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I, Shwetal V Ahire **,**

hereby submit this original work as part of the requirements for the degree of:

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Maternal adiposity and plasma concentrations of leptin and adiponectin.

Student Signature: Shwetal Ahire

This work and its defense approved by:

Committee Chair: Dr Debra Krummel, PhD, RD

Dr Seung-Yeon Lee,PhD

Approval of the electronic document:

I have reviewed the Thesis/Dissertation in its final electronic format and certify that it is an accurate copy of the document reviewed and approved by the committee.

Committee Chair signature: Dr Debra Krummel

Maternal adiposity and the plasma concentration of leptin and adiponectin.

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by

Shwetal V Ahire, MBBS

B J Medical College

Pune University, India

Committee Chair: **Debra Krummel, Ph.D., R.D.**

Abstract

Maternal adiposity and leptin and adiponectin levels

Objective: The purpose of this project was to determine the relationship of maternal adiposity to the adipokines, adiponectin and leptin.

Design: Prospective cohort study.

Subjects: Healthy, non-smoking pregnant women were recruited from the University Hospital's Obstetric Clinics (Cincinnati, Ohio) when they presented for their first prenatal visit in gestational week 8-16.

Main Outcome measure: Anthropometric parameters**,** plasma leptin and adiponectin

Methods: Healthy non-smoking pregnant women enrolled. Anthropometric parameters such as height, weight, mid-arm circumference, wrist circumference, skinfold thickness (biceps and thigh) were measured during early prenatal visit (8 to 16 weeks of gestation). Pre-pregnancy BMI (Body Mass Index) was obtained by self report. From the venous blood draw plasma leptin and adiponectin levels were analyzed (ELISA method). Bivariate correlation analysis was performed by using SPSS (Vs 14.0, Chicago, IL).

Results: A strong positive association between pre pregnancy BMI and plasma leptin levels and a strong negative association between pre-pregnancy BMI and plasma adiponectin levels were found. Mid-arm circumference had a positive association with plasma leptin and a negative association with plasma adiponectin. Wrist circumference, biceps skinfold and thigh skinfold had a negative association with plasma adiponectin

concentrations. Pre pregnancy BMI was significantly related to the anthropometric parameters measured in early pregnancy.

Conclusion: Adiposity prior to pregnancy and during early pregnancy period was associated with higher levels of leptin and lower levels of adiponectin in early to mid pregnancy.

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Introduction

Overweight and obesity are a global health problem, which has been increasing in prevalence in many countries over the last 40 years. Over the past three decades, the prevalence of obesity in the United States has more than doubled. Using Body Mass Index (BMI) as an indicator of adiposity, two-thirds of all Americans are classified as overweight (BMI of 25-29.9) or obese (BMI≥ 30). [1, 2] Prevalence of obesity is more in females than males. More and more women of childbearing age are being overweight or obese.[3-5] Consequently, most women enter pregnancy with excess body weight.

It is important to study the effects of adiposity during pregnancy. Since women with excess body weight, have increased risks, obese women have an increased incidence of pregnancy complications such as gestational diabetes and pre-eclampsia. [6, 7] The fetus of an obese mother has a greater chance of being born as still birth or having birth defects. Fetal overgrowth, defined as a birth weight greater then 90th percentile, is common in pregnancies of women with increased BMI, and results in the birth of a large-for-gestational age (LGA) infant.[8-12] LGA infants are more likely to have traumatic birth injuries due to shoulder dystocia and cause tearing of the birth canal.[13] Also babies born to obese women are more prone to develop obesity, diabetes, and hypertension in childhood and adulthood. [11]

Complications like gestational diabetes, pre-eclampsia, and fetal overgrowth are also related to changes in levels of the cytokines, such as adiponectin and leptin.[14-17] Leptin and adiponectin are adipose tissue derived hormones which are also expressed by the placenta in pregnancy. Leptin plays a key role in regulating energy intake and energy expenditure, including appetite and metabolism. Adiponectin modulates a number of metabolic processes, including glucose regulation and fatty acid catabolism. Earlier studies have found the relationship between higher level of leptin, lower levels of adiponectin and the complications like pre-eclampsia, gestational diabetes, preterm labor, and spontaneous abortion etc. [17-20]

Background

Obesity has joined malnutrition and infectious disease as one of the primary contributor to ill health in modern populations.[1, 2] World Health Organization categorizes obesity as a pandemic issue as it has higher incidence in developing as well as developed countries.

Over the past few decades, there has been a continual increase in the prevalence of obesity in the United States. Obesity-related healthcare costs have soared in the US and have now exceeded $100 billion annually. Obesity has higher prevalence in females than in males. In 2004, the prevalence of overweight and of obesity in American women of reproductive age was 52% and 29%, respectively. If these trends continue by 2030, 86.3% adults will be overweight or obese; and 51.1% obese. Amongst all females, black women (96.9%) would be the most affected.[2122] By 2048, all American adults would become overweight or obese, while black women will reach that state by 2034. Total health-care costs attributable to obesity/overweight would double every decade to 860.7-956.9 billion US dollars by 2030, accounting for 16-18% of total US health-care costs.[1, 2]

Obesity is a cost intensive disease because it is associated with numerous other health problems and diseases such as hypertension, type 2 diabetes mellitus, cardiovascular disease, and some cancers.[2] With the pandemic, we are moving away from the Healthy People 2010 objectives. Recent data from National Center for health

statistics indicates that there is no significant change in the prevalence of obesity since 2003-2004.[23] Timely, dramatic, and effective development and implementation of corrective programs/policies are needed to avoid the otherwise inevitable health and societal consequences if these projections are realized.

