values are 3-21ng/ml. Plasma folate does not give a true reflection of folate deficiency when used alone.

### **6.3 Consequences of folate deficiency**

Severe deficiency of folic acid may result in neonatal death (214,216). It may result in symptoms like megaloblastic anaemia, a sore tongue, angular stomatitis and abnormalities in skin and nail formation (210). A low folate level may also result in abortion, abruptio placentae, prematurity, low birth weight and neural tube defects (212,217). There is an increased risk of megaloblastic anaemia occurring in the neonate of a folate deficient mother, especially if delivery is pre-term (208). Low folate status has been associated with an increased risk of cervical cancer (209).

During pregnancy, low maternal folate status may impair cellular growth and replication in the fetus and/or placenta and thus increase the risk of pre-term delivery and low birth weight (218). In the study conducted by Scholl et al, women with low mean daily folate intakes and low serum folate concentrations at week 28 in pregnancy had twice the risk of preterm delivery and low birth weight after controlling for maternal characteristics, energy intake and other correlated nutrients (212). The question remains: will folate supplementation reduce the risk for preterm delivery among poor, urban women?

## **7. Neural tube defects and Folate Deficiency**

Neural tube defects (NTDs) are a group of brain and spinal cord malformations that develop at various points during gestation. The open neural tube defects (neural tissues are exposed to the surface) are those that occur when the neural tube, which forms the future brain, spinal cord, and spinal column, fails to close during neurulation (219). Normally, the tube is closed by the end of the sixth week (about 22 to 29 days) after the last menstrual period (220). Complete failure of closure at the rostral end results in anencephaly, localized defects in this location result in encephalocele, and failure of closure anywhere along the spine results in spina bifida. It is not easy to identify women who are at risk of NTD, by either dietary intake, serum, folate and red cell measures of folate status (221).

### **7.1 Prevalence of Neural Tube Defects**

It is estimated that every year, approximately 2500 infants are born with NTDs, and estimated 1500 affected fetuses are aborted in the USA (222). The prevalence of NTDs in the USA is approximately 6 per 1000 live births, although the rate varies geographically and seasonally (223). Most of the studies conducted in South Africa have shown that rates of NTDs among the rural black population are very high when compared to other ethnic groups. The seasonality of NTDs has also been observed (224,225). The incidence of NTDs in South Africa varies according to geographical location, socio-economic status and ethnicity. The prevalence of NTDs in South Africa during 1994-1995, ranged from 0.99 to 3.55 per 1000 live births (226). In the Limpopo Province, the incidence of NTDs was estimated at 3.55 per live births among blacks in 1995 (227). Venter confirmed the high prevalence of NTDs in the rural parts of the Limpopo Province and Delport confirmed a high prevalence in Pretoria (225,226,228).

## 7.2 Aetiology of Neural Tube Defects

The aetiology of neural tube defects appears to be multifactorial and includes both genetic and environmental factors, of which folate intake is the most firmly established (229). Female births are more prone to birth defects than male births. In women who have had a previous pregnancy affected by a NTD, the risk of an NTD increases to ten times the rate among the general population (230).

The secular and seasonal trends, variations in geographic area, social class, maternal age and reproductive history, and associations with drug ingestion, maternal illness, toxins, and nutrition underscore the effect of environmental factors on the aetiology of NTDs (222,224). Maternal exposure to heat during neurulation has also been associated with NTDs (231,232). Heat appears to be teratogenic. Women exposed to heat from hot tubs were approximately three times more likely to have an NTD-affected pregnancy than unexposed women.

The association between maternal diabetes mellitus and the risk of congenital birth defects is well documented. Investigators have confirmed at least a two to threefold increase in risk of major malformations among infants of mothers with insulin-dependent diabetes mellitus (233). Becerra and colleagues found that infants of diabetic were 15 times more likely to have anencephaly or spina bifida than infants of non-diabetic mothers (234). The teratogenic mechanism of diabetes mellitus is not well understood, although hypotheses include hyperglycaemia, ketoacidosis, and other biochemical abnormalities (234). The better the glycaemic control during the first trimester of pregnancy, the lower the risk of birth defects (235).

Researchers have demonstrated a relationship between pre-pregnancy weight and the risk of having offsprings with NTDs (236,237). Women with a body mass index of greater than  $29\text{kg/m}^2$  had twice the risk for an NTD-affected pregnancy than women whose BMI were less than or equal to  $29\text{kg/m}^2$ .

The underlying mechanism in the relationship between pre-pregnancy obesity and NTD risk is not known. Obese women may be deficient in other nutrients. Obesity also affects metabolic processes, which, in turn, may affect neural tube development.

Several therapeutic drugs and other substances may negatively affect folate status. Among them, anticonvulsants have been shown to be teratogenic. Maternal use of these medications during neurulation has been shown to increase the risk for offspring with NTDs to approximately 1-2% compared with 0.6% in the general population (238,239). Fertility drugs were associated with NTDs in one of the studies (240). Habits such as cigarette smoking and the use of alcohol, may also negatively affect folate status. One study demonstrated that smokers required more than three times the amount of folate intake per day to achieve a serum folate level equivalent to that of a non-smoker (241).

Several researchers have suggested an association between maternal exposure to organic compounds such as trihalomethanes in the workplace or in the home and an increased risk of NTDs (242). In a case-control study in Texas, Brender and Suarez (243) found that fathers employed in occupations associated with high solvent exposure were more likely to father offspring with anencephaly. Painters had three times more risk than men employed in other occupations. Paternal exposure to pesticides in agricultural occupations has also been linked to an increased risk of neural tube defects (244).

### **7.3 The Role of Folic Acid in Neural Tube Defects**

Nutritional deficiencies, especially vitamin deficiencies (C, B12, B6, folate and zinc) have been closely related to the failure of neural tube to close and the development of other defects. This was first postulated in the 1960s (245). Folate has a well-recognized role in cell division and growth. The link between folic acid antagonist medications and NTDs, and the association between factors related to

poverty and NTDs suggests that undernutrition, particularly folic acid deficiency, might be a mechanism in the pathogenesis of these defects. With a folate deficiency, DNA synthesis is inhibited, leading to defective cell proliferation and cell death (246).

Folate also is critical in the supply of methyl groups to the methylation cycle. A folate deficiency causes a shortage of the essential amino acid, methionine, and the cells are thus unable to methylate proteins, lipids and myelin (246). A dietary deficiency of folate or a genetic error in folate metabolism could cause NTDs by alterations in DNA synthesis and/or the methylation cycle. High levels of homocysteine may be toxic for the developing embryo (247). Mill and colleagues demonstrated that women with a pregnancy affected by an NTD, had significantly higher levels of homocysteine than control subjects (247). Folic acid may have synergistic effect with other nutrients, including vitamin C, B12, B6, pantothenic acid, and zinc (248).

#### **7.4 Prevention of folate deficiency and neural tube defects**

Current recommendations encourage pregnant women to consume foods rich in folic acid. Some of the dietary sources of folate are dried beans, lentils, ham, liver, roasted peanuts, oysters, watermelon and brewer's yeast (249). In the MRC (UK) vitamin study conducted at 33 centers in seven countries, it was found that folic acid supplementation had a 72% protective effect. This strengthens the current recommendation that folic acid supplementation be given to all women who have had an affected pregnancy (250). It is recommended that all women of reproductive age should consume 400 µg per day of folic acid and should continue until the 12<sup>th</sup> week of pregnancy (251). Folate supplementation should be given before conception because the malformation of neural tube defects occurs during the first four weeks of embryonic life (213).

The mechanism of folic acid in the prevention of NTD is not known. The effectiveness of multivitamin/folic acid supplementation in early pregnancy was also found to reduce the risk of NTD, since the absorption of folic acid in pregnant women with a history of NTDs is not impaired (252,253). Folic acid is widely considered non-toxic, even when consumed in large quantities; but concern has been raised for two groups: people with untreated vitamin B12 deficiency, and people with epilepsy who are on anticonvulsant treatment (221).

If folic acid supplements are administered to patients with undiagnosed vitamin B12 deficiency, there may be an exacerbation of the associated neurological disease. It is essential to eliminate the possibility of vitamin B12 deficiency before treatment with pharmacological doses of folic acid (254). High doses of folic acid administration to patients with epilepsy have been reported to induce seizure activities (217,255).

Many countries, including South Africa have accepted guidelines for the use of periconceptual folic acid by women (256,257,258,259). The recommendations are as follows:

1. Women who have had a pregnancy resulting in an infant or fetus with a neural tube defect should be counselled about the increased risk in subsequent pregnancies and should be advised that folic acid supplementation may reduce the risk for neural tube defects in subsequent pregnancies.
2. These women should take 5 mg folic acid daily for three months prior to conception and three months after conception in all subsequent pregnancies.
3. All women who might become pregnant should consume 0.4 mg of folic acid daily prior to and after conception for three months, to reduce the risk of having a child with an NTD.

An additional 100 or 200  $\mu\text{g}$  of folate are recommended daily for pregnant woman. The recommended total intakes range from 300 to 400  $\mu\text{g}$  (Panels from Canada, the EC and the USA) (Table 3). The UK Panel concludes that 100  $\mu\text{g}$  per day will maintain plasma and red cell folate levels at or above those of non-pregnant women. The USA panel states that the equivalent of 200  $\mu\text{g}$  of pteroylglutamic acid (PGA) will prevent folate deficiency in pregnancy among women who start with moderate folate stores. The WHO recommends a supplement of 200 to 300  $\mu\text{g}$  per day. Since 170  $\mu\text{g}$  per day is recommended for the non-pregnant woman, the total intake recommended for pregnancy is 370 to 470  $\mu\text{g}$  per day. Fortification of staple foods with folic acid has been advocated for some years (209,249,260). This appears to be the most effective public health strategy.

## **8. Motivation of the study**

Siloam hospital is a district hospital serving the rural areas of Nzhelele under Vhembe district. Siloam hospital is situated in the Limpopo Province and is about 160 kilometres from Polokwane City. It serves a rural population of about 200 000 people.

The paramount importance of nutrition of a woman during pregnancy for her own health and that of her baby is widely recognized and has received increasing attention in recent years. Data on the negative effects of poor nutrition during pregnancy on maternal health and reproductive performance, including birth weight, are available. The depletion of maternal nutritional reserves has been shown to reduce birth weight and increase perinatal complications and mortality of both mothers and infants.

A special case of nutrition and growth related to reproductive efficiency is that of pregnancy during adolescence, because two components of growth take place simultaneously. Increased nutritional needs at this juncture relate to the fact that adolescents gain up to 50% of their adult weight, more than 20% of their adult height, and 50% of their adult skeletal mass during this period. Micronutrients such as calcium, iron, folic acid, vitamin A, D, B6, and riboflavin and energy are the most likely to be inadequately supplied by the diets of female adolescents. Most of the adolescents' poor diets may have an adverse effect on outcome of their pregnancy. A number of studies of pregnancy in adolescence carried out in developed and developing countries indicate that rate of low birth weight and prematurity are higher in the young, physiologically immature adolescents than in the mature women. There is evidence suggesting that the incidences of eclampsia and pre-eclampsia are significantly higher among young adolescents than in any other group of reproductive age. The influence of iron and folic acid on the outcome of pregnancy cannot be overemphasized.

Pregnant adolescents are at a high risk for iron deficiency anaemia because of the high iron requirement for their own growth and the increased need for iron in pregnancy coupled with low dietary iron intake. South African dietary intake studies have concluded that the folic acid intake in rural black women of childbearing is insufficient. A deficiency of folate early in pregnancy, before the closure of the neural tube, may be related to neural tube defects in the fetus.

Given the documentation of low folate levels in women and pregnant women in the Limpopo Province, this was proposed as a possible aetiological factor involved in the relatively high NTD incidence previously recorded in the rural Limpopo Province. There is no data on the nutritional status of adolescent pregnant women especially for iron and folate in the Limpopo Province. More studies are essential to determine the nutritional status and the effect of micronutrient deficiency on the outcome of adolescent pregnancy in the Limpopo Province. For this reason a research project of this nature will assist the Provincial nutrition policy makers when they formulate objectives for nutrition education among teenagers and pregnant women.

# **METHODOLOGY**

## **AIM**

The aim of the study was to assess the nutritional status, with special emphasis on iron and folate status, and their relationship to the outcome of pregnancy and the newborn baby, of rural black, pregnant teenagers attending the antenatal clinic at Siloam Hospital.

## **OBJECTIVES**

The main objectives of the study were:

1. To determine by means of dietary questionnaire the mean dietary intake of the subjects.
2. To determine by means of the dietary questionnaire the mean intake of iron and folic acid.
3. To determine by means of anthropometric measurements, the nutritional status of the subjects.
4. To evaluate the iron and folic acid level of the subjects by means of biochemical analysis.
5. To evaluate the newborn babies in terms of weight status and neural tube defects.

## **METHODS**

### **Sample**

The study population was chosen from Siloam Hospital antenatal clinic in the Limpopo Province. The study population was consecutively selected by the researcher from the pregnant adolescents visiting the clinic for the first time. The study population comprised 40 rural black adolescent pregnant girls aged between 14 and 19 years.

A group of non-pregnant, non-lactating black adolescent girls (n=40) between the age of 14 and 19 was recruited to serve as controls. The control group was selected from nearby high schools or the community where the pregnant women came. The researcher first saw a group of pregnant adolescent girls in December 1999. The control group was interviewed the following year from February. A letter of permission was obtained from the Department of Education in the Vhembe district of the Limpopo Province. The control group was matched with the pregnant women. The matching criteria used were the age, educational status, marital status and socio-economic status. For the purpose of this study socio-economic status included employment history in the family, number of members of household, the type of house and the total household income. Women with serious medical complications such as hypertension, tuberculosis, diabetes mellitus, heart diseases and multiple pregnancies were excluded from the study.

### **Iron and folic acid supplementation at Siloam Hospital**

Iron and folic acid supplements were haphazardly distributed to pregnant women at Siloam Hospital when they were available. There was no policy dealing with supplementation for pregnant adolescents or teenagers. The nursing sisters used their own discretion as to who was given supplements. No explanation was given to the pregnant adolescents about why they were given supplements. The study did not interfere with the current policy in anyway.

### **Demographic information**

The subjects were interviewed separately by the dietitian in a private room during their antenatal visit. The dietitian introduced himself and explained the purpose of the study. The subject was reassured of the confidentiality of all the information that would be obtained and requested the subject to answer the questions as truthfully as possible. An informed consent was obtained from the subject. One

researcher conducted all interviews. Demographic data such as age, marital status, education, occupation, source of income and family structure were obtained from both the pregnant group and the control group using a pre-tested questionnaire (Annexure).

### **Questionnaires**

The demographic and dietary history questionnaire was piloted on pregnant adolescent girls (n=10) attending the antenatal clinic at Siloam Hospital. The demographic and dietary data questionnaire provided information on factors relevant to the household regarding the environment in which the subject lived. Questions that were not well understood were rephrased. Once the pilot had been completed, corrections and adjustments on the questionnaire were attended to before the study began.

The quantitative food frequency questionnaire (QFFQ) was also piloted on 15 pregnant adolescent girls. The QFFQ provided information on the eating patterns and intake over the previous six months for the pregnant adolescents and the control group. Food items that were not common or never consumed were excluded from the questionnaire. The questionnaire used had been documented to be a valid and reproducible instrument for the assessment of dietary intakes of the population of the North West Province (261, 262). Validity is the ability of an instrument to actually measure what it is intended to measure (263,264). The validity of a food frequency questionnaire depends to a great extent on items included in the food list and assumptions about portion size and nutrient content of the various groups (265).

