A COMPREHENSIVE STUDY ON ENERGY EXPENDITURE
AND BODY COMPOSITION IN DIABETES MELLITUS
COMPLICATING PREGNANCY

Dissertation done by
Dr. Geethu Antony (Registration No. 161521001),
The Tamil Nadu Dr. MGR Medical University, Tamil Nadu

in partial fulfilment of the rules and regulations for the degree of
DM Endocrinology examination to be held in in February 2019,
Christian Medical College, Vellore.
DECLARATION

I hereby declare that this dissertation titled “A comprehensive study on Energy Expenditure and body composition in diabetes mellitus complicating pregnancy” was carried out by me under the direct supervision and guidance of Dr. Nihal Thomas, Professor and Head, Unit-I and co-guidance of Dr. Thomas V Paul, Professor and Head, Unit-II, Department of Endocrinology, Diabetes & Metabolism, Christian Medical College, Vellore.

I also declare that this dissertation has not been submitted by me to any other university, or for the award of any other degree/diploma.

Vellore

Dr. Geethu Antony

Date: Christian Medical College, Vellore
Submission of Thesis for the award of DM in Endocrinology

This is to certify that Dr. Geethu Antony, MD, Specialty trainee for DM (Endocrinology), with Registration number 161521001 is hereby submitting her thesis titled “A comprehensive study on Energy Expenditure and body composition in diabetes mellitus complicating pregnancy”, which is the original work done by her under the guidance of Dr. Nihal Thomas, Professor and Head, Unit-I, and co-guidance of Dr. Thomas V Paul, Professor and Head, Unit-II, Department of Endocrinology, Christian Medical College, Vellore. This has not been submitted to any other University or Board of Examinations, in part or full.

Guide:
Dr. Nihal Thomas,
MD,MNAMS,DNB(Endo),FRACP(Endo),FRCP(Edin,Glasgow,London), PhD(Copenhagen)
Professor & Head, Unit-I,
Department of Endocrinology, Diabetes & Metabolism,
Christian Medical College, Vellore-632 004.
April 29, 2016

Dr. Geethu Antony,  
PG Registrar,  
Department of Endocrinology,  
Christian Medical College,  
Vellore 632 004.

Sub: Fluid Research Grant NEW PROPOSAL:  
A comprehensive study on Energy Expenditure and body composition in diabetes mellitus complicating pregnancy.

Dr. Geethu Antony, DM PG Registrar (Employment Number: 21250), Endocrinology, Diabetes and Metabolism, Dr Nihal Thomas, Endocrinology, Diabetes and Metabolism, Dr. Thomas V Paul, 20046, Dr. H.S Asha, 31848, Dr. DukhabanduNaik, 31624, Dr Riddhi Das Gupta, 20917, Endocrinology, diabetes and metabolism, Dr Annie Regi, 11190, Dr Gigi Elizabeth, Mathew, Dr Jessy Lionel, 14520, Dr Joe Fleming, V0002, Clinical Biochemistry, 13790, Dr. Visalakshi J, 31093, Biostatistics, Dr Mini Joseph, Endocrinology, diabetes and Metabolism, Mrs. Mercy Inbakumari, 90501, Endocrinology, diabetes and Metabolism, Dr. Vishalakshi J, Biostatistics.


Dear Dr. Geethu Antony,

I enclose the following documents:-

1. Institutional Review Board approval  
2. Agreement

Could you please sign the agreement and send it to Dr. Biju George, Addl. Vice Principal (Research), so that the grant money can be released.

With best wishes,

[Signature]

Dr. Biju George  
Secretary (Ethics Committee)  
Institutional Review Board

Cc: Dr Nihal Thomas, Professor, Endocrinology, CMC, Vellore.
April 29, 2016

Dr. Geethu Antony,
PG Registrar,
Department of Endocrinology,
Christian Medical College,
Vellore 632 004.

Sub: **Fluid Research Grant NEW PROPOSAL:**
A comprehensive study on Energy Expenditure and body composition in diabetes mellitus complicating pregnancy.

Dr. Geethu Antony, DM PG Registrar (Employment Number: 21250), Endocrinology, Diabetes and Metabolism, Dr Nihal Thomas, Endocrinology, Diabetes and Metabolism, Dr. Thomas V Paul, 20046, Dr H.S Asha, 31848, Dr Dukhabandu Naik, 31624, Dr Riddhi Das Gupta, 20917, Endocrinology, diabetes and metabolism, Dr Annie Regi, 11190, Dr Gigi Elizabeth, Mathew, Dr Jessy Lionel, 14520, Dr Joe Fleming, V0002, Clinical Biochemistry, 13790, Dr. Visalakshi J, 31093, Biostatistics, Dr Mini Joseph, Endocrinology, diabetes and Metabolism, Mrs. Mercy Inbakumari, 90501, Endocrinology, diabetes and Metabolism, Dr. Vishalakshi J, Biostatistics.


Dear Dr. Geethu Antony,

The Institutional Review Board (Blue, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed your project titled “A comprehensive study on Energy Expenditure and body composition in diabetes mellitus complicating pregnancy” on April 04 2016.

The Committee reviewed the following documents:

1. IRB Application format
2. Proforma
3. Information Sheet and informed Consent Form (English, Tamil and Telugu)
5. No. of documents 1 – 4
The following Institutional Review Board (Blue, Research & Ethics Committee) members were present at the meeting held on April 04th 2016 in the CREST/SACN Conference Room, Christian Medical College, Bagayam, Vellore 632002.

<table>
<thead>
<tr>
<th>Name</th>
<th>Qualification</th>
<th>Designation</th>
<th>Affiliation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Biju George</td>
<td>MBBS, MD, DM</td>
<td>Professor, Haematology, Research, Additional Vice Principal, Deputy Chairperson (Research Committee), Member Secretary (Ethics Committee), IRB, CMC, Vellore</td>
<td>Internal, Clinician</td>
</tr>
<tr>
<td>Dr. Anuradha Rose</td>
<td>MBBS, MD, MHSC (Bioethics)</td>
<td>Associate Professor, Community Health, CMC, Vellore</td>
<td>Internal, Clinician</td>
</tr>
<tr>
<td>Dr. Jayaprakash Muliyil</td>
<td>BSc, MBBS, MD, MPH, Dr PH (Epid), DMHC</td>
<td>Retired Professor, Vellore</td>
<td>External, Scientist &amp; Epidemiologist</td>
</tr>
<tr>
<td>Rev. Joseph Devaraj</td>
<td>BSc, BD</td>
<td>Chaplaincy Department, CMC, Vellore</td>
<td>Internal, Social Scientist</td>
</tr>
<tr>
<td>Ms. Grace Rebekha</td>
<td>M.Sc., (Biostatistics)</td>
<td>Lecturer, Biostatistics, CMC, Vellore</td>
<td>Internal, Statistician</td>
</tr>
<tr>
<td>Dr. Visalakshi. J</td>
<td>MPH, PhD</td>
<td>Lecturer, Biostatistics, CMC, Vellore</td>
<td>Internal, Statistician</td>
</tr>
<tr>
<td>Dr. Mathew Joseph</td>
<td>MBBS, MCH</td>
<td>Professor, Neurosurgery, CMC, Vellore</td>
<td>Internal, Clinician</td>
</tr>
<tr>
<td>Mr. Samuel Abraham</td>
<td>MA, PGDBA, PGDPM, M Phil, BL.</td>
<td>Sr. Legal Officer, CMC, Vellore</td>
<td>Internal, Legal Expert</td>
</tr>
<tr>
<td>Mrs. Pattabiraman</td>
<td>BSc, DSSA</td>
<td>Social Worker, Vellore</td>
<td>External, Lay Person</td>
</tr>
<tr>
<td>Dr. B. J. Prashantham</td>
<td>MA(Counseling Psychol, MA(Theology), Dr. Min(Clinical Counselling)</td>
<td>Chairperson, Ethics Committee, IRB. Director, Christian Counseling Center, Vellore</td>
<td>External, Social Scientist</td>
</tr>
<tr>
<td>Dr. Rajesh Kannangai</td>
<td>MD, PhD.</td>
<td>Professor, Clinical Virology, CMC, Vellore</td>
<td>Internal, Clinician</td>
</tr>
</tbody>
</table>
We approve the project to be conducted as presented.

The Institutional Ethics Committee expects to be informed about the progress of the project, any adverse events occurring in the course of the project, any amendments in the protocol and the patient information/informed consent. On completion of the study you are expected to submit a copy of the final report. Respective forms can be downloaded from the following link:


Kindly provide the total number of patients enrolled in your study and the total number of withdrawals for the study entitled: “A comprehensive study on Energy Expenditure and body composition in diabetes mellitus complicating pregnancy” on a monthly basis. Please send copies of this to the Research Office (research@cmcvellore.ac.in).
We approve the project to be conducted as presented.

The Institutional Ethics Committee expects to be informed about the progress of the project, any adverse events occurring in the course of the project, any amendments in the protocol and the patient information / informed consent. On completion of the study you are expected to submit a copy of the final report. Respective forms can be downloaded from the following link:


Kindly provide the total number of patients enrolled in your study and the total number of withdrawals for the study entitled: “A comprehensive study on Energy Expenditure and body composition in diabetes mellitus complicating pregnancy” on a monthly basis. Please send copies of this to the Research Office (research@cmcvellore.ac.in).
ACKNOWLEDGEMENT

Words are not enough to express my gratitude towards my guide Prof. Nihal Thomas. His work ethics, decisiveness and an irrepressible enthusiasm and zeal to think beyond the ordinary are qualities that have motivated me during my tenure in the department. I wholeheartedly thank him for his encouragement, understanding and support whenever I have found myself going through a difficult phase.

I owe my most sincere gratitude to the entire team of researchers, doctors, diabetic educators, technicians and the support staff who make up the “Metabolic Clamp Team” at CMC Vellore. I avail this opportunity to thank Dr. Riddhi Das Gupta, for his keen interest, unparalleled encouragement and enthusiasm during this study. Without his immense professional insight and guidance, I would not have completed this study. I would like to specially mention my friends Dr Roshna, Dr Shajith and Dr Mathews whose brilliant scientific mind and rational thinking has helped me understand the nuances of human physiology in a wholly different light.

I also sincerely thank Prof. M.S. Simon Rajaratnam who stressed the importance of simplifying complex clinical problems and the need to pay attention to the translational aspects of research to patient care. I wish to express my warm and sincere thanks to Prof. Thomas Paul. His ideals and concepts have had a significant influence during the entire period of my residency. I sincerely thank Dr Asha, Dr. Dukhabandhu Naik, Dr. Nitin Kapoor and Dr Felix for their patience, insightful observations, helpful advice and wisdom that has helped me have a clear concept of apparently difficult topics.
In my daily work, I feel extremely blessed to have a friendly and cheerful group of fellow colleagues. I would like to express my heartfelt thanks to all my seniors and juniors for all the times we have worked together.

I take this opportunity to sincerely acknowledge RSSDI and CMC Fluid Research for providing financial assistance that helped me to perform my work comfortably.

I owe much to the patients and to all the individuals who consented to be a part of my study and made this work possible.

My special thanks to all the staff members of the department of Endocrinology, for their cooperation during the period of my research.

I express my sincere thanks to my husband and my parents for their constant help, support, encouragement and cooperation in designing my dissertation.

Finally, I thank the Almighty for having all these wonderful people and pray for his continuous blessings.

Dr. Geethu Antony

Dr. Geethu Antony
ACKNOWLEDGEMENT

No words will be sufficient enough to express my gratitude towards my guide Prof. Nihal Thomas. His work ethics decisiveness and an irrepressible enthusiasm and zeal to think beyond the ordinary are qualities that have motivated me during my tenure in the department. I wholeheartedly thank him for his encouragement, understanding and support whenever I have found myself going through a difficult phase.

I owe my most sincere gratitude to the entire team of researchers, doctors, diabetic educators, technicians and support staff who make up the “Metabolic Clamp Team” at CMC Vellore. I avail this opportunity to thank Dr. Riddhi Das Gupta, for his keen interest, unparalleled encouragement and enthusiasm during this study. Without his immense professional insight and guidance I would not have completed this study. I would like to specially mention my friends Dr Roshna, Dr Shajith and Dr Mathews whose brilliant scientific mind and rational thinking has helped me understand the nuances of human physiology in a wholly different light.

I also sincerely thank Prof. M.S. Simon Rajaratnam who stressed the importance of simplifying complex clinical problems and the need to pay attention to the translational aspects of research to patient care. I wish to express my warm and sincere thanks to Prof. Thomas Paul. His ideals and concepts have had a significant influence during the entire period of my residency. I sincerely thank Dr Asha, Dr.Dukhabandhu Naik and Dr Felix for their patience, insightful observations and
helpful advice and wisdom that has helped me have a clear concept of apparently
difficult topics.

In my daily work, I feel extremely blessed to have a friendly and cheerful group of
fellow colleagues. I would like to express my heartfelt thanks to all my seniors and
juniors for all the times we were working together.

I take this opportunity to sincerely acknowledge RSSDI and CMC Fluid Research for
providing financial assistance that helped me to perform my work comfortably.

I owe much to patients and to all the individuals who consented to be a part of my
study and made this work possible.

My special thanks to all staff members of department of Endocrinology, for their
cooperation during the period of my research.

I express my sincere thanks to my husband and my parents for their constant help,
support, encouragement and cooperation in designing my dissertation.

Finally, I thank Almighty for making all these wonderful people to happen to me and
pray for his continuous blessings.

Dr. Geethu Antony
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AEE</td>
<td>Activity Energy Expenditure</td>
</tr>
<tr>
<td>BEE</td>
<td>Basal Energy Expenditure</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>BMR</td>
<td>Basal Metabolic Rate</td>
</tr>
<tr>
<td>CV</td>
<td>Co-efficient of Variation</td>
</tr>
<tr>
<td>CMC</td>
<td>Christian Medical College,</td>
</tr>
<tr>
<td>DEXA</td>
<td>Dual Energy X-ray Absorptiometry</td>
</tr>
<tr>
<td>DIT</td>
<td>Diet Induced Thermogenesis</td>
</tr>
<tr>
<td>DRI</td>
<td>Dietary Inference Intake</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
</tr>
<tr>
<td>ESR</td>
<td>Erythrocyte Sedimentation Rate</td>
</tr>
<tr>
<td>EGP</td>
<td>Endogenous glucose production</td>
</tr>
<tr>
<td>eg</td>
<td>for example</td>
</tr>
<tr>
<td>etc</td>
<td>et cetera</td>
</tr>
<tr>
<td>FBS</td>
<td>Fasting Blood Sugar</td>
</tr>
<tr>
<td>FFA</td>
<td>Free Fatty Acid</td>
</tr>
<tr>
<td>FFM</td>
<td>Fat Free mass</td>
</tr>
<tr>
<td>FM</td>
<td>Fat Mass</td>
</tr>
<tr>
<td>FGIR</td>
<td>Fasting glucose insulin ratio</td>
</tr>
<tr>
<td>FSIVGTT</td>
<td>Frequently sampled intravenous glucose tolerance test</td>
</tr>
<tr>
<td>GDM</td>
<td>Gestational Diabetes Mellitus</td>
</tr>
</tbody>
</table>
ABBREVIATIONS

GWG: Gestational Weight Gain
HDL: High Density Lipoprotein
HbA1c: Glycosylated Haemoglobin
Hg: Mercury
HOMA IR: Homeostatic model assessment for insulin resistance
HR: Heart Rate
IADPSG: International Association of Diabetes and Pregnancy Study Group
IC: Indirect Calorimetry
IV: Intravenous
IGT: Impaired Glucose Tolerance
KJ: Kilo Joule
LBM: Lean Body Mass
LPL: Lipoprotein Lipase
MNT: Medical Nutrition Therapy
NA: Not applicable
NGT: Normal Glucose Tolerance
NEAT: Non-Exercise Activity Thermogenesis
NEFA: Non Esterified Fatty Acid
OGTT: Oral Glucose Tolerance Test
OPD: Out Patient Department
ABBREVIATIONS

PA: Physical Activity
PPBS: Post Prandial Blood Sugar
PTE: Post meal Thermogenesis
PPT: Postprandial thermogenesis
PPAQ: Pregnancy Physical Activity Questionnaire
QUICKI: Quantitative Insulin Sensitivity Check Index
RCT: Randomised Controlled Trial
RDA: Recommended Daily Allowance
REE: Resting Energy Expenditure
RQ: Respiratory Quotient
SD: Standard deviation
SFT: Skin fold thickness
SPSS: Statistical Package for the Social Sciences
TEE: Total Energy Expenditure
T1DM: Type 1 Diabetes Mellitus
T2DM: Type 2 Diabetes Mellitus
WHO: World Health Organization
ABSTRACT

TITLE OF THE ABSTRACT:
“A comprehensive study on energy expenditure and body composition in diabetes mellitus complicating pregnancy”

DEPARTMENT : Endocrinology, Diabetes and Metabolism, Christian Medical College, Vellore.

NAME OF THE CANDIDATE : Dr Geethu Antony

DEGREE AND SUBJECT : D.M (Endocrinology)

NAME OF THE GUIDE : Professor Nihal Thomas

Keywords :
1. Gestational diabetes Mellitus
2. Postmeal theromogenesis
3. Insulin resistance
4. resting energy expenditure (REE)
5. leptin
AIM / OBJECTIVES:
Gestational diabetes mellitus (GDM) is associated with significant alterations in energy and fat metabolism. But there is paucity of longitudinal data in pregnancy. Our study aimed at assessing the longitudinal changes in resting energy expenditure (REE), body composition and postmeal thermogenesis (PTE) in a population of women with GDM and normoglycemia during pregnancy and postpartum.

MATERIAL & METHODS
A total of 34 subjects - 21 subjects with GDM and 11 pregnant women with normal glucose tolerance were included in the study. The subjects were assessed longitudinally at three visits – early pregnancy, late pregnancy and postpartum period. REE was estimated using indirect calorimetry. Subsequently PTE was calculated by energy expenditure over 3 hours following a mixed meal challenge test. The body composition was assessed using bio impedance analysis, diet by 24 hour recall and activity energy expenditure (AEE) using the PPAQ questionnaire.

RESULTS
In GDM subjects,

- Body fat percentage was higher than the controls in early pregnancy. The longitudinal increment in fat percentage was lower when compared to the control group.
- The GDM subjects had lower insulin sensitivity in early pregnancy.
The Resting Energy Expenditure (REE) adjusted to fat free mass was lower in the GDM subjects compared to the controls in early pregnancy.

The Post meal Thermogenesis (PTE) was higher in the GDM group than that in the control group at all the three visits.

The longitudinal decrement in PTE during late pregnancy and postpartum period was significantly higher.

The leptin levels showed a longitudinal increment in the GDM group in late pregnancy and in the postpartum period.

CONCLUSIONS:

Our study is the first of its kind in Indian mothers showing significant alterations in basal, thermogenic and activity induced energy expenditure in pregnancy and in the postpartum period. Decrement in postmeal thermogenesis is the main metabolic defect contributing to glucose intolerance in pregnancy and in the postpartum period.
## Contents

<table>
<thead>
<tr>
<th>S.No</th>
<th>PARTICULARS</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>2.</td>
<td>AIMS AND OBJECTIVES</td>
<td>4</td>
</tr>
<tr>
<td>3.</td>
<td>REVIEW OF LITERATURE</td>
<td>5</td>
</tr>
<tr>
<td>4.</td>
<td>MATERIALS AND METHODS</td>
<td>34</td>
</tr>
<tr>
<td>5.</td>
<td>RESULTS AND ANALYSIS</td>
<td>42</td>
</tr>
<tr>
<td>6.</td>
<td>DISCUSSION</td>
<td>77</td>
</tr>
<tr>
<td>7.</td>
<td>SUMMARY AND CONCLUSIONS</td>
<td>91</td>
</tr>
<tr>
<td>8.</td>
<td>LIMITATIONS OF THE STUDY</td>
<td>93</td>
</tr>
<tr>
<td>9.</td>
<td>BIBLIOGRAPHY</td>
<td>94</td>
</tr>
</tbody>
</table>

### APPENDIX

- ENGLISH VERSION –
  - PATIENT INFORM CONSENT FORM AND INFORMATION SHEET
- TAMIL VERSION –
  - PATIENT INFORM CONSENT FORM AND INFORMATION SHEET
- TELUGU VERSION –
  - PATIENT INFORM CONSENT FORM AND INFORMATION SHEET

### PROFORMA
INTRODUCTION:
Changes in nutrient metabolism occur in pregnancy to ensure adequate fetal growth and development. Extra dietary energy is required during pregnancy to make up for the energy deposited in maternal and fetal tissues and for the increase in energy expenditure due to increase in basal metabolic rate and due to the change in energy cost of physical activity and diet. The energy requirement of pregnancy remains controversial due to uncertainties regarding maternal fat deposition and changes in energy expenditure. As a result the recommendations for nutritional intake in pregnancy are diverse and depend on the study population. Gestational diabetes mellitus is carbohydrate intolerance first recognized during pregnancy. The changes in nutrient metabolism are accentuated in women who develop diabetes in pregnancy. Overall there is an increase in basal energy expenditure with a decline in activity associated energy expenditure. Although most women with diabetes mellitus in pregnancy return to normal glucose tolerance following delivery, they remain at substantially increased risk of diabetes mellitus. These patients remain a valuable model for the detection of early metabolic abnormalities associated with development of diabetes mellitus. Changes in energy expenditure and body composition are key to the understanding the metabolic milieu of pregnancy and pathogenesis of diabetes complicating pregnancy. Dietary recommendations and therapeutic interventions in diabetes mellitus complicating pregnancy should be adjusted according to the gestational variations in energy expenditure and body composition. The influence of leptin on nutrient metabolism in pregnancy remains a hitherto conflicting area with suggestions of a possible role in accentuating insulin resistance.
We therefore propose a study to assess the longitudinal changes in energy expenditure and body composition in pregnancy and postpartum. We will be comparing the energy expenditure and body composition in pregnant women with normal glucose tolerance and pregnant women with diabetes mellitus complicating pregnancy. Previous studies have shown that there is significant increase in energy expenditure, after adjusting for free fat mass as the pregnancy advances. But the studies fail to show any significant difference among patient with diabetes complicating pregnancy and normal pregnant population. However, there is a paucity of studies that have looked at both the basal and activity associated energy expenditure simultaneously in a population of pregnant women with and without diabetes.

Although nutritional intervention for overt diabetes and gestational diabetes is a fundamental treatment modality, there is a paucity of evidence-based data on this topic. This study may help in assessing the energy requirements in the different stages of gestation in an Indian population and will help in formulating adequate nutritional recommendations in our population with diabetes mellitus complicating pregnancy.

Post prandial thermogenesis is known to decrease both in normal pregnancy and in gestational diabetes mellitus. The decrease in PPT is accentuated in patient with diabetes mellitus compared to normal pregnant population. The persistence of this defect in the post-partum period might be one of the reasons by which these patients are predisposed to obesity and type 2 diabetes mellitus. Both insulin resistance and sympathetic system has been thought to play an important role in postprandial thermogenesis. Various
studies have shown conflicting results in this perspective. Therefore we intend to assess the changes in postprandial thermogenesis.

Our study also aims to assess the changes in body composition during gestation and postpartum in pregnant women with and without diabetes. Body composition and changes in fat free mass may be implicitly linked with the variations in energy metabolism and crucial to the development of abnormal glucose metabolism in pregnancy.

The changes in adipokines and leptin during the various phases of gestation and effect on nutrient metabolism remain controversial. Previous studies have been conflicting on the role of leptin and altered glucose metabolism in diabetes complicating pregnancy. Our study is the first of its kind that aims to assess changes in energy expenditure, body composition, postprandial thermogenesis and role of leptin simultaneously in the same population of pregnant women with and without diabetes mellitus.
AIMS AND OBJECTIVES:

Primary Objectives

1. To assess the longitudinal changes in energy expenditure during pregnancy and postpartum in women with normal glucose tolerance and diabetes mellitus complicating pregnancy.

2. To assess post meal thermogenesis during pregnancy and postpartum in women with normal glucose tolerance and diabetes mellitus complicating pregnancy.

Secondary Objectives

1. To assess the longitudinal changes in body composition during pregnancy and postpartum in women with normal glucose tolerance and diabetes mellitus complicating pregnancy.

2. To assess leptin levels and its role in energy metabolism during pregnancy in women with normal glucose tolerance and diabetes mellitus complicating pregnancy.
REVIEW OF LITERATURE:

Pregnancy is a dynamic, anabolic state. Various hormones start secreting from placenta from early pregnancy which brings changes in nutrient metabolism, in addition to changes in the anatomy and physiology of the mother. These metabolic changes ensure continuous supply of nutrients to the growing fetus, for adequate growth and development while maintaining maternal homeostasis. Depending on the energy intake one or more of the following adjustments occur: accretion in new tissue or deposition in maternal stores, redistribution among tissues, and increased turnover or rate of metabolism.1 These adjustments in nutrient metabolism are complex and evolve continuously throughout the pregnancy and postpartum. These metabolic changes are accentuated in women who develop gestational diabetes mellitus. Understanding the metabolic alteration and change in energy expenditure and deposition in normal pregnancy and gestational diabetes mellitus will help us in formulating the adequate weight gain and calorie intake in this population.

DIABETES MELLITUS COMPLICATING PREGNANCY

Gestational diabetes mellitus is defined as carbohydrate intolerance of variable severity with onset or first recognition during the present pregnancy. The pathophysiology of GDM remains controversial, GDM may reflect a predisposition to type 2 diabetes mellitus expressed under the metabolic conditions of pregnancy or it may represent the extreme manifestation of metabolic alterations that occur in pregnancy.

Although gestational diabetes is most often diagnosed in late gestation, metabolic dysfunction starts well before conception and possibly based on the Barker hypothesis,
when the women herself was developing in utero. Maternal insulin resistance, seen in these women is related to the metabolic syndrome of obesity, inflammation, insulin resistance resulting in hyperglycaemia and hyperinsulinaemia. Because of the 60% decrease in insulin sensitivity during gestation, the predisposing baseline insulin resistance is further exacerbated and, when associated with β-cell dysfunction, results in mild hyperglycemia, which we refer to as gestational diabetes mellitus.

**DIAGNOSTIC CRITERIA**

Endocrine society guidelines recommend universal testing for diabetes in pregnancy with fasting plasma glucose, HbA1C, or untimed random plasma glucose at the first prenatal visit (before 13 weeks gestation or as soon as possible thereafter) for those women not known to have diabetes. In the case of overt diabetes, but not gestational diabetes, a second test (a fasting plasma glucose, untimed random plasma glucose, HbA1C, or OGTT) must be performed in the absence of symptoms of hyperglycemia and found to be abnormal on another day to confirm the diagnosis. Pregnant women not previously identified before 24 weeks gestation with overt diabetes or gestational diabetes be tested for gestational diabetes by having a 2-hour, 75-g oral glucose tolerance test (OGTT) performed at 24 to 28 weeks gestation.