Obesity and Pregnancy

Obesity during pregnancy is considered a high-risk state because it is associated with many complications.[8, 24] Compared with normal-weight women, women who are obese have a higher prevalence of infertility. Once they conceive, they have higher rate of early miscarriage and congenital anomalies, including neural tube defects.[3, 25, 26] Besides the coexistence of pre-existing diabetes mellitus and chronic hypertension, obese women are more likely to have pregnancy-induced hypertension, gestational diabetes, thromboembolism, macrosomia, and spontaneous intrauterine demises in the latter half of pregnancy. Obesity in pregnancy is related to higher incidence of pre-eclampsia as well as placental abruption. Becker et al. showed a strong association between maternal weight in early pregnancy and higher risk of pre-eclampsia with odds ratio for pre-eclampsia as 4.1 in Canadian women.[6] Obesity in pregnancy is related to complications like thromboembolism. A prospective case-control study of antenatal pulmonary embolism undertaken through the UK Obstetric Surveillance System between February 2005 and August 2006 showed 94 cases, including several deaths. The adjusted odds ratio for obesity was 2.8 (95% confidence interval 1.12 to 7.02).[27]

Obese women also require instrument or Cesarean section delivery more often than average-weight women. A meta-analysis done by Chu et al. of 33 cohort studies showed unadjusted odds ratio of caesarian delivery of 1.46 (with 95% CI 1.34 to 1.60).[13] This provides quantitative estimation of requirement of caesarian section in women with higher BMI. Following Cesarean section delivery, obese women have a higher incidence of wound infection and disruption. Irrespective of the delivery mode, children born to obese mothers have a higher incidence of macrosomia and associated shoulder dystocia. [28, 29]

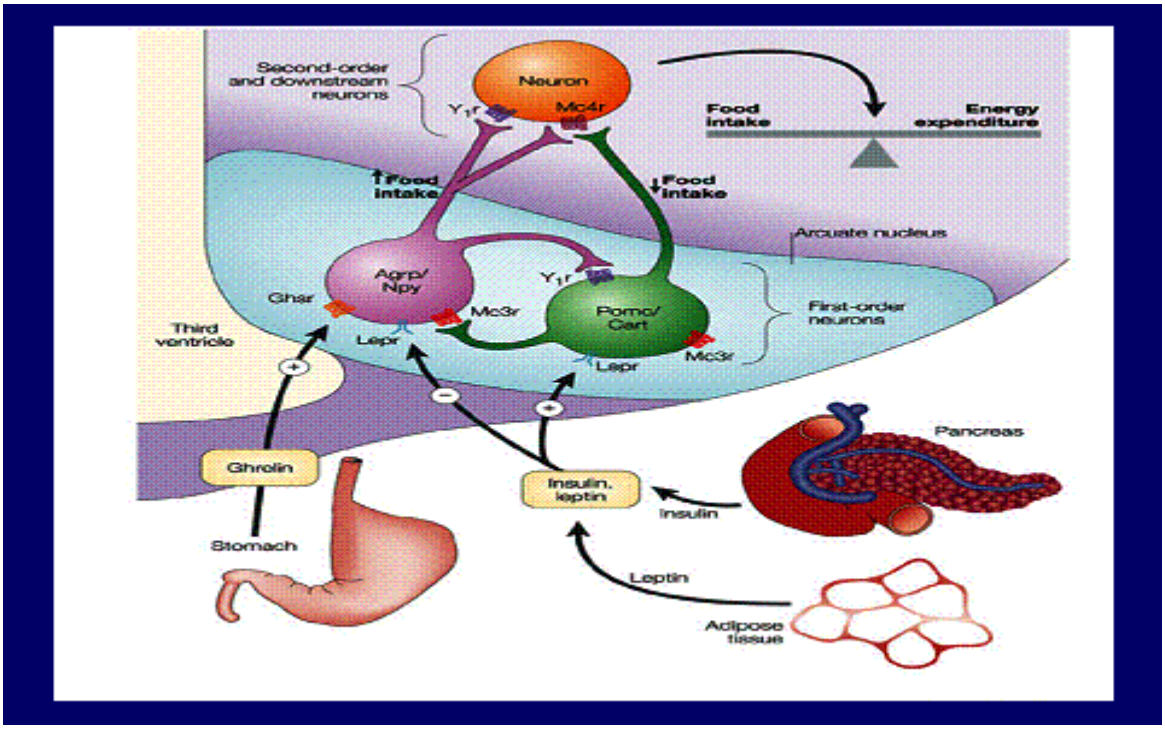
Women with a high BMI are more likely to give birth to babies who weigh greater than 90th percentile, a condition referred to as large for gestational age (LGA). [12, 30, 31]LGA babies have an increased risk of obesity, insulin resistance, and high blood pressure (key features of the "metabolic syndrome") in childhood as well as obesity, diabetes, and CVD in adult age. In the U S, a birth weight greater than 4000 g was recorded in 8% of all deliveries and the incidence is rising rapidly. In addition to being large at birth, children born to obese mothers are also more susceptible to obesity in adolescence and adulthood.[32] Prevention is the best way to address these problems.

Pregnancy and leptin

Leptin is a protein, which was initially identified as an adipocyte-derived hormone. It plays a key role in regulating energy intake and energy expenditure, including appetite and metabolism and cause decrease food intake and body weight via

its receptor in the hypothalamus. Administration of this hormone to leptin deficient mice decreases food intake and body weight. The molecular basis for this leptin resistance is still poorly understood in humans but most likely involves a dysfunction of leptin receptor and associated downstream signaling pathways.

Figure 1. Central nervous system receives signal from Leptin from adipose tissue to control the appetite. (Figure adapted from UMR 8090 2007 Research Report).[54]



Leptin plays an essential role in reproduction by regulating gonadotropin-releasing hormone secretion from the hypothalamus. It also modulates glucose metabolism by increasing insulin sensitivity and activates the sympathetic nervous system. In humans, leptin is also produced by placental trophoblasts and is secreted into

both the maternal and fetal circulation.[33] Leptin production in the placenta is increased in pregnancies complicated with several pathologic conditions.[15] Leptin gene expression in the placenta is augmented in severe preeclampsia, and maternal plasma leptin levels in severe pre-eclampsia are significantly higher than those in normotensive pregnant women. Adali et al. in the recent study done in Turkey showed that increased maternal levels of leptin may be involved in the pathogenesis of pre-eclampsia, and measurement of these adipokines may be useful in the assessment of the severity of disease.[34] Leptin production in the placenta is also increased in diabetic pregnancy with insulin treatment. Furthermore, leptin is proposed to play a functional role in implantation by virtue of its stimulatory effect on matrix metalloproteinase expression in cytotrophoblast. Dysregulation of leptin metabolism and/or function in the placenta may be implicated in the pathogenesis of various disorders during pregnancy, such as recurrent miscarriage, gestational diabetes, intrauterine growth retardation, and pre-eclampsia.[35, 36] Study done by Powers et al. at University of Pittsburgh showed the role of leptin in regulating amino acid transport in the human placenta.[37]