The infant anthropometric measurements questionnaire provided information on the infant and the outcome of birth. The anthropometric questionnaire provided anthropometric information for pregnant adolescents and the control group. A blood collection questionnaire provided information on the biochemical analysis

carried out. The following questionnaires were designed, tested and used in the project. (See also Annexures). The results of the pilot group were not included in the final analysis of the study results.

The interviewer (the dietitian) used the language (Venda), which was their mother tongue. Obstetric and medical histories were obtained from the subjects' hospital files. The clinic-nursing sister gave the files to the researcher. Information on tobacco smoking and alcohol intake was collected from the subjects. Prenatal history was obtained from the hospital cards of the pregnant women by the researcher. The intake of traditional or herbal medicines and pica were recorded.

### **Dietary intake**

The dietary intake of the subjects was collected by the completion of a pre-tested quantified food frequency questionnaire (QFFQ). The QFFQ provided information on the eating patterns and intake of the subjects over the previous six months. The QFFQ consisted of 118 food and drink items, including traditional dishes. The 118 food items included in the questionnaire were those commonly found and used in the area where the subjects come from. Standard household measuring utensils and actual food items were used to quantify food portion sizes. Other dietary aides used were a set of spoons, cups with measures, plates, a ruler, empty containers and packets of food items used locally. Food items like beans, samp, sugar, bread and water were used to estimate the exact amount of food consumed by the subjects. The dietitian requested each subject to measure the amount of food consumed using the utensils provided. Those food items measured were transferred to the measuring jugs and cups that had measurements written in millilitres. The QFFQ was completed during the first visit of the adolescent girls to the antenatal clinic, and for control group at the first visit to the schools. Personal interviews to complete the questionnaires lasted approximately 45 minutes for each subject.

## **Anthropometric measurements**

### ***Adult anthropometric measurements***

The dietitian performed all the anthropometric measurements. Anthropometric measurements were recorded on two occasions for the pregnant adolescent girls: on the first visit to the clinic and within 24 to 72 hours after delivery, but before discharge from the hospital. Anthropometric measurements were recorded only once for the control group. Standing height was measured to the nearest 0.5 cm with the subject barefoot, standing straight on a horizontal surface with heels together, shoulders relaxed, arms at the sides and the head in the Frankfurt horizontal plane (266). Height was measured (in centimetres) by means of Anthropometer ("Height 200cm N0.26SM, Hospital Products) measuring up to 2 metres. Body weight was measured to the nearest 0.5 kg with the subjects barefoot, in light clothing on a calibrated digital bathroom scale (Tanita model 1607, made in Japan). The scale was calibrated by weighing a standard 5 kg weight everyday it was used. Body weight was recorded twice for pregnant women, on the first visit to the clinic and within 24 to 72 hours after delivery. Body weight for the control group was recorded on one occasion.

Body mass index (BMI) for each subject was calculated using the formula: Weight (kg) divided by height squared ( $m^2$ ) (267). Mid-upper arm circumference (MUAC) was measured at the midpoint between the acromial and olecranon processes of the scapula and the ulna respectively. The arm was hanging relaxed at the patient's side. Mid-upper arm circumference was measured to the nearest 0.1 cm with a non-elastic tape measure, which was gently but firmly placed at a right angle to the long axis of the upper arm. Three measurements of the MUAC were taken and the average was recorded.

### ***Infant's anthropometric measurements***

A nursing sister measured body weight to the nearest 0.1 kg, using a calibrated paediatric beam balanced scale (Terrillon). The nursing sister was trained and standardized by the dietitian to take the anthropometric measurements. The scale was calibrated by weighing a standard 5 kg weight. The scale was positioned on a firm table. The scale has a tray on which the infant lies and a beam along which a weight is moved until it balances the weight of the infant. Length was measured using an Anthropometer for babies. Head circumference was measured to the nearest millimetre using a non-stretch tape made of fibreglass. The tape was placed just above the supra-orbital ridges covering the most prominent part of the frontal bulge, and over the part of the occiput. The tape was at the same level on each side of the head, perpendicular to the Frankfurt plane and pulled tightly (268). All measurements were taken within one hour after birth. The presence of any abnormality or neural tube defects was recorded for infants.

### **Blood collection**

The amount of 10 ml of venous blood was collected by venipuncture from each subject, by a registered nursing sister. Blood was drawn twice from the pregnant adolescent girls. The reason why blood was collected twice was to compare the biochemical values before and after delivery. A non-fasting blood sample was collected during the first visit to the antenatal clinic in the morning, and within 24 to 72 hours after delivery. Blood was collected once from control group. Blood was transferred into 5ml EDTA coated tubes for haematological analysis. The researcher, with the help of the laboratory technologist, analysed full blood count and haematocrit immediately. The full blood count and haematocrit were analysed by the Coulter machine (Coulter MD 11 Series Analyser) at Siloam Hospital. The machine was calibrated everyday by the medical technologist before use. The rest of the blood (5ml) was transferred into plain glass tubes, where it was allowed to clot, spun down and serum obtained for biochemistry. The centrifuge machine

was spun down at a speed of 1 500 rpm. Plasma for biochemistry was prepared by centrifugation for 15 minutes.

The amount of 100 µL whole blood was obtained from the EDTA tube to prepare for red blood cells folate. The amount of 0.002g of ascorbic acid was mixed with 2ml of distilled water in a separate tube. The ascorbic acid was freshly prepared. Blood was mixed with the ascorbic acid mixture by inversion several times in order to avoid foaming. The mixture was stored and frozen in the Wasserman tubes with other samples. The samples were shielded from light at all times. Plasma and serum were frozen immediately at  $-20^{\circ}$  Celsius until dispatched to the laboratory at Tygerberg Hospital for analysis. Blood was sent to Cape Town (Tygerberg) in a pre-cooled cooler box in batches within five weeks of collection by air. The following parameters were analysed from the frozen plasma and serum, namely: serum iron concentration, serum folate, red cell folate, serum ferritin, serum transferrin, transferrin saturation, and vitamin B12.

## **Analysis of data**

### ***Dietary data***

After completion of data collection, the questionnaires were re-checked for any missing information by the dietitian. If there was missing information, the subjects were followed up when they came to the antenatal clinic. The dietary intake was quantified using the National Research Institute for Nutritional Diseases (NRIND) food quantities manual (269). Dietary intake was expressed in grams per day for each subject and data was encoded. The average daily nutrient intake was calculated using the Statistical Analysis System (SAS) system for Windows (1999-2001) software package (270). Intakes were compared to the recommended dietary allowances (RDA) for pregnant women (79). Descriptive statistics (means, standard deviations, medians, ranges) and frequency distributions were calculated for all nutrients.

***Laboratory analysis***

The serum and red cell folate and vitamin B12 were determined using the SimulTRAC-SNB kit by ICN Pharmaceuticals (New York). Serum ferritin and transferrin were determined using specific immune reactions monitored using a nephelometer (Behring, Model BN 100). Iron levels were determined using atomic absorption spectrophotometry. The following cut-off points for biochemical and haematological values were used to analyse the results:

- Haemoglobin for pregnant girls: <11g/dl,
- Haemoglobin for non-pregnant girls: <12g/dl (126),
- MCV: <80 fl; MCH: <27 pg (271),
- MCHC: <32.5,
- Serum ferritin: <12 $\mu$ g/L (161),
- Serum iron: <6.6 $\mu$ mol/L,
- Transferrin saturation: <16% (162),
- Serum transferrin: <2.1g/L,
- Serum Folate: <4.1nmol/L; RBC Folate: <320nmol/L,
- Vitamin B12: <132pmol/L.

The coefficient of variation for each of the parameter were as follows:

Serum folate: 2.2 to 56.0 nmol/L; red cell folate: 13 to 856 nmol/L; Vitamin B12: 94 to 1170 pmol/L; Serum ferritin: <2 to 75  $\mu$ g/L; Serum transferrin: 2.91 to 6.46 g/L and Serum iron: 0.9 to 36.3  $\mu$ mol/L.

**Statistical analysis**

The results for the subjects between the age of 14 and 16 were analysed separately from those older than 16 years. The Statistical Analysis System package was used to analyze data. Means, standard deviations (SD) and medians of the subjects were calculated. Independent and Pairwise t-tests were used to compare the two groups of pregnant adolescent girls and the controls. A p-value of less than 0.05 was considered significant. Comparison of the various parameters in the two groups was done by administering Chi-square and Fisher's Exact test at p-values

of less than 0.05 level of significance. Chi-square was done on two by two or more tables, whereas Fisher exact test was only done on a two by two table. The Chi-square test was regarded invalid where cells had counts of less than 5. The Kruskal-Wallis test and the Wilcoxon two-sample test were used when results were being analysed separately based on age. The statistician was consulted for statistical analysis. Pearson's correlation coefficient was used to determine the relationship between infants' anthropometric data and pregnant adolescent girls' anthropometric data and dietary intake.

### **Ethical consideration**

The University of Stellenbosch Ethics Committee approved the study. Permission to conduct the study was also granted by the research committee of the Department of Health in the Limpopo Province. The purpose of the study was explained to all the participants who were asked to give a written informed consent. No one refused to participate in the study.

# RESULTS

## RESULTS

### Demographic information

Ninety-seven of both the control and pregnant adolescents were Venda speaking. A total of 97.5% of the pregnant girls were unmarried compared to only 75% of the control group ( $p = 0.0069$ ) (Table 7). The education status of both the pregnant adolescent girls and the control group ranged from grade four to grade twelve. In the control group, 60% were in grade 8 to 9; 33% in grade 10 to 11; and only 7.5% were in grade twelve. In the group of pregnant adolescent girls studied ( $n=40$ ), 7.5% were in grade 4 to 7; 50% in grade 8 to 9; and 5% in grade twelve. All subjects studied had some formal education.

Fifty-two percent of the control group and 85% of the pregnant adolescent girls lived in houses. A total number of 2.5% for the control and 12.5% of the pregnant adolescent girls lived in huts. The number of rooms available ranged from one to fourteen in both groups. Thirty two percent of the control group and 47.5% of the pregnant adolescent girls had one to four people living with them in their homes. The rest (67.5% of the control, 52.5% of the pregnant adolescents) had more than five family members living with them. A reasonable proportion of the control group (60% and fewer pregnant adolescent girls (47.5%) stayed with both parents. About 30% of the control and 15% of the pregnant adolescent girls lived with one parent. The majority of adolescent girls in both groups (92.5% of the control, 87.5% of the pregnant adolescents) were not owners of the houses in which they were living.

All subjects in both groups were unemployed. About 55% of the control group and 50% of the pregnant adolescent girls had one family member working. Only 2.5% of the control and 10% of the pregnant girls had family members not working. The main source of income in both groups was a disability grant and an old age pension (control=67.5%, pregnant=72.5%). A significant number of the control group (75%) and pregnant adolescent girls (50%) had a total monthly

income of between R501 to R2000. Only 5% of the control group and 22.5% of the pregnant adolescent girls had income above R2000.

**Table 7: Selected demographic information of control subjects and pregnant adolescents (numbers in brackets indicate the number of subjects)**

Question	Response	Control group (%) (n=40)	Adolescent girls (%) (n=40)	Chi-square p-value	Fisher's exact p-value (two-sided)
<b>Marital status</b>	Married	2.5(1)	25.0(10)	0.0035*	0.0069**
	Single	97.5(39)	75(30)		
<b>Educational status</b>	Grade 12	7.5(3)	5(2)	0.3095#	0.3446
	Grade 10-11	32.5(13)	37.5(15)		
	Grade 8-9	60(24)	50(20)		
	Grade 4-7	0	7.5(3)		
<b>Type of a house</b>	Hut	2.5(1)	12.5(5)	0.0004*	0.5373
	House	52.5(21)	85(34)		
	Shack	2.5(1)	2.5(1)		
	Hut and house	35(14)	0		
	House & shack	2.5(1)	0		
	Hut, house & shack	5(2)	0		
<b>Total monthly income</b>	R0 – R100	0	2.5(1)	0.2256#	0.1513
	R101 – R300	2.5(1)	2.5(1)		
	R301 – R500	15(6)	17.5(7)		
	R501-R1000	37.5(15)	32.5(13)		
	R1001-2000	37.5(15)	22.5(9)		
	R2000 and more	5(2)	22.5(9)		
	None	2.5(1)	0		
<b>Age distribution</b>	Below 16 years	25.0(10)	25.0(10)		
	Above 17 years	75.0(30)	75.0(30)		

#Chi-square test may not be a valid test due to cells with a count of less than 5

\* Chi-square p-value significant:  $p < 0.05$ \*\* Fisher's Exact Test p-value significant:  $p < 0.05$

### **Adult Anthropometric Measurements**

The body weight [mean (SD)] of the control group [61.6(9.9)] was slightly lower than that of pregnant women [62.6(8.1)] and at postpartum [57.8(8.0)] (Table 8). Body weight before delivery was significantly different ( $p=0.0001$ ) to body weight after delivery. The height in both groups of pregnant adolescent girls stayed the same during and after pregnancy. Height did not differ significantly between the two groups. The body mass index (BMI) [mean(SD)] of the control group [25.1(4.1)] was nearly equal to that of pregnant women [25.7(3.1)]. However, BMI was significantly lower postpartum ( $p=0.0001$ ). The mean mid-upper arm circumferences and age of the control group and pregnant women were not significantly different. The average gestational period was 28 weeks. The pregnant adolescent girls were followed up at post-partum.

The percentage of pregnant adolescent girls falling below the cut-off point of 19.6 for the BMI was 12.5% and 2.5% for the pregnant adolescent girls and control group respectively (Table 9). In none of the subjects was the height below the cut-off point.