**CARBOHYDRATE AND LIPID METABOLISM IN NORMAL PREGNANCY AND IN GESTATIONAL DIABETES MELLITUS**

Changes in carbohydrate and lipid metabolism occur during pregnancy to ensure a continuous supply of nutrients to the growing fetus despite intermittent maternal food
intake. The metabolic changes in pregnancy include impaired insulin response, decreased hepatic suppression of glucose production during insulin infusion and decreased insulin-stimulated glucose uptake in skeletal muscle, i.e. peripheral insulin resistance. During early pregnancy, glucose tolerance is normal or slightly improved. The peripheral sensitivity to insulin and hepatic basal glucose production is also normal in early pregnancy. The hyperinsulinemic euglycemic clamp studies done in pregnancy shows greater-than-normal sensitivity to the blood glucose–lowering effect of exogenously administered insulin in the first trimester than in the second and third trimesters. Insulin responses to oral glucose are also greater in the first trimester than before pregnancy. Longitudinal studies of glucose tolerance during gestation show a progressive increase in nutrient-stimulated insulin responses despite an only minor deterioration in glucose tolerance, consistent with progressive insulin resistance. The changes in insulin sensitivity from baseline, pre-gravid state through early pregnancy in lean women are inversely related to changes in maternal fat mass. The mechanisms, however, are not yet well defined. The hyperinsulinemic-euglycemic glucose clamp technique and intravenous-glucose-tolerance test indicate that insulin action in late normal pregnancy is 50–70% lower than that of normal, non-pregnant women. A progressive increase in basal and postprandial insulin concentrations is seen with advancing pregnancy. Obese pregnant women also develop peripheral and hepatic insulin resistance during the third trimester of pregnancy. The hyperinsulinemic-euglycemic glucose clamp technique indicates that insulin-stimulated glucose disappearance, carbohydrate oxidation, and suppression of endogenous glucose production in obese women are reduced in the third compared to the second trimester.
Glucose tolerance deteriorates in human pregnancy, but about 97-98% of all pregnant women retain a normal glucose tolerance and only 2-3% develops gestational diabetes. GDM is not due to defective secretion of insulin or due to disproportionate secretion of proinsulin or glucagon. Only quantitative differences in insulin secretion have been observed between women with GDM and normal pregnant women. Evidence supports the view that GDM is related to a pronounced peripheral resistance to insulin. Carbohydrate metabolism has been studied by using intravenous- glucose-tolerance test and hyperinsulinemic-euglycemic clamp with [6,6-2H] glucose before conception and in early and late gestation in non-obese women who were predisposed to and developed GDM. Basal endogenous glucose production increases similarly in patients with GDM and in control subjects throughout gestation. An increase in first-phase insulin response is observed in control subjects and in patients with GDM with advancing pregnancy; however, the increase is greater in control subjects. In late pregnancy, insulin suppression of hepatic glucose production is less in patients with GDM (80%) than in control subjects (96%). Catalano et al found that decreased insulin-stimulated glucose disposal preceded the development of decreased insulin response in women with GDM and was evident before pregnancy. The relative decrease in first-phase insulin response, as the first manifestation of beta cell dysfunction, and impaired suppression of hepatic glucose production becomes evident only after progressive decreased insulin sensitivity in late gestation, resulting in hyperglycemia.

GDM is accompanied by alterations in fasting, postprandial, and integrated 24-hour plasma concentrations of amino acids, glucose, and lipids. These changes include a 3-
fold increase in plasma triacylglycerol concentrations during the third trimester of pregnancy, elevation of plasma fatty acids, delayed postprandial clearance of fatty acids, and elevation of the branched-chain amino acids.\textsuperscript{11}

Changes in lipid metabolism promote the accumulation of maternal fat stores in early and mid-pregnancy and enhance fat mobilization in late pregnancy. In early pregnancy, increased estrogen, progesterone, and insulin favor lipid deposition and inhibit lipolysis. LPL activity in the adipose tissue from the femoral region, but not from the abdominal region, is elevated at 8–11 wk of gestation.\textsuperscript{12} Lipolysis in response to catecholamines is markedly higher in the abdominal than in the femoral region. The femoral cells are virtually unresponsive to catecholamines in pregnancy. In late pregnancy, HCS promotes lipolysis and fat mobilization. The increase in plasma fatty acid and glycerol concentrations is consistent with mobilization of lipid stores. This shift from an anabolic to a catabolic state promotes the use of lipids as a maternal energy source while preserving glucose and amino acids for the fetus. With prolonged fasting (48 h), as well as shorter periods of fasting (18 h), there is a rapid diversion of maternal metabolism to fat oxidation, with an elaboration of ketones.\textsuperscript{11} Decreases in plasma glucose, insulin, and alanine, and increases in plasma fatty acid and beta-hydroxybutyrate are seen in pregnant women hours before these changes are seen in non-pregnant women.\textsuperscript{13} The enhanced lipolysis and ketogenesis allow pregnant women to utilize stored lipid to subsidize energy needs and minimize protein catabolism.
ENERGY EXPENDITURE

The Total Energy Expenditure (TEE) is defined as the amount of heat energy used by the human body for daily functioning, and can be divided into 3 main components.

1. BEE (Basal Energy Expenditure) or REE (Resting Energy Expenditure)
2. DIT (diet induced thermogenesis): energy used during substrate metabolism
3. AEE (activity energy expenditure): energy used in physical activity

BEE or REE is the energy required to maintain the body’s basic cellular metabolic activity and organ functions, such as respiration and normal body temperature maintenance in the absence of recent food intake, physical activity, and psychological stress. BMR is the energy expended when an individual is lying at complete rest, in the morning after sleep in the post-absorptive state. In adults with sedentary life style BMR accounts for approximately 60% of the total daily energy expenditure and is mainly contributed by lean body mass. Resting energy expenditure, in general, is within 10% of the BMR. Diet induced thermogenesis is the energy expenditure associated with digestion, absorption and storage of food, and accounts for approximately 10% of the total energy expenditure. Diet induced thermogenesis has obligatory and facultative components. The obligatory component encompasses the fixed costs of digesting, absorbing, processing, and storing nutrients, and the facultative component is of variable magnitude. Activity thermogenesis is the thermogenesis that accompanies physical activities and is the most variable component of daily energy expenditure and can constitute 15 to 30% of 24-h energy expenditure. This can be divided into exercise and non-exercise activity thermogenesis (NEAT). NEAT or the ‘energy expenditure of spontaneous physical activity’ encompasses the combined energy costs of the physical
activities of daily living, fidgeting, spontaneous muscle contraction and maintaining posture when not recumbent. Other thermogenic variables may also need to be considered, such as the energetic costs of altered temperature, medications and emotion. Each of these components of energy expenditure is highly variable and the total effect of these variances determines the variability in daily energy expenditure between individuals.

A number of factors could either increase or decrease the measured REE and hence could act as potential sources of error. These should be thoroughly evaluated before applying the REE data to any clinical setting. A number of studies have shown that fat-free lean mass most closely correlates with REE independent of age, BMI, glycemic status and other metabolic variables. While REE is found to be higher in males, fever, cold exposure and hypothermia have also been implicated in causing an elevated REE. Studies have further shown 20-30% of diseased state initially cause decline in REE due to release of catabolic counter regulatory factors and drop in VO2 preceding hemodynamic instability. Subsequently around 65-75% of diseased states cause increase in REE. Interestingly, routine nursing procedures, such as a bed bath, dressing change, or repositioning, even in comatose patients, have all been documented to increase energy expenditure by 20% to 36%. The other important determinant of REE is concomitant drug usage. Of these agents like caffeine, nicotine and catecholamines can cause an increase in REE measuring up to 10% -20%. Decline in REE is usually associated with sedatives, analgesics and alpha and beta blockers.
Measurement of energy expenditure

The measurement of energy expenditure can be done using any of the 3 principles.

1. Indirect calorimetry
2. Direct calorimetry
3. Non calorimetric measures

Direct Calorimetry

Direct calorimeters measure the heat lost from the body. Radiative and convective heat losses account for approximately 80% of the total heat loss, while evaporative heat loss accounts for the remainder. Conductive heat loss is negligible in humans. There are three principal types of direct calorimeter: isothermal, heat sink and convection systems. These techniques require extreme expertise and are expensive to use in clinical and research settings.19

Non Calorimetric Methods

Non-calorimetric methods estimate energy expenditure by extrapolation from variables that relate to energy expenditure.19 These methods are often standardized against the calorimetric methods. Non calorimetric methods include

1. Isotope dilution doubly labelled water

Field-based measurements of total daily energy expenditure over 7–21 days can be obtained using doubly labelled water. The major advantage of doubly labelled water measurements is that accurate measurements (error of, 7%) of total daily energy expenditure are obtained in truly free-living individuals. There are important limitations; first, no information is obtained regarding the components of activity thermogenesis. Second, the thermic effect of food is not measured and is known to be variable (most
believe this to introduce only a small error). Third, O18 is expensive, thereby potentially limiting the number of subjects that can be studied.

2. Physiological methods

Heart rate monitoring,
Integrated electromyography,
Pulmonary ventilation volume
Thermal imaging

The role of newer technologies such as thermal imaging or global positioning remains to be determined but should be explored.

Indirect calorimetry

There are a number of modalities of indirect calorimetry being used in practice. One of the earliest tools used is a Douglas Bag which requires technical expertise and expensive analyser equipment, apart from being prone to frequent air leaks. The modern calorimeters are based on the multi-component metabolic carts\textsuperscript{20} that encompass the different devices like a hood/mouthpiece, gas analyzers and mixing chambers. Novel modalities involving heat flux sensors mounted on a small armband are being used experimentally in the ambulatory setting for lifestyle modification such as weight management, fitness improvement, and diabetes care.\textsuperscript{21} However, long term data validating these new techniques are lacking.

Pre-requisites for measurement

A number of studies have focused on the optimum conditions for carrying out an indirect calorimetry. Measurements must be conducted with strict adherence to resting conditions for accurate results.\textsuperscript{22} Measurements should be performed in a quiet
environment with the individual resting for 10–15 minutes before the measurement. The subject should be fasting for at least 6 hours, avoid exercise for at least 4 hours and avoid nicotine, caffeine and stimulatory nutritional supplements for at least 4 hours prior to the calorimetric assessment.

**Calculation of Resting Energy Expenditure: Weir’s Equation:**

Human energy stems from chemical energy, which is released from nutrients through the oxidation of food substrates. Carbon-based nutrients (i.e., fuels) are converted into carbon dioxide (CO2), water (H2O), and heat in the presence of oxygen (O2). Indirect calorimetry (IC) assesses the amount of heat generated indirectly according to the amount and pattern of substrate use and byproducts production. Specifically, energy expenditure can be calculated by measuring the amount of oxygen used and carbon dioxide released, by the body.

The specific amount of oxygen used is called oxygen consumption (VO2), whereas the amount of carbon dioxide gas produced by the cells is called carbon dioxide production (VCO2). The calculation of VO2 and VCO2 forms the inherent principle of indirect calorimetry. Total average daily energy expenditure in kcal is usually calculated using the modified Weir equation as follows: 23

\[
\text{Energy expenditure (kcal/d)} = [(\text{VO}_2 \times 3.941) + (\text{VCO}_2 \times 1.11) + (\text{uN}_2 \times 2.17)] \times 1440
\]

The urinary nitrogen component (uN2) is often excluded when calculating energy expenditure because it only accounts for around 4% of the true energy expenditure and contributes to a small error of 1%–2% in the calculation of final energy expenditure in both inpatients and outpatients. Thus the abbreviated equation is commonly used. 14

\[
\text{Energy expenditure (kcal/d)} = [(\text{VO}_2 \times 3.941) + (\text{VCO}_2 \times 1.11)] \times 1440
\]
Respiratory Quotient (RQ)

The estimation of Respiratory Quotient is integral to the calculation of energy expenditure by indirect calorimetry. RQ is defined as the ratio between VCO2 and VO2 (ie, VCO2/VO2) and reflects substrate use. The complete oxidation of glucose in a system yields an RQ of 1. However depending on the substrate oxidized, the value of RQ tends to vary. The physiological RQ tends to vary between 0.67-1.20. The RQ for lipids and proteins are approximately 0.69 and 0.82 respectively.

Factors influencing RQ:

An RQ value within the range validates the indirect calorimetry measurements. The value of RQ measured in an indirect calorimetry setting if found to lie outside the normal physiological range may have different connotations. The commonly encountered pitfalls in measurement of RQ include air leaks in the respiratory circuit, extreme pain or agitation during the measurement or subjects who have recently underwent procedures that affect gas exchange (eg., hemodialysis). Under or overfeeding can also affect RQ, as can the proportion of carbohydrate and fat in the diet.

Indirect Calorimetry offers a scientifically based approach for customizing a patient’s energy needs and nutrient delivery to maximize the benefits of nutrition therapy. Traditionally, IC has been underused, mostly due to costs, shortage of personnel, and lack of education or training. With recent advances in technology, indirect calorimeters are easier to operate, more portable, and affordable. Increased use of indirect calorimetry would facilitate individualized patient care and should lead to improved
treatment outcomes. Additionally, it facilitates the generation of energy expenditure data specific to different disease states, medical conditions, or patient subpopulations.

**BASAL METABOLIC RATE**

BMR should be measured between 06.00 and 09.00 hours in individuals who slept at the site of measurement overnight. The individuals should not have consumed food or energy-containing beverage for 9 hours prior to the measurement but may have consumed water. The measurement should be performed with the patient supine. A single pillow may support the subject’s head and/or the head of the bed should be at a vertical tilt. The subject should be in thermal comfort and the room should not be brightly lit. Subjects should be instructed to lie motionless and should not be allowed to talk or have other potentially stimulating distractions during the measurement. The measurement period should last for 20–40 minutes.

**RESTING ENERGY EXPENDITURE**

Resting energy expenditure should be performed in the post prandial state, at least 6 hours after consumption of any calories or performing any rigorous activity. Subjects should be fully rested while supine for 60 minutes prior to the measurement. The measurement is otherwise as described for BMR.

**THERMIC EFFECT OF FOOD**

Optimally, a measurement of BMR should be performed first, and then subjects should be provided with a meal of food. The energy content of the food should be known precisely and should be of 400 kcal or greater. Energy expenditure should then be measured for 400 minutes or until energy expenditure falls to within 5% of the BMR.
The thermic effect of food for the meals provided is calculated from the area under the energy expenditure above basal metabolic rate versus the time curve.

**BODY COMPOSITION**

Prevalence of overweight and obesity has been on rise over the past few years. Along with this increase in prevalence of obesity, there has been an increase in pregravid body mass index and gestational weight gain affecting maternal body composition changes in pregnancy. The body exhibits dynamic changes in composition during pregnancy to support the fetus. These changes are reflected in gestational weight gain (GWG), which includes gains in maternal and fetal fat mass (FM) and fat-free mass (FFM), as well as the placenta and amniotic fluid. During pregnancy, many of the assumptions inherent in body composition estimation are violated, particularly the hydration of fat-free mass, and available methods are unable to disentangle maternal composition from fetus and supporting tissues; therefore, estimates of maternal body composition during pregnancy are prone to error. Most methods theoretically divide the body into compartments from which an estimate of FM is derived. The two-compartment model divides the body into FM and FFM, while the three-compartment model further sub-divides the FFM compartment into water and a combination of mineral and protein. The four-compartment models further subdivide the FFM compartment into mineral, water and protein. The Institute of Medicine has indicated that these models are ‘satisfactory’ for estimating body composition changes in pregnancy, given that corrected values for hydration and density of FFM are applied. The available methods for assessing body composition changes in pregnancy, include
• Bioelectrical Impedance Analysis (BIA)

BIA is an inexpensive, rapid and non-invasive method for estimating body composition. BIA is based on the assumptions and relationships regarding electrical properties of various biological tissues at varying frequencies. BIA devices use an alternating current with very low amperage that uses the water content of the body as a conductor. The impedance, or opposition, of the electrical flow by tissues allows for estimation of TBW from which estimates of fat and FFM can be derived. Bio impedance analysis is considered safe in pregnancy. There are several factors which compromise the validity of this technique in pregnancy. First, estimates of TBW are influenced by the ratio of intracellular (ICW) to extracellular water (ECW), which changes markedly throughout pregnancy compared with a non-pregnant state and is likely to vary between women and by gestational age.27 The Model utilizing wrist-to-ankle BIS was developed in non-pregnant populations and may not be suitable for pregnancy, where greater water is located in the trunk region compared with non-pregnant populations, and therefore suggest development of a new model for BIS for assessment of body water in pregnancy.28

• Dual Energy X-ray Absorptiometry (DEXA)

This measures body fat and fat free mass with minimum radiation exposure (1 μ seivert). It uses the principle of measuring the attenuation difference between two x-ray beams of different strengths. The co-efficient of variation (CV) for total body fat measurement is <1% and for regional fat estimation <3% (precision error- 1kg). DXA is unsuitable in pregnancy due to radiation exposure; however, DXA is used before and after pregnancy to measure bone mineral content (BMC).
Review of literature

• Imaging – Magnetic Resonance Imaging and Computed Tomography

Imaging methods, including computed tomography and magnetic resonance imaging (MRI) and three-dimensional photonic scanning (3DPS), can be utilized to estimate body composition; however, 3DPS and MRI are still in the exploratory stages for pregnancy, while computed tomography is contraindicated due to radiation exposure and has not been utilized to evaluate changes from pre pregnancy to postpartum. There are no known risks to the use of MRI at low field strengths (for example, 1.5 Tesla) but its safety during the first trimester has not been sufficiently evaluated. There are no published studies to date that have used MRI to estimate changes in maternal body composition during pregnancy.

• Ultrasound

Several cross-sectional and longitudinal studies have used ultrasound measurements in pregnancy to measure maternal regional subcutaneous and visceral fat; however, standardized protocols for body fat assessment with ultrasound have not been developed.

• Densitometry

Body density can be estimated using hydrodensitometry (HD), otherwise known as underwater weighing, or air-displacement plethysmography (ADP) from which estimates of body composition of the combined, maternal–fetal unit can be derived. These methods are not suitable for field research and require specialized equipment.

• Total Body Water

TBW is typically measured using the dilution principle with isotope-labeled water labeled with deuterium (2H2O) or Oxygen 18 (18O), which provides an estimate of
TBW in the combined maternal and fetal unit. During pregnancy, TBW changes are highly variable. Several studies have reported TBW accretion of approximately 5–8 liters over the course of pregnancy. TBW measurements using stable isotope methods are considered safe in pregnancy, the correction factors for TBW estimates needed to derive body composition estimates may need to be population specific.

**Anthropometry**

Anthropometric measurements, particularly skin fold thickness (SFT) and mid-upper arm circumferences, have been used extensively to estimate changes in body composition in pregnancy. Typically FM changes are estimated using equations with body weight, SFT and often circumference measures. Estimates of body fat changes derived from skin folds are prone to measurement error, especially during pregnancy. Several factors, including initial size, parity, race and socioeconomic status, have been established as predictors of GWG; however, whether these factors independently predict overall body composition changes across pregnancy is unclear due to limited studies in this area. Although there is a growing body of research focusing on perinatal, offspring and maternal outcomes of GWG, there is a dearth of information on the short- and long-term outcomes of body composition changes during pregnancy on offspring and maternal outcomes and also whether these associations vary by initial BMI and body composition (FM, FFM). This is largely due to challenges in measuring body composition during pregnancy. Several studies have established that the overall composition of weight gain, specifically gains in body water and/or lean mass, are associated with greater offspring birth weight, whereas gains in fat are not associated with birth weight. Finally, studies that examine determinants and outcomes of body
composition changes in pregnancy are needed in order to guide future interventions and public health policies to optimize maternal health in pregnancy and maternal and offspring health postpartum.

**Energy Expenditure and Body Composition in Normal Pregnancy**

In pregnancy, as in the non-pregnant state, energy is required for basal metabolic requirements, growth, physical activity, and the metabolic response to food. Clearly the main factor differentiating energy balance during pregnancy from that in the non-pregnant state is the extra allowance necessary for the additional growth of fetal and maternal tissues as well as the extra energy required for maintaining this increased tissue mass. The energy cost of pregnancy and the amount of fat accretion during gestation vary considerably among published studies. The principal component of energy expenditure—that is, the basal metabolic rate—has been extensively investigated in pregnancy. Traditionally, the energy requirements of pregnant women have been derived from the increment in BMR and energy deposited in tissues. This factorial approach ignores potential energy expenditure changes in physical activity and the thermic effect of feeding. All the factors have to be considered while assessing the energy expenditure and energy requirement in pregnancy.

Estimates of the energy cost of pregnancy range from a cost of 80,000 kcal to a net savings of 10,000 kcal. Similarly, the increase in adipose tissue during gestation has a wide variation. Forsum et al reported a mean increase of 5 kg of adipose tissue in Swedish women, whereas Lawrence et al. found no increase in adipose tissue stores in women from the Gambia with their usual nutritional intake.
The total amount of energy required for pregnancy was calculated by Hytten and Leitch in 1964 by separating weight gain in pregnancy into the different chemical components on the basis of existing data and calculating the energy equivalents of these components from the heat of combustion. By this method the energy cost of 335 MJ (80000 kcal) for the whole pregnancy was reached. The recent studies have shown that the pregnant women do not significantly increase their energy intake above the non-pregnant level. The original calculation of Hytten and Leitch remains valid, and it seems more likely that the apparent energetic discrepancy is due to a physiological adaptation during pregnancy rather than any inherent error in the Hytten calculation. In attempting to investigate this problem different aspect of energy expenditure in pregnancy have to be measured, which includes basal metabolic rate, Physical activity expenditure and diet induced thermogenesis.

**Total and Basal Energy Expenditure**

During pregnancy, energy expenditure generally rises because of increases in maternal and fetal weight. However, the variability in metabolic response among women is striking and has been attributed to differences in body fatness. Declines in basal metabolic rate (BMR) and in the energy costs of exercise may indicate energy conservation or augmented metabolic efficiency in some pregnant women. Conflicting data have been reported on BMR in lactating women, with some authors reporting that it increased and others finding that it remained unchanged.

In a study done by Butt NF et al, and published in 1999; energy expenditure and body composition was compared between late gestation and post-partum. Energy expenditure, body composition, and hormone, metabolite, and catecholamine
concentrations in 76 women (40 lactating, 36 non-lactating) were assessed at 37 weeks of gestation and at 3 and 6 months postpartum. TEE and BMR were 15–26% higher during pregnancy than postpartum after being adjusted for FFM, fat mass, and energy balance. TEE and BMR were higher in lactating than in non-lactating women. Fasting serum insulin, insulin-like growth factor I, fatty acids, and leptin and 24-h urinary free norepinephrine, epinephrine, and dopamine correlated positively with TEE and BMR.

Another longitudinal study done by Butt et al assessed the longitudinal change in energy requirements of healthy underweight, normal-weight, and overweight pregnant women. The energy requirements of 63 women [17 with a low BMI, 34 with a normal BMI, and 12 with a high BMI] were estimated at 0, 9, 22, and 36 week of pregnancy and at 27 week postpartum. Basal metabolic rate (BMR) was measured by calorimetry, total energy expenditure (TEE) by double labelled water, and activity energy expenditure (AEE) was estimated as TEE-BMR. Energy deposition was calculated from changes in body protein and fat. Energy requirements were calculated as the sum of TEE and energy deposition. BMR increased gradually throughout pregnancy at a mean (SD) rate of 10.7(5.4) kcal/gestational week, whereas TEE increased by 5.2(12.8) kcal/gestational week, which indicated a slight decrease in AEE. Energy costs of pregnancy depended on BMI group. Although total protein deposition did not differ significantly by BMI group (mean for the 3 groups: 611 g protein), FM deposition did (5.3, 4.6, and 8.4 kg FM in the low-, normal-, and high-BMI groups; P_0.02). Thus, energy costs differed significantly by BMI group (P_0.02). In the normal-BMI group, energy requirements increased negligibly in the first trimester, by 350 kcal/d in the second trimester, and by 500 kcal/d in the third trimester.
Activity Induced thermogenesis

Physical activity and the related thermogenesis is known to decrease as pregnancy advances.\textsuperscript{34,38} The TEE of pregnant women remains controversial largely because of varying data on the extent of the reduction in PA in pregnancy. Reductions in the PA level in late pregnancy compared with the non-pregnant state may occur because of difficulties in movement related to larger body. The pregnancy physical activity patterns have so far been assessed through questionnaires\textsuperscript{28} or interviews.\textsuperscript{39} However, the reliability and validity of all self-reported methods is limited because of misreporting or miscoding of activities, inaccurate estimation of activity intensity or duration and differences in body mass. Various Questionnaires are available to assess the activity in different population. In pregnancy, the use of PPAQ has been validated. In the reproducibility and validity study of a self-administered PPAQ, moderate to high reproducibility was observed for total activity as well as for activities of varying intensities and types.\textsuperscript{40} The PPAQ was reasonably accurate in detecting sedentary, light, moderate, and vigorous intensity activities of a broad range of types (household/ care giving, occupational, and sports/exercise activities) among ethnically diverse pregnant women.\textsuperscript{40}

The doubly labelled water (DLW) method, considered the golden standard for measuring TEE and activity energy expenditure (AEE), does not provide specific information on the PA patterns. Accelerometry and heart rate (HR) recording have their own limitations when used alone. Although accelerometry is unable to account for increases in AEE during stepping, cycling, changing grade during walking or load bearing activities, HR measurements are affected by other factors, such as training state,
mental stress, dehydration or extreme ambient temperature. The combination of a HR and movement sensor was shown to give precise estimates of AEE and PA patterns during a wide range of activities (from low through moderate and high activities).