Pregnancy and adiponectin

Adiponectin is a hormone, which is secreted by adipose tissue into bloodstream and is very abundant in plasma relative to other hormones. Adiponectin modulates a number of metabolic processes, including glucose regulation and fatty acid catabolism. It is an important adipokine because of its beneficial effects on glucose and lipid metabolism. Low levels of adiponectin are associated with disease states such as

diabetes and cardiovascular disease. Adiponectin is found to play role in suppression of the metabolic derangements that may result in obesity, metabolic syndrome, atherosclerosis, type 2 diabetes and non alcoholic fatty liver disease. [20, 38, 39] Evidence suggests that adiponectin has anti-atherogenic properties by improving endothelial function and having anti-inflammatory effects in the vascular wall. In addition, adiponectin modifies vascular intracellular redox signalling and exerts indirect antioxidant effects on human myocardium. However, its clinical role in cardiovascular disease is obscure. Direct administration of adiponectin has been shown to be beneficial in animal models of diabetes, obesity and atherosclerosis.[39, 40] Adiponectin levels in humans can be increased through indirect methods such as weight loss or treatment with thiazolidinediones. Leptin and adiponectin appear to have complimentary actions in weight reduction.[39]

Lower levels of adiponectin during pregnancy are associated with gestational diabetes, pre-eclampsia and fetal overgrowth.[38, 41-43] Gestational diabetes is associated with lower levels of adiponectin during pregnancy as well as postpartum. Previous studies have found the relationship between lower levels of adiponectin in women with gestational diabetes. Study done in Athens, Greece found that the adiponectin levels were reduced in women with gestational diabetes during pregnancy as well as postpartum.[44] The mechanisms underlying fetal overgrowth in pregnancies complicated by maternal obesity are not known. Fetal growth is largely determined by nutrient transfer across the placenta, which is dependent on several factors, including maternal

nutrient levels and placental transport capacity. Maternal hormones like leptin and adiponectin are shown to interfere with placental transport capacity and hence may be related to fetal complications like overgrowth and metabolic disorders in later life. Higher levels of leptin and lower levels of adiponectin are shown to be related to fetal birth weight in previous studies. [45]

Significance

High concentrations of leptin and lower concentrations of adiponectin in early pregnancy are associated with higher occurrence gestational diabetes and pre eclampsia in later pregnancy. Higher levels of leptin and lower levels of adiponectin during early pregnancy are positively correlated with fetal adiposity at birth as well as metabolic disorders and obesity in adult life. The purpose of this thesis project was to determine if the pre-pregnancy and early pregnancy adiposity is related to the adipokines measured in early pregnancy. Finding this relationship will help us building preventive strategies against pregnancy complications like spontaneous abortion, preterm delivery, gestational diabetes, preeclampsia, fetal overgrowth etc.

The following hypotheses were tested in this study:

1. Pre-pregnancy BMI would be related to anthropometric measures (weight, mid arm circumference, wrist circumference, biceps skinfold, thigh skinfold) measured early to mid gestation.
2. Women entering pregnancy with excess adiposity (higher BMI) would have higher plasma levels of leptin and lower levels of adiponectin measured in early to mid gestation.
3. Anthropometric parameters of adiposity, weight, mid-arm circumference, wrist circumference, biceps skin fold, and thigh skin fold thickness measured in early to mid gestation would be related to the plasma levels of leptin and adiponectin.

Methods

This thesis project is a part of a larger research study named Lipid, Adipokines and Prenatal Programming (LAPP) study, a prospective cohort study led by Dr. Debra Krummel (Department of Nutritional Sciences) and Dr. Theresa Powell (Department of Obstetrics and Gynecology) at the University of Cincinnati. LAPP was proposed to study the effects of maternal nutritional status on maternal cytokine levels, placental function and inflammatory markers in mothers and infants. Although the LAPP study looks at various relationships like nutrients, anthropometric markers, cytokines, placental functions in mother as well as their infants, this thesis project will concentrate only on the relationship between maternal pre-pregnancy BMI and anthropometric measurements with serum levels of Leptin and Adiponectin during early pregnancy visit in LAPP study subjects.

Subjects

Healthy, non-smoking pregnant women were recruited to the study when they present for their first prenatal visit in gestational week 8-16. The inclusion criteria were that the women have a pre-pregnancy body mass index between 20 and 40, and are 18-35 years of age. Exclusion criteria include concurrent maternal disease (diabetes, hypertension, metabolic syndrome, eating disorders), tobacco or drug use, excessive weight gain prior to pregnancy, plans to leave the geographic area, and inability to travel to the General Clinical Research Center (GCRC) for the blood draw. Patients were recruited from the Ambulatory Center for Women's Health, Greater Cincinnati OB/Gyn

and the High Risk Clinic at University Hospital. These clinics provide prenatal care to women residing in the greater Cincinnati regional area and serving majority of African American low income women using Medicaid and WIC benefits.

Recruitment

Fliers were prominently displayed throughout the clinic (Appendix 1). Study personnel, physicians, nurses distributed a self-addressed stamped postcard to prospective participants (Appendix 2). List of the patients visiting the clinic were obtained from the clinic to look into medical records. Study coordinators approached pregnant women in the waiting room of the clinic once they checked in. Contact information was collected if they Sowed interest in the study, self-addressed stamped postcard was given if considering participation, and screening was done for a women with a definite interest in participation. Weekly recruitment goals were set. Disposition form is filled at the end of each week. Recruitment was done starting March 2007 to December 2007. Every week there were 16 patients scheduled for their early pregnancy visit in the clinic. However, on an average only 4-5 women actually showed up. About 261 women were approached in University hospital clinic. Out of 261, 51 women were not interested in participating in the study. The reasons were time constraints (4), does not like to participate in the studies (35), dos not like blood draw (8), other reason (4). 58 women were interested and passed screening. Out of 58, 14 women could not be reached by telephone to schedule, and 21 women were scheduled but did not show up for the study visit even after given a chance of rescheduling. Only 23 women showed up

and completed their first early pregnancy visit. Weekly recruitment goals were set and a disposition form was filled at the end of each visit.

Screening

Screening form was prepared with appropriate questions elaborating the eligibility criteria for the study. ***In person screening*** When approached in person by the recruiter, if the woman said that she is interested in participating in the study, screening was done. If woman passed screening, she was scheduled for her first visit in General Clinical Research Center of Cincinnati Children's hospital. ***Telephone screening*** Women approached in person by recruiter who said they needed more time to make a decision or who are in a hurry were given a post card. Study coordinator called women who returned post card with phone number and did telephone screening. Women who passed screening were scheduled for GCRC study visit.