**Table 8: Descriptive statistics of anthropometric parameters in controls and pregnant subjects (N=40 for control, N=40 for pregnant subjects)**

Variables	Mean	SD	CI 95%	Median	5 <sup>th</sup> percentile	95 <sup>th</sup> percentile	Probability
<b>Weight: (kg)</b>							P1=0.6374 P2=0.0610 P3=0.6558 P4=0.0775 P5=0.0001**
<b>Control</b>	61.6	9.9	58.5 – 64.8	60.7	46.5	77.3	
<b>Pregnant</b>	62.6	8.1	60.0 – 65.2	61.1	51.4	78.6	
<b>Postpartum</b>	57.8	8.0	55.3 – 60.4	56.6	46.8	73.5	
<b>Height (m)</b>							P1=0.2918 P2=0.3107 P3=0.2307 P4=0.2451 P5=0.7769
<b>Control</b>	1.58	0.1	1.56 – 1.59	1.6	1.5	1.7	
<b>Pregnant</b>	1.56	0.1	1.54 – 1.58	1.6	1.5	1.7	
<b>Postpartum</b>	1.56	0.1	1.54 – 1.58	1.6	1.5	1.7	
<b>Body mass index (kg/m<sup>2</sup>)</b>							P1=0.4732 P2=0.1801 P3=0.4954 P4=0.1090 P5=0.0001**
<b>Control</b>	25.1	4.1	23.8 – 26.4	24.2	20.4	32.8	
<b>Pregnant</b>	25.7	3.1	24.7 – 26.7	25.2	20.5	31.7	
<b>Postpartum</b>	23.7	3.1	22.7 – 24.7	23.2	19.1	29.7	
<b>MUAC (cm)</b>							P1=0.1533 P2=0.0913 P3=0.1845 P4=0.2170 P5=0.6047
<b>Control</b>	27.9	6.2	25.9 – 29.9	26.6	23.0	35.1	
<b>Pregnant</b>	26.4	2.4	25.6 – 27.1	26.6	22.2	30.3	
<b>Postpartum</b>	26.5	2.5	25.7 – 27.3	26.8	22.7	30.6	

P1 = independent t-test, compare control with pregnant girls

P2 = independent t-test, compare control with postpartum

P3 = pairwise t-test, compare control with pregnant girls

P4 = pairwise t-test, compare control with postpartum

P5 = pairwise t-test, compare pregnant girls with postpartum

\*\*p-value significant, p<0.01

**Table 9: Percentage of pregnant adolescent girls and control group falling below the BMI and Height cut-off points**

<b>Variable</b>	<b>Mean</b>	<b>SD</b>	<b>Cut-off point</b>	<b>% Below cut-off point</b>	<b>Probability</b>
<b>Body mass index (BMI) (kg/m<sup>2</sup>)</b>					
<b>Control</b>	25.1	4.1	19.8	2.5% (1)	P1=0.4732 P2=0.4954
<b>Pregnant</b>	25.7	3.1			
<b>Height (m)</b>					
<b>Control</b>	1.58	0.1	1.4m-1.5m	0.0	P1=0.2918 P2=0.2307
<b>Pregnant</b>	1.56	0.1			
<b>Pregnancy weeks</b>	28				
<b>Pregnant</b>		1.2			

P1 = independent t-test, compare control with pregnant girls

P2 = pairwise t-test, compare control with pregnant girls

### **Social Characteristics**

None of the members of neither the control group (100%) nor the pregnant adolescent girls (100%) smoked cigarettes (Table 10). Only one pregnant adolescent girl admitted drinking alcohol. However, the amount (one beer/day) was not significant. The use of traditional (herbal) medicine among the control group was 5.0% and among pregnant adolescent girls was 7.5%. The main use of these herbal medicines was for pain and digestive problems. Pica practices were the same in both the control group (42.5%) and among the pregnant adolescent girls (42.5%). Eating of soil was very common in both groups (control=32.5%, pregnant=42.5%). The control group ate other substances like chalk (7.5%), and ash (2.5%).

**Table 10: Selected social habits information of control subjects and pregnant adolescents (number in brackets indicates the number of subjects)**

Question	Response	Control group (%) (n=40)	Adolescent girls (%) (n=40)	Chi-square p-value	Fisher's Exact p-value (two sided)
<b>Do you smoke cigarette?</b>	Yes	0	0		
	No	100(40)	100(40)		
<b>Do you drink alcohol?</b>	Yes	0	2.5(1)	0.3143#	1.0000
	No	100(0)	97.5(39)		
<b>How much alcohol do you drink per day?</b>	One beer per day	0	2.5(1)	0.3143#	1.0000
	Don't drink	100(0)	97.5(1)		
<b>Use of traditional (herbal) medicine</b>	Yes	5(2)	7.5(3)	0.6651#	1.0000
	No	95(38)	92.5(37)		
<b>PICA practices</b>	Yes	42.5(17)	42.5(17)	1.0000	1.0000
	No	57.5(23)	57.5(23)		
<b>If Yes, specify</b>	Soil	32.5(13)	42.5(17)		
	Ash	2.5(1)	0		
	Chalk	7.5(3)	0		
	None	57.5(23)	57.5(23)		

#Chi-square test may not be valid due to cells with counts of less than 5

\*Chi-square p-value significant:  $p < 0.05$ \*\*Fisher's Exact Test p-value significant:  $p < 0.05$

### **Obstetric and Medical History**

About 2.5% of the control group had previously had tuberculosis and diabetes mellitus (Table 11). Only 2.5% of the pregnant adolescent girls had previously had tuberculosis and hypertension. A total of 7.5% of the control group indicated that they were using other medications such as paracetamol. About 55% of the pregnant adolescent girls also used supplements such as folic acid (7.5%), iron (7.5%), iron and folic acid (60%) and other forms of medication (2.5%). Only 2.5% of previous pregnancies in both the control and pregnant women ended in miscarriage. Normal deliveries in the previous pregnancies for the control group were 2.5% and 12.5% for the pregnant adolescent girls. None of the subjects had had more than one previous pregnancy.

Of the control group, 95% had never been pregnant while among the pregnant adolescent girls, 85% had never been pregnant before. When asked about their plans of having children in future, about 47.5% of the control group and 50% of the pregnant adolescent girls indicated their interest in having more children. About 22.5% of the control group and 50% of the pregnant adolescent girls showed that they did not want to have children. Of the control group, 30% were undecided. Those subjects who showed interest in having children in the future, indicated that they did not want more than four children.

**Table 11: Selected obstetric and medical history information of pregnant adolescents and the control group (number in brackets indicates the number of subjects)**

Question	Response	Control group (%) (n=40)	Adolescent girls (%) (n=40)	Chi-square p-value	Fisher's Exact p-value (two sided)
<b>Have you ever had any of the following diseases?</b>	Tuberculosis	2.5(1)	2.5(1)	0.1940#	0.0805
	Diabetes Mellitus	2.5(1)	0		
	Hypertension	0	2.5(1)		
	Others	27.5(11)	10(4)		
	None	67.5(27)	85(34)		
<b>Have you ever had any operation?</b>	Yes	7.5(3)	0	0.0775#	0.2405
	No	92.(37)	100(40)		
<b>If "Yes" what type of operation have you had?</b>	Abscess drainage	2.5(1)	0		
	Other (specify)	5(2)	0		
	None	92.5(37)	100(40)		
<b>Do you use other commercial medicines or tablets?</b>	Yes	7.5(3)	55(22)	0.0001#*	0.5241
	No	92.5(37)	45(18)		
<b>If "Yes" specify the medication type or tablets</b>	Folic acid	0	7.5(3)		
	Iron	0	7.5(3)		
	Iron & folic acid	0	37.5(15)		
	Paracetamol	7.5(3)	0		
	None	92.5(37)	47.5(19)		
<b>Are you planning to have more children in the future?</b>	Yes	47.5(19)	50(20)	0.0003#*	0.0610
	No	22.5(9)	50(20)		
	Don't know	30(12)	0		

#Chi-square test may not be valid due to cells with counts of less than 5

\*Chi-square p-value significant:  $p < 0.05$ \*\*Fisher's Exact Test p-value significant:  $p = 0.05$

### **Food Preparation and Diet History**

A total of 20% of the control group indicated that they prepared food at home themselves compared to 57.5% of the pregnant adolescent girls (Table 12). Among the control group 12.5% had three meals and two snacks; 35% had three meals and one snack; 45% had three meals and 5% had two meals a day. Among the pregnant adolescent girls (62.5%) had three meals and two snacks compared to 2.5% with two or one meal per day. The meals that the control group and the pregnant adolescent girls had were breakfast, lunch and supper. About 57.5% of the control group said they usually ate these meals on most weekdays compared to 62.5% of the pregnant adolescent girls.

The father or mother at home usually bought food. Firewood was the most commonly used fuel to prepare food (45% of the control group, 50% of the pregnant group) followed by paraffin with about 12.5% of both the pregnant and the control group. About 55% of the control group and 60% of the pregnant adolescent girls indicated that they prepared cooked meals once a day at home. Only 45% of the control group and 40% of the pregnant adolescent girls reported to have had two or more cooked meals per day. Almost all meals for the subjects were prepared at home. The subjects in both groups seldom went to eat at restaurants. A total of 77.5% of the control group bought their food from the supermarkets compared to 72.5% of pregnant adolescent girls. Most of the subjects (65% control, 47.5% pregnant) indicated that it took more than an hour to reach the supermarket.

Of the control group 77.5% and 57.5% of the pregnant adolescent girls usually bought food once a month ( $p=0.0072$ ). A significant number of pregnant adolescent girls (40%) bought food fortnightly. Of the control group 52.5% had gardens at home compared to 47.5% of the pregnant adolescent girls. Half of the control group (50%) had a refrigerator at home, whereas 65% of the pregnant adolescent girls did not have. When comparing their appetites, the

control group (82.5%) reported to have better appetite than the pregnant group (75%).

Few of the subjects had difficulty in swallowing or chewing food. Their bowel habits appeared to be normal with only 15% of the pregnant adolescent girls reporting to have occasional episodes of diarrhoea. Food intolerance or allergy appeared to be a common problem in the control group (42.5%) compared to adolescent girls (25%). They reported to be allergic to the following foods: beef (10% control), milk (7.5% pregnant) and fish (5% control, 2.5% pregnant). It was the people's perception that they had allergy, however the diagnosis was not confirmed. A total of 37.5% of the control group showed that they had never received nutrition education compared to 25% of pregnant adolescent girls. The main source of education in the control group was radio and television (25%) and school teachers (17.5%) in the case of pregnant adolescent girls.

**TABLE 12: Selected food preparation and dietary history information of pregnant adolescents and the control group (numbers in brackets indicate the number of subjects)**

Question	Response	Control group (%) (n=40)	Adolescent girls (%) (n=40)	Chi-square p-value	Fisher's Exact p-value (two sided)
<b>Meals eaten per day</b>	Five	12.5(5)	5(2)	0.5382#	0.5276
	Four	35(14)	27.5(11)		
	Three	45(18)	62.5(25)		
	Two	5(2)	2.5(1)		
	One	2.5(1)	2.5(1)		
<b>How often do you prepare meals at home?</b>	Two or more per day	45(18)	40(16)	0.6510	0.8213
	Once per day	55(22)	60(24)		
<b>Where are most of meals prepared?</b>	Home	100(40)	97.5(39)	0.3143#	1.0000
	Restaurant	0	2.5(1)		
<b>How often do you buy food at home?</b>	Everyday	2.5(1)	0	0.0355#*	0.0072**
	3-5 times per week	2.5(1)	0		
	Less than 2 times/week	5(2)	2.5(1)		
	Fortnight	12.5(5)	40(16)		
	Once a month	77.5(31)	57.5(23)		
<b>Vegetable garden</b>	Yes	52.5(21)	47.5(19)	0.5141#	0.6549
	No	47.5(19)	52.5(21)		
<b>Have you ever received nutrition education?</b>	Yes	37.5(15)	25(10)	0.2278	0.3348
	No	62.5(25)	75(30)		
<b>If "Yes", from what source?</b>	Clinic	2.5(1)	2.5(1)	0.0470#*	0.0166**
	Care group worker	0	2.5(1)		
	Radio/TV	25(10)	2.5(1)		
	Teacher	10(4)	17.5(7)		
	Never	62.5(25)	75(30)		

#Chi-square test may not be valid due to cells with counts of less than 5

\*Chi-square p-value significant:  $p < 0.05$ ; \*\*Fisher's Exact Test p-value significant:  $p < 0.05$

Maizemeal was staple food of all subjects. The most commonly used brand of maizemeal was “White Diamond” (control 47.5%, pregnant 32.5%), followed by “Induna” (control 15%, pregnant 17.5%). About 20% of the control group ground maizemeal themselves, compared to 12.5% of the pregnant adolescent girls. Most of the subjects preferred to stick to their brands (control 65%, pregnant 57.5%). Maize used by the subjects was unfortified and bought at nearby shops. Thirty eight percent of the control group indicated that they bought their chickens from shops, or slaughtered their own (7.5%). About 52.5% of the pregnant adolescent girls bought their chickens from shops and 5% preferred to slaughter their own. Meat was mostly bought from shops. Hawkers seemed to be the main providers of vegetables in the control group (45%), with 22.5% of pregnant adolescent girls who got theirs from their own farms. Hawkers (50% of the control, 35% of the pregnant group) mainly provided fruits. A total of 60% of the control group said they used spreads like fruit jam, margarine and peanut butter on their bread compared to 57.5% of the pregnant adolescent girls.

The most commonly eaten foods by both the pregnant adolescent girls and the control group, in order of frequency were: maizemeal, imifino (morogo), brown bread, atchaar, chicken feet, carbonated drinks, vetkoek, oranges, paw-paw, canned fish, pumpkin, and tea.

### **Dietary Intake of the Subjects**

The mean energy intake did not differ between the two groups (Table 13). The total protein intake of pregnant adolescent girls 60.9(15.8) was significantly lower when compared with the control group 70.4(21.6). Plant protein [control 34.4(1.05), pregnant 38.1(18.5) ] appeared to be the main source of total protein compared to animal protein from the two groups [control 31.9(19.3), pregnant 22.6(11.9) ]. The difference in amount of animal protein was significant between the two groups ( $p=0.0119$ ). The cholesterol intake was significantly higher in the control group. There was also a difference in polyunsaturated fatty acid intake which was significant ( $p=0.0097$ ).

The calcium intake [mean (SD)] of the pregnant adolescent girls [346.6(172.9)] was slightly lower than that of the control group [374.2(205.8)] (Table 14). The total iron intake [mean (SD)] of the pregnant girls was [10.4(2.8)] mg compared to [10.3(2.3)] mg of the control group. The intake of magnesium, phosphorus, selenium, zinc, iodine, vitamin A, thiamin, riboflavin, nicotinic acid, vitamin B6, and folate did not differ significantly between the two groups. The only significant difference between the two groups was vitamin D ( $p=0.0402$ ). When the pregnant adolescent girls were divided into two groups (<16 years ( $n=10$ ), >17 years ( $n=30$ ), their micronutrient intakes did not differ significantly with the exception of vitamin B12, with a significant difference in favour of the older group.