**Postprandial Thermogenesis or Diet Induced Thermogenesis**

The Diet induced thermogenesis consists of obligative and facultative components. Obligative costs are those incurred by the energy demands for digestion, absorption and storage of nutrients. Facultative expenditure is energy spent in excess of that required for the processing of nutrients. Obesity has been linked with a reduction in both obligative and facultative expenditure. Decreased obligative energy cost in obesity is associated with insulin resistance and altered glucose metabolism, whereas differences in facultative expenditure are associated with stimulation of the sympathetic nervous system, Na pumping, substrate recycling and protein synthesis. Various studies have shown pregnancy induces changes in obligative and facultative expenditure. An increased rate of synthesis of new tissue due to fetal and maternal growth could increase obligative expenditure. Alternatively, hormonal changes may enable the pregnant woman to reduce thermogenesis expenditure, at least in part, the increased energy requirement for basal metabolism and tissue deposition. For example, insulin resistance, which accompanies pregnancy, could reduce postprandial expenditure. Studies of the effect of pregnancy on thermogenesis have been inconsistent. Nagy & King et al failed to find a reduction in the TEF in early and late pregnancy following a 3.14 MJ (750 kcal) mixed meal challenge. Prentice et al, similarly noted that there was little variability in thermogenesis in eight women studied longitudinally over the course of pregnancy. There are conflicting results in other studies. Illingworth et al found a reduction in
postprandial expenditure in the second trimester (25-28 weeks gestation) of pregnancy, but not during early or late gestation. The energy saving during mid-gestation was small and amounted to a difference of only 22 kJ (5 kcal). Another study observed 29 kJ (7 kcal) and 55 kJ (13 kcal) savings in postprandial expenditure in the second and third trimesters of pregnancy respectively. The study estimated that 38.6 MJ of the total energy requirement was saved by the fall in thermogenesis during the latter two trimesters. In the study postprandial thermogenesis correlated positively with insulin sensitivity, as assessed by the decline in plasma glucose following a bolus of intravenous insulin. The authors concluded that insulin insensitivity was responsible for the reduction in thermogenesis. The exact role of insulin in the thermic effect of food remains controversial. An increase in serum insulin concentration leads to an increase in energy expenditure but similar studies have found no correlation between the insulin response to a meal and the thermic response. These findings in association with the observation that the two phenomena of suppressed energy expenditure and increasing insulin resistance are maximal at different times in the pregnancy suggest that the two processes are not causally related.

It is difficult to determine why the results of these studies differ. All pregnant women studied have been glucose tolerant despite differences in insulin sensitivity. It seems most likely that differences in study design and experimental methodology, along with the heterogeneity amongst pregnant women, explain the different results. These varying results stress the need of a similar in Indian population to understand the variation in metabolism.
Decrease in postpartum thermogenesis has been documented in both lean and obese type diabetes mellitus. It has been shown in different studies that the decrease in PPT which develop during gestational diabetes persists in to the post partum period\textsuperscript{50} and there is evidence that this reduced energy expenditure that results from decreased PPT might provide one of the mechanisms by which individuals are predisposed to obesity and type 2 diabetes.\textsuperscript{51} Similar study was conducted in a large group of European women with a history of GDM. These women were normoglycemic at the time of the study and were matched for ethnicity, age, parity, and time since delivery with a control population. The study assessed postprandial thermogenesis (PPT) for 3 h following a mixed meal in 29 normoglycemic European women with previous gestational diabetes (GDM), compared with 37 control women. Given the potential role of catecholamines and insulin in the regulation of PPT, the study assessed insulin and catecholamine responses to the meal. There were no significant differences in REE between the two groups whether assessed in absolute terms or after correction for LBM. Although mean values of total PPT were lower in the GDM group, this difference did not quite attain statistical significance ($P \leq 0.052$ one-sided). However, there was a marked difference in the shape of the PPT curve, suggestive of delay in PPT, between the two groups, as quantified by the lower PPT rate at 30 min post prandially in the GDM group. There was a consistent delay in insulin, and noradrenalin responses to the meal in the GDM group. Although the biological significance of the delayed PPT response is uncertain, one possibility is that this is an early metabolic manifestation that precedes an absolute decrease in PPT in these women with post-GDM which predisposes to diabetes mellitus. Due to the
variability in these studies with post meal thermogenesis larger studies are required which should include subjects from all ethnic groups, to arrive at a consistent result.

**Role of Leptin in Nurtient Metabolism in Pregnancy**

Although the source of leptin is well documented, the role of the increased maternal leptin concentrations during gestation has remained elusive. In addition to maternal adipose tissue the placenta produces leptin, and leptin concentrations fall within 48 h of delivery. Although leptin was originally thought to be related only to appetite suppression via central mechanisms, further reports pointed to a role of leptin in the control of energy expenditure. In obese subjects, leptin may have a stimulatory effect on fat oxidation by peripheral tissues. Minokoshi et al. have reported that leptin stimulates fat oxidation in skeletal muscle by activating AMP-activated protein kinase (AMPK) and then AMPK activation allows phosphorylation of acetyl-coenzyme A carboxylase, resulting in potent stimulation of fatty acid oxidation in muscle. Hyperleptinemia down regulates expression of lipogenic enzymes and up regulates enzymes of fatty acid oxidation. Hence, increase in maternal leptin, possibly from placental sources, may affect the increases in fat oxidation observed in obese subjects.

**Changes in Energy expenditure and Body composition in Diabetes Mellitus complicating Pregnancy**

As discussed earlier, not many studies have been done so far assessing the changes in energy expenditure and body composition in pregnant women with diabetes mellitus. Study done by Okereke et al; published in 2004 assessed the longitudinal changes in energy expenditure and body composition in obese pregnant women with normal glucose tolerance and diabetes mellitus. Fifteen obese women, eight with NGT and
seven with GDM, were evaluated before conception (P), at 12–14 wk (E), and at 34–36 wk (L). Energy expenditure and glucose and fat metabolism were measured using indirect calorimetry. Basal hepatic glucose production was measured using [6,6-2H2]glucose and insulin sensitivity by euglycemic clamp. Total weight gain in all of these women was 12.1 ± 3.9 kg. There was a significant (P < 0.0001) increase in weight and body composition over time in all subjects. There was a significant increase (6.6 kg, P < 0.0001) in fat mass from P to L. There was also a significant (P < 0.001) increase in the sum of the seven skin fold measurements. There were no significant differences in any of the body composition measurements between groups. There was a 30% increase in basal energy expenditure from P to L, whether expressed as basal VO2, milliliters per minute, or kilocalories per day. After adjustment for FFM, the increase in energy expenditure was 14% in the NGT and 21% in the GDM subjects. These differences did not reach statistical significance (P < 0.3 to 0.5) because of the great inter individual variability and may be due to small sample size. There were no significant changes in carbohydrate oxidation during fasting or storage from P to L. There was, however, a significant (P < 0.0001) 150% increase in basal fat oxidation (mg/min) from P to L. The study concluded that during pregnancy in obese women, there are significant alterations in body composition and energy expenditure among individuals but no difference was noted between women with NGT and those with GDM. There are significant increases in fat mass and basal metabolic rate and an increased reliance on lipid metabolism both in the basal state and during insulin infusion, unlike in lean subjects.

Similar study was done in lean subjects who had abnormal glucose tolerance before conception by Catalano et al.8 The study hypothesized that woman with decreased
insulin sensitivity before conception would have less fat accretion and smaller increases in energy expenditure. Six women with normal glucose tolerance and 10 women with abnormal glucose tolerance were evaluated before conception, and in early (12 to 14 weeks) and late (34 to 36 weeks) gestation. Body composition was estimated by hydrodensitometry, resting energy expenditure, and glucose and fat metabolism by indirect calorimetry, endogenous glucose production by infusion of [6-6 2H2] glucose, and insulin sensitivity using a hyperinsulinemic-euglycemic clamp (40 mU/m2/min). There was a smaller increase in fat mass (1.3 kg [P = .04]) in early pregnancy in women with abnormal glucose tolerance before pregnancy. Indirect calorimetry measured gestational age-related increases in basal oxygen utilization, with or without correction for fat-free mass (VO2, P = .002), resting energy expenditure (expressed in kilocalories, P = .0001), and carbohydrate oxidation (P = .0003). In early pregnancy, changes in fat mass correlated inversely with changes in insulin sensitivity (r= -0.52, P = .04). In early gestation, the changes in maternal fat mass and basal oxygen consumption are inversely related to the changes in insulin sensitivity. This response in lean women with decreased insulin sensitivity before conception may have survival value by providing a larger amount of available substrate to meet fetoplacental needs during gestation.

**Indian Data on Energy expenditure in Pregnancy**

A study was published in 1998 by Das et al comparing the longitudinal changes in basal energy expenditure in pregnant women and non-pregnant women. The mean ± SD of BEE were found to be 34.04 +/- 3.05, 35.85 +/- 2.60 and 39.69 +/- 2.75 Kcal/m2/hr during first, second and third trimesters of pregnancy respectively. BEE was progressively and significantly increased (P < 0.01). However, increase in BEE during
first trimester of pregnancy compared to that of luteal phase of menstrual cycle was insignificant. The results indicate that Indian pregnant women should maintain energy requirements by increasing caloric intake throughout the gestation.

Another study was done in South India which compared changes in energy expenditure, body composition and calorie intake in pregnant women. The study measured Basal metabolic rate (BMR), thermic effect of a meal (ThM), anthropometry, and dietary intakes in 18 control subjects and in 18 pregnant women at 12, 24, and 34 week gestation; and in 17 of these women at 12 and 24 week postpartum, to uncover any metabolic economy associated with either pregnancy or lactation. Energy expenditure was measured by respiratory gas exchange measurements in a ventilated hood. ThM was calculated by obtaining the mean increment in energy expenditure during the measurement period (30 mm) in each hour (which was considered representative for the entire hour) above pre-meal basal values. The post meal total energy output was calculated by obtaining the total energy expenditure during the 5 h after the ingestion of the test meal.

Mean weight gain from 12 week gestation to term was 11.4 ± 3.7 kg; mean birth weight of the infants was 3.06 ± 0.41 kg. Estimated gain in adipose tissue and fat mass were 3.1 ± 3.6 and 2.5 ± 2.9 kg, respectively. Energy cost of pregnancy was estimated to be 303 ± 171 MJ. The cumulative increase in energy intake over the last two trimesters of pregnancy was 290 ± 280 MJ, meeting a large part of the total estimated cost of pregnancy. In the study BMR was significantly higher (P < 0.05) in the pregnant and lactating group at 12 week gestation compared with the control group, and when expressed per kilogram body weight was almost 7% higher than in the control group,
although this was not significant. There was no significant increase in BMR per kilogram body weight with the progression of pregnancy. There was no significant reduction in TEM during pregnancy and its role as a possible adaptive mechanism to conserve energy during pregnancy was not supported by data in this study. The results indicated that the BMR and ThM were not associated with any energy saving either during pregnancy or lactation. The extra energy required during pregnancy and lactation appeared to have been met largely by increases in energy intake, rather than by any metabolic economy or increase in fat mobilization.

**Significance and novelty of the proposed study:**

Changes in energy expenditure and body composition are key to the understanding the metabolic milieu of pregnancy and pathogenesis of diabetes complicating pregnancy. Dietary recommendations and therapeutic interventions in diabetes complicating pregnancy should be made taking into account the gestational variations in energy expenditure and body composition. Previous studies have shown that there is significant increase in energy expenditure, after adjusting for free fat mass as the pregnancy advances. But the studies fail to show any significant difference among patient with diabetes complicating pregnancy and normal pregnant population. However, there is a paucity of studies that have looked at both the basal and activity associated energy expenditure simultaneously in a population of pregnant women with and without diabetes.

Although nutritional intervention for overt diabetes and gestational diabetes is a fundamental treatment modality, there is a paucity of evidence-based data on this topic. This study may help in assessing the energy requirements in the different stages of
gestation in an Indian population and will help in formulating adequate nutritional recommendations in our population with diabetes mellitus complicating pregnancy.
MATERIALS AND METHODS:

SALIENT ASPECTS OF THE STUDY DESIGN:

STUDY DESIGN: The study was a prospective study. Institutional review board approval was obtained. **IRB Min.No.10045, dated 04.04.2016**

STUDY PERIOD: The duration of the study was 2 years (2016-18).

STUDY SETTING:
The study was carried out at the Department of Endocrinology, Diabetes and Metabolism, Christian Medical College, Vellore, Tamil Nadu, India.

**Table-1: SAMPLE SIZE CALCULATION:**
Two Means - Hypothesis testing for two means

<table>
<thead>
<tr>
<th>Pre - test mean</th>
<th>27</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post - test mean</td>
<td>33</td>
</tr>
<tr>
<td>Standard deviation in group I</td>
<td>8</td>
</tr>
<tr>
<td>Standard deviation in group II</td>
<td>8</td>
</tr>
<tr>
<td>Effect size</td>
<td>0.75</td>
</tr>
<tr>
<td>Power %</td>
<td>80</td>
</tr>
<tr>
<td>Alpha error (%)</td>
<td>5</td>
</tr>
<tr>
<td>1 or 2 sided</td>
<td>2</td>
</tr>
<tr>
<td>Required sample size per group</td>
<td>16</td>
</tr>
<tr>
<td>Group 1</td>
<td>22</td>
</tr>
<tr>
<td>Group 2</td>
<td>11</td>
</tr>
</tbody>
</table>
Based on the data published from previous studies; the expected percentage of longitudinal change in Resting energy expenditure was 27% in pregnant women with normal glucose tolerance and 33% in pregnant women with diabetes mellitus complicating pregnancy. The sample size were calculated with alpha and beta errors at 5% and 80% respectively with varying differences. The sample size needed in each group was around 16. So the study recruited total of 34 subjects, 21 with diabetes mellitus complicating pregnancy and 13 with normal glucose tolerance.

**STUDY PARTICIPANTS AND RECRUITMENT:**
The study subjects were divided into the following two groups:
- Group 1: Pregnant women with gestational diabetes mellitus as diagnosed by the IADPSG criteria GDM (n=21)
- Group 2: Pregnant women with normal glucose tolerance (NGT) (n=13)

The subjects were assessed at three different time points which include:
- Visit 1: Early pregnancy (before 18 weeks)
- Visit 2: Late pregnancy (32-38 weeks)
- Visit 3: Postpartum 6-24 weeks

**SUBJECT ELIGIBILITY CRITERIA:**

**Inclusion criteria:**
- a. Women with gestational diabetes mellitus as diagnosed by the IADPSG diagnostic criteria
- b. Diagnosed before 18 weeks of gestation
- c. Able and willing to provide informed consent

**Exclusion criteria:**
- a. Pregestational diabetes mellitus
- b. BMI >35 kg/m² (1st trimester )
- c. Any chronic illness requiring medications interfering with glucose metabolism
- d. Multiple pregnancies
- e. Age >35 years
Materials & Methods

The following were performed and recorded during the screening visit:

a) Signed informed consent, date and time

b) Demography
   1. Name,
   2. Date of Birth
   3. Sex
   4. Medical History
   5. Concomitant Medications

c) Full history and physical examination

d) Laboratory Investigations
   1. Hemoglobin
   2. Serum Creatinine
   3. TSH
   4. HbA1C
   5. Fasting and Postprandial plasma glucose

Procedures performed at each visit

1. Measurement of Resting Energy Expenditure by Indirect Calorimetry
2. Assessment of Postmeal Thermogenesis by 3 hour indirect calorimetry following a mixed meal challenge test
3. Assessment of Activity energy expenditure by PPAQ
4. Assessment of dietary intake by 24 hour recall method
5. Body composition by Bio impedance analysis
6. Anthropometry –Height, weight, BMI, mid arm circumference, skin fold thickness at 6 sites (Biceps, Triceps, Subscapular, Supra iliac, thigh and Neck)
7. Assessment of serum leptin levels
Materials & Methods

Detailed diagrammatic Algorithm of the study

1. Pregnant women within 18 weeks of gestational age will be screened in Obstetric OPD, Endocrinology OPD and GDM clinic

2. According to the IADPSG diagnostic criteria divided into two groups

- Normal glucose tolerance
- Diabetes mellitus in pregnancy

3. Informed consent

4. First Visit: Before 18 weeks of gestation
   Second Visit: 32-38 weeks of gestation
   Third Visit: 6-24 weeks postpartum

5. Data Analysis and result

Investigations:
- Indirect Calorimetry at baseline and for 3 hours following a Mixed Meal Challenge
- Body Composition by bio impedance analysis
- PPAQ Questionnaire for activity energy expenditure
- Diet assessment
- Anthropometry – Skin fold thickness and mid arm circumference
- Serum Lipid levels at 1st and 2nd visit
ASSESSMENT METHODS

Resting Energy Expenditure

Resting Energy expenditure was measured by respiratory gas exchange measurements using open circuit indirect calorimeter with ventilated hood. The ventilated hood measurement system consists of a plastic hood that surrounds the subject’s head and a soft plastic collar round the neck and shoulders. A fixed flow of room air was maintained through the hood by connecting the outlet of the hood through a calibrated rotameter to a suction pump. A small sample of air (1 L/min) was drawn off from the outlet of the rotameter for minute-to-minute estimation of oxygen and carbon dioxide concentrations.

Subjects were instructed to complete their evening meal by 20.00 and to be in bed by 22.00 on the night before the metabolic measurements. On the morning of the metabolic measurement they woke up between 06.00 and 06.30, completed their dressing and grooming, and empty their bladder. The study started by 08.00am in a fasting state. All subjects were made to rest in bed for 30 minutes before the REE measurement began. At the end of the mandatory rest period, BMR was measured for 30 minutes within the ventilated hood after an initial 10 minutes period to allow VO2 to stabilize. The electrical outputs were interfaced with a desktop computer, and integrated measurements of oxygen consumption (VO2, measured in ml/min) and carbon dioxide production (VCO2 in ml/min), respiratory quotient (RQ), and total resting energy expenditure (REE, expressed as kcal/kg/min) were averaged and recorded over this 30-mm period. The oxygen consumption and carbon dioxide production were measured to calculate respiratory quotient \([(RQ) = VCO2 / VO2]\]. RQ within the normal physiological range confirmed a consistent calorie intake by the study subjects. The REE was then calculated using the abbreviated Weir equation:

\[
3.9 \times (VO2) + 1.1 \times (VCO2) \times 1.44 \quad [(VO2 – Oxygen intake (ml/minute), VCO2- Carbon dioxide output (ml/minute)].
\]
Materials & Methods

Activity energy expenditure

Activity logs and the factorial method is a frequently used approach for estimating activity thermogenesis. In the study we used PPAQ (Pregnancy Physical Activity Questionnaire) to assess the physical activity of the subjects. The PPAQ is a validated, self-administered questionnaire that takes on average 10–15 minutes to complete, and has been used to assess the physical activity levels of pregnant women. This questionnaire is composed of 32 questions, grouped into different types of activities. Specifically, this semi quantitative questionnaire asks women to estimate the duration and frequency spent per activity, during the current one month. Women will also be given the opportunity to provide 2 activities that are not listed in the questionnaire. In brief, an estimated average metabolic equivalent (MET-hr/wk) value was calculated using the duration of the time spent in each activity multiplied by the energy equivalent of the activity based on the PPAQ.

Assessment of Post Prandial thermogenesis with mixed meal challenge test:

Postprandial thermogenesis (PPT) represents the additional energy expenditure, above resting levels, that follows ingestion of food. In the study we measured the increment in energy expenditure after a mixed meal challenge test. The use of a mixed meal has been previously validated as a physiological thermogenic stimulus. The metabolic rate was measured for 3 hours following the meal while the subject remained resting. Post meal thermogenesis was calculated as the increment in energy expenditure over baseline (i.e. post meal energy expenditure minus REE). This mixed meal consists of a balanced mixture of carbohydrates, protein and fat in percentages that are identical to a normal balanced diet. Following the overnight fast, subjects were administered a the mixed meal of ensure nutritional powder (carbohydrate 54%, fat 32% and protein 14%) in a 10Kcal per lean body weight to be drank over 5-10 minutes Subsequently the energy expenditure was estimated using an indirect calorimeter for 3 hours. The second 30 mm in each hour was considered as the measurement period and to be representative of the energy expenditure for the entire hour; the initial 30 minutes was designated as the rest period. The subjects remained awake and motionless in the recumbent position during
the measurement periods. Between measurement periods, i.e., during the rest periods, some movement and reading was permitted. The subjects took standard meal and snacks the night prior to the test and fasted after 10 pm, in order to minimize changes in metabolism between the tests.

Blood sampling was performed through an indwelling intravenous catheter, and blood was drawn at fasting and at 60,120 and 180 minutes following the meal, Samples for estimation of glucose, insulin, and non-esterified fatty acids (NEFA) were taken at 0, 1, 2 and 3 hour intervals during the test.

**Diet Assessment**

Calorie intake of the patients was assessed at each visit by a 24 hour Recall method.

**Anthropometry and Body Composition**

The most common method used to measure maternal body composition changes in pregnancy is anthropometry. Body weight and height were measured with an electronic balance and stadiometer. Skin fold thickness was measured at 6 sites; triceps, biceps, subscapular and suprailliac and thigh region. Mid arm circumference and neck circumference was measured. Skin fold thickness was taken on healthy, undamaged and uninfected dry skin as moist skin is harder to grasp and can influence the measurement. Subjects were instructed to keep the muscles relaxed during the test. The skin fold site was marked using a pen with water soluble ink. The skin fold was firmly grasped by the thumb and index finger, using the pads at the tip of the thumb and finger and the skin fold gently pulled away from the body. The Harpenden skin fold caliper was placed perpendicular to the fold, on the site marked, at approximately 1cm below the finger and thumb. While maintaining the grasp of the skin fold, the caliper was released so that full tension was placed on the skin fold. The dial was read to the nearest 0.50mm, 1 to 2 seconds after the grip has been fully released. Two measurements were taken at each site. If repeated tests varied by more than 1 mm, the measurements were repeated. Triceps skin fold thickness was measured at the midpoint between the acromion and
olecranon processes on the left side with the arm hanging by the side. Biceps skin fold thickness was measured at the anterior surface of the biceps midway between the anterior axillary fold and the antecubital fossa. Sub scapular region skin fold thickness was measured below the inferior angle of the scapula.

Body composition was measured with bio impedance analysis at each visit to assess the longitudinal changes in fat mass and free fat mass. Bioelectrical impedance analysis is a non-invasive, inexpensive and technically precise modality. It works on the principle that the aqueous tissues of the body due to their dissolved electrolytes are major conductors of electric current, fat and bone have poor conductance properties. It estimates fat free mass from the resistivity of water and electrolyte rich compartment against an electric current. Fat mass was calculated as body weight minus fat free mass. The measured whole body impedance is largely determined by the limbs, and hence bioelectrical impedance analysis is insensitive to changes in the trunk. A weak A/C current is passed through the outer pair of electrodes, while the voltage drop across the body is measured using the inner pair of electrodes from which the body’s impedance is derived. In this study Body fat was calculated by bio impedance instrument “bodystat version 2/02”.

**Statistical methods:**

Descriptive statistics of the variable were presented in terms of mean and standard deviation. Normality of the data was tested using Shapiro Wilk test and Q-Q plot. For variables with normal distribution, parametric tests were used: independent student t test and ANOVA for comparison of means among groups. Generalised linear model was used for comparing measurement repeated at different time points. For variables without normal distribution, non-parametric tests were used: Kruskall Wallis test for comparison of means among groups Mann-whitney U test was used for post hoc analysis. A p -value of <0.05 is taken as statistically significant in all cases. All statistical analysis was performed using IBM SPSS (Statistical Package for the Social Sciences) version 21.0.
Results

A total of 46 subjects were recruited into the study. The number of cases (pregnant women with Gestational diabetes mellitus) was twenty one and controls (pregnant women with normal glucose tolerance) were thirteen. The subjects were followed up longitudinally through three time points, early pregnancy (before 18 weeks of gestation), late pregnancy (between 32 -38 weeks of gestation) and the postpartum phase (4-12 months after delivery). Out of the forty six subjects, thirty four subjects (GDM n=21, NGT n=13) completed the second visit and twenty four subjects (GDM n=16, NGT n=8) completed all the three visits. The data was compared for thirty four subjects during pregnancy and twenty four subjects in all the three visits including postpartum period.