Enrollment

Pregnant women were enrolled at week 8-16 of gestation once they show up for their first study visit at GCRC of Cincinnati Children's Medical Center and sign the informed consent form.

Once women show up for their early pregnancy visit at GCRC, informed consent was obtained after explaining all the details of the study and answering any concerns subject might have.

Height and weight is measured and blood draw is done by GCRC staff. ***Height*** was measured without shoes on a stadiometer affixed to the wall at the GCRC clinic by GCRC staff. Measurements were recorded to the nearest 1/8 inch. If the measurement is less than 1/8 inch, then it was rounded up. ***Weight*** was measured on a digital scale in women wearing light clothing, without shoes. The weight will be measured in the GCRC clinic by GCRC staff. Weight was recorded to the nearest 0.1 kg.

Anthropometric measurement protocol was prepared by referring to National Health and Nutrition Examination Survey (NHANES) standard protocol for taking measures of height, weight, body mass index, arm circumference, and skinfold thickness measurements at bicep and thigh locations). The NHANES video were viewed the study team and training was done with the help of mock subjects prior to study. The protocol was followed for taking measurements of arm circumference, wrist circumference, and biceps skinfold, thigh skinfold in all the subjects. Following instructions from the protocol were followed to obtain the anthropometric measurements.

Arm Circumference

The midpoint of the right upper arm was determined and marked. This was calculated with the subject standing upright, shoulders relaxed, and the right arm flexed at elbow joint. The distance between uppermost edges of posterior border of acromian process to the tip of olecrenon process was measured. This distance was divided by 2

and the midpoint was obtained. The arm circumference was calculated with subject standing upright, shoulders relaxed, and right arm hanging lose on the side. Measuring tape was placed around the midpoint, perpendicular to the long axis of arm. By holding measuring tape gently on skin surface, the measurement is taken on the lateral aspect of the arm. The arm circumference was recorded to the nearest 0.1 cm. Measurements are taken three times and the average of them is taken as a final arm circumference.

Wrist circumference

The subject stood with the right arm flexed at elbow so that the palm is uppermost and hand muscles relaxed. The measuring tape was placed distal to the styloid process of the radius and ulna, which are located by palpating with the index and middle finger of each hand. The tape was positioned perpendicular to the long axis of forearm and in the same plane on the anterior and posterior aspects of the wrist. The whole circumference was measured without compressing the soft tissue and recorded to the nearest 0.1cm. Measurements were taken three times and the average of them is taken as a final wrist circumference.

Skin-folds

A Lange caliper (β-technologies Inc., Cambridge MD), pen, and physician's ordered data collection sheet were used. Skinfolds were measured on two sites (biceps & thigh). All measurements were done on right side of the body. The caliper was placed 1 cm away from the thumb and finger perpendicular to the skin-fold and halfway

between the crest and the base of the fold. The pinch was maintained while reading the caliper. The caliper was read within 1-2 seconds. Three measures were taken at each site and retested if duplicate measurements were not within 1 mm.