**Table 13: Mean (SD) macronutrient intake of the subjects (N=40 for control, n=40 for pregnant)**

Variables	Mean	SD	CI 95%	Median	5 <sup>th</sup> ptcl	95 <sup>th</sup> ptcl	Probability
<b>Tot. energy (kJ)</b> Control Pregnant	9241.5 9122.5	2274.9 2139.9	8513.9-9969.0 8438.1-9806.8	8819.1 5152.1	5644.9 5152.1	13280.0 12681.2	P1=0.8102 P2=0.8197
<b>Tot. Protein (g)</b> Control Pregnant	70.4 60.9	21.6 15.8	63.5 – 77.3 55.8 – 66.0	66.4 57.7	42.9 39.1	107.3 95.7	P1=0.0276* P2=0.0723
<b>Animal protein(g)</b> Control Pregnant	31.9 22.6	19.3 11.9	25.7 – 38.1 18.8 – 26.4	26.3 20.8	11.5 7.3	77.3 22.1	P1=0.0119** P2=0.0123**
<b>Plant protein (g)</b> Control Pregnant	38.4 38.1	10.5 9.8	35.1 – 41.7 35.0 – 41.2	38.4 39.2	20.9 22.2	57.3 55.4	P1=0.8958 P2=0.8987
<b>Total fat (g)</b> Control Pregnant	55.8 48.4	22.1 18.5	48.7 – 62.9 42.4 – 54.3	49.0 45.0	30.1 22.9	97.8 88.2	P1=0.1079 P2=0.0917
<b>Sat. Fatty acid(g)</b> Control Pregnant	12.7 12.8	5.6 5.1	10.9 – 14.5 11.2 – 14.4	11.4 11.3	5.5 7.3	24.8 22.1	P1=0.9351 P2=0.9262
<b>Monounsaturated Fatty acid (g)</b> Control Pregnant	17.9 15.7	9.0 7.3	15.0 – 20.8 13.3 – 18.0	14.6 13.9	9.0 7.4	36.7 30.9	P1=0.2297 P2=0.2150
<b>Polyunsaturated fatty acid (g)</b> Control Pregnant	18.5 14.5	6.7 6.7	16.4 – 20.7 12.4 – 16.7	18.5 13.5	9.3 4.7	30.4 29.7	P1=0.0097** P2=0.0137**
<b>Cholesterol (mg)</b> Control Pregnant	289.0 196.6	202.3 141.4	224.3 – 353.7 151.4 – 241.8	244.0 129.4	80.6 36.1	631.1 479.0	P1=0.0207* P2=0.0272*
<b>Total carbohydrate(g)</b> Control Pregnant	326.1 344.7	91.3 89.8	296.8 – 355.3 316.0 – 373.5	320.7 341.0	159.9 200.0	492.8 511.1	P1=0.3597 P2=0.3750
<b>Total fibre (g)</b> Control Pregnant	25.6 25.6	6.6 7.8	23.5 – 27.7 23.2 – 28.1	23.8 26.8	17.1 12.6	40.2 38.1	P1=0.9643 P2=0.9662
<b>Added sugar (g)</b> Control Pregnant	24.6 29.2	19.1 16.7	18.5 – 30.7 23.8 - 34.5	23.2 26.9	2.7 8.3	55.0 66.1	P1=0.2598 P2=0.1849

P1 = independent t-test, compare control with pregnant

P2 = pairwise t-test, compare control with pregnant (matched pairs)

\*\*p-value significant, p&lt;0.01

\*p-value significant, p&lt;0.05

ptcl = percentile

**Table 14: Mean (SD) micronutrient intakes of the subjects (N = 40 for control, N = 40 for pregnant)**

<b>Variables</b>	<b>Mean</b>	<b>SD</b>	<b>CI 95%</b>	<b>Median</b>	<b>5<sup>th</sup> ptcl</b>	<b>95<sup>th</sup> ptcl</b>	<b>Probability</b>
<b>Calcium (mg)</b>							
Control	374.2	205.8	308.4 – 440.0	302.8	193.6	680.2	P1=0.5186
Pregnant	346.6	172.9	291.4 – 401.9	304.3	116.5	672.1	P2=0.5029
<b>Total iron (mg)</b>							
Control	10.3	3.1	9.2 – 11.3	9.3	6.7	16.0	P1=0.9092
Pregnant	10.4	3.8	9.2 – 11.6	9.2	5.7	17.7	P2=0.9190
<b>Nonhaem iron (mg)</b>							
Control	3.1	2.3	2.4 – 3.8	2.6	1.3	6.7	P1=0.2552
Pregnant	3.8	2.8	2.9 – 4.6	2.8	0.4	9.1	P2=0.2619
<b>Magnesium (mg)</b>							
Control	387.9	98.7	356.3 – 419.5	377.2	226.0	569.7	P1=0.5922
Pregnant	375.7	103.0	342.8 – 408.7	374.3	204.9	548.3	P2=0.6082
<b>Phosphorus (mg)</b>							
Control	1085.5	295.2	991.0 – 1179.9	1010.3	652.3	1603.1	P1=0.1807
Pregnant	1002.3	254.0	921.0 – 1083.5	975.3	563.4	1448.2	P2=0.2072
<b>Selenium (mg)</b>							
Control	11.3	11.2	7.8 – 14.9	8.2	2.7	40.6	P1=0.4271
Pregnant	9.5	9.3	6.5 – 12.5	6.2	1.2	31.4	P2=0.4473
<b>Zinc (mg)</b>							
Control	8.0	2.3	7.3 – 8.8	7.5	4.9	12.5	P1=0.5801
Pregnant	7.7	2.0	7.1 – 8.4	7.6	4.8	11.4	P2=0.6132
<b>Iodine (mg)</b>							
Control	17.4	16.8	12.1 – 22.8	12.0	3.6	48.8	P1=0.7851
Pregnant	16.4	16.6	11.1 – 21.7	13.8	1.7	47.6	P2=0.7872
<b>Vitamin A (RE)</b>							
Control	696.2	423.4	560.8 – 831.6	671.5	201.6	1534.5	P1=0.8508
Pregnant	720.1	677.4	503.4 – 936.7	545.3	56.3	2169.7	P2=0.8540
<b>Thiamin (mg)</b>							
Control	1.3	0.4	1.1 – 1.5	1.2	0.7	2.1	P1=1.0000
Pregnant	1.3	0.4	1.2 – 1.5	1.3	0.7	2.1	P2=0.9999
<b>Riboflavin (mg)</b>							
Control	0.9	0.4	0.8 – 1.0	0.8	0.4	1.8	P1=0.0615
Pregnant	0.8	0.3	0.7 – 0.8	0.7	0.4	1.4	P2=0.0491
<b>Nicotinic acid(mg)</b>							
Control	12.1	3.9	10.9 – 13.3	11.1	7.1	20.6	P1=0.8508
Pregnant	12.3	4.7	10.8 – 13.8	11.4	6.6	22.6	P2=0.8491
<b>Vitamin B6 (mg)</b>							
Control	1.2	0.5	1.1 – 1.4	1.1	0.6	2.3	P1=0.8113
Pregnant	1.2	0.6	1.0 – 1.4	1.1	0.7	2.4	P2=0.8136
<b>Folate (ug)</b>							
Control	229.1	68.2	207.3 – 250.9	215.4	124.2	354.9	P1=0.4466
Pregnant	215.5	89.0	187.1 – 244.0	192.9	117.7	368.0	P2=0.4595
<b>Vitamin B12 (mg)</b>							
Control	4.8	3.2	3.8 – 5.8	4.3	1.6	11.4	P1=0.5788
Pregnant	4.4	4.4	2.9 – 5.8	3.3	0.6	11.2	P2=0.5530
<b>Vitamin C (mg)</b>							
Control	91.5	45.6	76.9 – 106.1	85.6	27.0	174.3	P1=0.5484
Pregnant	99.9	75.3	75.8 – 123.9	76.0	20.2	269.9	P2=0.5680
<b>Vitamin D (mg)</b>							
Control	5.7	4.2	4.4 – 7.1	4.5	1.5	11.8	P1=0.0562
Pregnant	4.1	3.3	3.0 – 5.1	3.1	0.4	11.1	P2=0.0402*
<b>Vitamin E (mg)</b>							
Control	11.3	5.0	9.7 – 12.9	10.2	4.7	22.8	P1=0.1028
Pregnant	9.5	5.1	7.8 – 11.1	8.1	2.7	20.0	P2=0.1046

\*p-value significant, p&lt;0.05

p1 and p2 = p-value

Sixty seven percent (67%) of the RDA was used as a cut-off point (78) (Table 15). When the energy intake of the pregnant adolescent girls (n=40) was compared to the RDA (10500kj), 95% were above 67%, with only 5% falling below the 67% range. The total protein intake of pregnant adolescent girls (97.5%) was above the 67% RDA, with only 2.5% below range. Calcium intake in all age groups was below 67% of RDA. Total iron intake for all the pregnant adolescent girls was 45% above 67% of the cut-off point, with 55% below 67% of the RDA. This dietary iron intake did not include intake from the supplements

A large percentage of the pregnant adolescent girls (95%) had an intake of selenium far below the cut-off point. The intake of zinc (62.5%) in all pregnant adolescent girls was below 67% compared to 37.5% who were above the recommended intake. Fifty-three percent of pregnant adolescent girls had a vitamin A intake above 67% compared to 30% who were below the cut-off point.. Many pregnant adolescent girls (from 56.7% to 76.7%) had an intake of vitamin B6 and C above 67% of the RDA. Folate intake did not appear to be a problem for the pregnant adolescent girls (95% above range). Only 5% of pregnant adolescent girls had an intake of folate below the cut-off point. This folate intake did not include the supplements.

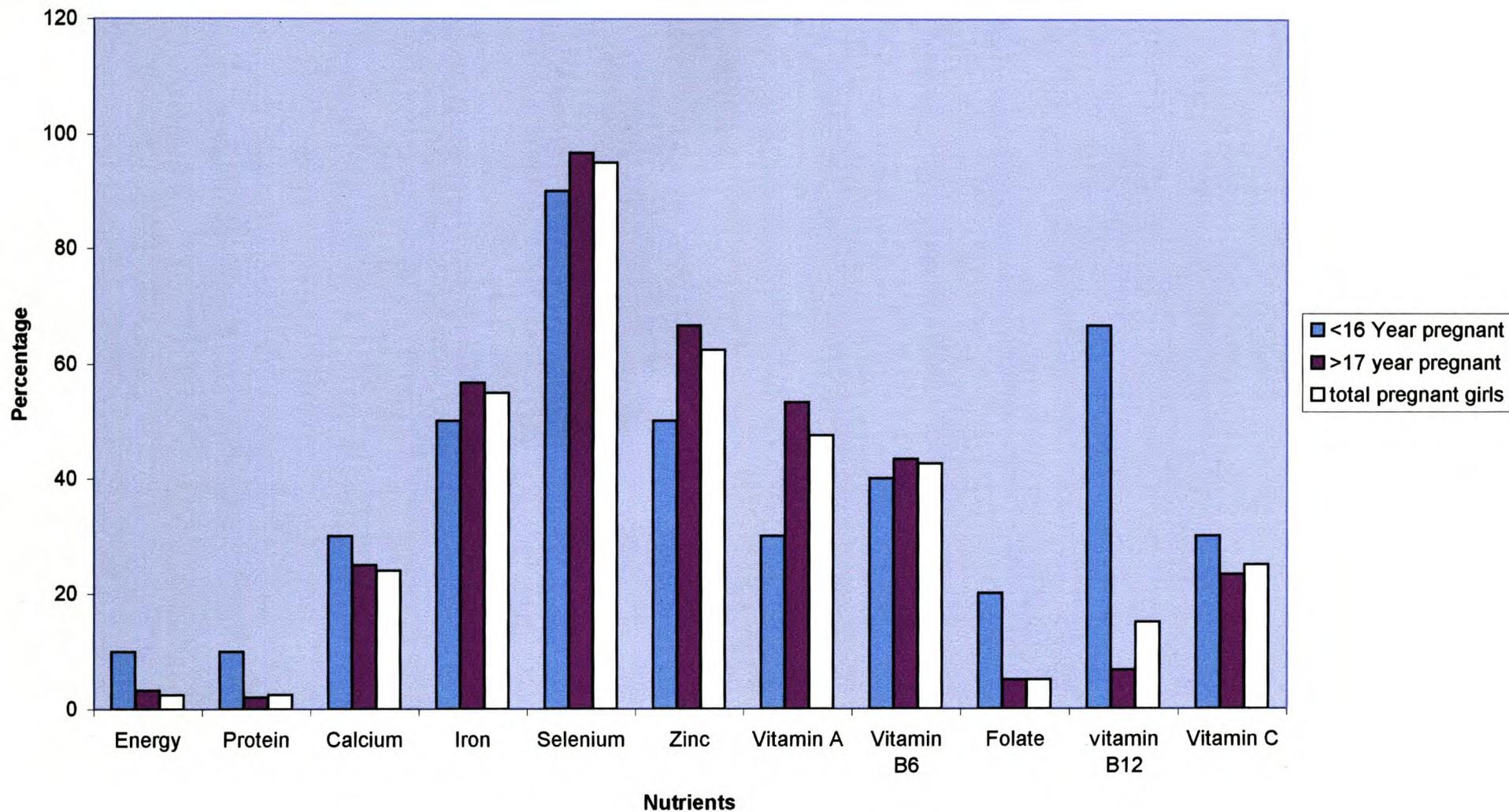
When dietary intake information was divided per age group, in the under 16 year olds, the percentage of those below and above 67% of RDA was equal (50%) for iron intake (Figure 1). About 43.3% of pregnant adolescent girls (>17 years) were above 67% compared to 56.7% who were below the 67% cut-off point. More individuals (53.3%) in the 17-year old category seemed to have a vitamin A intake below the range. The pregnant adolescent girls (<16 years) seemed to have a problem with vitamin B12 deficiency, with 66.7% below the 67% of RDA.

**Table 15: The percentage of total number of pregnant adolescent girls falling below the cut-off value of the RDA**

<b>Variable</b>	<b>Mean</b>	<b>SD</b>	<b>RDA</b>	<b>&lt;67% of RDA</b>	<b>Chi-square p-value</b>	<b>Fisher's Exact</b>
<b>Energy</b>	9122.5	2139.9	10500	5.0	0.4022#	0.4423
<b>Protein (g)</b>	60.9	15.8	60	2.5	0.0794#	0.2500
<b>Calcium (mg)</b>	346.6	172.9	1200	25.0		0.3546
<b>Iron (mg)</b>	10.4	3.8	30	55.0	0.7136#	0.7307
<b>Selenium (µg)</b>	9.5	9.3	65	95.0	0.4022#	0.4423
<b>Zinc (mg)</b>	7.7	2.0	15	62.5	0.3458#	0.4568
<b>Vitamin A (RE)</b>	720.1	677.4	800	52.5	0.2007#	0.8120
<b>Vitamin B6 (mg)</b>	1.2	0.6	2.2	42.5	0.8535#	1.0000
<b>Folate (ug)</b>	215.5	89.0	400	5.0	0.0120#	0.0577
<b>Vitamin B12 (mg)</b>	4.4	4.4	2.2	15.0	0.0106#	0.0258
<b>Vitamin C (mg)</b>	99.9	76.3	70	25.0	0.6733#	0.6893

#Chi-square may not be valid due to cells with counts less than 5

**Figure 1: Percentage of subjects with micronutrient intakes below 67% of the RDA**



### **Haematological Data**

The mean red blood cell count in the control group [4.5(0.4)] was significantly higher than that of pregnant adolescents ( $p=0.0001$ ) (Table 16). The pregnant adolescents' red blood cell count was significantly higher at postpartum than the red cell count during pregnancy ( $p=0.0051$ ). The mean haematocrit in the control group [39.3(4.3)] was significantly higher than that of pregnant adolescents ( $p=0.0001$ ) and at postpartum ( $p=0.0026$ ). Mean haemoglobin concentration in the control group [12.5 (1.7)] was significantly higher than that of the pregnant adolescents and higher at postpartum ( $p=0.0001$ ). In the control group the haemoglobin concentration was significantly higher than the haemoglobin concentration of pregnant adolescents at postpartum ( $p=0.0078$ ). The mean corpuscular volume (MCV), MCH and MCHC did not differ significantly between the two groups.

In the pregnant adolescents, the mean serum ferritin was significantly higher at postpartum than the serum ferritin during pregnancy ( $p=0.0054$ ). The mean serum iron in the control group [9.7(7.5)] was significantly lower compared to pregnant adolescents at postpartum ( $p=0.0165$ ) (Table 17). In the pregnant adolescents, mean serum iron was significantly higher at postpartum than the serum iron during pregnancy and the control group. The transferrin saturation did not differ significantly between the two groups. The mean serum transferrin in the pregnant adolescents [4.5(0.8)] was significantly higher than the control group ( $p=0.0001$ ) and higher at postpartum ( $p=0.0005$ ). In the pregnant adolescents serum transferrin was significantly higher at postpartum when compared to the serum transferrin of the control group ( $p=0.0089$ ). In the pregnant adolescents, serum folate was significantly higher at postpartum than the serum folate in the control group ( $p=0.0028$ ). The mean red cell folate among the pregnant adolescents [367.5(178.6)] was significantly higher than that of the control group ( $p=0.0002$ ). In the pregnant adolescents, red cell folate was significantly higher at postpartum than the red cell folate of the control group ( $p=0.0008$ ).