46 subjects were willing to participate in the study after informed consent (GDM – 28 subjects and NGT -17 subjects)

First visit assessment done in early pregnancy
Subjects participated: GDM -28, NGT -17

Second visit assessment done in late pregnancy
Subjects participated: GDM -21, NGT -13

Third visit assessment done in postpartum
Subjects participated: GDM -16, NGT -8

Total of 34 patients were included in the data analysis
GDM -21, NGT -13

Figure 1: Study overview flowchart
Table 1: Baseline characteristics

<table>
<thead>
<tr>
<th>Variables</th>
<th>GDM (n = 28) Mean ± SD</th>
<th>NGT (n = 17) Mean ± SD</th>
<th>*P  value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>31.4 ± 7.5</td>
<td>30.0 ± 8.2</td>
<td>0.61</td>
</tr>
<tr>
<td>Period of gestation at first visit (weeks)</td>
<td>13.5 ± 3.5</td>
<td>12.01 ± 2.7</td>
<td>0.15</td>
</tr>
<tr>
<td>Body mass index (BMI) (kg/m²)</td>
<td>26.1 ± 3.5</td>
<td>24.2 ± 4.3</td>
<td>0.07</td>
</tr>
<tr>
<td>Gravida [N (%)]</td>
<td></td>
<td></td>
<td>0.33</td>
</tr>
<tr>
<td>Primi - 15 (53.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multi - 13 (46.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family h/o of diabetes [N (%)]</td>
<td>19(67.8)</td>
<td>4 (23.5))</td>
<td>0.08</td>
</tr>
<tr>
<td>Past h/o of GDM [N (%)]</td>
<td>6(21.4)</td>
<td>1 (5.8)</td>
<td>0.35</td>
</tr>
<tr>
<td>FPG at diagnosis (mg/dL)</td>
<td>101.6±8.6</td>
<td>81.8±6.7</td>
<td>0.001#</td>
</tr>
<tr>
<td>HbA1c at diagnosis (%)</td>
<td>5.4±0.5</td>
<td>5.1±0.3</td>
<td>0.03#</td>
</tr>
<tr>
<td>Hemoglobin(gm/dL)</td>
<td>12.1±0.9</td>
<td>11.4±1.7</td>
<td>0.07</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>0.51± .14</td>
<td>0.48±.07</td>
<td>0.64</td>
</tr>
<tr>
<td>Serum TSH (IU/L)</td>
<td>1.67±1.29</td>
<td>1.56±1.26</td>
<td>0.76</td>
</tr>
</tbody>
</table>

Abbreviations – FPG –fasting plasma glucose, TSH –Thyroid stimulating hormone, BMI –Body mass index, GDM –Gestational diabetes mellitus, NGT -Normal glucose tolerance

# P < 0.05-considered as statistically significant

*Student t test
Table 2: Baseline anthropometric indices

<table>
<thead>
<tr>
<th>Variables</th>
<th>GDM (n= 28) Mean ± SD</th>
<th>NGT (n = 17) Mean ± SD</th>
<th>*P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waist circumference (cm)</td>
<td>94.8 ±9.5</td>
<td>81.7 ± 11.9</td>
<td>0.006#</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>96.1± 10.76</td>
<td>93.4 ± 10.8</td>
<td>0.44</td>
</tr>
<tr>
<td>Waist -Hip ratio</td>
<td>0.95 ± 0.16</td>
<td>0.81 ± 0.10</td>
<td>0.03#</td>
</tr>
<tr>
<td>Waist -Height Ratio</td>
<td>0.61 ±0.06</td>
<td>0.53±0.08</td>
<td>0.01#</td>
</tr>
<tr>
<td>Biceps skinfold (cm)</td>
<td>1.5 ± 0.58</td>
<td>1.3 ± 0.61</td>
<td>0.23</td>
</tr>
<tr>
<td>Triceps skinfold (cm)</td>
<td>2.7 ± 0.65</td>
<td>2.1± 0.64</td>
<td>0.004#</td>
</tr>
<tr>
<td>Subscapular skinfold (cm)</td>
<td>2.7 ± 0.68</td>
<td>1.9 ± 0.45</td>
<td>0.02#</td>
</tr>
<tr>
<td>Supra-iliac skinfolds (cm)</td>
<td>2.7 ± 0.63</td>
<td>2.0 ± 0.39</td>
<td>0.02#</td>
</tr>
<tr>
<td>Thigh skinfolds (cm)</td>
<td>4.5 ± 0.96</td>
<td>3.5 ± 0.78</td>
<td>0.01#</td>
</tr>
<tr>
<td>Neck skin fold (cm)</td>
<td>0.72± 0.16</td>
<td>0.60± 0.12</td>
<td>0.02#</td>
</tr>
<tr>
<td>Neck circumference(cm)</td>
<td>32.5± 2.1</td>
<td>30.6± 1.7</td>
<td>0.007#</td>
</tr>
<tr>
<td>Mid arm circumference (cm)</td>
<td>29.6± 2.9</td>
<td>26.9± 3.9</td>
<td>0.02#</td>
</tr>
</tbody>
</table>

GDM – Gestational diabetes mellitus, NGT - Normal glucose tolerance
*Student t test
# P < 0.05-considered as statistically significant
The mean age of the subjects with GDM was 31.4 years (± 7.5) while the control group was slightly younger, with a mean age of 30 years (±8.2). The HBA1c was in the non-diabetic range (less than 5.7%) in both the groups confirming onset of glucose intolerance in pregnancy for GDM subjects. The family history of diabetes was higher in the GDM group (67.8% versus 23.5%). Twenty two out of forty six subjects were primigravida and a past history of GDM were present in seven subjects, six (21.4%) in the GDM group and one in control group. There were no significant differences in haemoglobin, TSH, and creatinine between the groups. The baseline mean BMI of the GDM group was higher than the control which approached statistical significance (26.1 ± 3.5 versus 24.2± 4.3kg/m², P value 0.07). Figure 2 shows the distribution of BMI among the groups. At the time of recruitment, the GDM group had a higher proportion of obese (BMI ≥25kg/m²) and overweight (BMI 23.0-24.9kg/m²) subjects when compared to the controls. Among

![Figure 2: Baseline Distribution of BMI](chart)

The mean age of the subjects with GDM was 31.4 years (± 7.5) while the control group was slightly younger, with a mean age of 30 years (±8.2). The HBA1c was in the non-diabetic range (less than 5.7%) in both the groups confirming onset of glucose intolerance in pregnancy for GDM subjects. The family history of diabetes was higher in the GDM group (67.8% versus 23.5%). Twenty two out of forty six subjects were primigravida and a past history of GDM were present in seven subjects, six (21.4%) in the GDM group and one in control group. There were no significant differences in haemoglobin, TSH, and creatinine between the groups. The baseline mean BMI of the GDM group was higher than the control which approached statistical significance (26.1 ± 3.5 versus 24.2± 4.3kg/m², P value 0.07). Figure 2 shows the distribution of BMI among the groups. At the time of recruitment, the GDM group had a higher proportion of obese (BMI ≥25kg/m²) and overweight (BMI 23.0-24.9kg/m²) subjects when compared to the controls. Among
the anthropometric measures baseline waist circumference, waist-hip ratio, waist-height ratio, neck and mid arm circumference were significantly higher in the GDM group. There was a significant difference in the skin fold thicknesses (triceps, subscapular, supra-iliac, thigh, neck) between the GDM subjects and controls in early pregnancy. The biceps skin fold was similar in both the groups unlike the other sites.

Table 3: Follow up and outcome of pregnancy

<table>
<thead>
<tr>
<th>Variables</th>
<th>GDM (n = 21) Mean ± SD</th>
<th>NGT (n = 13) Mean ± SD</th>
<th>*P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age at first visit in weeks</td>
<td>13.6 ± 3.6</td>
<td>11.8 ± 2.9</td>
<td>0.13</td>
</tr>
<tr>
<td>Gestational age at second visit in week</td>
<td>33.8 ± 1.3</td>
<td>34.4 ± 1.7</td>
<td>0.18</td>
</tr>
<tr>
<td>Gap between delivery and third visit in weeks</td>
<td>32.3 ± 13.5</td>
<td>29.68 ± 8.1</td>
<td>0.56</td>
</tr>
<tr>
<td>Treatment N (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MNT alone</td>
<td>12 (57.14)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Metformin</td>
<td>6 (28.57)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>1 (4.76)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metformin + insulin</td>
<td>2 (9.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mode of delivery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal N (%)</td>
<td>14 (66.67)</td>
<td>8(61.54)</td>
<td></td>
</tr>
<tr>
<td>Caesarean section N (%)</td>
<td>7 (33.33)</td>
<td>5(38.4)</td>
<td></td>
</tr>
<tr>
<td>Birth weight</td>
<td>2.8 ±0.4</td>
<td>2.9±0.3</td>
<td>0.37</td>
</tr>
</tbody>
</table>

**Diabetes mellitus in the postpartum period N (%)**

<table>
<thead>
<tr>
<th></th>
<th>GDM (n=16) N (%)</th>
<th>NGT (N)=8 N (%)</th>
<th>*P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFG</td>
<td>8 (50)</td>
<td>1(12.5)</td>
<td>0.49</td>
</tr>
<tr>
<td>IGT</td>
<td>2 (12.5)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>IFG+IGT</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Overt DM</td>
<td>2 (12.5)</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: MNT –medical nutrition therapy; IFG –impaired fasting glucose; IGT- impaired glucose tolerance; DM –diabetes mellitus; GDM – Gestational diabetes mellitus; NGT –Normal glucose tolerance, NA –Not applicable

*Student t test

# P < 0.05-considered as statistically significant
** In postpartum GDM( N )=16, NGT (N)=8
The mean gestational age at the time of first and second visit was similar in both the groups. The mean duration of post-partum visit was 31 weeks. Among the GDM subjects, thirteen subjects were on medical nutrition therapy, eight subjects were on combined MNT and metformin. The total dose of metformin ranged from 500mg to 2gm. Two patients were started on insulin along with metformin in late pregnancy. One patient was on a basal bolus regimen and the other was on basal insulin at night. Mode of delivery and neonatal births weight was similar in both the groups. Macrosomia (birth weight > 3.5kg) was seen in four babies, two each in both the groups. Low birth weight (less than 2.5 kg) was seen in two babies with GDM mothers. None of the babies in the control group had low birth weight. No neonatal complications were noted. Nearly 50% of subjects with GDM were found to have persistent glucose intolerance (IFG or IGT or both) during the postpartum period. Two patients had overt diabetes mellitus and were started on metformin. One patient in the control group developed new onset impaired fasting glucose (IFG).
<table>
<thead>
<tr>
<th>Variables</th>
<th>Group</th>
<th>Early pregnancy</th>
<th>*P value</th>
<th>Late pregnancy</th>
<th>**P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>GDM (N)=21</td>
<td>64.28 ± 10.2</td>
<td>0.04#</td>
<td>69.34 ± 9.31</td>
<td>0.13</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>NGT (N) =13</td>
<td>55.96 ± 11.7</td>
<td></td>
<td>62.92 ± 12.61</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>GDM (N)=21</td>
<td>26.55 ± 4.14</td>
<td>0.06</td>
<td>28.52 ± 3.87</td>
<td>0.17</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>NGT (N) =13</td>
<td>23.44± 4.47</td>
<td></td>
<td>26.32± 4.66</td>
<td></td>
</tr>
<tr>
<td>Change in weight (kg)</td>
<td>GDM (N)=21</td>
<td>2.0(-4 -12)</td>
<td>0.43</td>
<td>4.5(-1 -16)</td>
<td>0.21</td>
</tr>
<tr>
<td>Median (range)</td>
<td>NGT (N) =13</td>
<td>1.7 (-6 – 5.7)</td>
<td></td>
<td>7.1 (-0.7-15.10)</td>
<td>1.26</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>GDM (N)=21</td>
<td>38.1± 6.1</td>
<td>0.04#</td>
<td>37.4 ± 6.5</td>
<td>0.27</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>NGT (N) =13</td>
<td>31.5±8.5</td>
<td></td>
<td>34.8± 6.3</td>
<td></td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>GDM (N)=21</td>
<td>24.7 ± 6.7</td>
<td>0.03#</td>
<td>26.2± 6.7</td>
<td>0.10</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>NGT (N) =13</td>
<td>17.5± 7.2</td>
<td></td>
<td>22.5± 8.1</td>
<td></td>
</tr>
<tr>
<td>Lean mass (%)</td>
<td>GDM (N)=21</td>
<td>61.9± 6.1</td>
<td>0.03#</td>
<td>62.5 ± 6.5</td>
<td>0.11</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>NGT (N) =13</td>
<td>68.4± 8.5</td>
<td></td>
<td>65.2 ± 6.3</td>
<td></td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>GDM (N)=21</td>
<td>39.7± 6.3</td>
<td>0.51</td>
<td>43.1± 6.3</td>
<td>0.47</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>NGT (N) =13</td>
<td>38.3± 5.2</td>
<td></td>
<td>40.5 ± 5.4</td>
<td></td>
</tr>
</tbody>
</table>

BMI – body mass index, GDM – Gestational diabetes mellitus, NGT – normal glucose tolerance
*denotes P value between GDM and NGT group in early pregnancy, Student t test
** denotes P value between GDM and NGT group in late pregnancy, Student t test
# P < 0.05 considered as statistically significant
Table 5: Longitudinal changes in body composition indices during pregnancy and postpartum

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group</th>
<th>Early pregnancy Mean ± SD</th>
<th>Late pregnancy Mean ± SD</th>
<th>Postpartum Mean ± SD</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>GDM (N)=16</td>
<td>63.1 ± 10.5</td>
<td>67.2 ± 9.4</td>
<td>64.4 ± 8.4</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>NGT (N)=8</td>
<td>52.7 ± 10.2</td>
<td>58.1 ± 10.7</td>
<td>57.5 ± 12.5</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>GDM (N)=16</td>
<td>26.2 ± 3.9</td>
<td>27.7 ± 3.4</td>
<td>26.6 ± 3.1</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>NGT (N)=8</td>
<td>22.4 ± 3.9</td>
<td>24.6 ± 3.8</td>
<td>24.4 ± 4.5</td>
<td></td>
</tr>
<tr>
<td>Body fat %</td>
<td>GDM (N)=16</td>
<td>38.2 ± 6.9</td>
<td>37.3 ± 6.9</td>
<td>38.0 ± 5.4</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>NGT (N)=8</td>
<td>30.2 ± 7.0</td>
<td>34.1 ± 6.7</td>
<td>36.2 ± 5.2</td>
<td></td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>GDM (N)=16</td>
<td>24.3 ± 7.4</td>
<td>25.3 ± 6.7</td>
<td>24.5 ± 5.7</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>NGT (N)=8</td>
<td>16.3 ± 6.7</td>
<td>20.4 ± 7.4</td>
<td>21.1 ± 6.9</td>
<td></td>
</tr>
</tbody>
</table>

BMI – Body mass index, GDM – Gestational diabetes mellitus; NGT – Normal glucose tolerance
* Denotes P value of longitudinal change in body composition indices between GDM and NGT group
# P < 0.05 - considered as statistically significant
The body composition as assessed by the bio-impedance analyser showed significantly higher body fat content and percentage in the GDM group in the early phase of pregnancy (p-0.04 and 0.03 respectively) whereas in late pregnancy, the difference was not significant (p-0.26 and 0.19 respectively). In subjects with normal glucose tolerance, the body fat percentage increased by 3.3% from early to the late phase of pregnancy, whereas the body fat percentage remained unchanged in GDM subjects. In the control group; among the patients who came for the third visit (figure 3) the body fat has increased from 30.2% in early pregnancy to 34.1% in late pregnancy and 36.1% in the postpartum period where as in the GDM group there was no increment in body fat percentage. On comparing the longitudinal change in body fat percentage through pregnancy and postpartum phase between the groups,
the increment in body fat percentage in the control group did not reach statistical significance (p=0.16) The GDM subjects had lower degree of weight gain during pregnancy compared to control (7.1 kg versus 4.5kg, p=.21)

Table 6: Longitudinal changes in biochemical parameters in pregnancy and post-partum

<table>
<thead>
<tr>
<th>Variables</th>
<th>Groups</th>
<th>Early pregnancy</th>
<th>Late pregnancy</th>
<th>Postpartum</th>
<th>p* value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting Glucose (mg/dl) Mean ±SD</td>
<td>GDM (N)=16</td>
<td>90.8±8.3</td>
<td>86.2±6.46</td>
<td>100 ± 14.3</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>NGT (N) =8</td>
<td>78.5±7.1</td>
<td>81.0±7.8</td>
<td>93.7± 9.2</td>
<td></td>
</tr>
<tr>
<td>Fasting Insulin µIU/ml Median (range)</td>
<td>GDM (N)=16</td>
<td>7.3 (1.90-23.60)</td>
<td>4.9(1.9-52.5)</td>
<td>7.4(1.9-21.3)</td>
<td>0.04#</td>
</tr>
<tr>
<td></td>
<td>NGT (N) =8</td>
<td>3.35(1.90-9.30)</td>
<td>5.1(1.9-51.0)</td>
<td>8.1 (1.9-31)</td>
<td></td>
</tr>
<tr>
<td>Fasting FFA (meq/dl) Mean ± SD</td>
<td>GDM (N)=16</td>
<td>561.6±161.8</td>
<td>444.1±108.72</td>
<td>453.4 ± 216.5</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>NGT (N) =8</td>
<td>465.8±144.1</td>
<td>406.5±156.99</td>
<td>416.3 ± 122.2</td>
<td></td>
</tr>
<tr>
<td>Serum leptin (ng/ml) Median (range)</td>
<td>GDM (N)=16</td>
<td>7.0(1.4- 46)</td>
<td>11.0(2.2-17.8)</td>
<td>17.7(8.4-33.0)</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>NGT (N) =8</td>
<td>12.6(3.6-48.5)</td>
<td>8.6(2.4 -30.4)</td>
<td>10.1(5.5-34.1)</td>
<td></td>
</tr>
</tbody>
</table>

FFA –free fatty acid, GDM – Gestational diabetes mellitus, NGT –normal glucose tolerance
* Denotes P value of longitudinal change in biochemical parameters between GDM and NGT group using general linear model
# P < 0.05-considered as statistically significant
Table 7: Comparison of biochemical parameters between groups in pregnancy

<table>
<thead>
<tr>
<th>Variables</th>
<th>Groups</th>
<th>Early pregnancy</th>
<th>*P value</th>
<th>Late pregnancy</th>
<th>**P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Groups</td>
<td>Mean ±SD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting Glucose (mg/dl)</td>
<td>GDM (N)=21</td>
<td>87.7 ± 9.7</td>
<td>0.006#</td>
<td>85.6±5.9</td>
<td>0.03#</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>NGT (N)=13</td>
<td>79.7±6.4</td>
<td></td>
<td>79.2 ±8.9</td>
<td></td>
</tr>
<tr>
<td>Fasting Insulin (µIU/ml) Median (range)</td>
<td>GDM (N)=21</td>
<td>6.8(1.90-119)</td>
<td>0.31</td>
<td>5.3(1.9-52.8)</td>
<td>0.90</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>NGT (N)=13</td>
<td>3.5(1.9-49.2)</td>
<td></td>
<td>5.2(1.9-51.0)</td>
<td></td>
</tr>
<tr>
<td>Fasting FFA (meq/dl)</td>
<td>GDM (N)=21</td>
<td>527.7±148.6</td>
<td>0.06</td>
<td>446.12±108.72</td>
<td>0.27</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>NGT (N)=13</td>
<td>425.1±134.8</td>
<td></td>
<td>394.51±140.1</td>
<td></td>
</tr>
<tr>
<td>Serum leptin (ng/ml)</td>
<td>GDM (N)=21</td>
<td>9.1(1.4-46.0)</td>
<td>0.78</td>
<td>14.17(3.6-54.1)</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>NGT (N)=13</td>
<td>11.2(2.1-21.0)</td>
<td></td>
<td>9.21(2.4-43.1)</td>
<td></td>
</tr>
</tbody>
</table>

FFA – free fatty acid, GDM – Gestational diabetes mellitus; NGT – Normal glucose tolerance
*denotes P value between GDM and NGT group in early pregnancy, Student t test
** denotes P value between GDM and NGT group in late pregnancy, Student t test
# P < 0.05 - considered as statistically significant
### Table 8: Insulin sensitivity indices during pregnancy

<table>
<thead>
<tr>
<th>Variables</th>
<th>Groups</th>
<th>Early pregnancy</th>
<th>*P Value</th>
<th>Late pregnancy</th>
<th>**P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HOMA IR</strong></td>
<td>GDM (N=21)</td>
<td>1.4 (0.43-6.0)</td>
<td>0.54</td>
<td>1.0 (0.41-12.5)</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>NGT (N=13)</td>
<td>0.67 (0.35-9.4)</td>
<td></td>
<td>1.0 (0.3-11.4)</td>
<td></td>
</tr>
<tr>
<td><strong>QUICKI</strong></td>
<td>GDM (N=21)</td>
<td>0.36 ± 0.04</td>
<td>0.03</td>
<td>0.37 ± 0.05</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>NGT (N=13)</td>
<td>0.40 ± 0.05</td>
<td></td>
<td>0.38 ± 0.06</td>
<td></td>
</tr>
<tr>
<td><strong>FGIR</strong></td>
<td>GDM (N=21)</td>
<td>12.9 (3.8-53.1)</td>
<td>0.15</td>
<td>15.3 (1.8-46.3)</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td>NGT (N=13)</td>
<td>22.2 (1.5-44.7)</td>
<td></td>
<td>15.1 (1.7-43.1)</td>
<td></td>
</tr>
</tbody>
</table>

HOMA – homeostatic model assessment, QUICKI – quantitative insulin sensitivity check index, FGIR – fasting glucose insulin ratio, GDM – Gestational diabetes mellitus, NGT – normal glucose tolerance

*denotes P value between GDM and NGT group in early pregnancy, Student t test
** denotes P value between GDM and NGT group in late pregnancy, student t test
#: P < 0.05 – considered as statistically significant
Table 9: Longitudinal changes in the insulin sensitivity indices in pregnancy and postpartum

<table>
<thead>
<tr>
<th>Variables</th>
<th>Groups</th>
<th>Early pregnancy</th>
<th>Late pregnancy</th>
<th>Postpartum</th>
<th>*p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HOMA IR</strong></td>
<td>GDM (N)=16</td>
<td>1.4 (0.43 – 6.0)</td>
<td>1.3 (0.41 – 12.5)</td>
<td>1.8 (0.44-5.8)</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>NGT (N) =8</td>
<td>0.60(0.35 -1.9)</td>
<td>0.98(0.33-11.4)</td>
<td>1.7 (0.4-8.5)</td>
<td></td>
</tr>
<tr>
<td><strong>QUICKI</strong></td>
<td>GDM (N)=16</td>
<td>0.36±0.05</td>
<td>0.34±0.05</td>
<td>0.35 ± 0.04</td>
<td>0.03#</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>NGT (N) =8</td>
<td>0.42±0.04</td>
<td>0.39±0.06</td>
<td>0.36± 0.06</td>
<td></td>
</tr>
<tr>
<td><strong>FGIR</strong></td>
<td>GDM (N)=16</td>
<td>11.9(3.8 -53.2)</td>
<td>11.30(1.8-46.3)</td>
<td>14.1 (5.2-52.9)</td>
<td>0.65</td>
</tr>
<tr>
<td>Median (range)</td>
<td>NGT (N) =8</td>
<td>28.5(9.13 -43.7)</td>
<td>16.9(1.7-37.3)</td>
<td>11.1(3.6-47.4)</td>
<td></td>
</tr>
</tbody>
</table>

HOMA –homeostatic model assessment, QUICKI –quantitative insulin sensitivity check index, FGIR –fasting glucose insulin indices, GDM – Gestational diabetes mellitus; NGT –Normal glucose tolerance
* Denotes P value of longitudinal change in insulin sensitivity indices between GDM and NGT group
The baseline fasting insulin and free fatty acids levels were higher in the GDM subjects (6.8 versus 3.5 µIU/ml, p=0.31 and 527.7versus 425.1meq/dl, p=0.06), the difference approached statistical significance for free fatty acid levels. The fasting insulin levels increased from early to late phase of pregnancy and postpartum in the control group, while subjects with GDM showed a decrease in insulin levels in late phase of pregnancy; this longitudinal change in insulin levels were statistically significant between the two groups (p=0.04). The fasting free fatty acids were higher in GDM subjects when compared to the control group in early phase of pregnancy (p=0.06). The FFA levels declined in late pregnancy and postpartum period in both the groups. Among the insulin sensitivity indices; the QUICKI was significantly lower in subjects with GDM indicating a higher insulin resistance (p=0.03). HOMA – IR and FGIR also showed a lower degree of insulin sensitivity in the GDM subjects in early phase of pregnancy, though the difference was not statistically significant. On longitudinal follow up; the QUICKI index showed a significant decrease in late pregnancy and postpartum phase in controls compared to the GDM subjects (p=0.03). The serum leptin levels were comparable among GDM and NGT patients in early phase of pregnancy (9.1 versus 11.2ng/ml, P=0.78). The leptin levels increased in late pregnancy and postpartum period in GDM subjects whereas the levels remained stable in subjects with normal glucose tolerance. The longitudinal increase in leptin levels between the groups did not reach statistical significance (p=0.38).
ASSESSMENT OF RESTING ENERGY EXPENDITURE (REE)

Table 10– Resting energy expenditure

<table>
<thead>
<tr>
<th>Variables</th>
<th>Groups</th>
<th>Early pregnancy</th>
<th>( P^* ) Value</th>
<th>Late pregnancy</th>
<th>( P^{**} ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting energy expenditure (kcal/day)</td>
<td>GDM(N=21)</td>
<td>2435.0 ± 496.6</td>
<td>0.62</td>
<td>2853.6 ± 592.8</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td>NGT(N=13)</td>
<td>2539.3 ± 646.3</td>
<td></td>
<td>2822.1 ± 758.9</td>
<td></td>
</tr>
<tr>
<td>Resting energy expenditure (kcal/kg/day)</td>
<td>GDM(N=21)</td>
<td>62.2 ± 13.1</td>
<td>0.39</td>
<td>67.1 ± 15.6</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>NGT(N=13)</td>
<td>67.0 ± 17.1</td>
<td></td>
<td>69.5 ± 13.7</td>
<td></td>
</tr>
</tbody>
</table>

REE – Resting energy expenditure, FFM – Fat free mass, GDM – Gestational Diabetes mellitus, NGT – Normal glucose tolerance
*denotes \( P \) value between GDM and NGT group in early pregnancy, Student t test
** denotes \( P \) value between GDM and NGT group in late pregnancy, Student t test
# \( P < 0.05 \)-considered as statistically significant

Table 11: Longitudinal changes in REE in pregnancy and postpartum

<table>
<thead>
<tr>
<th>Calorimetry indices</th>
<th>Group</th>
<th>Early pregnancy</th>
<th>Late pregnancy</th>
<th>Postpartum</th>
<th>*p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total REE (kcal)</td>
<td>GDM (n=16)</td>
<td>2417.0±478.7</td>
<td>2809.9±621.6</td>
<td>2670.0±709.8</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>NGT (n=8)</td>
<td>2466.0±593.8</td>
<td>2487.5±512.2</td>
<td>2470.3±667.3</td>
<td></td>
</tr>
<tr>
<td>REE adjusted to FFM (kcal/kg)</td>
<td>GDM (n=16)</td>
<td>62.9±13.3</td>
<td>68.3±17.1</td>
<td>69.8±21.4</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>NGT (n=8)</td>
<td>68.3±16.0</td>
<td>66.3±13.5</td>
<td>69.2±18.6</td>
<td></td>
</tr>
</tbody>
</table>

REE – Resting energy expenditure, FFM – Fat free mass, GDM – Gestational diabetes mellitus, NGT – Normal glucose tolerance
* Denotes \( P \) value of longitudinal change in REE between GDM and NGT group
# \( P < 0.05 \)-considered as statistically significant
The resting energy expenditure (REE) was similar among subjects with GDM and controls both in early and late phase of pregnancy. When adjusted to fat free mass; the REE was lower in GDM subjects in early pregnancy; the difference was not statistically significant. (62.2 versus 67.0, \( p=0.39 \)). The REE adjusted to fat free mass was similar in both groups in the postpartum period. The lower REE adjusted to fat free mass seen in early pregnancy in subjects with GDM was not seen in the postpartum period. During pregnancy the longitudinal increase in total REE and REE adjusted to fat free mass was higher in women with GDM when compared to...
subjects with normal glucose tolerance (17% versus 11% and 8% versus 4% respectively, p=0.65). From late pregnancy to postpartum, REE showed longitudinal decrease in GDM subjects and remained unchanged in controls. The REE adjusted to fat free mass was higher in the postpartum phase compared to early and late phase of pregnancy in both the groups.

**Figure 5: Longitudinal changes in REE -total and adjusted to FFM across groups**
Correlation with REE (resting energy expenditure)

Figure 6: Correlation between REE and serum leptin levels in early pregnancy a) GDM, b) NGT

Figure 7: Correlation between total body fat and REE in GDM subjects in early pregnancy
In the study, the resting energy expenditure (REE) was found to have positive correlation with serum leptin levels in early phase of pregnancy in both the groups. The REE correlated with body fat content in GDM subjects in early pregnancy. The REE negatively correlated with fasting glucose insulin ration in all the three visits, maximum correlation was found in the postpartum period ($r=-0.71, p=0.03$).