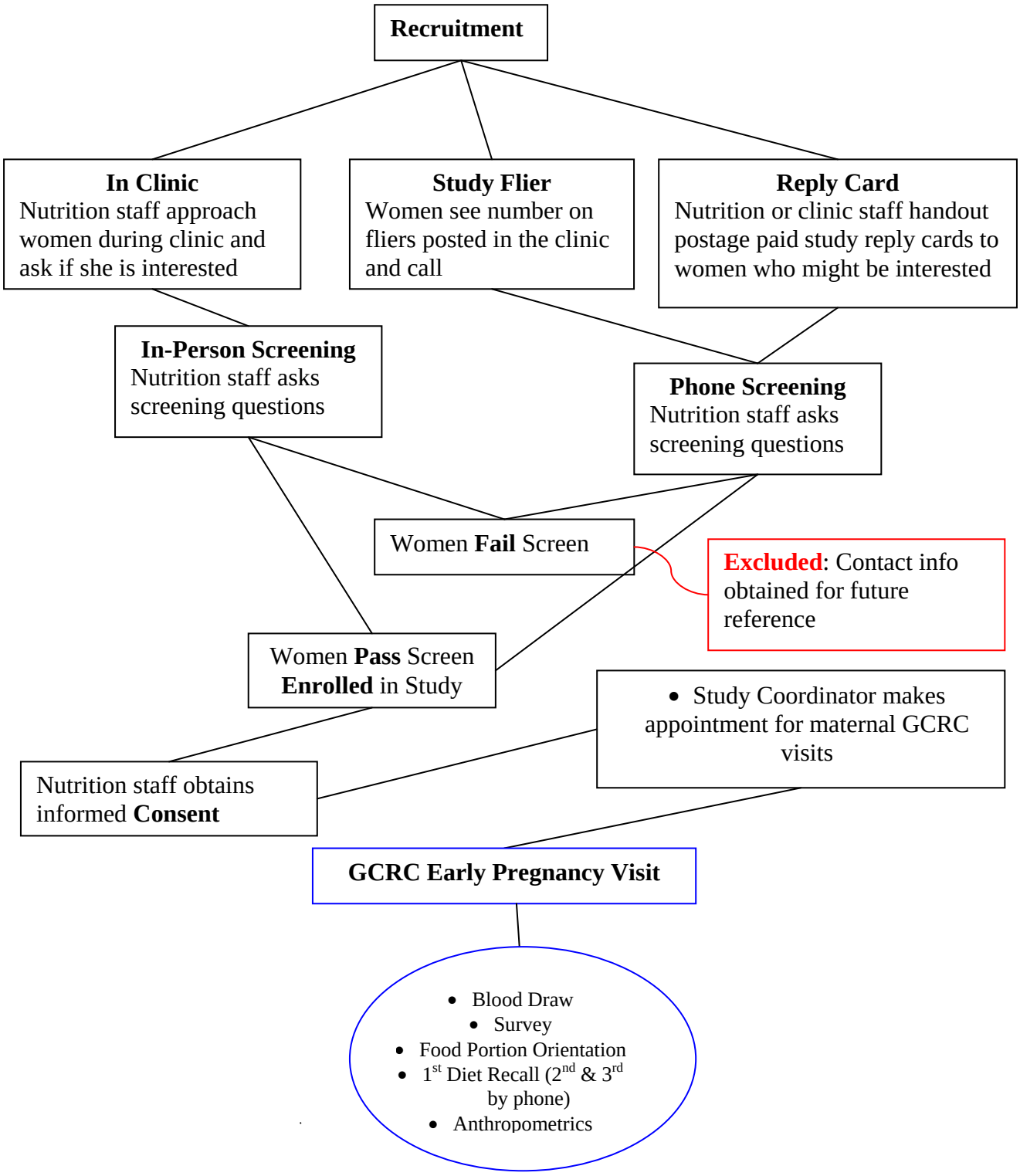
Biceps skinfold

The subject stood erect with shoulders relaxed and arms hanging loosely at the side and palms directed anteriorly. Skinfold was raised 1cm superior to the line marked for arm circumference and on a vertical line joining the anterior border of the acromian and the center of the antecubital fossa. The caliper jaws were applied at the marked level. Measurement were recorded to the nearest 0.1 cm.

Thigh skinfold

The measurement site was located at the midline of the anterior aspect of thigh, midway between the inguinal crease and the proximal border of the patella. The subject flexed the hip to assist location of the inguinal crease. The proximal reference point was on the inguinal crease at the midpoint of the long axis of thigh. The distal reference point which was proximal border of patella is located while the knee of the subject is extended. The vertical fold was measured while subject is standing. The body weight was shifted to other foot while the leg on the side of measurement is relaxed while knee is slightly flexed and foot flat on floor. The caliper jaws were applied 1 cm distal to the fingers holding the fold. The thickness of the fold was recorded to the nearest 0.1 cm.

Figure 3. Patient Flow chart for LAPP study



Blood draw

A fasting venous blood sample was obtained in the GCRC. Blood was spun in GCRC and stored at -80C until analyzed in batch. Plasma leptin and adiponectin were analyzed using an ELISA kit by the LAPP study personnel.

Safety

The University of Cincinnati Institutional Review Board (IRB) and the Cincinnati Children's Hospital Medical Center IRB reviewed the proposed LAPP research study to ensure that the subjects' rights and welfare were adequately protected. The principal investigators, co-investigators (physicians), research scientists, and a study coordinator, all had completed HIPAA confidentiality training before start of the study. Only these members monitored all components of the program.

Data

All study data on paper was maintained in locked filing cabinets in a locked study office. Participant ID numbers were used on all data and identifiable information was removed whenever possible. All computerized data was maintained on a secure server that is HIPAA compliant and maintained in a locked office that is limited in access only to the research team. Study data was gathered on paper forms and then entered into a database of Microsoft Excel and SPSS v14.0.

Statistical Analysis

Body Mass Index (BMI) was calculated as Body Mass Index = Weight in kg / height in meters^2. Pregnant women were divided in two groups, one with BMI greater than 25 and one with BMI less than 25.

Descriptive univariate analysis including mean and standard error of mean were used to describe age, height, weight, weight gain during pregnancy and the serum leptin and adiponectin levels of the pregnant women of both the BMI categories. To test the hypothesis that women with higher BMI do have high serum leptin and low serum adiponectin levels, we derived mean of the leptin and adiponectin levels for the two groups and ran two tailed significance test to test the hypothesis. Relationship between pre-pregnancy BMI and anthropometric measurements during early pregnancy visit and serum leptin and adiponectin levels was determined by bivariate analysis by using Pearson correlation coefficient. Statistical analysis was done by using statistical software (version 14.0).

Results

Maternal demographic data

Table 1. Demographic characteristics of LAPP subjects

Variable	N
Age	
19 - 24 years	11
25 - 29 years	4
30 – 34	4
Ethnicity	
Black, not Hispanic	16
White, not Hispanic	3
Food Stamps	
Yes	10
No	9
WIC	
Yes	13
No	6

Maternal demographic properties of 19 pregnant women are listed in Table 1. All 19 women were from low socioeconomic class. Out of 19, 16 were African American and 3 were Caucasians. When we look at the sample characteristics, the mean maternal age is 24.78. The mean pre-pregnancy weight is 77.97, and mean maternal height is 166.40. Mean maternal weight gain is 15.4.

Table 2. Characteristics of LAPP subjects

Variable	Mean
Height (cm)	165.7
Weight (kg)	82.4
Gestational Age (wks)	15
Percent Body Fat	55
Biceps Skinfold (mm)	24
Thigh Skinfold (mm)	41

When compared with the demographic data obtained in other studies, the mean values of age, height, weight and weight gain during pregnancy are consistent. [45]

Table 1. Mean Values of Age, Weight, Height, BMI and Weight Gain

	Maternal Age (yrs) (n=23)	Pre-pregnancy Weight (kg) (n=23)	Pre-pregnancy BMI (n=23)	Gestational Wt Gain (kg) (n=23)
Mean	24.78	77.97	28.35	15.40
SEM	1.07	5.10	1.94	1.53

Maternal serum leptin and adiponectin concentrations

The mean leptin and adiponectin levels found in this study in pregnant women in their early pregnancy visit are consistent with the findings from previous studies. We

categorized the sample in two groups, one with BMI more than 25 and one with BMI less than 25, and calculated the mean values of leptin and adiponectin. Table 2 shows the comparison between the mean leptin and adiponectin levels for the two categories of the women. In the table below, we can clearly see that mean leptin levels are more in women with BMI more than 25 and low in women with BMI less than 25 and can see the statistical significance by two tailed statistics. Similarly, adiponectin levels are lower in women with BMI more than 25 and higher in women with BMI less than 25.

Table 3. Mean Adiponectin and Leptin levels in women categorized by BMI

Maternal Blood Levels		**BMI ≤ 25**	**BMI > 25**	**Sig. (2-tailed)**
Adiponectin ug/ml	Mean	12.61 (N=8)	6.93 (N=11)	.002
	SEM	.78	1.21	
Leptin ng/ml	Mean	13.01 (N=8)	39.08 (N=11)	.001
	SEM	2.87	5.72	

Relationship between pre-pregnancy BMI and anthropometric measurements during early pregnancy visit with maternal serum leptin and adiponectin levels

Bivariate correlation analysis was done to understand the relationship between the two different continuous variables. Different variables to be correlated with plasma levels of leptin and adiponectin are – pre-pregnancy BMI, wrist circumference, mid-arm circumference, and biceps skinfold and thigh Skinfold measurements done at two different intervals during pregnancy (early pregnancy visit and late pregnancy visit). For

these continuous variables we used Pearson correlation analysis to obtain the significance direction and magnitude of the association.