The mean serum vitamin B12 in the control group [466.4(237.9)] was significantly higher than that of pregnant adolescents ( $p=0.0001$ ) and at postpartum ( $p=0.0002$ ). In the pregnant adolescents serum vitamin B12 was significantly higher at postpartum than the serum vitamin B12 during pregnancy ( $p=0.0168$ ).

**Table 16: Descriptive statistics of biochemistry data (n=40 for control, n=40 for pregnant)**

Variables	Mean	SD	CI 95%	Median	5 <sup>th</sup> percentile	95 <sup>th</sup> percentile	Probability
<b>Red blood cell count (<math>\times 10^{12}/L</math>)</b>							P1=0.0001** P2=0.0118* P3=0.0001** P4=0.0176* P5=0.0051**
<b>Control</b>	4.5	0.4	4.4 – 4.7	4.5	4.0	5.1	
<b>Pregnant</b>	4.0	0.5	3.9 – 4.2	4.0	3.4	5.0	
<b>Postpartum</b>	4.2	0.6	4.0 – 4.4	4.1	3.3	5.5	
<b>Haematocrit (%)</b>							P1=0.0001** P2=0.0026** P3=0.0001** P4=0.0027** P5=0.1262
<b>Control</b>	39.3	4.3	37.9 – 40.7	40.7	30.4	44.6	
<b>Pregnant</b>	34.7	4.8	33.1 – 36.2	35.3	27.8	41.1	
<b>Postpartum</b>	35.5	6.5	33.4 – 37.5	35.8	26.8	46.1	
<b>Haemoglobin (g/dl)</b>							P1=0.0001** P2=0.0044** P3=0.0001** P4=0.0078** P5=0.0554
<b>Control</b>	12.5	1.7	12.0 – 13.0	13.0	9.2	14.6	
<b>Pregnant</b>	10.9	1.5	10.4 – 11.3	10.9	8.6	13.2	
<b>Postpartum</b>	11.2	2.1	10.6 – 11.9	10.9	8.3	14.9	
<b>MCV (fl)</b>							P1=0.8385 P2=0.7740 P3=0.8315 P4=0.7572 P5=0.0172*
<b>Control</b>	85.0	13.2	80.7 – 89.2	89.2	63.3	97.8	
<b>Pregnant</b>	34.7	8.5	82.7 – 88.2	86.6	69.3	98.1	
<b>Postpartum</b>	35.5	8.2	81.6 – 86.9	84.4	70.2	96.7	
<b>MCH (pg)</b>							P1=0.2367 P2=0.2534 P3=0.2713 P4=0.2816 P5=0.6926
<b>Control</b>	29.1	11.2	25.5 – 32.7	28.6	19.8	32.3	
<b>Pregnant</b>	26.9	3.1	25.9 – 27.9	27.5	21.6	31.9	
<b>Postpartum</b>	27.0	3.1	26.0 – 28.0	27.2	21.6	31.9	
<b>MCHC (g/dl)</b>							P1=0.4442 P2=0.8649 P3=0.4168 P4=0.8592 P5=0.1944
<b>Control</b>	31.7	1.5	31.2 – 32.2	31.9	29.3	33.8	
<b>Pregnant</b>	31.4	1.4	31.0 – 31.9	31.0	29.5	34.2	
<b>Postpartum</b>	31.8	1.7	31.2 – 32.3	31.8	29.0	34.3	

P1 = independent t-test, compare control with pregnant girls; p2 = independent t-test, compare control with girls at postpartum

P3 = pairwise t-test, compare control with pregnant girls; p4 = pairwise t-test, compare control with girls at postpartum

P5 = pairwise t-test, compare pregnant girls with girls at postpartum; \*\*p-value significant, p<0.01; \*p-value significant, p<0.05

**Table 17: More descriptive statistics of biochemistry data (n=40 for control, n=40 for pregnant)**

Variables	Mean	SD	CI 95%	Median	5 <sup>th</sup> ptcl	95 <sup>th</sup> ptcl	Probability
<b>Serum ferritin (ng/ml)</b>							P1=0.0599 P2=0.1901 P3=0.0931 P4=0.1167 P5=0.0054**
<b>Control</b>	16.1	12.3	12.1 – 20.0	13.5	2.0	36.5	
<b>Pregnant</b>	11.6	8.5	8.9 – 14.3	9.0	2.0	25.0	
<b>Postpartum</b>	20.7	18.2	14.8 – 26.5	15.5	2.0	64.5	
<b>Serum iron (µmol/L)</b>							P1=0.0674 P2=0.0165* P3=0.0840 P4=0.0244* P5=0.3864
<b>Control</b>	9.7	7.5	7.3 – 12.1	7.7	1.0	25.0	
<b>Pregnant</b>	12.7	6.8	10.5 – 14.9	12.7	3.9	21.3	
<b>Postpartum</b>	13.9	7.8	11.4 – 16.4	13.4	4.0	30.2	
<b>Transferrin saturation (%)</b>							P1=0.5529 P2=0.1037 P3=0.5685 P4=0.1093 P5=0.1405
<b>Control</b>	12.1	9.3	9.2 – 15.1	9.7	1.8	31.3	
<b>Pregnant</b>	13.3	8.0	10.7 – 15.9	12.4	3.2	26.0	
<b>Postpartum</b>	15.4	8.4	12.7 – 18.1	13.7	2.6	31.1	
<b>Serum transferrin (mg/ml)</b>							P1=0.0001** P2=0.0089** P3=0.0001** P4=0.0078** P5=0.0005**
<b>Control</b>	3.7	0.5	3.5 – 3.8	3.6	3.0	4.5	
<b>Pregnant</b>	4.5	0.8	4.2 – 4.8	4.4	3.4	5.9	
<b>Postpartum</b>	4.1	0.8	3.8 – 4.3	3.8	3.0	5.7	
<b>Serum folate (ng/ml)</b>							P1=0.0103* P2=0.0028** P3=0.0138* P4=0.0029** P5=0.1181
<b>Control</b>	5.5	2.4	4.7 – 6.2	4.7	2.5	9.8	
<b>Pregnant</b>	8.6	7.0	6.4 – 10.8	5.9	2.9	25.3	
<b>Postpartum</b>	12.6	13.9	8.1 – 17.0	6.4	2.2	45.5	
<b>RBC Folate (ng/ml)</b>							P1=0.0002** P2=0.0008** P3=0.0007** P4=0.0015** P5=0.8145
<b>Control</b>	229.6	131.9	187.4-271.8	201.0	73.5	519.0	
<b>Pregnant</b>	367.5	178.6	310.4-424.7	314.0	125.5	700.0	
<b>Postpartum</b>	359.7	194.6	297.4-421.9	297.5	116.0	770.5	
<b>Serum vitamin B12 (pg/ml)</b>							P1=0.0001** P2=0.00028* P3=0.0001** P4=0.0001** P5=0.0168*
<b>Control</b>	466.4	237.9	390.3-542.4	375.0	192.5	980.0	
<b>Pregnant</b>	230.1	98.2	198.6-261.5	222.5	93.5	380.0	
<b>Postpartum</b>	289.3	156.2	239.3-339.2	257.5	97.0	560.0	

P1 = independent t-test, compare control with pregnant girls

P2 = independent t-test, compare control with girls at postpartum

P3 = pairwise t-test, compare control with pregnant girls

P4 = pairwise t-test, compare control with girls at postpartum

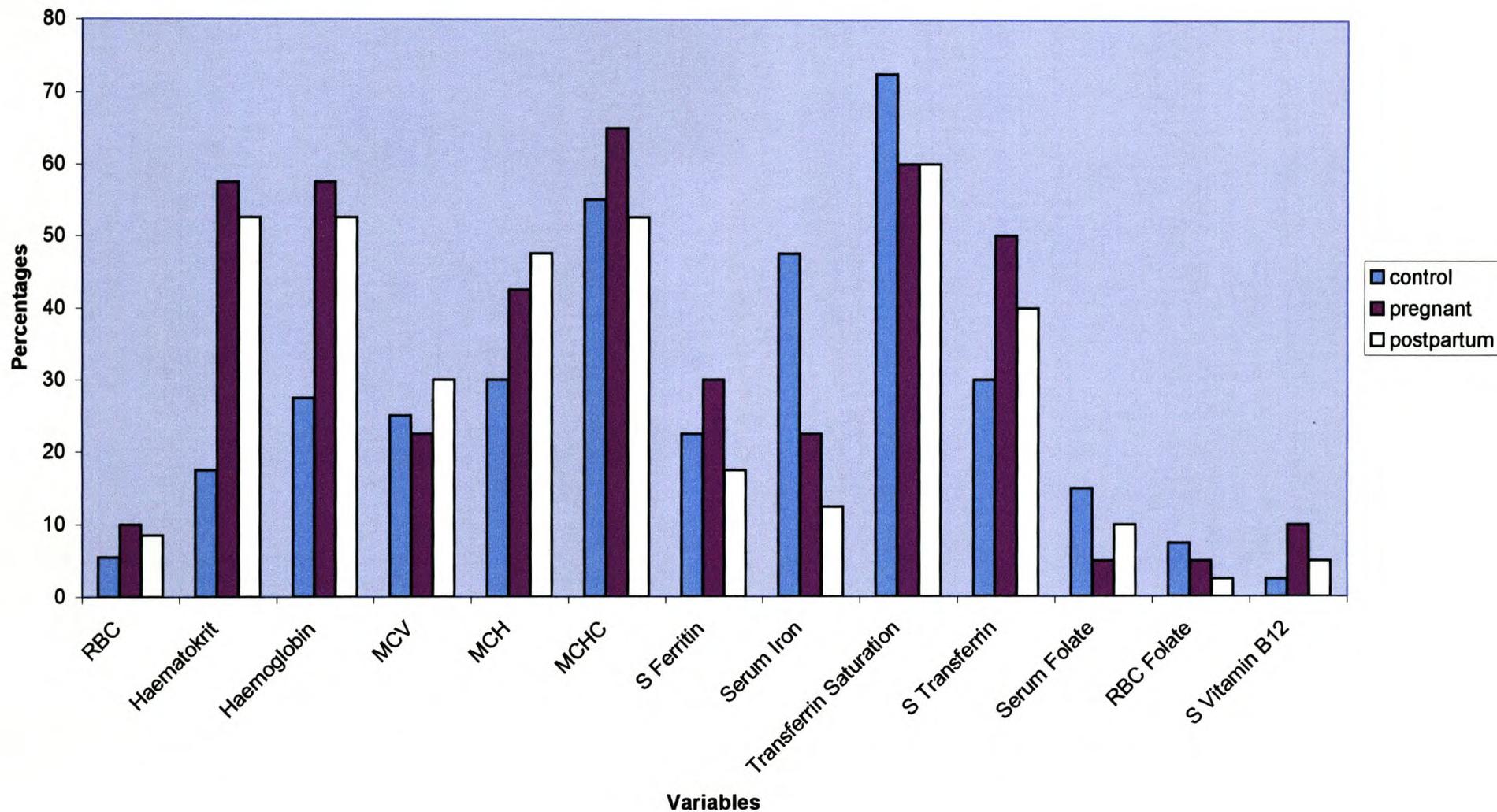
P5 = pairwise t-test, compare pregnant girls with girls at postpartum

\*\*p-value significant, p<0.01

\*p-value significant, p<0.05

The percentages of haematological values of those with ranges below the normal values did not differ a lot between values of pregnant adolescent girls and values at postpartum (Figure 2). Significant differences were observed in serum iron of the control group and pregnant girls. There was a significant difference between the haematocrit of the control group and the pregnant adolescent girls ( $p=0.0002$ ) (Table 18). A significant number of control group had haemoglobin (72.5%) below the cut-off point compared to the pregnant women (42.5%) ( $p=0.0066$ ). About 30% of the pregnant women had serum ferritin below the cut-off point compared to 22.5% of the control group. Table 19 also shows percentages of pregnant girls and the control group with values below the cut-off point

**Figure 2: Haematological data of the subjects with values below the normal ranges**



**Table 18: The haematological values of pregnant adolescent girls and control group below the cut-off point**

Variable	Mean (SD)	Cut-off value	% Below cut-off value	Chi-square p-value
<b>Red blood cell count (<math>\times 10^{12}/L</math>)</b>				P= 1.0000
<b>Control</b>	4.5(0.4)		0.0	
<b>Pregnant</b>	4.0(0.5)	<3.9	0.0	
<b>Haematocrit (%)</b>				P=0.0002**
<b>Control</b>	39.3(4.3)	<36%	17.5	
<b>Pregnant</b>	34.7(4.8)	<33%	57.5	
<b>Haemoglobin (g/dl)</b>				P=0.0066**
<b>Control</b>	12.5(1.7)	<12 g/dl	72.5	
<b>Pregnant</b>	10.9(1.5)	<11 g/dl	42.5	
<b>MCV (fl)</b>				P=0.7928
<b>Control</b>	85.0(13.2)		25.0	
<b>Pregnant</b>	85.5(8.5)	<80 fl	22.5	
<b>MCH (pg)</b>				P=0.4981
<b>Control</b>	29.1(11.2)		30.0	
<b>Pregnant</b>	26.9(3.1)	<27 pg	42.5	
<b>MCHC (g/dl)</b>				P=0.3613
<b>Control</b>	31.7(1.5)		55.0	
<b>Pregnant</b>	31.4(1.4)	<32.5 g/dl	65.0	

\*p-value significant,  $p < 0.05$ \*\*p-value significant,  $p < 0.01$

**Table 19: Iron, ferritin, vitamin B12 (serum) and folate (serum and red blood cells) values of pregnant adolescent girls and control group below the cut-off point**

Variable	Mean (SD)	Cut-off value	% Below cut-off value	Chi-square p-value
<b>Serum ferritin (ng/ml)</b>				P=0.4459
<b>Control</b>	16.1(12.3)	<12 ug/L	22.5	
<b>Pregnant</b>	11.6(8.5)		30.0	
<b>Serum iron (µmol/L)</b>				P=0.0617
<b>Control</b>	9.7 (7.5)	<6.6 µmol/L	47.5	
<b>Pregnant</b>	12.7(6.8)		22.5	
<b>Transferrin saturation (%)</b>				P=0.2371
<b>Control</b>	12.1(9.3)	<16 %	72.5	
<b>Pregnant</b>	13.3(8.0)		60.0	
<b>Serum transferrin (mg/ml)</b>				P=0.0004**
<b>Control</b>	3.7(0.5)	<2.1 g/L	0.0	
<b>Pregnant</b>	4.5(0.8)		0.0	
<b>Serum folate (ng/ml)</b>				P=0.1315
<b>Control</b>	5.5(2.4)	<4.1 nmol/L	15.0	
<b>Pregnant</b>	8.6(7.0)		5.0	
<b>RBC Folate (ng/ml)</b>				P=0.0815
<b>Control</b>	229.6(131.9)	<320 nmol/L	7.5	
<b>Pregnant</b>	367.5(178.6)		2.5	
<b>Serum vitamin B12 (pg/ml)</b>				P=0.0485*
<b>Control</b>	466.4(237.9)	<132 pmol/L	0.0	
<b>Pregnant</b>	230.1(98.2)		10.0	

\*p-value significant, p&lt;0.05

\*\*p-value significant, p&lt;0.01

Red blood cell counts of pregnant adolescent girls supplemented with folic acid 4.4(0.5) were slightly above those of pregnant girls with no therapy 4.2(0.8), iron therapy 3.6(0.6) and iron and folic acid therapy 4.0(0.4) (Table 20). Haemoglobin was slightly different in all groups. Serum iron was lower in a group without therapy 12.9(8.1) than in a group that had iron and folic acid supplementation 13.1(5.5). Serum folate was also very low in a group with no therapy compared to the group that had iron and folic acid supplementation. Red blood cell folate was more elevated in the group that had iron and folic acid therapy than in the group that had no therapy. The mean serum ferritin levels of pregnant girls and at postpartum with no therapy were lower than those who had had iron and folic acid. Differences were also noticed in haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, serum transferrin, transferrin saturation and serum vitamin B12 in supplemented and unsupplemented groups. However, none of the differences were significant.