**Figure 8: correlation between REE and fasting glucose insulin ratio (FGIR) in GDM patients in the postpartum period**
## POST-MEAL THERMOGENESIS (PTE)

### Table 12: Postmeal thermogenesis in pregnancy

<table>
<thead>
<tr>
<th>Variables</th>
<th>Groups</th>
<th>Early pregnancy</th>
<th>P* Value</th>
<th>Late pregnancy</th>
<th>P ** Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTE (kcal/day)</td>
<td>GDM (N=21)</td>
<td>65.1 (-15.2 - 366.0)</td>
<td>0.04#</td>
<td>32.9 (-18.3 - 120.1)</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>NGT (N=13)</td>
<td>16.6 (-23.45 - 81.5)</td>
<td></td>
<td>12.3 (-13 - 123)</td>
<td></td>
</tr>
<tr>
<td>PTE adjusted to FFM (kcal/kg/day)</td>
<td>GDM (N=21)</td>
<td>1.8 (-0.42 - 7.1)</td>
<td>0.02</td>
<td>0.76 (-0.7 - 2.9)</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td>NGT (N=13)</td>
<td>0.47 (-0.55 - 1.99)</td>
<td></td>
<td>0.31 (-0.68 - 2.8)</td>
<td></td>
</tr>
</tbody>
</table>

PTE – postmeal thermogenesis, FFM – Fat free mass, GDM – Gestational diabetes mellitus, NGT – normal glucose tolerance

* denotes P value between GDM and NGT group in early pregnancy, Student t test

** denotes P value between GDM and NGT group in late pregnancy, Student t test

### Table 13: Longitudinal changes in PTE during pregnancy and postpartum

<table>
<thead>
<tr>
<th>Calorimetry indices</th>
<th>Group</th>
<th>Early pregnancy Median (range)</th>
<th>Late pregnancy Median (range)</th>
<th>Postpartum Median (range)</th>
<th>*P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total PTE (kcal)</td>
<td>GDM (n=16)</td>
<td>63.6 (-15-366.0)</td>
<td>36.6 (-18.1-120.6)</td>
<td>27.9 (-28.3-207.0)</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td>NGT (n=8)</td>
<td>22.9 (-5.5-58.3)</td>
<td>18.3 (-13.0 - 123.6)</td>
<td>20.0 (-70.81-103.8)</td>
<td></td>
</tr>
<tr>
<td>PTE adjusted to FFM (kcal/kg)</td>
<td>GDM (n=16)</td>
<td>1.8 (-0.42 - 7.1)</td>
<td>0.84 (-0.31 - 2.9)</td>
<td>0.78 (-0.69 - 4.7)</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>NGT (n=8)</td>
<td>0.60 (0.13 -1.9)</td>
<td>0.48 (-0.28 -2.9)</td>
<td>0.63 (-1.57 -3.1)</td>
<td></td>
</tr>
</tbody>
</table>

PTE – postmeal thermogenesis, FFM – Fat free mass, GDM – Gestational diabetes mellitus, NGT – normal glucose tolerance

* denotes P value of longitudinal change in PTE between GDM and NGT group

# P < 0.05 - considered as statistically significant
Figure 9: Longitudinal change of PTE in GDM (n=21) and NGT (n=13) subjects

a) PTE  b) PTE adjusted to lean body mass
Figure 10. Correlation between PTE and HOMAIR in early pregnancy

Results

\[ r = -0.52, p = 0.01 \]
Figure 11: Changes in post meal thermogenesis over 3 hours during MMCT a) Early pregnancy b) Late pregnancy c) Post-partum

In the study; PTE was calculated as an increment in energy expenditure from REE during mixed meal challenge test over a period of 3 hours. The total PTE and PTE adjusted to fat free mass was significantly higher in the GDM subjects when compared to controls in early phase of pregnancy (p =0.04 and 0.02 respectively). The total PTE positively correlated with HOMA IR in GDM subjects in early pregnancy (r=-0.52, p=0.01). The PTE showed a progressive decrease in late pregnancy and postpartum period when compared to early pregnancy in both the groups. The decrease in PTE from early to late pregnancy was lower in GDM patients compared to normal subjects, the difference approached statistical significance (p=0.08). Figure 11 shows the changes in PTE during MMCT. Both the groups followed same pattern of PTE during MMCT. The PTE showed an increase in first and second hour and a decrease in the third hour. In late phase of pregnancy in controls PTE showed an increment in third hour unlike in other visits.
Results

Figure 12: Changes in plasma glucose over 3 hours during MMCT a) Early pregnancy b) Late pregnancy c) Post-partum
Figure 13: Changes in serum insulin levels over 3 hours during MMCT a) Early pregnancy b) Late pregnancy c) Post-partum
Figure 14: Changes in serum free fatty acid levels over 3 hours during MMCT a) Early pregnancy b) Late pregnancy c) Post-partum
The utilisation of substrates was similar in GDM and normal pregnant women in both the phases of pregnancy and postpartum period. The area under the curve (AUC) for serum insulin and glucose levels (as shown in figure 12 and 13) showed an increment in the first and second hour followed by a decline in the third hour. The AUC for free fatty acid levels showed a constant decline from baseline. The decrease in FFA (shown in figure 14) was lower in GDM subjects compared to control and the change approached statistical significance in late pregnancy (p=0.06)
### Results

**ACTIVITY ENERGY EXPENDITURE**

*Table 14: Activity energy expenditure assessed by PPAQ questionnaire*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Early pregnancy</th>
<th>Late pregnancy</th>
<th>Postpartum</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mets.hour/week</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>GDM N=21</strong></td>
<td><strong>NGT N=13</strong></td>
<td><strong>GDM N=21</strong></td>
<td><strong>NGT N=13</strong></td>
</tr>
<tr>
<td><strong>Total activity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>85.7±40.9</td>
<td>104.4±50.2</td>
<td>95.4±45.7</td>
</tr>
<tr>
<td>Median(range)</td>
<td>13.4 (1.2 -66.7)</td>
<td>18.4 (3.7- 59.5)</td>
<td>19.7 (1.8 -58.4)</td>
</tr>
<tr>
<td><strong>Sedentary</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median(range)</td>
<td>44.5 (8.4 –82.4)</td>
<td>61.33 (18.2-89.2)</td>
<td>47.4 (18.4-113.9)</td>
</tr>
<tr>
<td><strong>Light</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median(range)</td>
<td>18.1 (2.4-91.3)</td>
<td>23.2 (0.8-94.5)</td>
<td>21.0 (0.8-67.1)</td>
</tr>
<tr>
<td><strong>Moderate</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median(range)</td>
<td>18.1 (2.4-91.3)</td>
<td>23.2 (0.8-94.5)</td>
<td>21.0 (0.8-67.1)</td>
</tr>
<tr>
<td><strong>Heavy</strong></td>
<td>0</td>
<td>0</td>
<td>9.75 (1/21)</td>
</tr>
<tr>
<td><strong>Household</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median(range)</td>
<td>78.6 (17.5 -116.1)</td>
<td>51.04 (8.4-158.2)</td>
<td>60.4 (17.6-120.7)</td>
</tr>
<tr>
<td><strong>Occupational</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median(range)</td>
<td>22.05 (7- 56.3)</td>
<td>14.0 (2.8-32.0)</td>
<td>9.1 (2.8-68.4)</td>
</tr>
<tr>
<td><strong>Sports</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median(range)</td>
<td>4.8 (1.9-19.2)</td>
<td>3.0 (0.8- 4.8)</td>
<td>4.8 (0.8-12.4)</td>
</tr>
</tbody>
</table>

Met –metabolic equivalent of task, GDM –gestational diabetes mellitus, NGT –normal glucose tolerance

*denotes P value between GDM and NGT group in early pregnancy; student t test ** denotes P value between GDM and NGT group in late pregnancy; student t test ***denotes P value between GDM and NGT group in postpartum period, student t test

# P < 0.05-considered as statistically significant
Figure 15: Longitudinal changes in activity energy expenditure between groups

The activity energy expenditure (AEE) assessed by PPAQ questionnaire was expressed in mets.hour per week. The AEE was lower in GDM subjects when compared to controls in the early phase of pregnancy whereas it was higher in late pregnancy and postpartum period in subjects with GDM; though the difference was not statistically significant. The total AEE was highest in the postpartum period in both the groups. In GDM subjects; the AEE increased in late phase of pregnancy when compared to early pregnancy, whereas in normal subjects, the AEE decreased in late phase of pregnancy. The AEE was categorised into various groups based on the mets required for each activity- sedentary (0-1.9 mets), light (2-2.9mets) moderate (3-5.9mets) and heavy intensity (≥6mets). The maximum proportion of energy expenditure was in light intensity activity followed by sedentary activity in early and late phase of pregnancy. In the postpartum period majority of AEE was
Results

derived from light and moderate intensity activity. Only one patient was doing high intensity activity in late pregnancy and postpartum period

ENERGY INTAKE

**Figure 16: Distribution of energy intake among groups.**
Calorie intake was assessed by 24 hour recall method. In late pregnancy the total calorie intake was significantly lower in GDM subjects when compared to normal pregnancy (1588 versus 1932kcal/day, p=0.02). This decrease will be the reflection of nutritional intervention in GDM subjects. The total calorie intake was similar in both the groups in early pregnancy and postpartum period. The carbohydrate proportions were higher and protein intake was inadequate in both the groups at all visits (9-11%).
Table 15: Comparison of GDM subjects with impaired and normal glucose tolerance in the postpartum period

<table>
<thead>
<tr>
<th>Variables</th>
<th>Groups</th>
<th>Early pregnancy</th>
<th>*P value</th>
<th>Late pregnancy</th>
<th>**P value</th>
<th>Postpartum</th>
<th>***P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>DM</td>
<td>26.2 ± 3.6</td>
<td>0.96</td>
<td>27.6 ± 3.3</td>
<td>0.81</td>
<td>27.3 ± 3.7</td>
<td>0.54</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>NGT</td>
<td>26.2 ± 4.4</td>
<td></td>
<td>27.9 ± 3.7</td>
<td>0.59</td>
<td>26.1 ± 2.6</td>
<td>0.29</td>
</tr>
<tr>
<td><strong>Fat percentage (%)</strong></td>
<td>DM</td>
<td>36.6 ± 7.7</td>
<td>0.32</td>
<td>36.0 ± 8.5</td>
<td>0.47</td>
<td>40.1 ± 6.7</td>
<td>0.29</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>NGT</td>
<td>40.0 ± 5.4</td>
<td></td>
<td>38.7 ± 4.7</td>
<td>0.47</td>
<td>36.4 ± 6.9</td>
<td>0.29</td>
</tr>
<tr>
<td><strong>Fasting insulin (µIU/L)</strong></td>
<td>DM</td>
<td>6.6 (1.9-236)</td>
<td>0.54</td>
<td>6.5 (2.5-52.8)</td>
<td>0.23</td>
<td>11.2 ± 4.8</td>
<td>0.02</td>
</tr>
<tr>
<td>Median (range)</td>
<td>NGT</td>
<td>8.3 (1.9-21.30)</td>
<td></td>
<td>4.9 (1.9-8.2)</td>
<td>0.47</td>
<td>5.5 ± 3.4</td>
<td>0.47</td>
</tr>
<tr>
<td><strong>Serum Leptin (ng/ml)</strong></td>
<td>DM</td>
<td>3.4 (1.6-46)</td>
<td>0.83</td>
<td>12.0 (3.6-48.5)</td>
<td>0.53</td>
<td>19.5 ± 8.2</td>
<td>0.47</td>
</tr>
<tr>
<td>Median (range)</td>
<td>NGT</td>
<td>11.8 (1.4-23.0)</td>
<td></td>
<td>12.6 (4.6-27.0)</td>
<td>0.83</td>
<td>18.1 ± 9.1</td>
<td>0.47</td>
</tr>
<tr>
<td><strong>REE (kcal/day)</strong></td>
<td>DM</td>
<td>2382.2 ± 354.6</td>
<td>0.82</td>
<td>2725.1 ± 502.7</td>
<td>0.59</td>
<td>2595.5 ± 767.4</td>
<td>0.47</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>NGT</td>
<td>2438.1 ± 603.5</td>
<td></td>
<td>2906.5 ± 765.4</td>
<td>0.59</td>
<td>2719.7 ± 635.5</td>
<td>0.47</td>
</tr>
<tr>
<td><strong>REE/ FFM (kcal/kg/day)</strong></td>
<td>DM</td>
<td>60.2 ± 14.1</td>
<td>0.42</td>
<td>64.1.2 ±13.5</td>
<td>0.32</td>
<td>60.0 ± 30.4</td>
<td>0.51</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>NGT</td>
<td>65.7 ± 12.7</td>
<td></td>
<td>73.1 ± 20.5</td>
<td>0.32</td>
<td>69.7 ± 16.2</td>
<td>0.51</td>
</tr>
<tr>
<td><strong>Total PTE (kcal)</strong></td>
<td>DM</td>
<td>78.4 (13.1–114.4)</td>
<td>0.21</td>
<td>38.7 (-3.1 –89.6)</td>
<td>0.76</td>
<td>63.6(11.5 – 207.)</td>
<td>0.20</td>
</tr>
<tr>
<td>Median (range)</td>
<td>NGT</td>
<td>44.5 (-15.1 – 79.6)</td>
<td></td>
<td>29.1 (-28.2 –120.6)</td>
<td>0.76</td>
<td>17.5(-28.3 – 117.1)</td>
<td>0.20</td>
</tr>
<tr>
<td><strong>PTE / FFM(kcal/kg)</strong></td>
<td>DM</td>
<td>1.8 (0.28 – 3.0)</td>
<td>0.28</td>
<td>1.1 (-0.08 – 2.1)</td>
<td>0.78</td>
<td>1.6(0.34 – 4.7)</td>
<td>0.19</td>
</tr>
<tr>
<td>Median (range)</td>
<td>NGT</td>
<td>1.3 (-0.42 -2.0)</td>
<td></td>
<td>0.73(-0.71 -2.9)</td>
<td>0.78</td>
<td>0.4(-0.60 -2.7)</td>
<td>0.19</td>
</tr>
</tbody>
</table>

DM – GDM subjects who had overt or pre-diabetes in the postpartum period; (N=8)
NGT – GDM subjects who had normal glucose tolerance in the postpartum period (N=8)
PTE – Postmeal thermogenesis, FFM – Fat free mass, REE – Resting energy expenditure, BMI – Body mass index
* denotes P value between subjects with DM and NGT in early pregnancy; ** denotes P value between subjects with DM and NGT in late pregnancy
*** denotes P value between subjects with DM and NGT in postpartum
SUBGROUP ANALYSIS

Among the subjects with GDM who came for postpartum visit, eight patients were found to have impaired glucose tolerance (pre-diabetes, n=6 and overt diabetes mellitus, n=2). On comparison between the subjects who had impaired and normal glucose tolerance in the postpartum period; fasting insulin levels were significantly higher in patients with glucose intolerance in the postpartum period. But the fasting insulin levels were lower in these patients in early pregnancy. The BMI and fat mass were not significantly different between the groups in all three visits. The REE in total and adjusted to fat free mass was lower in all the three visits in subjects who remained glucose intolerant in the postpartum period. PTE in total and adjusted to fat free mass was higher in subjects with glucose intolerance. The differences in REE and PTE between the groups were not statistically significant.
Table 16: Comparison of GDM subjects with past history of GDM and without past history of GDM

<table>
<thead>
<tr>
<th>Variables</th>
<th>group</th>
<th>Early pregnancy</th>
<th>P* value</th>
<th>Late pregnancy</th>
<th>P** value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²) Mean ±SD</td>
<td>H/o GDM</td>
<td>24.9±3.7</td>
<td>0.40</td>
<td>27.1±3.3</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>No h/o GDM</td>
<td>26.9±4.3</td>
<td></td>
<td>28.9±3.8</td>
<td></td>
</tr>
<tr>
<td>Leptin (ng/ml) Median (range)</td>
<td>H/o GDM</td>
<td>2.8 (1.6-11.0)</td>
<td>0.03#</td>
<td>16.2 (8.6-23.1</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>No h/o GDM</td>
<td>10.3 (1.4-46.0)</td>
<td></td>
<td>17.0 (3.6-54.1)</td>
<td></td>
</tr>
<tr>
<td>REE (kcal/day) Mean ±SD</td>
<td>H/o GDM</td>
<td>2186.0±652.2</td>
<td>0.03#</td>
<td>2615.3±59.0</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>No h/o GDM</td>
<td>2503.7±525.5</td>
<td></td>
<td>2913.1±602.2</td>
<td></td>
</tr>
<tr>
<td>REE/FFM (kcal/kg/day) Mean ±SD</td>
<td>H/o GDM</td>
<td>52.7±16.2</td>
<td>0.24</td>
<td>59.4±15.9</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>No h/o GDM</td>
<td>64.5±11.6</td>
<td></td>
<td>69.1±15.4</td>
<td></td>
</tr>
<tr>
<td>PTE (kcal) Median (range)</td>
<td>H/o GDM</td>
<td>78.8 (13.0-95.4)</td>
<td>0.41</td>
<td>31.3 (2.2-89.6)</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>No h/o GDM</td>
<td>64.3 (-15.1-366)</td>
<td></td>
<td>32.9 (-14.1-120)</td>
<td></td>
</tr>
<tr>
<td>PTE/FFM (kcal/kg) Median (range)</td>
<td>H/o GDM</td>
<td>1.8 (0.28-2.6)</td>
<td>0.39</td>
<td>0.8 (0.04-2.1)</td>
<td>0.84</td>
</tr>
<tr>
<td></td>
<td>No h/o GDM</td>
<td>1.7 (-42-7.1)</td>
<td></td>
<td>0.81 (-0.72-2.9)</td>
<td></td>
</tr>
</tbody>
</table>

Subjects with past history of GDM n=4, Subjects without past history of GDM n=17
PTE –Postmeal thermogenesis, FFM –Fat free mass, REE –Resting energy expenditure ,
BMI –Body mass index, h/o –history of
*denotes P value between subjects with h/o of GDM and no h/o of GDM in early pregnancy , student t test
** denotes P value between subjects with h/o of GDM and no h/o of GDM in late pregnancy, student t test
# P < 0.05-considered as statistically significant

Among the GDM subjects, four subjects had history of GDM in the previous pregnancy and 17 did not have history of GDM in previous pregnancies. On comparison between the two groups, patients with past history of GDM had lower REE and REE adjusted to fat free mass in early and late phase of pregnancy. The difference in REE adjusted to FFM was between the groups was statistically significant in early pregnancy (p=0.03). The PTE did not show significant difference between the groups. BMI and serum leptin levels were lower in subjects with past
history of GDM in early pregnancy and the difference in leptin level was statistically significant (p=0.03)

**Table 17: Comparison of GDM subjects on metformin with MNT and MNT alone**

<table>
<thead>
<tr>
<th>Variables</th>
<th>group</th>
<th>Late pregnancy</th>
<th>Post- partum</th>
<th>P* value</th>
<th>Post- partum</th>
<th>P** value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m^2)</td>
<td>Metformin</td>
<td>27.3±3.97</td>
<td>25.9±3.1</td>
<td>0.18</td>
<td>27.2±3.2</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>No metformin</td>
<td>29.9± 3.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>Metformin</td>
<td>11.4 (3.6 -48.5)</td>
<td>16.1(8.4-31.5)</td>
<td>0.17</td>
<td>19.3 (8.6-33.0)</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td>No metformin</td>
<td>24.2(4.6 -54)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>REE (kcal/day)</td>
<td>Metformin</td>
<td>2763.6±480.2</td>
<td>2745.7±882.9</td>
<td>0.64</td>
<td>2583.7±336.2</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>No metformin</td>
<td>2765.5± 631.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>REE/FFM (kcal/kg/day)</td>
<td>Metformin</td>
<td>69.5±9.1</td>
<td>70.8±13.2</td>
<td>0.27</td>
<td>68.9±23.8</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>No metformin</td>
<td>65.9±17.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTE (kcal)</td>
<td>Metformin</td>
<td>37.7(-3.6-83.5)</td>
<td>63.6(-14.3-207)</td>
<td>0.73</td>
<td>17.5 (-28.3-53)</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>No metformin</td>
<td>28.8(-14.1-120)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTE/FFM (kcal/kg)</td>
<td>Metformin</td>
<td>0.91(0.81-2.1)</td>
<td>1.64(-.35-4.7)</td>
<td>0.78</td>
<td>0.43(-.69 -1.5)</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>No metformin</td>
<td>0.71(-0.7-1.7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In late pregnancy Metformin N=8, No metformin N = 12, In postpartum Metformin N =7, No metformin N=9
1 subject only on insulin is not included in the analysis
PTE –Postmeal thermogenesis, FFM –Fat free mass, REE –Resting energy expenditure, BMI –Body mass index
* denotes P value between metformin and no metformin group in late pregnancy
** denotes P value between metformin and no metformin group in postpartum period
# P < 0.05-considered as statistically significant

Among the GDM subjects; eight were on metformin and MNT and 12 subjects were on MNT alone and one subject was on MNT with insulin. On comparison between the subjects who were on metformin versus not on metformin, weight gain and BMI were lower in patients who were on metformin both in late pregnancy and in the the postpartum period. Serum leptin levels were lower in the metformin group, total REE was comparable between the groups in late pregnancy, but the REE adjusted to fat free mass was higher in subjects on metformin. The PTE in total and adjusted to
FFM was higher in the metformin group in late pregnancy, but this difference in PTE was not seen in the postpartum period.
This was a comprehensive study assessing all the domains of energy expenditure and body composition in subjects with diabetes mellitus complicating pregnancy. In the study we assessed longitudinal changes in resting energy expenditure (REE), Post-meal thermogenesis (PTE), activity energy expenditure (AEE), energy intake, body composition, and leptin levels in subjects with gestation diabetes mellitus in pregnancy and in the postpartum period. We compared these parameters with the control group (pregnant subjects with normal glucose tolerance). Our study comprised of 21 subjects with GDM and 13 subjects with normal glucose tolerance. 16 subjects in the GDM group and 8 subjects in the control group were assessed in the postpartum period, excluding subjects who were lost to follow up.

**BASELINE CHARACTERISTICS**

The mean (SD) age of the GDM group was 28 years (4.3) whereas the mean age of controls was 25.7(5.0) years. Majority of subjects was multigravida in both the groups (52% and 70% in GDM and NGT group respectively). The mean BMI of the GDM subjects were significantly higher than the normal pregnant mothers. The past history of GDM was present in 4 subjects in the GDM group and 1 subject in the NGT group. Older age, obesity, past history of GDM and family history of diabetes mellitus are considered as traditional risk factors for GDM. Several Indian and global studies have shown a strong association between family history and the prevalence of GDM. Our study has identified family history of diabetes in both the groups. The family history of diabetes was more in the GDM group (60% versus 30%) compared to the controls. In the GDM group 12 (57%) subjects were on medical nutritional therapy alone, 6 subjects (28.5%) were on metformin, 1 patient was on insulin alone, 2 patients were on combined metformin and insulin. All the patients had good glycemic control as assessed by self-monitoring of blood glucose.
Discussion

levels. The rate of vaginal delivery was similar in both the groups (66% in GDM, 61% in NGT group). All the subjects had term delivery (Gestational age > 37 weeks). A study from south India highlights that more than 32% of the gestational diabetes patients had to undergo caesarean section to terminate their pregnancy.\textsuperscript{57} Our study had similar rate of caesarean section (35%). The mean birth weight was similar in both the groups (2.88kg in infants born to GDM mothers and 2.99kg in infants born to control group). Macrosomia (birth weight >3.5 kg) was seen in 4 babies (2 in GDM subjects and 2 in control group). 8 out of 16 subjects in GDM group had persistent glucose intolerance in the postpartum period and 2 patients who had blood glucose levels in the diabetic range were restarted on metformin along with medical nutritional therapy. A systematic review of 20 studies found a seven fold increase in the risk of developing T2DM, when comparing women with a pregnancy complicated by GDM to women with a normoglycemic pregnancy.\textsuperscript{58}

RESTING ENERGY EXPENDITURE

The key findings from the study:

The resting energy expenditure (REE) in total was similar in subjects with GDM and normal glucose tolerance in early and late phase of pregnancy where as it was slightly higher in subjects with GDM in the postpartum period. When adjusted to fat free mass (FFM), REE was lower in subjects with GDM subjects when compared to the controls in the early phase of pregnancy. The lower REE adjusted to fat free mass in the GDM subjects were not maintained in the late phase of pregnancy and the postpartum period. There was longitudinal increase in REE, expressed as kcal per day in both the groups from early phase of pregnancy to late phase, but the percentage change was higher in subjects with GDM (17% versus 11%, P value 0.55). REE adjusted to fat free mass was similar in both the groups in the post-
partum period and was slightly higher than the early phase of pregnancy. The REE in the early phase of pregnancy in subjects who had history of GDM in previous pregnancy was significantly lower, when compared with subjects without history of GDM.

Previous studies reported that the resting energy expenditure does not change in first and second trimester of normal pregnancy and increases gradually from third trimester to term. The theoretical increases in total energy expenditure of pregnancy had been estimated to be 80,000 kcal, or 300 kcal/day. The additional energy costs include the increases in maternal and fetoplacental tissue and the energy costs of pregnancy such as increased maternal cardiac output. REE represents around 60% of total energy expenditure. It is important to estimate the change in resting energy expenditure both in terms of total energy expenditure (kilocalories per day) and adjusted for FFM. FFM represents metabolically active tissue (for example, skeletal muscle), whereas fat mass is proportionately less metabolically active. Several studies have documented conflicting data on REE of pregnant women with normal glucose tolerance and with diabetes mellitus complicating pregnancy. In a previous study published by Catalano et al there was 30% increase in basal energy expenditure from preconception to late pregnancy, when expressed as kilocalories per day. After adjustment for FFM, the increase in energy expenditure was 14% in the NGT and 21% in the GDM subjects. These differences did not reach statistical significance (P 0.3 to 0.5) because of the great inter individual variability. Our study also showed a higher increase in GDM patients from early to late pregnancy. In our study the change in REE adjusted to FFM was 8% in the GDM group and 4% in the control group.
The influence of BMR and REE on GWG and GDM has been studied extensively in the past and the results have been conflicting. Several studies state that a low prepregnancy BMR is associated with increased GWG, which increases the risk for GDM. Others claim that excessive GWG, in part due to increased maternal circulatory, respiratory and renal functions, is associated with increased risk for GDM. In this case, the higher BMR observed appear to be a mere epiphenomenon of the increased GWG and few studies showed no significant BMR variation between women with and without GDM. In few studies outside pregnancy, T2DM has been positively associated with higher BMR levels in various ethnicities after adjustment for FFM, FM, age, and sex, indicating a possible positive relation of BMR with GDM. In our study the REE in early pregnancy was lower in the GDM subjects despite the higher BMI and fat mass than the control group. However this difference in REE did not persist in the late phase of pregnancy and postpartum visits. In late pregnancy and postpartum period; GDM group had higher REE and similar REE adjusted to FFM when compared to the NGT group. The weight gain in pregnancy and postpartum period was lower in subjects with GDM compared to the controls. The mean weight gain during pregnancy was 4.5kg in the GDM group and 7.1kg in the NGT group, whereas in postpartum period it was 0.95kg and 5kg respectively. On assessing body composition, the fat mass was significantly higher in early pregnancy in the GDM subjects, whereas lean body mass was almost similar in both the groups. There was significant increase in fat mass and fat mass percentage in NGT subjects during pregnancy and postpartum, where as in GDM subjects the fat mass and fat mass percentage remained almost unchanged. Lean body mass showed increase from early to late pregnancy in both groups. The lesser increment in REE in normal
pregnant women may contribute to the greater weight and fat mass gain in this group during pregnancy and postpartum.