Our primary hypothesis is that that maternal obesity increases maternal serum levels of leptin and decreases levels of adiponectin in pregnant women. Pearson correlation analysis show strong positive association between pre-pregnancy BMI and plasma leptin and strong negative association between pre-pregnancy BMI and plasma adiponectin levels (Maternal plasma leptin: r = +0.820, p=.000; Maternal Plasma Adiponectin: r = -0.807, p=.000).

Pre-pregnancy BMI is also associated with anthropometric parameters measured in early pregnancy visit (Weight in kg early pregnancy visit: r = +0.923, p=.000; Mid-arm circumference: r = +0.951, p=.000; Wrist circumference: r = +0.748, p=.000; Bicep skinfold: r = +0.732, p=.000; Thigh skinfold: r = +0.770, p=.000).

Anthropometric parameters like mid-arm circumference, wrist circumference, biceps skinfold, thigh skinfold measured during early pregnancy visit in pregnant women do show association with the levels of leptin and adiponectin. Mid-arm circumference (Maternal Plasma Leptin: r=0.785, p=.000; Maternal Plasma Adiponectin: r= -0.730, p=.000) has strong positive association with plasma leptin and strong negative association with plasma adiponectin. Wrist circumference (Maternal Plasma Leptin: r=.460, p=.048; Maternal plasma adiponectin: r= -0.640, p=.003), biceps skinfold (Maternal Plasma Leptin: r= 0.534, p=.019; Maternal Plasma Adiponectin: r= -0.839, P=0.000), and thigh skinfold (Maternal Plasma Leptin: r= 0.666, p=0.002; Maternal

Plasma Adiponectin: r= -0.712, P= 0.001) have strong negative association with plasma adiponectin levels.

Discussion

Very few studies have been done to see the relationship between adiposity and adipokine levels during pregnancy. Most of the studies are concentrated on the levels of adipokines with pregnancy related complications or levels of adipokines with fetal adiposity.[15, 38, 41, 44, 46, 47, 47-49] Also previous studies focus on nutrition during pregnancy and the fetal growth or obesity during pregnancy and fetal growth or obesity during pregnancy and complications like preeclampsia and gestational diabetes.[25, 50] This study is unique in a way it tries to find out the relationship between maternal adiposity and leptin and adiponectin levels. Our primary hypothesis is that maternal adiposity does have effect on the serum levels of adiponectin and leptin. We propose that maternal adiposity is the root cause of changes in the levels of adipokines which does further may cause pregnancy related complications.

This thesis study has several strengths. First, it is a part of LAPP study which is prospective cohort study which enabled us to collect extensive and detail data. Second, the study population consisted of homogeneous population with respect to socioeconomic background. All subjects were non smokers, teetotaler, and had no pathological conditions. Third, study visits were done in General clinical research center which was very close to the University hospital which was source of recruitment.

The primary limitation of this study is that relatively small number of subjects were studied (n=23). This decreases our ability to detect biologically relevant

differences. Subjects were recruited at University Hospital considering the large volume of patients but there were some issues. Some reasons to have fewer subjects in this study was low compliance and many women at University Hospital showing late (>20 weeks) for their first pregnancy visit. We tried to solve this problem by more advertisement, in person recruitment and by involving the clinic staff in our recruitment efforts. All subjects were given reminder phone calls and ample chances to reschedule if interested to participate in study. Although even after all these efforts the study population remained on lower side (23 subjects).

This study provides new information about the changes in leptin and adiponectin in overweight and obese women. This is significant because changes in leptin and adiponectin levels may affect the outcome of the pregnancy and may have marked effect on fetal growth in rest of the pregnancy. We found a strong positive relationship between maternal pre-pregnancy BMI and anthropometric measurements during pregnancy with plasma leptin and adiponectin levels. This evidence supports the concept of adiposity as a high risk state during pregnancy. Most previous studies concentrate on maternal complications due to obesity or pregnancy related complications due to high leptin and low adiponectin levels or effect of changes in leptin and adiponectin levels on fetal birth weight. To best of our knowledge none of the studies except a Swedish study has focused on maternal adiposity and the levels of leptin and adiponectin. The study in Sweden has shown strong positive correlation between maternal BMI and levels of leptin and negative correlation between BMI and

adiponectin in early pregnancy.[51] This study is focused on body mass index and dietary intake of pregnant women with various metabolic hormones like leptin, resistin, adiponectin, insulin etc. Our study is unique that it does find out the relationship between body mass index as well as other measures of adiposity like biceps and thigh skinfold thickness, arm and wrist circumference with the levels of adipokines. Our study does concentrate of various measures of adiposity and try to find out the relationship between them and the adipokine levels. The strong positive correlation between various anthropometric measurements and plasma leptin levels and negative correlation with adiponectin levels fund in our study support the proposed mechanism of effect of maternal obesity on fetal growth by Swedish study. [51]

If we look at the demographic characteristics of the subjects, the mean maternal age is 24.78. The mean pre-pregnancy weight is 77.97, and mean maternal height is 166.40. The mean height and weight is found to be similar to the ones observed in previous other studies. [20, 49, 51-53] The observed maternal weight is too high for the height as well as this reproductive age group. More and more women are entering into pregnancy overweight. This is very alarming and as we know the various complications due to obesity during pregnancy, steps should be taken to deal with this problem. Women in reproductive age group should be offered more health education and should be encouraged to lose weight. Prenatal education can play vital role in this and is a must for all the women in reproductive age group.