**Table 20: The mean (SD) haematological data of pregnant girls divided according to micronutrient supplements**

Variables	No micronutrient supplement (N = 19)		Iron and folic acid supplementation (N = 15)	
	Mean	SD	Mean	SD
<b>Red blood cell count</b> ( $\times 10^{12}/L$ )				
Pregnant	4.2	0.8	4.0	0.4
Postpartum	4.1	0.6	4.2	0.5
<b>Haemoglobin (g/dl)</b>				
Pregnant	10.8	1.7	10.9	1.3
Postpartum	11.0	2.5	11.5	1.7
<b>Haematocrit (%)</b>				
Pregnant	34.6	5.8	34.4	3.7
Postpartum	35.1	7.7	36.1	5.3
<b>MCV (fl)</b>				
Pregnant	84.6	9.8	86.6	7.7
Postpartum	83.7	8.5	84.6	8.4
<b>MCH (Pg)</b>				
Pregnant	26.4	3.2	27.5	3.1
Postpartum	26.3	2.9	27.6	3.2
<b>MCHC (g/dl)</b>				
Pregnant	31.2	1.3	31.7	1.4
Postpartum	31.2	1.3	32.4	1.8
<b>Serum ferritin (<math>\mu g/l</math>)</b>				
Pregnant	10.8	9.7	12.5	8.1
Postpartum	14.1	12.1	23.7	20.5
<b>Serum iron (<math>\mu mol/L</math>)</b>				
Pregnant	12.9	8.1	13.1	5.5
Postpartum	15.4	8.6	11.5	5.4
<b>Transferrin saturation (%)</b>				
Pregnant	13.4	9.3	14.3	6.9
Postpartum	16.6	8.1	13.2	7.1
<b>Serum transferrin (mg/ml)</b>				
Pregnant	4.6	0.8	4.2	0.8
Postpartum	4.1	0.9	4.0	0.7
<b>Serum folate (ng/ml)</b>				
Pregnant	5.7	3.1	10.3	7.1
Postpartum	8.7	12.1	16.0	17.0
<b>RBC Folate (ng/ml)</b>				
Pregnant	296.7	157.3	436.5	147.5
Postpartum	293.3	204.1	383.9	126.1
<b>Serum vitamin B12 (pg/ml)</b>				
Pregnant	195.9	77.7	272.6	118.4
Postpartum	250.1	103.0	298.5	160.8

SD = Standard Deviation  
N = number of subjects

The iron status of the subjects was classified as follows:

- Category I (Iron deficiency) – Iron deficiency was classified as subjects with normal haemoglobin, low transferrin saturation and low serum ferritin.
- Category II (Iron deficiency anaemia) – Iron deficiency anaemia was classified as subjects with low haemoglobin, low transferrin saturation and low serum ferritin.
- Category III (Anaemia) – subjects with haemoglobin below the cut-off point were classified as having anaemia.

Anaemia was observed in 27.5% of the control group and 57.5% of the pregnant girls (Table 21). Anaemia was also observed in 52.5% of the subjects at postpartum. Iron deficiency and iron deficiency anaemia were observed in 35% and 22.5% of the control subjects respectively. Iron deficiency in the pregnant adolescent girls was 45% compared to 30% at postpartum. When low haemoglobin, transferrin saturation and serum ferritin were used to diagnose the subjects with iron deficiency anaemia, 30% of the pregnant girls had iron deficiency anaemia, a prevalence that decreased to 20% postpartum.

**Table 21: Iron status of pregnant adolescent girls and the control group (N = 40 for the control group, N = 40 for the pregnant girls)**

<b>Variables</b>	<b>Anaemia (%)</b>	<b>Iron deficiency (%)</b>	<b>Iron deficiency anaemia (%)</b>
<b>Control subjects</b>	11 (27.5%)	14 (35%)	9 (22.5%)
<b>Pregnant subjects</b>			
<b>Pregnant</b>	23 (57.5%)	18 (45%)	12 (30%)
<b>Postpartum</b>	21 (52.5%)	12 (30%)	8 (20%)

### **Infants' Anthropometric Measurements**

The mean birth weight was 2770g (633.5), and the median was 2800.0. Twenty-five percent of the pregnant adolescent girls had infants with low birth weight (<2500g). The mean baby length was 47.8cm (5.1), with a head circumference of 35.5cm (3.4) (Table 22). The head circumference of about 10% of the infants was below the third percentile when compared to their gestational age. The mean gestational age was 38.7 weeks. No birth defects were reported. No children showed signs of neural tube defects. About 60% of the 16 year old had normal deliveries and 40% delivered by caesarean section. A total of 76.7% (>17yrs) had normal delivery and 23.3% (>17 yrs) delivered by caesarean section. The difference in birth weight between babies with mothers under 16 years and babies with mothers 17 years and older was not significant. Pearson's correlation coefficient was used to determine the relationship between the infant's anthropometric measurements and pregnant adolescent dietary intake and anthropometric data. There was no correlation between the mean weight of the baby and the weight of the pregnant girls ( $r=0.1317$ );  $p=0.4178$ ). The body mass index of the pregnant girls did not correlate with the mean birth weight ( $r=0.0770$ ;  $p=0.6368$ ) and the gestational age ( $r=0.0896$ ;  $p=0.5827$ ).

**Table 22: Infants' anthropometrical measurements (n=40)**

<b>Variables</b>	<b>Mean</b>	<b>Standard deviation</b>	<b>CI 95%</b>	<b>Median</b>	<b>5<sup>th</sup> percentile</b>	<b>95<sup>th</sup> percentile</b>
<b>Birth weight (g)</b>	2770.7	633.5	2568.1-2973.3	2800.0	1510.0	3680.0
<b>Baby length (cm)</b>	47.8	5.1	46.1 – 49.4	48.0	39.5	58.5
<b>Head circumference (cm)</b>	35.5	3.4	34.4 – 36.6	48.0	39.5	58.5
<b>Gestational age (weeks)</b>	38.7	1.2	38.3 – 39.1	39.0	37.0	40.5

# **DISCUSSION**

## **Discussion**

Most of the subjects recruited for research were not married and still at school (Table 7). Almost half of the subjects were between grade eight and nine. Since they were still at school, they had neither employment nor any property of their own. It is interesting to note that pregnant adolescent girls seem to be well off because most of them lived in houses and had one family member who earned a salary. This may make this study different from others that found that pregnant teenagers were predominantly from low socio-economic status (12). Both the pregnant adolescent girls and the control group were from the same background. Although every effort was made to match the subjects to controls, there were some statistical differences on marital status, age and type of house they lived in.

Smoking appeared not to be fashionable among these adolescents, because none admitted to smoking (Table 10). Only one pregnant adolescent girl reported to have consumed alcohol. A total abstinence from alcohol consumption by other subjects may be an anomaly. Feelings of distrust or embarrassment towards the interviewer, as well as the stigmatisation associated with alcohol consumption in rural areas among teenage girls, may have resulted in under-reporting. Pica practices in both control and adolescent pregnant was very common. Soil consumption seems to be fashionable in both groups, especially among pregnant adolescent girls. The consequence of pica practices on pregnancy outcome has not been analysed. The effect of pica practices on micronutrients like calcium and iron has been observed in African American women (272). Pica lowers the plasma iron and calcium levels (273). The impact of pica on serum ferritin, mean corpuscular haemoglobin (MCH) and serum folate in pregnant women is of major concern. The control group consumed other substances like chalk and ash. The consumption of these substances have often been associated with iron deficiency.

Only 2.5% of the subjects have had tuberculosis previously (Table 11). There were no other diseases that could have interfered with the outcome of their pregnancy. The cause of some adolescents' previous miscarriages was not known. About 2.5% of pregnant girls had had preterm babies previously. About 22.5% of the control group and 50% of the pregnant adolescent girls showed that they did not want to have children anymore. The reasons why they did not want to have children were not given. Having a child out of wedlock could be the reason why many of them have decided not to have another child.

About 12.5% of the pregnant adolescent girls had a body mass index (BMI) below the cut-off point of 19.8 as recommended by the Institute of Medicine (Table 9) (20). Only one person had a BMI below 18.5 in the control group. Both the pregnant adolescents and the control group were nutritionally compromised. Since only a small proportions (12.5%) of the girls were nutritionally compromised, it may explain the favourable outcome of most pregnancies.

Pre-pregnancy BMI has been used to predict women who are at risk of having low birth weight baby. The mean weight of pregnant adolescents after delivery was 57.8 kg, which indicated that adolescents did not gain enough weight. The mean mid-upper arm circumference of both pregnant adolescents and the control group were within the normal range. The mid-upper arm circumference is almost stable throughout pregnancy even if measured at a late stage of pregnancy (205). A decrease in mid-upper arm circumference reflects either a reduction in muscle mass or a reduction in subcutaneous tissue (24). Because almost all the pregnant adolescents and control group had enough energy from their diets, muscle mass and subcutaneous tissues were not affected.

Pregnant anthropometric measurements such as weight ( $p=0.4178$ ), MUAC ( $p=0.9539$ ) and BMI ( $p=0.6368$ ) did not correlate with infant anthropometric measurements such as birth weight in this study. Twenty percent of babies born to pregnant adolescent girls were of low birth weight (<2500g). The head

circumference is an important screening procedure to detect abnormalities of head and brain growth, especially in the first year of life (275). Children who had low birth weight also had small head circumferences. Dietary intake, especially the intake of nutrients such as iron and folic acid might have influenced the outcome of pregnancy. Iron deficiency in pregnant women has been associated with low birth weight (274). Low birth weight babies have a higher risk of dying in infancy and early childhood. The influence of diet during pregnancy on the newborn could be linked to this. The impact of iron deficiency anaemia on developmental delays and behavioural disturbances has been well documented (276,277). Trace elements in fetal tissue and blood have been found to correlate with birth weight and head circumference, including the essential fatty acids needed for neural tissue growth.

Dietary intake has been associated with the outcome of pregnancy in previous studies. Eating three meals (breakfast, lunch and supper) appeared to be common in all subjects (Table 15). The food frequency questionnaire was used to assess the nutrient intake of the subjects. A main methodological criticism of the food frequency questionnaire is that it contains only a specific number of food items, which limits the responses of the interviewee (278). The quantitative food frequency questionnaire developed for use in this study, which included 118 food and beverage items, and incorporated traditional dishes, was considered to be comprehensive. Pregnant adolescent girls appeared to consume enough energy from the diet when compared to the recommended dietary allowance (Table 12) (279). This was in line with African American adolescent pregnant girls, studied in the USA (22). The main source of energy was maize meal, which was consumed by all the subjects. Other studies showed that increased energy and protein intake resulted in the increase in maternal weight and fetal growth (280).

The main source of protein in the diet of both pregnant adolescent girls and the control group was plant protein. Vegetable protein lacks certain essential amino acids such as phenylalanine, tryptophan, methionine and lysine. If they are not

combined correctly with other vegetable proteins, a deficiency of other essential amino acids may occur. Maize contains large amount of methionine and beans are rich in lysine (281). The animal protein intake was significantly low in both groups. The pregnant adolescent girls and the control group did not commonly use red meat and chicken, because they had to be bought. However, dietary intake of the pregnant girls was sufficient for a favourable pregnancy outcome.

The cholesterol intake of the pregnant adolescents was significantly low because of low intake of animal proteins such as red meat which have a high cholesterol content. Cholesterol is an essential component of the structural membranes of all cells and is a major component of brain and nerve cells (282). Calcium and vitamin D were the nutrients supplied inadequately in the diet, as explained by low intake of milk and other dairy products by the subjects.

The dietary intake of vitamin C was adequate in both groups. Vitamin C has a role in enhancing the absorption of iron (139). Adequate intake of vitamin C among the pregnant adolescents and the control group could be attributed to the fact that the dietary information was collected during summer when foods that are rich in vitamin C are in abundance in the region. About 47.5% of pregnant adolescent girls had intake of vitamin A below 67% of RDA. Vitamin A can be fatal if the fetus is exposed to either too little or too much in its early development (283). The decreased intake of vitamin A in both groups could be attributed to the low intake of foods such as carrots, oranges, sweet potatoes, paw paw, broccoli and foods of animal origin.

The mean total iron intake of pregnant adolescent girls (10.4 mg/day) fell below the RDA (30mg/day), and 55% of the women had low intakes (<67% of the RDA) (Figure 2). This iron intake does not include supplements. Both pregnant adolescents and the control group consumed iron rich foods in small quantities. Low dietary iron intake might be the main reason for iron deficiency. During the second half of pregnancy a woman has to absorb 3.0 mg iron per day to balance

her requirements if her pre-pregnancy iron stores were adequate and 6.0 mg iron per day if they were inadequate (284).

Iron deficiency is an important cause of anaemia in pregnant women. Serum ferritin is considered the most reliable method of assessing iron status during pregnancy (285). During pregnancy, a combination of low haemoglobin and low serum ferritin has been recommended to detect iron-deficiency anaemia because, apart from iron deficiency, other common causes of anaemia during pregnancy such as infection are not characterized by low ferritin concentration (286).

A single indicator alone cannot measure iron status. However, when two or more indices are present there is a much higher probability that the anaemia is, in fact, due to iron deficiency. A high percentage (57.5%) of the pregnant girls had anaemia (Hb <11g/dl) when measurements were taken during pregnancy compared to 27.5% of the control group (Table 21). After delivery, a significant number of the pregnant adolescent girls (52.5%) were anaemic. A high percentage of pregnant girls with haemoglobin of below 11g/dl could be attributed to haemodilution, because haemoglobin concentration declines throughout the first and second trimester and rises again nearer the term (287). However, the haemoglobin concentration did not rise significantly after the birth of the child and that clearly indicates anaemia. The prevalence of anaemia was higher than that reported by other studies in Gazankulu (17%), Soweto (42-50%) Coloureds (25%) in Johannesburg (117,122,288). This study indicated that the prevalence of anaemia in non-pregnant, non-lactating women was half of that of the pregnant adolescent girls in developing countries (289). In a study conducted in the United States comparing black and white women, blacks were found to have significantly lower haemoglobin than whites and a higher prevalence of anaemia regardless of education, income, smoking status or iron status (290).

In this study, a significant number of pregnant adolescent girls (30%) had low serum ferritin during pregnancy (Table 18). After delivery, only 17.5% had low

serum ferritin compared to 22.5% of the control group. This was lower than the prevalence of 68% in pregnant Coloured women in Johannesburg, as reported by Lamparelli et al (120). The presence of infections and inflammations might have contributed to the elevated values of ferritin concentration (161,162).