Several factors have been implicated in the variability of BMR in pregnancy, including pre-pregnancy body weight and body fatness, lean body weight, reduced daily activity during pregnancy, changes in serum concentration of metabolism related-hormones, and cardiac output changes.\textsuperscript{65,66} During late pregnancy, the fetus contribution to the BMR increase is about 50\%.\textsuperscript{67} Nonetheless, controversy exists in regard to the magnitude of the contribution of each factor. Our study has shown that REE positively correlated with the fat mass and leptin levels in early phase of pregnancy in GDM subjects. REE found a negative correlation with fasting glucose insulin ratio in all the three visits. Among the GDM subjects; the REE adjusted to FFM was higher in subjects who were on metformin when compared to subjects on MNT alone and the weight gain in metformin group was significantly lower (2 kg versus 6.5 kg, p=0.07).

**MIXED MEAL CHALLENGE TEST - POSTMEAL THERMOGENESIS**

The key findings from the study:

There was a longitudinal decrease in postmeal thermogenesis (PTE) in total and when adjusted to fat free mass from early phase of pregnancy to late pregnancy in both the groups. The reduction in PTE was greater in subjects with GDM when compared to the controls (42\% versus 20\%). The difference was showing trend towards statistically significance (p=0.08). The reduction in PTE through pregnancy persisted into the postpartum period in the GDM group, but not in the control group. On comparing PTE among both groups at each visit, GDM subjects had higher PTE in total and when adjusted to fat free mass. On subgroup analysis we found that
subjects on metformin had higher PTE compared to subjects on MNT alone in late pregnancy, but this difference was not seen in the postpartum period, off metformin.

On comparing PTE in subjects with past history of GDM, PTE was comparable in both the groups (with or without past history of GDM). In GDM subjects the total PTE positively correlated with HOMAIR (r=-0.52, p=-0.01) in the early phase of pregnancy. On assessing hourly change in PTE after the mixed meal, the total PTE showed an increment in first and second hour and then started decreasing, but did not touch the baseline at the end of third hour. The pattern was similar in both groups except in late pregnancy where the PTE continued to increase at third hour in the controls, whereas it showed a decrement in subjects with GDM.

Reduction in post meal thermogenesis has been demonstrated previously in normal pregnancy especially after 24 weeks.68 Illingworth et al were the first to suggest a possible energy saving role for PTE during the second trimester of pregnancy.69 The resultant energy saving if continued over 24 weeks of pregnancy contributes to 13% of the additional energy expenditure of pregnancy.69 An even greater reduction of PTE was observed in women with GDM in various studies though there are contradicting data which shows no significant difference in post meal thermogenesis between GDM subjects and normal glucose tolerance.70 As we know insulin sensitivity decreases as pregnancy advances and may contribute to development of diabetes in the pregnancy. Previous studies have found no statistical association between the insulin insensitivity and reduced postprandial thermogenesis within the women with gestational diabetes.71 Our study showed reduction in PTE in the late pregnancy in both the groups compared to the early phase of pregnancy. The percentage decrease in PTE from early to late phase of pregnancy was higher in GDM subjects compared to the controls (42% versus 20%) and the PTE correlated
Discussion

with HOMA IR in subjects with GDM. However contradictory to the literature; our study showed that the absolute PTE was higher in GDM subjects compared to NGT group at each visit. The GDM subjects in our study had higher BMI, fat mass, leptin levels and higher insulin levels. Also around 50% of them were on metformin. Also they had lesser weight gain and they were on intense life style interventions compared to the control group. Many of these factors could have influenced the higher PTE, further large sample size studies are required to assess the factors contributing to higher PTE in GDM subjects.

Another study by Kousta et al compared PTE postmeal thermogenesis following a mixed meal in 29 normoglycemic European women with previous gestational diabetes compared with 37 control women. Although mean values of total PTE were lower in the GDM group, this difference did not quite attain statistical significance. However, they observed a difference in the shape of the PTE curve between groups and by applying a mathematical model, there was a consistent delay in PTE, insulin, and noradrenaline responses to the meal in the GDM group. Although the biological significance of the delayed PTE response is uncertain, one possibility is that this is an early metabolic manifestation that precedes an absolute decrease in PTE in these women with post-GDM. In our study; the 4 patients with past history of GDM had comparable PTE with subjects without history of GDM.

Utilisation of substrates during MMCT

We assessed the response of glucose, insulin and free fatty acid during mixed meal challenge tests. The AUC for glucose and insulin were slightly higher in GDM subjects compared to control group in all three visits, but the difference was not statistically significant. Free fatty acids levels decreased uniformly in both the groups in all visits. The change in substrate utilisation (glucose and free fatty acids)
and insulin secretion rates did not show any correlation to the PTE. In the study by Kousta et al; the delay in PTE response was associated with a delay in the insulin response to the meal in the GDM group. Individual patterns of PTE and insulin response to the meal were correlated: This may reflect a causal relationship, with the delayed metabolic response responsible for the delayed thermogenic response in subjects with GDM. Previous studies have shown that catecholamine’s modulates PTE. There is evidence to suggest that insulin resistance leads to decreased thermogenesis, especially in obese subjects. The insulin, glucose and fatty acid disposal were similar in both groups in our study, which fail to explain the high postmeal thermogenesis in GDM subjects. The assessment of catecholamine response was not done in our study which is a significant factor in determining PTE.

**BODY COMPOSITION AND ANTHROPOMETRIC INDICES**

GDM subjects in our study had higher BMI compared to NGT. In GDM subjects twelve were obese, three were overweight and six had normal BMI in the early phase of pregnancy. In the control group, majority had normal BMI (Eight), four were obese and one was overweight. The mean weight gain in GDM subjects was lesser in late pregnancy and in postpartum when compared to the controls (4.5kg versus 7.1kg). There was no significant increase in mid arm and neck circumference in both the groups. The neck fold thickness was significantly higher in GDM group throughout pregnancy and postpartum. Other skinfold thicknesses were also higher in the GDM subjects, not all were statistically significant. On assessment of body composition by bio impedance analyser, fat mass and fat mass percentage and were significantly higher in subjects with GDM in the early phase of pregnancy. Fat mass content and fat mass percentage increased in late pregnancy and postpartum period.
in the control group whereas in GDM subjects, the fat mass and fat mass percentage showed minimal increase in late pregnancy and postpartum. Previous studies have shown significant increase in the fat mass in both lean and obese subjects with normal glucose tolerance and gestational diabetes during pregnancy. The increases in fat mass in early pregnancy were more apparent in lean women with gestational diabetes, most likely related to the significant decrease in insulin sensitivity in these women. Distribution of fat mass accretion during pregnancy in various studies ranged from 2.0 to 13.1 kg. In our study the lesser weight gain and fat mass accretion may be related to the higher REE, PTE, AEE and lesser calorie intake in the GDM subjects compared to normal pregnant women. Further studies are needed to determine the contribution of each factor in gestational weight gain.

**INSULIN SENSITIVITY INDICES**

Fasting insulin levels progressively increased in subjects with normal glucose tolerance in the late pregnancy and postpartum period. However the trend of increase in insulin resistance was not seen in GDM subjects. Insulin sensitivity indices like HOMA IR, QUICKI and FGIR also showed similar trends of increased insulin resistance in normal subjects. The fasting insulin levels in subjects with GDM were higher than control in early phase of pregnancy, though the difference was not statistically significant (p=0.31). QUICKI was significantly lower in the GDM group in early pregnancy (p=0.04). Fasting insulin levels in late pregnancy decreased from early pregnancy. We also assessed the fasting free fatty acids which was higher in GDM subjects in all the visits and in both the groups, FFA showed a longitudinal decrease in late pregnancy and postpartum period when compared to early pregnancy.
Previous studies have shown a progressive rise in insulin secretion as the pregnancy advances, indirectly signifying an increase in insulin resistance. Catalano P et al., found a significant 65% increase in both basal insulin and C-peptide concentrations in all subjects with advancing gestation. Significant decrease in FGIR in 3rd trimester shows increase in insulin requirement to maintain the similar plasma glucose concentration during pregnancy. In normal pregnancy, there is an approximate 50% decrease in insulin mediated glucose disposal and a 200% to 250% increase in insulin secretion to maintain euglycemia in the mother. QUICKI has proved to be a versatile tool in measuring IR. It has shown linear relation with other gold standard technique such as euglycemic hyperinsulinemic clamp testing and frequently sampled intravenous glucose tolerance test (FSIVGTT). In our study, QUICKI showed a significant longitudinal decrease in late pregnancy and postpartum period from the early pregnancy in control group. In GDM subjects QUICKI did not differ significantly at each visit.

GDM is characterised by insulin resistance and failing beta cell compensation for that resistance. Previous studies have shown a significant decrease in insulin sensitivity in late gestation in women with gestational diabetes in comparison with a matched control group which is a reflection of the decreased insulin sensitivity that exists prior to pregnancy. The changes in insulin sensitivity from baseline or pre-gravid phase through early pregnancy are inversely related to changes in maternal fat mass. In our study the insulin sensitivity did not worsen in GDM subjects in late pregnancy, which may be related to the lesser weight gain and decrease in fat mass and treatment modalities like metformin.
**SERUM LEPTIN**

Serum leptin levels were comparable in both the groups in early phase of pregnancy. The leptin levels increased during late pregnancy and postpartum period in GDM subjects; whereas similar increase was not seen in the control group. In early pregnancy leptin levels correlated with the REE, but not with PTE in both the groups. In both the groups serum leptin levels were higher in obese subjects in early and late pregnancy and in the postpartum period. In our study leptin correlated with HOMA IR, QUICKI index and fasting insulin levels in normal pregnancy\((r=-0.60, -0.82, 0.62, p<0.05)\). Patients on metformin had a lower leptin levels compared with patients on MNT in late pregnancy.

Although the source of leptin is well documented, the role of the increased maternal leptin concentrations during gestation has remained elusive. In addition to maternal adipose tissue the placenta produces leptin.\(^8\) Although leptin was originally thought to be related only to appetite suppression via central mechanisms, further reports pointed to a role of leptin in the control of energy expenditure and intake.\(^5\) Most studies have found that hyperleptinemia in early pregnancy appears to be predictive of an increased risk to develop GDM later in pregnancy, independent of maternal adiposity.\(^8\)

**ACTIVITY ENERGY EXPENDITURE**

The findings from our study

The activity energy expenditure was assessed using PPAQ questionnaire in the study. We found that the total derived AEE expressed in METS hour per week was lower in the GDM subjects compared to controls in early pregnancy. In late pregnancy and postpartum the total AEE was higher in GDM subjects reflecting the
increased awareness among GDM subjects regarding physical activity for control of diabetes. The total AEE increased by 11% in GDM subjects from early to late pregnancy, whereas in normal pregnant women AEE decreased by 14.5%. The total AEE was highest in postpartum period in both the groups, though the difference was not statistically significant. On assessing the intensity of activity, majority of the AEE was contributed from light intensity activity (2-2.9mets) in both the groups throughout pregnancy and postpartum (40-60%). There was a significant increase in moderate intensity activity (3-5.9 METS) in the postpartum period in GDM subjects compared to early and late phase of pregnancy. In the early phase of pregnancy sixteen out of twenty one GDM subjects and eleven out of thirteen NGT controls were doing moderate intensity activity whereas all patients were doing moderate intensity activities in late pregnancy and postpartum period. None of the subject except one in control group was doing high intensity activity in the pregnancy and postpartum period. (≥6 mets). These may be related to the cultural practices in the society. On assessing the domains of physical activity majority of the work was from household activities (60-90%). The exercise or sports related activities contributed to less than 10% of the total AEE.

Questionnaires are a commonly used, inexpensive, and acceptable method to determine physical activity levels. PPAQ questionnaire has been validated in pregnancy and there has been high quality evidence that it has sufficient reliability in assessing total physical activity. The results from systematic review and meta-analyses indicate that greater total physical activity before pregnancy or during early pregnancy was significantly associated with a lower risk of GDM. Similarly in our study subjects with diabetes mellitus complicating pregnancy had lower AEE in early pregnancy, though it did not reach statistical significance (p=0.27). The studies
have shown that the magnitude of this association was greatest for pre-pregnancy physical activity with women in the highest quantiles of activity experiencing a 55% reduction in risk, compared with that for women with the lowest activity. The inverse association observed between physical activity and development of GDM is biologically plausible. Research among non-pregnant individuals has shown that exercise-induced improvements in glycemic control may be due to increases in GLUT4, a glucose transport protein. Researchers have demonstrated that physical activity may also have an indirect and potentially more long-term role in glucose tolerance through favourable changes in body composition. Decrease in fat mass and increases in muscle mass have been shown to have positive effects on glycaemic control. Our study has shown lesser weight gain and fat mass gain during pregnancy and postpartum period in subjects with GDM. The increased AEE might be contributing to this which would have translated into better glycemic control.

**DIETARY ASSESSMENT**

The total energy intake was significantly lower in the GDM subjects in late pregnancy, whereas it was comparable in early pregnancy and postpartum period. According to the recommended dietary allowance for Indians, a woman requires an additional 350 kcal/day during the second and third trimester of pregnancy and 550 kcal in early postpartum period. Thus, a sedentary Indian pregnant mother requires 1900 kcal in early pregnancy, 2250 kcal/day in late pregnancy and 2450 kcal/day in postpartum. Energy-related studies agree that caloric restrictions are necessary for the overweight or obese mother. Various guidelines recommend 30% calorie restriction for obese subjects with diabetes mellitus in pregnancy with minimum intake of 1600-1800 kcal/day. A randomized controlled trial (RCT) (n = 124) compared a diet with moderate energy restriction providing 70% of the dietary
reference intake (DRI) for pregnancy (1590–1776 kcal/day) vs. a diet that was unrestricted (2010–2220 kcal/day). After taking into account the estimated intake analysis, no significant difference was found between the groups in various outcomes (frequency of insulin use, mean birth weight, ketonemia). No adverse effects were reported with any of the energy restriction. In our study the average calorie intake in early pregnancy was 90% and 85% of RDA (1900kcal) in GDM and control subjects respectively. This seems to be adequate considering the mean BMI of our study population was in the obese range. The GDM subjects had a significant decrease in calorie intake in late pregnancy (decrease of 148kcal from early pregnancy) owing to the intense nutritional interventions, whereas the controls had a mean increase in calorie intake of 318kcal.

The meal composition of macronutrients did not change during pregnancy and postpartum period in spite of dietary advice. The meal composition was inappropriate with a high quantity of calories from carbohydrate and fat and an inadequate contribution from proteins. The contribution from carbohydrate was more than 60% in both groups, when carbohydrate restricted diet has been recommended for glycemic control (recommended carbohydrate -40-50% of total calorie). Similar findings were reported in a South Indian study on patients with type 1 diabetes mellitus patients. A Korean study found that the GDM group had an undesirable macronutrient composition and obtained 56.6% of their calories by carbohydrate intake, which exceeded the recommended levels. Our study did not look into the glycemic index, fibre content and micronutrient intake which can influence the maternal weight gain, development of GDM and fetal outcomes including birth weight and prematurity.
SUMMARY AND CONCLUSIONS:

A total number of 34 subjects which included 21 subjects with Gestational Diabetes Mellitus (GDM) and 13 healthy pregnant controls were included in this study over a period of 2 years 2016 -2018. The subjects were followed longitudinally in early and late pregnancy and postpartum period.

To summarize our conclusions at the end of analysis:

- Body fat percentage in the GDM group was higher than the controls in early pregnancy. The longitudinal increment in fat percentage was lower in the GDM subjects, when compared to the control group.

- The GDM subjects when compared with the controls had lower insulin sensitivity in early pregnancy. GDM subjects did not show a significant change in insulin sensitivity unlike the control subjects, whom showed a significant decrement in insulin sensitivity during late pregnancy and postpartum period.

- The Resting Energy Expenditure (REE) adjusted to fat free mass was lower in GDM subjects than the controls in early pregnancy. The REE was similar among the groups in late pregnancy and postpartum period.

- The Post meal Thermogenesis (PTE) after the mixed meal challenge test was greater in the GDM group than that in the controls in all the three visits.

- There was a significant decrement in PTE during late pregnancy and postpartum period in both the groups, when compared to early pregnancy.
The longitudinal decrement in PTE was significantly higher in subjects with GDM than that in the control group.

The baseline serum leptin levels were higher in GDM subjects than the normal pregnant women. The leptin levels showed a longitudinal increment in the GDM group in late pregnancy and postpartum period, whereas leptin levels in the control group did not show any significant change.

The pattern of change in serum insulin, free fatty acids and plasma glucose levels during MMCT was similar in both the groups.

The Activity Energy Expenditure (AEE) was lower in the GDM group than the controls in early pregnancy.

The AEE showed an increment in GDM subjects in late pregnancy and postpartum. The AEE in postpartum period was higher than during pregnancy in both the groups.

The total calorie intake was significantly lower in subjects with GDM in late pregnancy than the control group. During early pregnancy and postpartum period the calorie intake was comparable between the groups.

The REE in the GDM group was found to have a significant correlation with total body fat and serum leptin levels in early pregnancy.

The REE in both the groups had significant correlation with Fasting insulin glucose ratio (FGIR) in all three visits.

The PTE in the GDM group was found to have significant correlation with HOMA-IR in early pregnancy.
LIMITATIONS OF THE STUDY

- The body composition was analysed using bio impedance method in the study. We were not able to assess the differential changes in central and peripheral fat composition, which could have influenced the energy expenditure and insulin sensitivity.

- The previous studies have shown that sympathetic system plays an important role in mediating energy expenditure. In our study we did not measure the influence of catecholamines in post meal thermogenesis during MMCT.

- The impact of various adipocytokines and inflammatory mediators during pregnancy in body composition, insulin sensitivity and energy expenditure is well known. The role of adipokines other than leptin was not assessed in our study.


22. Compher C, Frankenfield D, Keim N, Roth-Yousey L, Evidence Analysis Working Group. Best practice methods to apply to measurement of resting


60. Regulation of basal metabolic rate in uncomplicated pregnancy and in gestational diabetes mellitus. HORMONES. 2017;13(3). doi:10.14310/horm.2002.1743


71. Piers LS, Diggavi SN, Thangam S, van Raaij JM, Shetty PS, Hautvast JG. Changes in energy expenditure, anthropometry, and energy intake during the


75. Catalano PM, Drago NM, Amini SB. Longitudinal changes in pancreatic beta-cell function and metabolic clearance rate of insulin in pregnant women with normal and abnormal glucose tolerance. *Diabetes Care.* 1998;21(3):403-408.


1. You are being called to join this research study.

The title of the study is:

“A comprehensive study on Energy Expenditure and body composition in diabetes mellitus complicating pregnancy”.

2. The study is being done by

**Principal Investigator:** Dr. Geethu Antony(Research Study Doctor)
Dept of Endocrinology, Diabetes and Metabolism,
Christian Medical College, Vellore
Tamil Nadu- 632004.
Phone: +91-416-2282528, +917094355646
Email ID: geethuantony86@gmail.com

3. **Do I have to take part in this research study?**

The purpose of this research is to study the changes in the amount of energy you utilize and distribution of body fat during your pregnancy and after delivery. We will also assess how these changes are different in pregnant women with diabetes mellitus and without diabetes mellitus.

Your participation is entirely voluntary.

If you decide to take part you will be asked to sign this consent form. Your signature means that you agree to take part in this research. After reading this form and having a discussion about what it says, you can decide whether you would like to be a part of this study.
If you decide not to participate, the care providers at this facility will continue to give you all of the standard care that is appropriate for you. You will be given a copy of this form whether or not you agree to participate in this study. Even after deciding to take part, you are free to withdraw at any time without giving any reason. This will not affect your care and you will continue to be treated at this hospital as before.

The form discusses:

a) What the researchers will learn from the research?

b) What will happen to you during the research?

c) What risks and/or discomforts you might expect/experience

d) As a research subject if you can expect any benefits, and are there any alternatives to this research for your condition.

4. Why have I been asked to take part in this research study?

You are being asked to participate as a subject as you have developed diabetes mellitus during pregnancy/ or as you are pregnant. If you agree to take part in this study you will have tests and examinations to be sure that you qualify for the study. You will be assessed for your body fat distribution, we will also assess the amount of energy you spend each day while resting and after food intake. Also we will assess the amount of activity you do using a questionnaire and also your eating pattern.

Identifying these things will help us in deciding the appropriate nutritional recommendations during pregnancy and lactation in normal pregnant women and in pregnant women with diabetes mellitus.

5. Why is this research study being done?

The rationale for this research is based on previous studies that have been done previously which show varying data regarding the changes in energy utilization and body fat changes during pregnancy and after delivery. Previous data have shown that these changes might predispose to diabetes mellitus during pregnancy. These changes may vary depending upon the ethnicity of the study population. Not many studies have been done in India which studied these parameters. Hence this research study is being undertaken in an attempt to identify the changes in energy expenditure in body
composition and energy expenditure which happens during pregnancy and postpartum in women with diabetes mellitus complicating pregnancy and in normal pregnant women.

6. How many people will take part in the research study?

You will be one of approximately 33 pregnant women who will be participating in this study. The study will be conducted at Christian Medical College, Vellore.

7. What will happen if I take part in this research study?

You will undergo medical testing to determine your eligibility at the Clinical Research Center (CRC), located at Department of Endocrinology Diabetes and Metabolism, Christian Medical College, Vellore-632004.

This visit will include a full history and physical examination, and some blood investigations and blood pressure, measurement done as part of standard of care. If you meet all eligible study criteria, you will be invited to participate in the study. There will be about 33 patients participating in this study and it is important that you complete all parts of the study.

If you are enrolled in the study, you have to come for the study at 3 different time points. First visit will be in your early gestational period, before 18 weeks of pregnancy. The second visit will be in your late gestational period, 32-38 weeks of pregnancy. Third visit will be in postpartum, 6-24 weeks after your delivery. The visits can be scheduled depending on your convenience.

For the procedures you will have to come to the CRC in a fasting state in the morning. You will be undergoing the following procedures in each visit:

Physical examination will include measurement of height, weight, mid arm circumference, skin fold thickness at 5 different sites (biceps, triceps, thigh, upper back and lower back). Skin fold thickness will be measured by a caliper and is not painful. Body composition will be assessed using a bio impedance analyser called bodystat. It is a small hand held battery operated machine and the test is non invasive. It has been found to be safe in pregnancy.
You will also undergo indirect calorimetry to assess the energy production. Indirect calorimeter, consist of a plastic hood that surrounds your head and a soft plastic collar round the neck and shoulders. A fixed flow of room air is maintained through the hood by connecting the outlet of the hood to a suction pump. The hood will be properly disinfected before reuse. One relative of you and a staff will be present in the same room during the entire period of study to make you comfortable. The study will start by 08.00 am in a fasting state. You have to take dinner by 8.00pm the previous day and have to be in fasting from midnight. You have to rest in bed for 30 minutes before the test begins. After 30 minutes of the calorimeter, you will be given a drink which gives you around 400 Kcal which is equivalent to the breakfast you take. The drink is prepared by a mixing powder which consists of carbohydrate, protein and fat in standard ratio with water. This powder is found to be safe to use in pregnancy. After the mixed meal, indirect calorimetry test will be repeated for 30minutes every hour for 3 hours. Blood samples will be taken at 0, 1, 2 and 3 hours along with the test. During the visit you will be asked about your diet and physical activity using a questionnaire.

These visits will be approximately 4-5 hours long.

8. What are the possible side effects, discomforts, risks or inconveniences i can expect from being in this research study?

1. Blood withdrawal: The total amount of blood drawn from your body will not exceed 10ml in each visit. You will have mild pain like that of a pin prick while blood samples are being drawn.

2. Bio impedance analyser –This device is used to measure the body composition. This has been proved to be safe in pregnancy

3. Mixed meal challenge test
You will be given a drink which gives you around 400 Kcal which is equivalent to the breakfast you take. The drink is prepared by a mixing powder with water which consists of carbohydrate, protein and fat in standard ratio. This powder is found to be safe to use in pregnancy.

9. Are there likely to be any benefits to taking part in this research study?
This study will help us to increase our understanding of changes in energy expenditure and body composition in normal pregnancy and in diabetes mellitus complicating pregnancy. This might help in future to decide on the nutritional interventions in this population.

10. **What other choices do I have if I do not take part in this research study?**

You may choose not to participate in this study.

11. **Who may see my records?**

The research records will be stored in a password protected computer and your name will not be used in any written or verbal reports. Your research records and medical records may be inspected by members of the research team.

The people who review this research study as members of the Christian Medical College Institutional Review Board (IRB) may also review your research and medical records. The Office of Human Research Protections (OHRP) may also review your research study records.

All of these groups have been requested to maintain confidentiality.

12. **Who can answer my questions about the study?**

If any questions arise related to this research project, you may call the Principal Investigator (Researcher Study Doctor):

Dr. Geethu Antony.,
Senior Registrar,
Department of Endocrinology, Diabetes & Metabolism
Christian Medical College, Vellore
(Telephone: 7094355646, email –geethuantony86@gmail.com)

13. **Use of identified specimens for future research:**

In addition to the research, you are consenting for, Dr Geethu Antony or other researchers at this institution may wish to study the samples in future research projects.

Information about the research may be shared with other researchers, your identity will not be revealed.
14. Participant:
Please indicate your choice by initialing one (1) of the following options

___ I consent to have my specimens used for future research studies.
___ I consent to have my specimens used for future research studies only for the study of ____________________________.
___ I do NOT consent to have my specimens used for future research studies. The specimens will be destroyed at the end of the study.

15. Participant:
For future contact, please initial your choices below

I consent to be contacted in the future to learn about:
___ New research protocols that I may wish to join.
___ General information about research findings.
___ Information about the test on my sample that may benefit me or my family members in relation to choices regarding preventive or clinical care

_____ I do not agree to be contacted in the future, even if the results may be important to my health or my family's health.