Previous studies done to study the adverse effects of higher levels of leptin and lower levels of adiponectin during pregnancy were done on women with normal range BMI.[1,20,25,26] As compare to the mean leptin and adiponectin levels from other studies, the observed leptin levels are higher and adiponectin levels were lower in our study. This can be explained by higher mean weight and pre-pregnancy BMI than the other studies. The observed mean leptin and adiponectin levels are consistent with the ones found in women in Swedish study.[51]

After categorizing the sample in two groups, we can clearly see that the mean leptin levels are more in women with BMI more than 25 and low in women with BMI less than 25. Similarly, adiponectin levels are lower in women with BMI more than 25 and higher in women with BMI less than 25. Also bivariate correlation analysis show strong association between pre pregnancy BMI and plasma leptin levels and strong negative association between pre-pregnancy BMI and plasma adiponectin levels. Pre pregnancy BMI was found to be related to the anthropometric parameters measured in early pregnancy showing that women who enter pregnancy with higher BMI tend to gain more weight during pregnancy. Mid-arm circumference has strong positive association with plasma leptin and strong negative association with plasma adiponectin. Wrist circumference, biceps skinfold and thigh skinfold have strong negative association with plasma adiponectin levels. These strong correlations further strengthen our hypothesis that adiposity is related to the low adiponectin and high leptin levels. Various studies have shown the relationship between obesity and various pregnancy related

complications. Studies also have shown the relationship of plasma leptin and adiponectin levels with the complications during pregnancy and to the fetus. By showing the strong relationship between the leptin and adiponectin levels with obesity, this study further high lightens the severity of adverse effects of obesity. This can be the missing link between obesity and pregnancy related complications like pre eclampsia, premature birth etc. The adverse effects are not only limited to pregnancy but also can be seen in fetus. Also in the fetus the complications large birth weight, increased incidence of type 1 diabetes mellitus etc are seen in childhood. Such children are more prone to metabolic syndrome and disorders like diabetes mellitus and hypertension in adulthood. Prevention is a best way to handle this issue. Women in reproductive group do need to be educated and overweight women should be encouraged to lose some weight before entering into pregnancy. Similar studies need to be done to further strengthen it because of limitations due to sample size.

Appendix 1

Study Post card

Nutrition and Pregnancy Study

You can help future generations to grow up healthy!

What: Study looking at blood tests in pregnant women and their babies.

Why: To learn more about how to prevent diseases like diabetes and high blood pressure.

Who: Healthy women ages 18 to 35 who are less than 20 weeks pregnant may be eligible to participate in this study

How: For information, call study coordinator at 513-558-7034 or fill out this card and drop in the mail - no postage needed.

Free Gift Cards! Free Diet Analysis

Appendix 2

Nutrition and Pregnancy Research Study

Free Gift Cards! **Free Diet Analysis!**

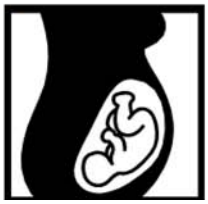
What: This is a research study to look at blood tests in pregnant women and their babies

Why: To learn more about how to prevent diseases like diabetes and high blood pressure

Who: Healthy women between 8 and 20 weeks pregnant are eligible to participate in this exciting study

How to participate:

Contact Study coordinator at 513-558-7034



Appendix 3

SCREENING FORM **Date_____**

Name _____ Phone # _____

Interviewer *I am going to ask you some questions to see if you meet the criteria for our study. All answers are confidential. They will only be used to see if you meet the criteria for our study.* Circle or write their answers.

Are you pregnant?

YES NO

Do you know how far along you are? _____

What is the date of your last period? _____ Weeks gestation: _____wks.

Is participant less than 16 weeks? **YES NO**

Have you ever been told that you have diabetes? **YES NO**

Have you ever been told that you have high blood pressure? **YES NO**

Do you smoke cigarettes or any other drugs? **YES NO**

Have you smoked within the past 2 months? **YES NO**

Do you use any street drugs? **YES NO**

Are you currently taking any medications to lower cholesterol or blood pressure? **YES NO**

Do you plan to leave Cincinnati before your baby is three months old? **YES NO**

Did you gain or lose weight in the 6 months before you became pregnant?

YES

NO

If YES, *Do you know how much?* _____

Weight change exceeds 20 pounds? **YES NO**

What is your date of birth? _____

Is birth date between today's date 1989 to today's date 1972? **YES NO**

What is your current weight? _____

What is your current height? _____

Circle BMI on table _____ BMI between 20 and 40? **YES** **NO**

If NOT all answers circled are underlined responses – say
Thank you so much for taking the time to talk to me, but you don't qualify for this study. Would you like me to take down your contact information in case you qualify for another study?

If all answers circled are the underlined responses - Participant Qualifies - say
Congratulations! You qualify to participate in our study.
(if in person) *You may pick an item from our "Thank You" basket.*

- *The next step is for me to get your contact information and when is the best times for me to call you.*
- *I will call you as soon as possible to schedule your first visit to the General Clinical Research Center located at Children's Hospital.*
- *You will need to make sure you don't eat for 12 hours before your blood draw so you may want it to be in the morning.*

Circle Best Day(s) for clinic M T W H F Best Time(s)
_____

Address: _____

- *Here is your study folder.* Read through instruction sheet
- *Here is the map and directions to the GCRC at Children's Hospital.*
- *Here is your survey that if you have time you can complete before your clinic visit and bring back with you.*
- *Thank you so much for participating and I look forward to talking with you soon.*

►

Appendix 4

Anthropometric Measurement Protocol

I. Purpose

The purpose of doing anthropometric measurements is to obtain measures of adiposity in pregnant women throughout their pregnancy.

II. Measurements

- **Subject Preparation**

  Have participant wear easily removable or loose fitting clothing (shorts, sport bra, bathing suit etc) or offer them gowns and have them change into any of these items in the private rooms at GCRC. Participants will be asked to remove shoes.

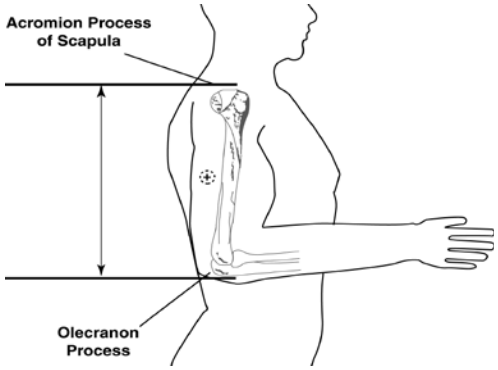
- **Height** is measured without shoes on a stadiometer affixed to the wall at the GCRC clinic by GCRC staff. Measurements are recorded to the nearest 1/8 inch. If the measurement is less than 1/8 inch, then it is rounded up. Height will be measured at entry into the study.

- **Weight** will be measured on a digital scale in women wearing light clothing, without shoes. The weight will be measured in the GCRC clinic by GCRC staff. Weight will be recorded to the nearest 0.1 kg. At all clinic visits, weight is recorded.