Hypochromia was more common in the pregnant adolescent girls (MCH: 42.5% and 47.5%) than in the control group (MCH: 30%). Microcytosis was more common in pregnant adolescents after delivery than during pregnancy. Microcytosis was also very common in the control group (25%). Red blood cell microcytosis and hypochromia are late indicators of iron deficiency. These parameters are not sensitive enough to diagnose iron deficiency anaemia during pregnancy. The high level of MCHC (55%) in the control group was of concern. The high percentage of low mean corpuscular haemoglobin concentration of 65% and 52.5% in pregnant adolescent girls during pregnancy and after birth respectively, confirmed the presence of iron deficiency anaemia in this study.

The serum ferritin levels were almost the same in the second and third trimester of pregnant adolescent girls. This could be attributed to plasma volume expansion and haemodilution as these can cause ferritin concentrations to decrease during the second and third trimester (163). The haemodilution may persist during the first three days post-delivery. The second blood specimen was taken during this time and may still reflect the haemoglobin value during pregnancy. The amount of blood loss during delivery will also influence this value.

Serum iron in the control group was lower than in the pregnant adolescent girls. Low serum iron does not diagnose iron deficiency and is not reliable in pregnancy due to the increased plasma volume (130). It could be that many pregnant adolescents had depleted iron stores at the onset of pregnancy. This was supported by the high prevalence of low serum ferritin (22.5%) in the control group.

The high prevalence of low percentage transferrin saturation (60%) is in agreement with other studies (120,122). Transferrin saturation declines in the presence of iron deficiency (160). Transferrin saturation may be normal or lower in the presence of chronic infections or malignancies. The prevalence of low serum ferritin concentration was lower than the prevalence of low transferrin concentrations in this study. This was an unexpected finding, since a decrease in serum ferritin usually precedes the decrease in transferrin saturation in the development of iron deficiency (291). This may be partly due to false positive identification of low iron status by transferrin saturation when using cut-off values for non-pregnant women.

For the purpose of this study iron deficiency was defined as the condition where there is absence of iron stores with or without anaemia. The subjects with iron deficiency were classified as having normal haemoglobin with depleted transferrin saturation and serum ferritin levels. The prevalence of iron deficiency in the pregnant adolescent girls was high (45%) compared to 35% of the control subjects (Table 21). Iron deficiency anaemia (depleted haemoglobin, depleted transferrin saturation and serum ferritin) was found in 30% of the pregnant girls and 22.5% of the control subjects. The prevalence of iron deficiency anaemia at postpartum was 20%. Iron deficiency anaemia during pregnancy may increase perinatal risks for the mothers and neonates; and increases overall infant mortality. This is a point of serious concern especially in the adolescent girls around Siloam Hospital area.

Forty five percent of pregnant adolescent girls reported to have had iron supplementation during pregnancy (Table 11). However, it decreased neither the prevalence of anaemia nor the prevalence of low iron status sufficiently. It could be that many pregnant adolescent girls did not take their iron tablets. The side effects of iron supplementation such as constipation, diarrhoea and nausea might have contributed to the low intake, and this can be improved by counselling about the diet and supplement use. It is also possible that at 3 or 4 months the pregnant

teenagers would not yet have had sufficient supplements to make a difference to their nutritional status. The importance of taking iron and folate supplements was not emphasized at the antenatal clinic. Siloam Hospital did not have a policy on the supplementation of pregnant women. Supplementation was done at the discretion of the nursing sister.

The WHO considers pregnant women as a priority group for iron supplementation and recommends two tablets of ferrous sulphate (each providing 60 mg elemental iron) per day in the second half of pregnancy (292). The effectiveness of iron supplementation during pregnancy has always been a difficult task to accomplish. Routine supplementation seems to be the most favourable method of supplementing pregnant women, especially those in the developing countries (178).

The social profile of the pregnant adolescent girls did not seem to influence their iron and folate status. Most of the subjects came from good families even though they were all unemployed. They did not have their own income or the same claim to household resources as family members. The dietary intake of both pregnant adolescent girls and the control group was inadequate in terms of nutrients such as iron, folate and vitamin B12. This is a point of serious concern especially during pregnancy.

The mean dietary intake of folate (215.5  $\mu\text{g}/\text{day}$ ) for pregnant adolescent girls was below the cut-off point of 67% RDA (400 $\mu\text{g}/\text{day}$ ) (Table 14). Folate needs increase during pregnancy in response to the demands of maternal erythropoiesis and fetal placental growth (293). The primary causes of folic acid deficiency in pregnant adolescent girls could be attributed to inadequate food rich in folate such as organ meats and the lack of fresh, slightly cooked foods. Cooking methods used in food preparation are more significant, because exposure to heat, air, ultraviolet light and over-boiling may contribute to folate loss (294).

The prevalence of biochemically diagnosed folate deficiency in the pregnant adolescent girls (2.5%) in the present study was lower than that of the control group (7.5%) and much lower than that of the non-pregnant women in Johannesburg (21%) (295). About 20% of the pregnant adolescent girls had a low dietary intake of folate (<67% RDA). Forty five percent of the pregnant adolescent girls had folate supplementation during pregnancy.

When the contribution of folate supplementation was taken into account, only 22.5% had low folate intakes (<67% RDA). The low prevalence of low red blood cell folate concentration in the pregnant adolescent girls is partly due to the folate supplementation. The pregnant adolescent girls had a high prevalence of vitamin B12 deficiency. Vitamin B12 is associated with macrocytic anaemia. The main source of protein from the diet of pregnant adolescent was mostly of plant origin, and vitamin B12 is present only in foods of animal origin. The deficiency is associated with the low intake of animal foods by both the pregnant adolescents and the control group.

# **CONCLUSIONS AND RECOMMENDATIONS**

## Conclusions and Recommendations

Concurrent pregnancy and growth has been found to have a detrimental effect on the iron and folate status of adolescent girls. Dietary studies in adolescents have shown serious shortfalls in intakes. The findings in this study showed that pregnant adolescent girls appeared to be similar to the control group socio-economically, anthropometric and nutritionally. The difference in micronutrient intakes between the two groups did not differ much. The high prevalence of iron deficiency in the control group of childbearing age suggests that periodic screening for anaemia is indicated among adolescent girls and pregnant adolescents at the first antenatal visit. Most pregnant adolescent girls in this study came to the clinic only in the second and third trimesters. None of the girls came in the first trimester.

Educational programmes, as part of lifeskills programmes targeted at adolescents and teenagers should reach girls that could potentially fall pregnant, especially in the Siloam area. An educational programme must include:

- Reproductive needs (avoidance of sex or safe sex);
- Nutritional needs, especially targeted at improving nutritional status to meet future reproductive needs;
- Early booking, if pregnant;
- Effective supplementation during pregnancy.

Nutrition education at schools would help improve food choices of all young adolescent girls before they become pregnant, whether they become pregnant as teenagers or later in life. During their visit to the antenatal clinic, pregnant adolescent girls should receive nutrition education on how to increase their iron intake and, especially on iron absorption early in pregnancy. To increase dietary iron intake more iron-rich foods like organ meat (especially liver), meat, poultry, legumes, green leafy vegetables and nuts should be included. If possible, the intake of the highly absorbable haem iron should be increased by increasing the

intake of meat, poultry or fish. The absorption of non-haem iron can be increased several times when it is ingested simultaneously with vitamin C-rich foods and/or meat, fish and poultry.

Other substances such as phytates, tannins from tea, which are known to decrease the absorption of non-haem iron, should be avoided during and after meals. The greatly elevated iron requirements of pregnant adolescent girls indicate the need for increasing pre-pregnancy reserves during adolescence and the provision of iron supplements during pregnancy. The impact of iron supplementation on pregnancy outcome is still debatable as is the question of when supplementation should begin. Many researchers have recommended that iron and folate should be routinely supplemented to all pregnant women in sub-economic communities. Many studies have showed that iron supplementation during pregnancy reduces the prevalence of iron deficiency anaemia.

Proper nutrition during pregnancy influences the growth and development of the fetus and the well-being of the pregnant woman. Inadequate dietary intake by adolescent girls should be a point of serious concern to policy makers. The sample size of the study was too small to can draw a conclusion about the nutritional status of all South African pregnant adolescent girls. We recommend that all pregnant adolescent girls should be supplemented with iron and folate during pregnancy when iron requirements are increased. There is a need to strengthen nutrition education in schools and all health care facilities and develop nutrition messages that target adolescents. Health education at schools should also focus on the importance of nutrition in adolescent girls.

Nutrition education messages must be tailored to the currently prevailing consumption patterns and desired changes therein, including the improvement of the nutrient density of adolescents' diets as well as good eating habits. When appropriate, home grown crops and the use of foods of animal origin from domestic animal production should be encouraged. Information on the importance

of iron to the pregnant woman and her unborn child, on how to take the iron supplements and how to handle the side effects will improve their willingness to take the supplements and their effectiveness. At present there are no specific nutrition intervention strategies targeted at adolescents.

The need to improve the dietary and nutrient intake of adolescent girls should be addressed within the current framework of the Integrated Nutrition Programme (INP). Food fortification with essential micronutrients such as iron, folic acid, vitamin A and zinc to improve micronutrient status of adolescents should be implemented. Studies of this nature should be encouraged to cover the whole population for the purpose of establishing baseline data for adolescent nutrition.

## **LIMITATIONS OF THE STUDY**

## **Limitations of the study**

The study sample was too small to draw any conclusion about the nutritional status of the adolescent pregnant girls. The age group below 17 years was too small to allow meaningful statistics. The study did not look at the pattern of weight gain and its impact on pregnancy outcome. The timing of baseline measurements and the administration of the quantitative food frequency questionnaire might have influenced the results. Dietary intake data was collected during the summer months when food was in abundance. Taking the haematological and biochemical parameters at 24-72 hours postnatal is also a limitation to this study, because it is an unstable period for most of the parameters measured. Although every effort was made to match subjects and controls as closely as possible, there were differences in the age, educational and socio-economic status between the two groups. The study group reflects the nutritional status of the pregnant teenagers served by the antenatal clinic of Siloam Hospital. However, the sample size was too small to relate nutritional status to pregnancy outcome.

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# **ANNEXURES**

# **DEMOGRAPHIC AND DIETARY HISTORY QUESTIONNAIRE**

## DEMOGRAPHIC AND DIETARY HISTORY QUESTIONNAIRE

Interviewer: \_\_\_\_\_ Date of interview:  
\_\_\_\_\_

Subject CODE: \_\_\_\_\_

### DEMOGRAPHIC INFORMATION

NAME: \_\_\_\_\_

AGE (IN YEARS): \_\_\_\_\_

#### Ethnic origin:

Venda	1
Northern Sotho	2
Tsonga	3
Other (specify)	4

#### What is your marital status?

Married	1
Single	2
Divorced	3
Widow	4

#### Educational status

Tertiary	1
Grade 12	2
Grade 10 – 11	3
Grade 8 – 9	4
Grade 4 – 7	5
No formal education	6

#### Are you employed?

Yes	1
No	2

**The type of employment**

Part time	1
Full time	2
Self-employed	3
Other (specify)	4

**What type of a house do you live in?**

Hut	1
House	2
Shack	3
Double storey	4
Other (specify)	5

**Number of rooms available**

--	--

**Number of people sleeping in the house**

--	--

**Mother's family structure**

Stay with both parents	1
Stay with one parent	2
Stay with relatives	3
Stay with non-relatives	4
Other (specify)	5

**Are you the owner of the house?**

Yes	1
No	2

**Is the house you are living in?**

Rented	1
Hired	2
Built by myself	3

**How many people contribute to the income?**

--	--

**Which of the following is the source of income in the family?**

Pension	1
Disability grant	2
Salary	3
Other (Specify)	4

**What is the total monthly income at home?**

R0 – R100	1
R101 – R300	2
R301 – R500	3
R501 – R1000	4
R1001 – R2000	5
R2000 and more	6

**SOCIAL HABITS****Do you smoke cigarettes?**

Yes	1
No	2

**If Yes, how many cigarettes do you smoke per day?**

--	--

**Do you smoke dagga or other drugs?**

Yes	1
No	2

Specify: \_\_\_\_\_

**How often do you smoke cigarette, dagga or other drugs?**

Everyday	1
Once a week	2
On and off	3
Sometime on weekends	4

**Do you drink alcohol (e.g. spirits, beer, wine)?**

Yes	1
No	2

**Do you prefer to drink?**

Beer	1
Wine	2
Whisky/Vodka	3
Other (specify)	4

**How often do you drink beer or wine or strong drinks?**

Everyday	1
Once a week	2
On and off	3
Sometime on weekends	4

**How much alcohol do you drink per day?**

Tots of spirit Specify:	1
Number of beers per day Specify:	2

**Do you use traditional (herbal) medicine?**

Yes	1
No	2

If Yes, specify: \_\_\_\_\_

**Do you take substances like soil, chalk and ash (pica)?**

Yes	1
No	2

If Yes, specify: \_\_\_\_\_

**OBSTETRIC AND MEDICAL HISTORY****Do you suffer from any of the following diseases?**

Tuberculosis	1
Diabetes mellitus	2
Hypertension	3
Heart diseases	4
Other (specify)	5

**Have you ever had any operation?**

Yes	1
No	2

If Yes, specify: \_\_\_\_\_

**Do you use other medication or tablets at home?**

Yes	1
No	2

If Yes, specify: \_\_\_\_\_

**Previous pregnancy outcome**

Miscarriage	1
Stillborn	2
Preterm birth	3
Abnormal child	4
Low birth weight	5
Neural tube defects	6
Healthy/normal	7

**Number of gravidity**

Zero	1
One	2
Two	3
Three	4

**Number of children at home**

--	--

**Are you planning to have more children in the future?**

No	2
No	2

If Yes, how many: \_\_\_\_\_

**FOOD PREPARATION AND DIETARY HISTORY****What do you use to prepare food at home?**

Electric stove	1
Hot plate	2
Paraffin stove	3
Gas stove	4
Fire wood	5
Coal stove	6
Other (specify)	7

**How often do you prepare meals at home?**

Two or more per day	1
Once per day	2
Three to five per week	3
Once per week	4

**Where are most of your meals prepared?**

Home	1
Restaurant	2
Wimpy	3
Cafes	4
Fast food shop	5
Road/steak houses	6

**How often do you eat at restaurants, steak houses, cafes, take away?**

Daily	1
Weekly	2
Monthly	3
Less than once a month	4
Never	5

**Who prepares food at home?**

Yourself	1
Mother	2
Sister	3
Caretaker	4
Other (specify)	5

**How many meals do you usually eat per day?**

Five	1
Four	2
Three	3
Two	4
One	5

**At what times do you usually, eat your meals?**

Breakfast	1
Snack	2
Lunch	3
Supper	4
Snack	5

**Do you usually eat these meals on most weekdays?**

Yes	1
No	2

**Who buys food at home?**

Mother	1
Father	2
Yourself	3
Brother	4
Sister	5
Other (specify)	6

**Where do you buy your food?**

Supermarket	1
Café	2
Spazashop	3
Hawkers	4
Other (specify)	5

**How long does it take to get to the place where you buy your food?**

Less than 15 minutes	1
15 – 30 minutes	2
30 – 60 minutes	3
More than an hour	4

**How often do you buy food at home?**

Everyday	1
3 – 5 times a week	2
Less than 2 times a week	3
Fortnight	4
Once a month	5

**Do you have a vegetable garden at home?**

Yes	1
No	2

**Do you have a refrigerator at home?**

Yes	1
No	2

**How is your appetite?**

Good	1
Fair	2
Poor	3

**Do you have any problem with swallowing or chewing of food?**

Yes	1
No	2

**Bowel habits**

Normal	1
Constipated	2
Diarrhoea	3

**Do you have any food intolerance or allergies?**

Yes	1
No	2
If Yes, specify	3

**Have you ever attended any educational talk about nutrition?**

Yes	1
No	2

**If Yes, through which type of source?**

Dietician	1
Clinic	2
Care group worker	3
Radio/TV	4
Nutrition adviser	5
Other (specify)	6

# **QUANTITATIVE FOOD FREQUENCY QUESTIONNAIRE**

**NUTRITIONAL STATUS OF PREGNANT WOMEN (<20 YEARS) WITH SPECIAL EMPHASIS ON IRON AND FOLIC ACID**

**CODE:**

**INTERVIEW DATE:**

**QUANTITATIVE FOOD FREQUENCY QUESTIONNAIRE**

Thank you for accepting the invitation to participate in this study. We want to find out what adolescent girls eat in this area. This information is important to know as it will tell us if people are eating enough and if they are healthy.