Your wish does not constitute a guarantee that you will be contacted.

16. Will I be asked to stop participating in this study before the study is finished?
Your study doctor may discontinue your participation in this study. Reasons for discontinuation could be, for example:
You experience an unexpected side effect or you have abnormal laboratory results that would be considered unsafe.
You develop, during the course of the study, symptoms or conditions that are excluded in the study
You do not follow instructions.
You use a medication or drug not allowed by the study (except: medications for arthritis, hypertension and hyperlipidemia

17. What if new information becomes available?
If the research study doctor obtains new information that might lead you to change your mind about continuing in this study, the research study doctor will tell you about it. If you decide to withdraw, the research study doctor and your personal doctor will make arrangements for your care to continue.

18. Can I stop the study at any time?
Your participation in this study is voluntary, and you can withdraw from the study at any time without giving a reason. If you decide to withdraw, you should talk with the research study doctor to see how best to complete the withdrawal process.
If you agree to participate and withdraw at a later time, some of your information may have already been entered into the study and that will not be removed.
In addition, you may be asked to return to the research study doctor again for any final tests in order to close the record and tests or monitoring that are necessary for your health as a result of your participation. These results may be recorded.
Your treatment by doctors and staff at the institution involved in this study, now and in the future, will not be affected in any way if you agree to participate and withdraw later.

19. What are my rights if I take part in this research study?
Your participation in this study is voluntary. You do not waive any of your legal rights by participating in this research study. Your treatment by doctors and staff at the institution(s) involved in this study, now and in the future, will not be affected in any way if you refuse to participate or if you enter the study and withdraw later.
You must tell the research study doctor about any past and present diseases or allergies you are aware of and about all medications you are taking including over-the-counter remedies and nutritional supplements or herbs.
If any other doctor recommends that you take any medicine, please inform him/her that you are taking part in a research study. You should give the other doctor the research study doctor's name and phone number.
You may carry out all your normal daily activities.

Research Team: (kindly fill other author’s name)
1. Dr. Geethu Antony, Department of Endocrinology, Christian Medical College, Vellore
2. Dr. Nihal Thomas, Department of Endocrinology, Christian Medical College, Vellore
3. Dr Thomas Paul, Department of Endocrinology, Christian Medical College Vellore
4. Dr H. S Asha, Department of Endocrinology, Christian Medical College, Vellore
5. Dr Dukhabandu Naik, Department of Endocrinology, Christian Medical College, Vellore
6. Dr Riddhi Das Gupta, Department of Endocrinology, Christian Medical college, Vellore.
7. Dr Annie Regi, Department of Obstetrics and Gynecology, Christian Medical College,
8. Dr Gigi Elizabeth Mathew, Department of Obstetrics and Gynecology, Christian Medical College, Vellore
9. Dr Jessy Lionel, Department of Obstetrics and Gynecology, Christian Medical College, Vellore
10. Dr Joe Fleming, Department of Clinical Biochemistry, Christian Medical College, Vellore
11. Dr Vishalakshi J, Department of Biostatistics, Christian Medical College, Vellore
12. Dr Mini Joseph, Department of Endocrinology, Christian Medical College, Vellore
13. Mrs. Mercy Inbakumari, Department of Endocrinology, Christian Medical College, Vellore
INFORMED CONSENT FORM

DEPARTMENT OF ENDOCRINOLOGY DIABETES AND METABOLISM

CHRISTIAN MEDICAL COLLEGE, Vellore.

Study title: A Comprehensive Study on Energy Expenditure and Body composition in pregnant women with diabetes mellitus complicating pregnancy

Principal investigator: Dr. Geethu Antony

Study number:

Please tick the following as appropriate:

(i)  I confirm that I have read and understood the information sheet for the above study and have had the opportunity to ask questions.

(ii) I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

(iii) I understand that my identity will not be revealed in any information released to third parties or published.

(iv) I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s).

(v)  I agree to take part in the above study.

The following is a list of items we discussed about this research study. If you have any questions about any of these items, please ask the person who is discussing the study with you for more information before agreeing to participate.

What the study is about.
What I must do when I am in the study.
The possible risks and benefits to me.
Whom to contact if I have questions or if there is a research related injury.
Any costs and payments that I may need to make.
I can discontinue participating in the study at any time without penalty.
Other choices.
All written and published information will be reported as group data with no reference to my name.
INFORMED CONSENT FORM

If there is a schedule explaining how the study medicines are to be taken, I will be given the time schedule.
I have been given the name of the researcher and others to contact.
I have the right to ask any questions.

The signature section is specially formatted - please do not modify.

Name of Participant                     Signature/ thumb impression of participant

__________________                         ____________________

Date ______________________

Name of the witness: ______________________

Signature of the witness: ______________________

Name of Person conducting the Informed Consent Process                     Signature of Person conducting the Informed Consent Process

__________________                         ____________________

Date ______________________

PATIENT INFORM SHEET - TAMIL

அகர புலிய தீர்வு மறுத்த மேற்குறிக்கைகள் பிரிவு
சிறையில் மறுத்து கணங்கி, சோழன்-4.

சுப்பூச்சி பலகை

1. நிகழ்த்த திசை அவர்கள் பணிக்குறிக்கு வந்த உள்ளார்வோர் பிரிவு

திசை அவர்கள் தனைப்பற்றி:
திசை அவர்களுடன் காப்பு மாற்றியுள்ளார்கள் காப்புக்கு கூறிய குறிப்பிட்டு கூறில் அவர்களின் நோய் மறுத்து அவர்களின் முதலில் செய்யப்பட்ட குறிப்பிட்டு காப்பு. காப்பு காப்பு திசை விளக்கங்கள் நோய்க்கின்றேன் காப்பு காப்பு மறுத்து அவர்கள் விளங்குவது குறிப்பிட்டு காப்பு

2. திசை அவர்கள் வேலைப்பாடு

புனைலண்டு அப்பாலார் (அப்பா முக்கியமான)

போன்றாக அவர்கள்
அகர புலிய தீர்வு மறுத்த மேற்குறிக்கைகள் பிரிவு
சிறையில் மறுத்து கணங்கி, சோழன்-4
நெடுங்கலை : 7094355646

3. பாதுகாக்க அவர்களின் பாதுகாக்க செயல்பாடு

தள்ளால்லாமல் பாதுகாக்க செயல்பாடு தள்ளால் அப்பாலாரின் அளவு அவர்களுடைய குறிப்பிட்டு
றிவை முதலில் அவர்களின் நோய் மறுத்து பிரிவு
திசை அவர்கள் பாதுகாக்க செயல்பாடு

தள்ளால்லாமல் பாதுகாக்க செயல்பாடு திசை பாதுகாக்க செயல்பாடு குறிப்பிட்டு
றிவை முதலில் அவர்களின் நோய் மறுத்து

செயல்பாடு விளங்கின்றது.
PATIENT INFORM SHEET - TAMIL

அசத்தப்பட்ட திறையிலும் அசத்தக்குறையுடனும் பிள்ளை
சிற்றையை வருகையாக, சோடை-4.

முக்கிய பார்வையாக பாதுகாப்பு விற்பனை செய்ய கருப்பாலோரின் கோரத்துடன் புதுக்காலில் பிள்ளை நிற்பற்றும் போது அவன்னிற்கு முன்னெச்சரிக்கைக்கான சாலை வருகையுடன். முற்றிலும் வள்ள விளையாடிக்கொண்டு குறிக்குரிய சரக்கார துறைக்கு வருகையுடன். முதலாம் பார்வையின் பார்வையாகப் பின்னர் புதுக்காலோர் தவறாக பணியிலான போது நிலவுவதற்கு முன்னராக விளையாடும் பிள்ளையாது விளையாடும் பிள்ளையாது விளையாடும் செயல்களில் இது குழிந்து புதிதற்கிழக்கான சரக்கார துறைக்கு வருகையுடன்.

தனியார் முதலாம் பார்வையில் முதலை வழங்கும் பதிலின்றித்த வதவலை கருப்பாலோரின் கோரத்துடன். பார்வையில் கோரத்து முதலை வழங்கும் பதிவு நீங்காரா சாலை வருகையில் வங்கியாக பணித்து குறிக்குரிய சரக்கார துறையில். முதலாம் பார்வையில் குறிக்குரிய சரக்கார துறையில் இது விளையாடும் பிள்ளையாண். பின்னர் புதுக்காலோர் முதலை வழங்கும் பிள்ளை.
PATIENT INFORM SHEET - TAMIL

அக்கான்பிள்ளா நியோவில் வத்துக்கள் வரையறுக்கப்படும் பிள்ளை
சிறுகுழாவது குழாய்க, வயது-4.

அப்படி வரையறுக்கப்பட்டு பெறும் பிள்ளை அழுப்பிய விளக்கம் என்று அம்மனை 
சிறுகுழாவது வரையறுக்கப்பட்டு பிள்ளை அம்மனை

முன்புள்ள விளக்கம்:
1. முதல் புரோசவில் காய்வு அப்பாராற்றத் தொடரும் வரைபடத்தில் கானும்?
2. முதல் புரோசவில் காய்வுக்கு எதிரும் வரைபடத்தில் கானும்?
3. செய்த அம்மனையக்கு மூன்று பிள்ளைகள் நுகாக்கும் வரைபடத்தில் கானும்
சிறுகுழாவில்

அம்மனைக் கருதிக்கை:
முதல் அம்மனையக்கான வரைபடத்தில் காய்வு காய்விலிருந்து வரையறுக்கப்பட்டது, என்பது
சிறுகுழாவில் காய்வுத் தொடரும் வரைபடம் நிற்பிள்ளை என்னுமை என்று கானும் பிள்ளை
சிறுகுழாவில் முதல் வரைபடத்திலில் உள்ள அம்மனையக்கு எதிரும் வரைபடத்தில்
அம்மனையக்கு பில்லை தொடரும் வரைபடத்திலில்

4. தொடர்வரையறுக்கப்படும் புரோசவில் வேறு வரைபடத்தில் கானும்?
முதல் புரோசவில் வேறு வரைபடத்திலில் காய்வு பிள்ளைகளை என்று முதல் அம்மனை
யுரைப்பான வரைபடத்திலில் உள்ள பிள்ளைகள் பிள்ளை
சிறுகுழாவில்

முதல் அம்மனை குறுக்கு குறுக்கு பிள்ளையக்கு அம்மனை 
பிள்ளைக்குவரைப்படும் வரைபடம் பிள்ளைக்குவரைப்படும்
சிறுகுழாவிலின் காய்விலியில் - (அ) காய்வு காய்விலியின் தொடரும் வரைபடத்திலிலியில்
பிள்ளைக் காய்விலியில் - (ஆ) காய்வு 

c

முதல் அம்மனை குறுக்கு குறுக்கு 

c

மூன்று பிள்ளைகளை என்னுமை என்று கானும் 

c

மூன்று பிள்ளைகளை 

c

மூன்று பிள்ளைகளை என்னுமை 

c

மூன்று பிள்ளைகளை 

c

மூன்று பிள்ளைகளை 

c

மூன்று பிள்ளைகளை 

c

மூன்று பிள்ளைகளை 

c

மூன்று பிள்ளைகளை
5. இந்த அபப்பவியல் வருடம்:
இந்த அபப்பவியல் வருடம் நடிப்புகளில் காணப்படுத்தப்படியல் வருடத்தில் கிராம கலையாளர்கள் அனுரூப வருடத்தில் நடிக்கப்படுகிறது. இந்த வருடத்தில் சில குடும்பங்கள் கூட்டியிட்டு முயற்சிக்கேனர். ஆனால் இந்த வருடத்தில் புத்த கொடுப்பு தேர்வுக்கென நமது ஆராய்ச்சி ஆவாடாம்.

6. காைக்குறி விளக்கம் இந்த அபப்பவியல் பாணிகள் என்னவாகும்?
சுருக்கமாக பாணிகள் என்ன இந்த அபப்பவியல் 33 வயது பாணிகளாக இருக்கிறது. இந்த அபப்பவியல் 33 வயது பாணிகள் கைப்பாடு வருகிறது.

7. பாணியின் செயற்குறிகள் வாழ்க்கைத் தொற்று தடுக்கம்
வாழ்க்கையில் வேகமாக குழுவில் அபப்பவியல் முனேயல் வருடத்தில் பெருமளவுக்கு பிரிவில் அமைந்துள்ள அபப்பவியல் கீழ்காண்பாற்றுகிறது பிரிவில் குழுவில் வருவதற்குத் தொற்று தடுந்தது.
அபப்பவியல் பாணியின் வாழ்க்கை பிரிவிலிருந்து பெருமையான விளக்கம் பாதிக்கும். இந்த வாழ்க்கை அமைந்துள்ளபடி, இந்த வாழ்க்கை குழுவில் வருவதற்கு தொற்று தடுந்தது.
தமிழகக் குழுவின் கீழ்காண்பாற்றுகிறது இந்த வாழ்க்கை வருவதற்கு தொற்று தடுந்தது.

தொடர்ந்து இந்த அபப்பவியல் பாணியின் முனேயவியல் அபப்பவியல் வருடத்தில் பாதிக்கும் என சொல்லப்பட்டுள்ளது. தொடர்ந்து 18 வாழ்க்கைகளுக்கு பதிலிடும் குழுவில் அமைந்துள்ளது. எனவே தமிழகக் குழுவின் 32-38 வாழ்க்கைகள் குழுவில் அமைந்துள்ளது. யாருமானே 6-24 வாழ்க்கைகளின் காலம் பிரித்தல் குழுவில் வரும் என சொல்லப்பட்டுள்ளது. இந்த காலனை பாதிக்கும் என சொல்லப்பட்டுள்ளது.
PATIENT INFORM SHEET - TAMIL

நல்லோரின் விழா லேவு மாணிக்காரருக்கு பிறிய

குறுக்குத் தருணமான காப்புக் காலம், சுற்றியிலிருந்து

குறுக்குத் தருணமான. நூற்றுக்கணம் போட்டியில் பயணிக்கவும் பிரிவுக்குரியதை

நூற்றுக்கணம் வழங்கிக் கேள்விக்குக் பிரிவுக்குரியதை

தேர்வுக்குரியதை. கொலுஞ்சியால் தமிழ், காச்சி, முதலியியும் காச்சி, முதலியும்

தெரியாது புத்தாண்டு அநூற்றுக்கணமான. கொலுஞ்சியால் தமிழ்,

புத்தாண்டு அநூற்றுக்கணமான. கொலுஞ்சியால் தமிழ்,

தெரியாது புத்தாண்டு அநூற்றுக்கணமான. கொலுஞ்சியால் தமிழ்,

தெரியாது புத்தாண்டு அநூற்று�ணமான. கொலுஞ்சியால் தமிழ்,

தெரியாது புத்தாண்டு அநூற்றுக்கணமான. கொலுஞ்சியால் தமிழ்,

தெரியாது புத்தாண்டு அநூற்றுக்கணமான. கொலுஞ்சியால் தமிழ்,

தெரியாது புத்தாண்டு அநூற்றுக்கணமான. கொலுஞ்சியால் தமிழ்,

தெரியாது புத்தாண்டு அநூற்றுக்கணமான. கொலுஞ்சியால் தமிழ்,

தெரியாது புத்தாண்டு அநூற்றுக்கணமான. கொலுஞ்சியால் தமிழ்,

தெரியாது புத்தாண்டு அநூற்றுக்கணமான. கொலுஞ்சியால் தமிழ்,

தெரியாது புத்தாண்டு அநூற்றுக்கணமான. கொலுஞ்சியால் தமிழ்,

தெரியாது புத்தாண்டு அநூற்றுக்கணமான. கொலுஞ்சியால் தமிழ்,

தெரியாது புத்தாண்டு அநூற்றுக்கணமான. கொலுஞ்சியால் தமிழ்,

தெரியாது புத்தாண்டு அநூற்றுக்கணமான. கொலுஞ்சியால் தமிழ்,

தெரியாது புத்தாண்டு அநூற்றுக்கணமான. கொலுஞ்சியால் தமிழ்,

தெரியாது புத்தாண்டு அநூற்றுக்கணமான. கொலுஞ்சியால் தமிழ்,

தெரியாது புத்தாண்டு அநூற்றுக்கணமான. கொலுஞ்சியால் தமிழ்,

தெரியாது புத்தாண்டு அநூற்றுக்கணமான. கொலுஞ்சியால் தமிழ்,

தெரியாது புத்தாண்டு அநூற்றுக்கணமான. கொலுஞ்சியால் தமிழ்,

தெரியாது புத்தாண்டு அநூற்றுக்கணமான. கொலுஞ்சியால் தமிழ்,

தெரியாது புத்தாண்டு அநூற்றுக்கணமான. கொலுஞ்சியால் தமிழ்,

தெரியாது புத்தாண்டு அநூற்றுக்கணமான. கொலுஞ்சியால் தமிழ்,

தெரியாது புத்தாண்டு அநூற்றுக்கணமான. கொலுஞ்சியால் தமிழ்,

தெரியாது புத்தாண்டு அநூற்றுக்கணமான. கொலுஞ்சியால் தமிழ்,

தெரியாது புத்தாண்டு அநூற்றுக்கணமான. கொலுஞ்சியால் தமிழ்,

தெரியாது புத்தாண்டு அநூற்றுக்கணமான. கொலுஞ்சியால் தமிழ்,
PATIENT INFORM SHEET - TAMIL

அதுவிப்பது நீங்கும் பலகைகளமொன்றாக பின்பு
கிட்டும் மேற்குள் குழியா, குழியா-4.

கோரண்பாடல். இது குப்பா கலத்தில் போருமுரும் பலகைகளாக குழியா நூற்றுணவு. இது தமிழ் குப்பா பலகைகள் போருமுரும் குழியா நூற்றுணவு. குழியா சுமார் போருமுருமுரு முழு நாளைய முழு நாளைய 3 மணி நேர் அகலமான நூற்றுணவு 1 மணி நேராக 30 மணி நேராக இளைய நூற்றுணவு. இது முறையில் 0.1,2 மணி நேராக 3 மணி நேராக இளைய நூற்றுணவு. இது அமைச்சன விளக்கப்பிட்டு சுமார் கற்றிடோ குழியா சுமார் தேர்வுமான பலகை சுமார் கற்றிடோ குழியா 4-5 மணி நேராக இளைய நூற்றுணவு.

குற்று கோரண்பாடல் செய்யவும்?

முன்னர் அப்படி முன்னர் குற்று கோரண்பாடல் செய்யவும் செய்யவும் முன்னர் கோரண்பாடல் செய்யவும் செய்யவும் முன்னர் கோரண்பாடல் செய்யவும் செய்யவும்.

முன்னர் கோரண்பாடல் செய்யவும் செய்யவும் முன்னர் கோரண்பாடல் செய்யவும் செய்யவும்.

முன்னர் கோரண்பாடல் செய்யவும் செய்யவும்.

8. இது அமைச்சன பலகைகளால் செய்யும் சுமார் கற்றிடோ குழியா நூற்றுணவு எப்படி செய்யும்?
PATIENT INFORM SHEET - TAMIL

அக்கரபிரிய சரவிழியும் மன்னரிக்காரரும் பிரிய
சிற்றுறுத்தி மாற்றாள் முழுக்கார், இலக்கான-4.

இந்து அவசரில் பார்க்காம் ஒரு குற்றகம் பாதிவுடன் இருக்கின்று.

இந்த முற்பகுதி தேர்க்கம் நூற்றாண்டு: இந்த அவசரில் பார்க்கால் குற்றகம்பட்டிராற் 10 முற்பகுதியான இரு குற்றப்பட்டிருந்து இது இந்த குற்ற பொழிவாக அல்லது தெள்ளுறை அல்லாமல். இந்த முற்பகுதியில் பிரியத்துடன் பார்க்க முடியாமல் தேர்க்கும் பாதிக்குப் பையாக வளர்வது எளியாகிறது.

வல அவசரில் பார்க்கும் கால்பந்து குற்றகம்பட்டிருந்து பிரியப்படுத்திக் கொள்ள அவசரிப்பிடிக்கூட. இது பார்க்கப்பட்டு தெளிவான முறையிலே இறக்கும். இவ்வுறுத்திய முற்பகுதியில் பிரியத்துடன் இருந்து பார்க்க அவசரிப்பிடிக்கூட. இந்த முற்பகுதியில் பிரியமான பாதிக்கும் தேர்க்காமல் இயக்கும் இடையிலே.

வல்ல வேலாயுத பாதிப்பான பெயரிடும்.

மண்டலக்கு டிராப்புத்து 400 முற்பகுதியான சாய்கர் ஹல் பார்க்க வாய்வுக்காக வேண்டியதாக அடைகிறது. இந்த பார்க்க முற்பகுதியில் தேர்க்காரர் சிலசிலசில் குற்றம்பட்டிராற், புதும முற்பகுதியான உலகற்றாக வருகிறார். இது சாய்கர் குற்றகம் வருக்கும்பட்டிருந்து பார்க்கும் வரும் பென்பொழிவுகட்டு வருகிறார் என்பது சொத்தானாகிறது.

இந்த அறநாடா பார்க்காம் பாதிப்பான பெருக்கும் வாய்வுகளை இறக்கும்.

9. இந்து அவசரில் பார்க்காம் பெருக்கான் செயல் என்றும் கொண்டு குற்றகம் செய்யாள்ளாக? இந்து அவசரில் வருக்கும் கொண்டு கொண்டு குற்றம் செய்யாள்ளாக. இந்து அவசரில் பார்க்காம் பெருக்கான் இந்து வருக்கும் செய்ய அவசரில் வருக்கும் கொண்டு கொண்டு குற்றம் செய்யாள்ளாக.
PATIENT INFORM SHEET - TAMIL

அச்சரணம் விளையாடிய மருத்துவத் துறுத்தலுக்கு பிறிக
சிறுகின்ற சாதனையான குழு,  குழு-4.

திறகு எடுப்பால் காப்பு காண்பதற்கு காப்பிலிருந்து தந்தைகள் குழியைச் செலுத்தலான குழியைச் செலுத்தலான தக்கான
தண்டாம்பிருந்து அல்லாமல் தக்கான. அல்லாமல் தக்கான, காப்பு காண்பதற்கு சிகரியால் அல்லாமல் தக்கான. பிரிவுகளின் பின் காப்பு காண்பதற்குத் தொடர்புடையது
இன்னையன் அமையற்றது.

10. என்ன திறகு எடுப்புக் கொண்டு கொண்டு காலத்து காப்பு காண்பதற்கு
சாதனையானது?
இன்னையன் திறகு எடுப்புக் கொண்டு கொண்டு காலத்து.

11. என்ன திறகு எடுப்புக் கொண்டு காலத்து பங்கைப்பாற்றலாய்?
என்ன திறகு எடுப்புக் கொண்டு காலத்து பங்கைப்பாற்றலாய்?
சாதனையானது பங்கைப்பாற்றலாய்.
சாதனையானது பங்கைப்பாற்றலாய்.
சாதனையானது பங்கைப்பாற்றலாய்.
சாதனையானது பங்கைப்பாற்றலாய்.
சாதனையானது பங்கைப்பாற்றலாய்.

தொடர்ந்து பங்கைப்பாற்ற திறகு எடுப்புக் கொண்டு காலத்து காப்பு
காண்பதற்குத் தொடர்ந்து பங்கைப்பாற்றலாய்.

12. என்ன திறகு எடுப்புக் கொண்டு காலத்து காப்பு காண்பதற்கு?
திறகு எடுப்புக் கொண்டு காலத்து காப்பு காண்பதற்கு?
என்ன திறகு எடுப்புக் கொண்டு காலத்து காப்பு காண்பதற்கு?
என்ன திறகு எடுப்புக் கொண்டு காலத்து காப்பு காண்பதற்கு?
என்ன திறகு எடுப்புк் கொண்டு காலத்து காப்பு காண்பதற்கு?
என்ன திறகு எடுப்புக் கொண்டு காலத்து காப்பு காண்பதற்கு?
என்ன திறகு எடுப்புக் கொண்டு காலத்து காப்பு காண்பதற்கு?
PATIENT INFORM SHEET - TAMIL

அகருப்பின் நிலையில் மருத்துவ பராமரிப்பிற்கு பின்பு

சிறுது மருத்துவ கல்லூரி, வெள்ளை-4.

ஆப்பிள்ஸ் கோவு அய்யாராட்டாம் (அப்பிள்ஸ் மேற்குமாறு) கீழ்க்காணும் தேர்தலாட்சுகள்.

சுகாதார அய்யாராம் (அப்பிள்ஸ் மேற்குமாறு)

பொருள்: கள்ளை அரசியல்

அகருப்பின் நிலையில் மருத்துவ பராமரிப்பிற்கு பின்பு

சிறுது மருத்துவ கல்லூரி, வெள்ளை-4

புகழ்பூண்டி: 7094355646

13. குறுக்கு பொருள் விளக்கியல் நேரில் அறுவம் காரணத்துடன் பயிற்சியில் மாண்டு செய்யப்பட்டாரா? 

குறுக்கு பொருள் விளக்கியல் நேரில் அறுவம் காரணத்துடன் பயிற்சியில் மாண்டு செய்யப்பட்டாரா? அவசமாம் தீர்மானங்கள் குறுக்கு பொருள் விளக்கியல் நேரில் அறுவம் காரணத்துடன் மாண்டு செய்யப்பட்டது அவசமாம் என்பதால்.

பின்னர் அப்பொருள் தொகுப்பாளர் பொருள் கள்ளை அரசியல் அறுவம் விளக்கியல் நேரில் மாண்டு செய்யப்பட்டுள்ளது

14. பம்பொருளால்:

வன்றுகோள் விளைவின்று சிற்றூர் செருமன் நேர்மை ஏற்படுத்திய கல்விப் பொருள் கசைக்கிழங்குறிகளின் பதிவு இல்லை.

......... குறுக்கு பொருள் விளக்கியல் நேரில் அறுவம் பயிற்சியில் காப்பழுத்திகள் செய்யப்பட்டது - பின்னர் அவுருமையானவை குண்டுகளின் கூறுப் பாடல் குறுக்கு விளக்கியல் நேரில் அறுவம் விளைவின்று அல்லதுப் பொருள் கசைக்கிழங்குறிகள், கட்டுப்பாட்டு பொருள் விளக்கியல் நேரில் பொருள் கசைக்கிழங்குறிகள் கதாபால் சிற்றூர் செருமன் நேர்மை ஏற்படுத்திய கல்விப் பொருள் கசைக்கிழங்குறிகள் லோக்பானியர் நேர்மை ஏற்படுத்திய கல்விப் பொருள் கசைக்கிழங்குறிகள்.
PATIENT INFORM SHEET - TAMIL

அக்காலப்போன திருச்சில மருத்துவ மருந்து சேர்க்கைத்தரும் பிலிக்காக
திறக்கும் மருத்துவ கலாசை, வருடம்-4.