- Rest of the measurements, arm circumference, wrist circumference, biceps skinfold, thigh skinfold, are done at the GCRC clinic by Powell-Krummel study staff. Hands are washed with soap & measuring tape is wiped with alcohol swab before taking each measurement.

- **Arm Circumference**

1. Determine the midpoint of the right upper arm. This is calculated with the subject standing upright, shoulders relaxed, and the right arm flexed at elbow joint (see the figure below).
2. Measure the distance between uppermost edges of posterior border of acromian process to the tip of olecrenon process.
3. Divide this distance by 2; this is the midpoint.
4. Now the arm circumference is calculated with subject standing upright, shoulders relaxed, and right arm hanging lose on the side. Place measuring tape around the midpoint, perpendicular to the long axis of arm. By holding measuring tape gently on skin surface, measurement is taken on the lateral aspect of the arm. The arm circumference is recorded to the nearest 0.1cm. Measurements are taken three times and the average of them is taken as a final arm circumference.



- **Wrist circumference**

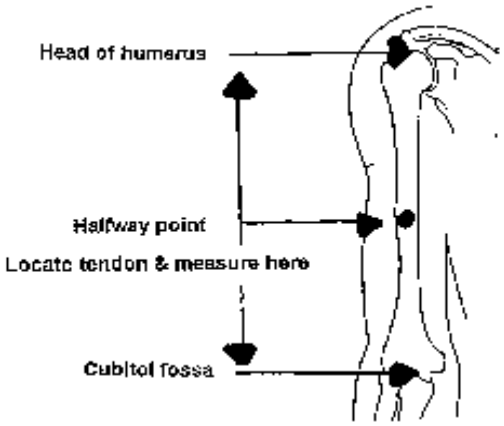
1. Have subject stand with right arm flexed at elbow so that palm is uppermost and hand muscles relaxed.
2. Place measuring tape distal to the styloid process of the radius and ulna, which are located by palpating with the index and middle finger of each hand. The tape is positioned perpendicular to the long axis of forearm and in the same plane on the anterior and posterior aspects of the wrist (see figure below).
3. Measure around the whole circumference without compressing the soft tissue. The wrist circumference is recorded to the nearest 0.1cm. Measurements are taken three times and the average of them is taken as a final wrist circumference.

- **Skin-folds**
  - **Equipment –** Lange caliper (β-technologies Inc., Cambridge MD), pen, Physician's ordered data collection sheet. Skinfolds will be measured using two sites (biceps & thigh). All measurements will be done on right side of the body.

- Caliper should be placed 1 cm away from the thumb and finger perpendicular to the skin-fold and halfway between the crest and the base of the fold.
- Pinch should be maintained while reading the caliper.
- Read the caliper within 1-2 seconds.
- Take three measures at each site and retest if duplicate measurements are not within 1 mm.
- Rotate through measurement sites or allow time for skin to return to normal texture and thickness.
- Skinfolds will be measured at both clinic visits (8 to 16 weeks of gestation and at 32 to 36 weeks of gestation).
- Measurements will be recorded in Physician's ordered data collection sheet.

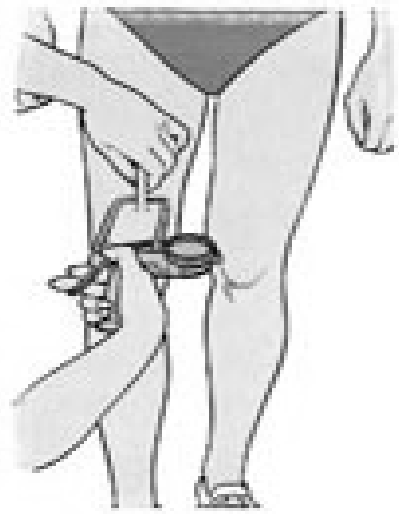
- **Biceps skinfold**

1. Have subject stand erect with shoulders relaxed and arms hanging loosely at the side and palms directed anteriorly.
2. Skinfold is raised 1cm superior to the line marked for arm circumference and on a vertical line joining the anterior border of the acromian and the center of the antecubital fossa. The caliper jaws are applied at the marked level. Record measurement to the nearest 0.1 cm.



- **Thigh skinfold**

1. Locate measurement site at the midline of the anterior aspect of thigh, midway between the inguinal crease and the proximal border of the patella. The subject flexes the hip to assist location of the inguinal crease. The proximal reference point is on the inguinal crease at the midpoint of the long axis of thigh. The distal reference point which is proximal border of patella is located while the knee of the subject is extended.
2. Measure the vertical fold while subject is standing. The body weight is shifted to other foot while the leg on the side of measurement is relaxed while knee is slightly flexed and foot flat on floor. If balancing is a problem, the subject holds the top of measurer's shoulder, a counter top, or high backed chair.
3. The caliper jaws are applied 1 cm distal to the fingers holding the fold.
4. Record the thickness of the fold to the nearest 0.1 cm.



III. Anthropometric Equations

Equations, developed in pregnant women, will be used to estimate the changes in maternal adiposity from the first trimester of pregnancy to the last trimester of pregnancy. {{307 Paxton,A. 1998; }}

- To predict change in maternal adiposity, i.e., body fat storage, from 14 to 37 weeks of gestation, we will use the following equation:

  Fat change (kg) = 0.77 (weight change, kg) + 0.07 (change in thigh skin-fold thickness, mm) – 6.13

- To predict the actual "fat mass" at term/ 37th week of gestation, the anthropometric equation used is as follows

  Fat mass (kg) = 0.40 (weight at 37th week of gestation, kg) + 0.16 (biceps skinfold thickness at 37th week of gestation, mm) + 0.15 (thigh skinfold thickness at 37th week of gestation, mm) + 0.09 (wrist circumference at 37th week of gestation, mm) + 0.10 (prepregnancy weight) – 6.56

IV. Data Entry Form

Height, weight, arm circumference, wrist circumference, biceps skinfold, thigh skinfold readings will be entered in early and late pregnancy GCRC order form. The data is analyzed in anthropometry analysis form where the equations in III will be applied to the data to get the estimation of changes in fat mass during pregnancy.

V. Training

- The NHANES 3 video on Anthropometric Measurement Procedures was viewed by both research assistants that will be obtaining measures.
- The Anthropometric Standardization Reference Manual was read.

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