Please think carefully about the food and drink you have consumed during the past month. I will go through a list of foods and drinks with you and I would like you to tell me:

- if you eat the food
- how the food is prepared
- how much of the food you eat at a time
- how many times a day you eat it and if you do not eat it every day, how many times a week or a month you eat it.

To help you describe the amount of a food you eat, I will show you the utensils of different sizes and food items. Please measure the amount which is the closest to the amount you eat.

Please remember that there are no right or wrong answers. Everything you tell me is confidential.

Is there anything you want to ask now?

Are you willing to go on with the questions?

**INSTRUCTION:** Circle the subject's answer. Fill in the amount and times eaten in the column. I shall now ask you about the amount of food you have been eating in the last few months.

**CODE**

Do you eat maize meal porridge? (enriched)	YES	1					
Nothing added	NO	2					
If YES, what type do you have at home now?							
Brand name:							
Do not know							
Grind self:							
	YES	1					
	NO	2					
If brand name given, do you usually use this brand?							
	YES	1					
	NO	2					
	Don't know	3					
Where do you get your maize meal? (may answer more than one)							
Shop							
Employer							
Harvest and grind self							
Other: specify							
Do not know							
FOOD	DESCRIPTION	Amount	TIMES EATEN			AMOUNT PER DAY	CODE
			Per Day	per week	per month	seldom/ never	
Maize-meal porridge	Stiff (pap)						3400
Maize-meal porridge	Soft						3399
Do you pour milk on your soft porridge?			YES 1 NO 2				
If YES, what type of milk (whole fresh, sour, 2%, fat free, milk blend,) _____							
<b>INSTRUCTION: Show subject examples.</b>							





FOOD	DESCRIPTION	Amount	TIMES EATEN			AMOUNT PER DAY	CODE
			Per Day	per week	per month		
Beef	With fat						2945
	With fat trimmed						2945
	Fried without bone						2941
	Stewed with bone						2909
	Stewed without bone						2909
	Grilled with bone						2908
	Grilled without bone						2908
	Minced						A012
Mutton	Fried with bone						2994
	Fried without bone						2974
	Stewed with bone						3039
	Stewed without bone						2039
	Grilled with bone						3038
	Grilled without bone						3038
	Minced						A013
Pork	Fried with bone						2930
	Fried without bone						2930
	Stewed with bone						A014
	Stewed without bone						A014
	Grilled with bone						2992
	Grilled without bone						2992
	Minced						A015
	Vegetable in stew:						A016
Beef offal's	Intestines: boiled						A017
	Stewed with vegetables						
	Tripe						A018
	Heart						2968
	Lungs						3019
	Liver						2920
	Kidneys						2923
	Other specify:						
Wors/ sausage	Fried						3053
	Grilled						
	Stewed:						
Bacon						2906	
Cold meats	Polony						2919
	Ham						1564
	Viennas						1531
	Other: specify						
Canned meat	Bully beef						2940
	Other:						
Meat pie	Home made						2939
	Bought						
Hamburger	Home made						A019
	Bought						

FOOD	DESCRIPTION	Amount	TIMES EATEN				AMOUNT PER DAY	CODE
			Per Day	per week	per month	seldom/ never		
Dried beans/peas/lentils	How do you prepare them?							3205
Soya products	Brand at home now Don't know:							3188
Canned fish	Specify brand name:							3102
	How do you prepare it?							
Fried fish	With batter/crumbs							3072
	Without batter/crumbs							3060
Eggs	Boiled							2867
	Fried							2869
	Poached							2869
	Scrambled							2869
Other: specify								
Where do you get your vegetables from? (may answer more than one)								
							Own vegetable garden	1
							Employer's farm	2
							Own farm	3
							Shops/supermarket	4
							Hawkers	5
							Veld	6
							Gift	7
							Other (specify)	8
Cabbage	How do you cook cabbage?							
	Boiled, nothing added							3756
	Boiled with potato, onion and fat							3815
	Fried, nothing added							3812
	Fried with onion and tomato							A020
	Boiled with tomato and onion							3810
	Other: Don't know							
Spinach	How do you cook it?							
	Boiled, nothing added							3761
	Boiled fat added							3899
	Boiled with onion and tomato							3786
	Fried with onion and tomato							2786
	With potato added							A021
	Other:							

FOOD	DESCRIPTION	Amount	TIMES EATEN			AMOUNT PER DAY	CODE
			Per Day	per week	per month		
Morogo/ other green leafy	How do you cook it?						
	Boiled, nothing added						3980
	Boiled, fat added						
	Boiled with tomato and onion added						
	With potato added						A021
	- with peanuts						A022
	Other:						
	Don't know						
Tomato and onion 'gravy'	Home made-with fat						3910
	Home made-without fat						
	Canned						8221
Pumpkin	How do you cook it?						
	Cooked in fat and sugar						3893
	Boiled, little sugar and fat						3893
	Boiled, nothing added						4164
	Other:						
	Don't know						
Carrots	How do you cook it?						
	Boiled, sugar and fat						3819
	With potato and onion						3757
	Raw, salad						3709
	Raw with cabbage						A023
Mealies	How do you eat it?						3724
	On cob-with fat Without fat						
	Off cob – with fat Without fat						3724
Beetroot salad	Home made						3699
	Bought						
	Mixed with onion						A024
Potatoes	How do you cook it?						
	Boiled/baked with skin						4155
	Without skin						3737
	Mashed						3876
	Mixed with tomato and onion						A024
	French fries						3740
	Potato salad						3928
	Other:						
Sweet potato	How do you cook it?						
	Boiled/baked with skin						3748
	Without skin						3903
	Mashed						3748
Other:							

FOOD	DESCRIPTION	Amount	TIMES EATEN			seldom/ never	AMOUNT PER DAY	CODE																							
			Per Day	per week	per month																										
Vegetable salads	Raw tomato							3921																							
	lettuce							3927																							
	Cucumber							3921																							
Other vegetables Specify:																															
<b>FRUITS</b> Where do you get your fruits from? (may answer more than one) <table style="width: 100%; border: none;"> <tr> <td style="width: 70%;"></td> <td style="width: 20%;">Own fruit trees</td> <td style="width: 10%; text-align: center;">1</td> </tr> <tr> <td></td> <td>Employer's farm</td> <td style="text-align: center;">2</td> </tr> <tr> <td></td> <td>Own farm</td> <td style="text-align: center;">3</td> </tr> <tr> <td></td> <td>Shops/supermarket</td> <td style="text-align: center;">4</td> </tr> <tr> <td></td> <td>Hawkers</td> <td style="text-align: center;">5</td> </tr> <tr> <td></td> <td>Veld</td> <td style="text-align: center;">6</td> </tr> <tr> <td></td> <td>Gift</td> <td style="text-align: center;">7</td> </tr> <tr> <td></td> <td>Other (specify)</td> <td style="text-align: center;">8</td> </tr> </table>									Own fruit trees	1		Employer's farm	2		Own farm	3		Shops/supermarket	4		Hawkers	5		Veld	6		Gift	7		Other (specify)	8
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	Veld	6																													
	Gift	7																													
	Other (specify)	8																													
Apples	Fresh Canned							3532																							
Pears	Fresh Canned							3542																							
Bananas								3540																							
Oranges								3560																							
Grapes								3550																							
Peaches	Fresh Canned							3565																							
Apricots								3556																							
Mangoes								3551																							
Guavas	Fresh Canned							3563																							
Pawpaw																															
Wild fruit/berries	Specify:							7070																							
Dried fruit	Types:																														
Other fruit:	Specify:																														
<b>BREAD AND BREAD SPREADS</b>																															
Bread	White							3210																							
	Brown							3211																							
	Whole wheat							3212																							
Do you spread anything on the bread? If YES, what do you spread?			Always 1 sometimes 2 never 3																												

FOOD	DESCRIPTION	Amount	TIMES EATEN			AMOUNT PER DAY	CODE
			Per Day	per week	per month		
Margarine	What brand do you have at home now? <hr/> Don't know: _____						3484
Butter	What brand do you have at home now? <hr/> Don't know						3479
Peanut butter							3485
Jam							3985
Honey							
Cheese	Type:						
Achaar							3117
Avocado							3656
Polony							
Other spreads: specify							
Dumpling							A027
Vetkoek							3257
Mayonnaise							3488
Provita/ Crackers							
<b>FATS:</b>							
What fats do you use and where do you use them?							
Margarine	Where used? Number of spoons:						3521
Oil	Where used: Number of spoons						3507
Holsum	Where used: Number of spoons:						A028
<b>DRINKS:</b>							
Tea							4038
Milk/cup tea	What type of milk do you use in tea? Specify:						
Sugar/cup tea							
Coffee							4037
Sugar/cup coffee							
Milk/cup coffee	What type of milk do you use in coffee? Specify:						



FOOD	DESCRIPTION	Amount	TIMES EATEN			AMOUNT PER DAY	CODE
			Per Day	per week	per month		
Jelly							3983
Custard	Homemade Commercial						2716
Ice cream							3519
Raisins							3354
Pudding	Specify:						
Other Specify:							
<b>SAUCES, GRAVIES, CONDIMENTS</b>							
Tomato sauce							3139
Packet soups	Minestrone Royco Vegetables Other:						3154 3159 3162
<b>INSECTS, WILD BIRDS AND ANIMALS (hunted in bushes and farms)</b>							
Locusts							
Mopani worms							
Wild birds							
Wild animals							
Others:							
<b>MISCELLANOUS: Please mention any other foods used more than once/two per week which we have not talked about:</b>							

**Do you use any of the following?**

	Name of product	Amount/day
Vitamins		
Vitamins and minerals		
Tonics		
Health foods		
Body building preparations		
Dietary fibre supplement		
Aloe Vera		
Enteral feeds		
Other:		

# **CONSENT FORM**

**PARTICIPATION IN RESEARCH PROJECT**

**NUTRITIONAL STATUS OF PREGNANT WOMEN (BELOW 20 YEARS OF AGE) WITH SPECIAL EMPHASIS ON IRON AND FOLIC ACID STATUS**

**DECLARATION BY PATIENT**

I, the undersigned: \_\_\_\_\_

ID NO: \_\_\_\_\_

Of \_\_\_\_\_ the \_\_\_\_\_ following \_\_\_\_\_ address:

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

**Confirm that:**

1. I was invited to take part in a research project in the antenatal clinic (Siloam Hospital). The project will be under the guidance of the Department of Human Nutrition of the University of Stellenbosch.

**2. It was explained to me that:**

2.1 The main aim of the study is to assess the nutritional status of pregnant women and the outcome of pregnancy. The main reason for this study is to find out if there is a relationship between the dietary intake (especially folic acid and iron) of the pregnant women and the health of the newborn.

**2.2 The procedures will as follows:**

My body weight will be measured by means of the electronic scale on the first visit to the clinic. Body height and mid-upper arm circumference will be measured. The entire procedure will take approximately 10 minutes. A small amount of 10ml of blood (approximately 2 teaspoons) will be drawn twice

from me, i.e. on the first visit and on admission for delivery. The following parameters will be analyzed from the blood, i.e. serum iron concentration, serum folate, serum transferrin, serum ferritin, red cell folate, transferrin saturation, haemoglobin, MCV, MCH, haematocrit and vitamin B12. I shall be required to complete questionnaires regarding general information and dietary intake.

3. The confidentiality of the project was explained to me, namely that all information will be regarded as strictly confidential and accessible to members of the research team only.
4. Although the results may be published in scientific journals or presented at congresses, my name will not be made known at any stage.
5. It was explained to me that participation is voluntary and that I may withdraw at any stage. My withdrawing from the study will not in anyway affect the quality of treatment or my newborn baby will receive.
6. It was explained to me that all measurements will be done during my routine visits to the hospital, therefore no additional visits or expenses will be expected from me.
7. I was given the opportunity to ask questions, and all my questions were answered to my satisfaction.
8. I hereby accept participation in the project on a voluntary basis.

**Signed at:** \_\_\_\_\_ **on** \_\_\_\_\_ **19** \_\_\_\_\_

**Patient:** \_\_\_\_\_

**Witness:** \_\_\_\_\_

**Researcher:** \_\_\_\_\_

**Signature:** \_\_\_\_\_ **Date:** \_\_\_\_\_

# **BIOCHEMICAL AND HAEMATOLOGICAL DATA QUESTIONNAIRE**

## BIOCHEMICAL AND HAEMATOLOGICAL DATA OF PREGNANT AND NON-PREGNANT ADOLESCENT GIRLS

N.B. Blood will be collected twice for pregnant girls (i.e. on the first visit and 24 to 72 hours postpartum)

PARAMETERS	FIRST VISIT	SECOND VISIT
Red blood cells (		
Haemoglobin (g/dl)		
Haematocrit (%)		
Mean corpuscular volume (fl)		
Mean corpuscular haemoglobin (g/dl)		
Serum iron (umol/L)		
Serum folate (ng/ml)		
Red blood cell folate (ng/ml)		
Serum transferrin (mg/ml)		
Serum ferritin (ng/ml)		
Transferrin saturation (%)		
Transferrin saturation (%)		
Serum vitamin B12 (pg/ml)		

# **INFANTS ANTHROPOMETRIC MEASUREMENTS**

**INFANTS' ANTHROPOMETRIC MEASUREMENTS****CODE:****MOTHER'S CODE**

<b>Measurements</b>	<b>After birth</b>
Weight (g)	
Length (cm)	
Head circumference (cm)	

1. Gestational age: \_\_\_\_\_

2. Presence of neural tube defects (specify)

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3. Other abnormalities (specify)

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**4. Type of birth (e.g. normal or caesarean)**

Normal	1
Caesarean	2

**5. Birth weight classification**

	<b>Cut-off points</b>	<b>Codes</b>
Low birth weight		
Very low birth weight		
Normal		
Overweight		

**ANTHROPOMETRIC MEASUREMENTS (PREGNANT AND CONTROL GROUP)**

NAME: \_\_\_\_\_ CODE: \_\_\_\_\_

<b>Measurements</b>	<b>First visit</b>	<b>Second visit</b>
Weight (kg)		
Height (m)		
mid-upper arm circumference (cm)		
Body mass index (BMI)		