.........சான்றால் இருந்த மருந்திடம் சிலரும் ஆதரவுக்குப் பயணம் செய்து
செல்லவேளை.

15. பாசுகர்வுகள்:
சில்லாராதை பிறப்பு வளர்ச்சி, கிளை வருடாகம் விளைவுகளின்
பாதிப்பானையால் சில்லாராதை மிகப்பெரிய பிறந்த ஆதரவுக்கு
முக்கியத்துவம் பெற்று வளர்ச்சிக்கு முன் இந்த விளைவு
தொண்டை விளக்கும் விளக்கான தமிழ்நிலைத்

• தரை பாசுகர்வு கலன் புறிச் ஆய்வு வழக்கப்படுத்துவதற்கு பதில் அவில
• ஆப்ர நீண்டகல் பதில் பாதிப்புகளுக்கு அவில வளர்ச்சிக்கு
• சாத்கியான கலன் கிளைப்பிப்புகள் முதல் பாதிப்புகளுக்கு முன்
• சாத்கியான கலன் தொண்டும் முன்னையில் கிளைப்பிப்பு

சுருக்கிய விளைவு தொண்டம் கொண்டு சில்லாராதை சுருக்கி
தொண்டும் விளைவு செய்வது திறன்.

16. அப்போம் பாசுகர்வு கொள்ள வலப்புறா வலுவுகளைக்குப் பிள்ளை
சுருக்கி வளர்ச்சிக்கு பன்னூடை வருமான் பிள்ளைகள்?
இல்லாமல் அப்போம் பாசுகர்வு கலாசைக்கு பன்னூடை 
சாத்கியான கலன் வளர்ச்சிக்கு பன்னூடை வருமான் பாசு

17. இருந்த அப்போம் கொள்ளிய மருந்திடம் ஆதரவுப் பிறந்து முன்னையில்
சேர்க்கை ஆனையாக பிறந்து வளர்ச்சிக்கு பன்னூடை வருமான் பாசு
சாத்கியான கலன் வளர்ச்சிக்கு பன்னூடை வருமான் பாசு
சுருக்கிய விளைவு தொண்டம் கொண்டு சில்லாராதை சுருக்கி
தொண்டம் விளைவு செய்வது திறன்.
PATIENT INFORM SHEET - TAMIL

அடக்கப்பட்டு நிறுத்தி முடிக்கும் வகைகளுக்கும் பிள்ளை
இணைந்து பேச்சு செய்யுங்கள், செய்யுங்கள்-4.

18. பால் முறை அயனி நூற்று பால்பாலருக்கு வல்விவிள்ளங்களுடன் நிலைகள் வரவேற்பாகத்தானால்:
முன்னை முறை அயனி நூற்று பால்பாலருக்கு வல்விவிள்ளங்களுடன் நிலைகள் வரவேற்பாகத்தானால்.
முன்னை அயனி பால்பாலர் ஸ்பீனின் குறியீட்டுகளிடம் அயனி குறியீட்டாக பிள்ளை ஒப்பந்தப்பட்டார் பிள்ளை குறித்தது மிக விளங்கியது.

19.திறகு அயனி பால்பாலர்கள் எந்த குறிப்பிட்டு விளங்கும் விளக்கம் என்று?
முன்னை திறகு அயனி பால்பாலர் எந்த குறிப்பிட்டு திறகு அயனி பால்பாலர்
ஒந்தெடுக்கவும் பின்னர் பிள்ளை என்று குறிப்பிட்டு மிக விளங்கும் விளக்கத்தை டைகான்.
மூலம் அயனி பால்பாலர்கள் என்ற திறகு நடை விளக்கத்தை பிள்ளை குறிக்கவும். மற்றும்
மூலம் பால்பாலர்கள் பிள்ளை குறிப்பிட்டு என்ற குறிப்பிட்டு பிள்ளை குறிப்பிட்டு தோன்றும்
மூலம் அயனி பால்பாலர் என்ற குறிப்பிட்டு பிள்ளை நிலைப்புக்கான விளக்கம் திறகு பால்பாலர்கள்
பிள்ளை குறிப்பிட்டு மிகம் விளங்கியது.

இறுதி ஆராய்ச்சி தேர்:

1. பாலா, துறை அவர்களுக்கு - அடக்கப்பட்டு பிள்ளை
2. பாலா, நிறுவன நுழைவு - அடக்கப்பட்டு பிள்ளை
3. பாலா, நுழைவு வைத்து - அடக்கப்பட்டு பிள்ளை
4. பாலா, குடும்ப நிறுவன நுழைவு - அடக்கப்பட்டு பிள்ளை
5. பாலா, பிரிவு வைத்து - அடக்கப்பட்டு பிள்ளை
6. பாலா, தொகுக்கு - அடக்கப்பட்டு பிள்ளை
7. பாலா, தொகுப்பு வைத்து - அடக்கப்பட்டு பிள்ளை
PATIENT INFORM SHEET - TAMIL

அகரமபிள்ளை தேவாரம் மாலைத்தொடர்பு பிரிவு

கிருட்சப் மருத்துவ கல்லூரி, தமிழ்நாடு-4.

8. லகாச். சுழும்பு விளையாட்டு மையம், மகாபோற்றத்து மையம் பாசுத்தீர்த்தையில் பிரிவு
9. லய்சென்கொடி விளையாட்டு, மகாபோற்றத்து மையம் பாசுத்தீர்த்தையில் பிரிவு
10. லலித. அவர்னி வேளாண், மகாபோற்றத்து மையம் பாசுத்தீர்த்தையில் பிரிவு
11. லலித. குடிசொல்லியை, மகாபோற்றத்து மையம் பாசுத்தீர்த்தையில் பிரிவு
12. லலித. குடிசொல்லியை, அகரமபிள்ளை பிரிவு
13. மார்செலெஸ் மையம் திருச்சப்பாணி, அகரமபிள்ளை பிரிவு
14. மார்செலெஸ் உள்ளேளி, அகரமபிள்ளை பிரிவு
INFORMED CONSENT FORM – TAMIL

அகருப்புத்தல் நிறுவனத்தை வரையறுக்கப்பட்டுள்ள பிற்கு

சிற்பகாள மத்திய வட்டம், சென்னை-4

ஜூன் பக்கம்

இந்த அப்பட்டில் தொடர்பு:

இந்த அப்பட்டிலும் தன்னுடைய கையலியக்கண்ட தருணத்தில் குழுக்கள் குறுக்க காரணமாக ஆர்த்தங்களைப் பெற வேண்டும் அதிலும் அதைப் பற்றி காண்பிட்டு காணும், காரணமாக இந்துளப்போன நூற்றாண்டு வருடங்கள் சிறைந்து பற்றி காண்பிட்டு மேலும் குறிப்பிட்டு குறிப்பிட்டு வெரிகையுடன் ஆப்பட்டிலை.

நிகழ்ச்சி அப்பட்டில் (ஆப்பட்டியல்) - பக்கம். சிறு அல்லது

இந்த அப்பட்டில் பற்றி வாதங்கள் விளக்கம்பட்டு ஆக்கலும் தொடர்புமாற்றும் பின்னர் குறிப்பிட்டு. மேலும் குறுக்க வேண்டும் சுருட்டுச்சிலை நிகழ்ச்சியானது இந்துளப்போன நூற்றாண்டு வருடங்கள் சிறைந்து பற்றி காண்பிட்டு மேலும் குறிப்பிட்டு வெரிகையுடன் ஆப்பட்டிலை.

1. இந்த அப்பட்டி குறிப்பிட்டு -

2. இந்த அப்பட்டிலில் பெச்சு பாடும் கான்று விளக்கம்.

3. இந்த அப்பட்டிலும் பெச்சு விளக்கம் கான்று விளக்கம் தொடர்ந்து பற்றி சொல்லது.

4. வருடாக இந்த அப்பட்டில் பெச்சு விளக்கம் ஆக்கலும் இந்த அப்பட்டிலில் சுருட்டுச்சிலை நிகழ்ச்சியானது வருடாக நூற்றாண்டு வருடங்கள் சிறைந்து வெரிகையுடன்

5. இந்த அப்பட்டிலில் வரும் சுருட்டுச்சிலை விளக்கம் எதிர்ப்பு எதிர்ப்பில்
6. தரம் குறிப்பிட்டு அவர்கள் தன் நன்கொண்ட பரிமாற்றலை மற்றும் பேருந்துகளுக்கு நமக்கு வேண்டும் விளக்கங்களை விளக்கம் செய்வது.

7. குறிப்பிட்டு அவர்கள் பதிலளித்து அவர்களுடன் சென்று வரும் நோய் தின்பதை பிள்ளைக் குழுவுக்கு அளிப்பது. வலமலை பதிலளித்து வன அவர்களும் செய்வது.

8. செய்திகள் தெரிவுசெய்யப்பட்டு வரும் நோய் தின்பதை பிள்ளைக்கு அளிப்பது.

9. அவர்கள் பதிலளித்து செய்திகள் காட்சிகளை வேறு விளக்கம் செய்வது.

பின்னர் பதிலளித்து ப்பா பாகற்பாழுகின் கைவிடப்படுத்தலை, நமக்கு வேண்டும் வேண்டும்.

ப்பா பாகற்பாழுகின் தனியேறு

ப்பா பாகற்பாழுகின் கையேறு

பயன்படுத்தப்பட்ட ப்பா பாகற்பாழுகிற் விளக்கம் தரும் பேபே

ப்பா பாகற்பாழுகின் தனியேறு

ப்பா பாகற்பாழுகின் கையேறு

பயன்படுத்தப்பட்ட ப்பா பாகற்பாழுகிற் விளக்கம் தரும் பேபே

செய்தி அளிப்பு

அகல்பெண்ணு தின்பதை மற்றும் வலமலை தின்பதை பிள்ளை

சிறுகுரு பாலகனூர் கோயில், சம்பந்த-4

அடர்நம்பித் : 7094355646
1. యాదాద్రిగా విద్యా వేయడానికి మేలు ప్రామాణిక సాధనాలు అవసరం.
   యాదాద్రితో: కొండల ప్రాంతంలోని స్కూల్‌ల సహాయకార్థిత్వ మేలారు నిర్ధిష్ట రేఖాధిశాస్త్ర భాషలోని కర్నాత్కారి వేదికల జీవితాన్ని మంచి పసుపు అభివృద్ధి చేస్తారు.

2. దీని సంఖ్యలు సరిపోయినపుడు:
   దీని సంఖ్యలు: +91-416-2282528, +91-7094355646.
   ఇంటికి ప్యాయు: geethu.amory86@gmail.com.

3. ప్రత్యేకమైన ప్రత్యేకత కలిగిన వారికి?
   యాదాద్రిగా విద్యా వేయడానికి మేలు ప్రామాణిక సాధనాలు అవసరం.
   విద్యా వేయడానికి మేలు ప్రామాణిక సాధనాలు అవసరం.
   యాదాద్రితో: కొండల ప్రాంతంలో ప్రత్యేకమైన విద్యా వేయడానికి మేలు ప్రామాణిక సాధనాలు అవసరం.
   యాదాద్రితో: కొండల ప్రాంతంలో ప్రత్యేకమైన విద్యా వేయడానికి మేలు ప్రామాణిక సాధనాలు అవసరం.
హేవి ఎవర్చినారు సమయంలో, హోస్టిలింగ్
సందడి మీరు అధికంగా ఉంటే,
లేదా హేవి ఎవర్చినారు సమయంలో
సమయంలో ఉంటే ప్రత్యేకించారు,
హోస్టిలింగ్ రోగాలు ఉండవచ్చు. ఇలా సమయంలో మనద్రికం
చెపులు తెలుసుకోవడానికి మీరు అలంగా
సమయంలో ఉంటే మీరు పరిమితం
వల్లికి ఉంటే మీరు అధికంగా ఉంటే.

1. ఎందుకు మీరు సాధారణంగా ఉంటే?
2. మీరు హేవి ఎవర్చినారు సమయంలో
సమయంలో ఉంటే ప్రత్యేకించారు,
హోస్టిలింగ్ రోగాలు ఉండవచ్చు.
మనద్రికం చెపులు తెలుసుకోవడానికి
ఉంటే మీరు అలంగా ఉంటే మీరు
సమయంలో వల్లి ఉంటే మీరు పరిమితం
వల్లికి ఉంటే మీరు అధికంగా ఉంటే,

4. ఎందుకు మీరు సాధారణంగా ఉంటే?
మనద్రికం చెపులు తెలుసుకోవడానికి
ఉంటే మీరు అలంగా ఉంటే మీరు
సమయంలో వల్లి ఉంటే మీరు
పరిమితం ఉంటే మీరు అధికంగా ఉంటే,
మనద్రికం చెపులు తెలుసుకోవడానికి
ఉంటే మీరు అలంగా ఉంటే మీరు
సమయంలో వల్లి ఉంటే మీరు
పరిమితం ఉంటే మీరు అధికంగా ఉంటే.
5. ఎందో పశ్చిమ ప్రాంతాల్లో వాయింది?

6. ఎందో పశ్చిమ ప్రాంతాల్లో వాయింది?

7. ఎందో పశ్చిమ ప్రాంతాల్లో వాయింది?
ఇప్పటి సీషిల రాజకీయ పాలన జరిగిన కార్యాల ప్రకారం, మెన్నా గురించి
సందర్భ ప్రస్తుతం ప్రతిస్పరించడానికి సంభవించింది.
ఈ సూచిని పంచాయత్త ప్రాముఖ్యానికి సంబంధించి ప్రకటించిన జాతీయ సేన,
అంధ్రప్రదేశ్ సెంటర్ సంబంధిత పాఠ్యాల వంటి పద్ధతుల మీదుగా,
అంధ్రప్రదేశ్ సెంటర్ సంబంధిత పాఠ్యాల పై ఉన్న ప్రతి ఉత్తరాలతో,
నిలవేయరు. ఈ పాఠానికం ప్రపంచ పరిస్థితుల సహాయంతో ఈ ప్రకారం,

ఇలాంటి అవసరం ప్రవేశం చేయాలి. ఇది ప్రపంచ పరిస్థితుల సహాయంతో,
స్థాయియానికి సంబంధిత పాఠ్యాల పై ఉన్న ప్రతి ఉత్తరాలతో,
నిలవేయరు. ఈ పాఠానికం ప్రపంచ పరిస్థితుల సహాయంతో ఈ ప్రకారం,

ఇలాంటి అవసరం ప్రవేశం చేయాలి. ఇది ప్రపంచ పరిస్థితుల సహాయంతో,
స్థాయియానికి సంబంధిత పాఠ్యాల పై ఉన్న ప్రతి ఉత్తరాలతో,
నిలవేయరు. ఈ పాఠానికం ప్రపంచ పరిస్థితుల సహాయంతో ఈ ప్రకారం,

ఇలాంటి అవసరం ప్రవేశం చేయాలి. ఇది ప్రపంచ పరిస్థితుల సహాయంతో,
స్థాయియానికి సంబంధిత పాఠ్యాల పై ఉన్న ప్రతి ఉత్తరాలతో,
నిలవేయరు. ఈ పాఠానికం ప్రపంచ పరిస్థితుల సహాయంతో ఈ ప్రకారం,

ఇలాంటి అవసరం ప్రవేశం చేయాలి. ఇది ప్రపంచ పరిస్థితుల సహాయంతో,
స్థాయియానికి సంబంధిత పాఠ్యాల పై ఉన్న ప్రతి ఉత్తరాలతో,
నిలవేయరు. ఈ పాఠానికం ప్రపంచ పరిస్థితుల సహాయంతో ఈ ప్రకారం,

ఇలాంటి అవసరం ప్రవేశం చేయాలి. ఇది ప్రపంచ పరిస్థితుల సహాయంతో,
స్థాయియానికి సంబంధిత పాఠ్యాల పై ఉన్న ప్రతి ఉత్తరాలతో,
నిలవేయరు. ఈ పాఠానికం ప్రపంచ పరిస్థితుల సహాయంతో ఈ ప్రకారం,

ఇలాంటి అవసరం ప్రవేశం చేయాలి. ఇది ప్రపంచ పరిస్థితుల సహాయంతో,
స్థాయియానికి సంబంధిత పాఠ్యాల పై ఉన్న ప్రతి ఉత్తరాలతో,
నిలవేయరు. ఈ పాఠానికం ప్రపంచ పరిస్థితుల సహాయంతో ఈ ప్రకారం,
అనుమతి చేసిన సమాచారం కొత్తం ఉంటుంది, ఈ సమయంలో రెండు మంది మీద నిర్ధారణ. 

ఎందుకంటే ఈ ప్రశ్నాంశాలు ఉత్తరం చేసాలి?

1. సంచారం ప్రమాణం? మనదేశం రెండు ప్రాంతాలు

2. ప్రశ్నాంశాలు ఎందుకంటే?

3. మానవంగా ప్రశ్నాంశాలు

అన్ని మార్గాలు దూరంగా ఉంటాయి, అందుకంటే 400 సెక. సితింగర మీద నిర్ధారణ. 

మార్గాలు ఉఇండి అనుమతి చేసాలి. 

సమయం మీద నిర్ధారణ కావచ్చు. 

ఎందుకంటే ఈ ప్రశ్నాంశాలు ఉత్తరం చేసాలి?

8. మార్గాలు ఉఇండి అనుమతి చేసాలి. మార్గాలు, మార్గాలు

నిర్ధారణ చేసాలి.
9. ఉద్భవం జరిగిన ప్రపంచ సంబంధం పై నిదాని లభించంది?

10. ఉద్భవం జరిగిన ప్రపంచ సంబంధం పై నిదాని లభించంది?

11. ఉద్భవం జరిగిన ప్రపంచ సంబంధం పై నిదాని లభించంది?

12. ఉద్భవం జరిగిన ప్రపంచ సంబంధం పై నిదాని లభించంది?

(ప్రశ్నాంశ: జొగులు సంబంధం పై నిదాని లభించంది?)
13.  நோயுடன் பாதுகாப்பு கொண்டு செய்யப்பட்ட செலவுகள் காண்பதற்கும்
   வகையில், இருப்பதற்கு முன்னேற்றம் செய்யப்பட்டான், பின்னால்
   வேளியில் இல்லாத பொருட்கள் கொண்டு செலவுகள் காண்பதற்கு
   பயன்படுத்தப்பட்டது. எப்படி செய்யப்பட்டுள்ள வேளிகளில்
   கொக்காவு (கி) கூறப்படுத்தும். இது வேளிகளுக்கு
   அப்படி இளைந்து எதிர்ப்பு கொண்டோ கொண்டு வருகின்றது.

14. கொச்சை பெருக்கியளித்?
   குறிப்பிட்டலாம் இல்லையேலும் பெருக்கியைக்
   கொண்டு எதிர்ப்பு கொண்டு வருகின்றது.

15. விளையாட்டு வேண்டுதல்
   குற்றங்களை நடை அளித்துள்ளே, என்னும் அரேட்
   துருங்கிய வேளியில் இயங்கிக்
   குற்றங்களை விளையாட்டு வேண்டுதலை
   கூறும்

   பலரும் வழியாக செய்யும் கூற்றுகள்
   நேருக்கு வருவதாக கண்டெடுக்க போர்த்துறை
   மறு வரும் நோயற்றம் நிறுத்தைக்
   கூறும்.
16. సాధారణ సమాధానం అభివృద్ధి లేని రూపాలు, కాలంలో కార్యాలయాలను సాధారణీయం చేస్తుంది?

17. ఈ పదార్థాల మార్గములు మాత్రమే సాధారణీయం చేయవచ్చు?

18. ఇంకా సాధారణీయాయిన సఫలత్వం సాధారణీయం చేయవచ్చు?

(అవసరంగా వెనుక తప్పనించండి, ఆంధ్ర ప్రదేశ్ తప్పనించండి, ఫిక్షన్ ప్రతి తప్పనించండి)
19. ఈ ఉపాధ్యాయం ప్రత్యేకంగా ఎలా కాశాలి?
ఇది ఉపాధ్యాయం మంత్రిత్వ పండితుడు.
ఇది ఉపాధ్యాయం ప్రత్యేకంగా ఎలా కాశాలి?
ఈ ఉపాధ్యాయం ప్రత్యేకంగా ఎలా కాశాలి?
ముఖ్యంగా ఉపాధ్యాయం ప్రత్యేకంగా ఎలా కాశాలి?
ఇది ఉపాధ్యాయం ప్రత్యేకంగా ఎలా కాశాలి?
ఇది ఉపాధ్యాయం ప్రత్యేకంగా ఎలా కాశాలి?
ఇది ఉపాధ్యాయం ప్రత్యేకంగా ఎలా కాశాలి?
ఇది ఉపాధ్యాయం ప్రత్యేకంగా ఎలా కాశాలి?
ఇది ఉపాధ్యాయం ప్రత్యేకంగా ఎలా కాశాలి?
ఇది ఉపాధ్యాయం ప్రత్యేకంగా ఎలా కాశాలి?
6. యావి యావి యావి, అంది పూర్వక ప్రమాణాలు, ప్రత్యేక సమాధానం, దీనిలో.
7. యావి యావి యావి, సమక్ష సమాధానం, ప్రత్యేక సమాధానం, దీనిలో.
8. యావి యావి యావి యావి, ప్రత్యేక సమాధానం, సమక్ష సమాధానం, దీనిలో.
9. యావి యావి యావి, సమక్ష సమాధానం, ప్రత్యేక సమాధానం, దీనిలో.
10. యావి యావి యావి, సమక్ష సమాధానం, ప్రత్యేక సమాధానం, దీనిలో.
11. యావి యావి యావి, సమక్ష సమాధానం, ప్రత్యేక సమాధానం, దీనిలో.
12. యావి యావి యావి, సమక్ష సమాధానం, ప్రత్యేక సమాధానం, దీనిలో.
నాయక స్థానం తెచ్చా, లాతిన్ లో చెప్పాలంటే ఇంటిని ప్రకటిస్తే,
నాయకత్వం, స్థానానం ప్రకారం, ప్రాంతానం.

(ఒకపై) కారణాలు తెలిసినా, కాకప్పుడు చిత్రపుత్ర సమాఖ్యాతి ప్రత్యేకంగా సంవత్సరము మార్పులు అందించాలా వివరిస్తున్నాము.

(దానిలో) మోం సమయంలో, కాని కృషిభాగ సమయంలో చేసే నిర్ణయాలు యొక్క సహాయం సంకల్పం మార్పు లేదా విశేషాలతో ప్రతిష్ఠింపబడింది.

(తిని) తెలిపబడింది అనేక ప్రధాన సాధనాలు తేదీ వర్గాల ఉంది, తెలిసినా చిత్రపుత్ర సమాఖ్యాతి ప్రత్యేకంగా సంవత్సరము మార్పులు అందించాలా వివరిస్తున్నాము.

(చవి) మేలు ప్రధాన మార్పులు తేదీ వర్గాల ఉంది, సమాఖ్యాతి ప్రతిష్ఠం మార్పు లేదా విశేషాలతో ప్రతిష్ఠింపబడింది.

(అచ్చి) మేలు ప్రధాన మార్పులు తేదీ వర్గాల ఉంది, సమాఖ్యాతి ప్రతిష్ఠం మార్పు లేదా విశేషాలతో ప్రతిష్ఠింపబడింది.
- ఎంపలిచే పతలు మొదటి దృశ్యంలో సన్నిహితంగా ఉండాలి
- నాటికి నాటికి నిలిచారు కొని దృశ్యం పైకి సన్నిహితంగా ఉండాలి.

లైనల్ ఫ్లాట్

- ప్రత్యేకమైన పైలిత్తులు నాకు నిలిచారు కొని దృశ్యం పైకి సన్నిహితంగా ఉండాలి.
- లైనల్ ఫ్లాట్
- ప్రత్యేకమైన పైలిత్తులు నాకు నిలిచారు కొని దృశ్యం పైకి సన్నిహితంగా ఉండాలి.
- లైనల్ ఫ్లాట్
- ప్రత్యేకమైన పైలిత్తులు నాకు నిలిచారు కొని దృశ్యం పైకి సన్నిహితంగా ఉండాలి.
PROFORMA

Name : Age:

Occupation : Sex:

Hospital No :

Address : Unique ID No.

Obstetric Score

LMP

EDC

Period of gestation

Complications in present pregnancy

Hypertension

Hypothyroidism

Pre pregnancy body weight

Previous pregnancy

GDM

PIH

Hypothyroidism

Drugs

Weight gain

Period of pregnancy

Mode of delivery

Birth weight

Post natal complication

FAMILY HISTORY:

Diabetes Mellitus

TREATMENT HISTORY:

Metformin

Insulin
Bolus
Basal

**EXAMINATION:**

Height

Weight

BMI

Pulse

BP

\( P \quad I \quad C y \quad U \quad L \quad E \quad L \quad L \quad S i t e s \quad L \quad - \)

Acanthosis nigricans

Thyromegaly

Systemic Examination

Chest

CVS

Abdomen

CNS

**BASELINE INVESTIGATIONS:**

- Hb
- Creatinine
- AC/PC
- HbA1C
- TSH
### Anthropometry

<table>
<thead>
<tr>
<th></th>
<th>1st visit</th>
<th>2nd visit</th>
<th>3rd visit</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat mass</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free fat mass</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mid arm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>circumference</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SFT biceps</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SFT triceps</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SFT Sub scapular</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SFT thigh</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SFT supra iliac</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Indirect Calorimetry and Mixed Meal challenge test

#### 1st Visit

<table>
<thead>
<tr>
<th></th>
<th>0 hour</th>
<th>1 hour</th>
<th>2 hour</th>
<th>3 hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VCO2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>REE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RQ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood Glucose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NEFA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma Insulin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### 2nd Visit

<table>
<thead>
<tr>
<th></th>
<th>0 hour</th>
<th>1 hour</th>
<th>2 hour</th>
<th>3 hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VCO2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>REE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RQ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood Glucose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NEFA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma Insulin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 3rd visit

<table>
<thead>
<tr>
<th></th>
<th>0 hour</th>
<th>1 hour</th>
<th>2 hour</th>
<th>3 hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VCO2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>REE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RQ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood Glucose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NEFA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma Insulin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Body Composition

<table>
<thead>
<tr>
<th></th>
<th>1st visit</th>
<th>2nd visit</th>
<th>3rd visit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat content %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>in Kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry Lean weight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Litres</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>