The Contribution of Intra-organ Fat Deposition to Insulin Resistance in Normal Pregnancy and Gestational Diabetes

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A thesis submitted for degree of Doctorate of Medicine

Institute of Cellular Medicine

September 2017

ABSTRACT

Gestational diabetes (GDM) affects 3-5% of pregnancies and is associated with stillbirth, accelerated fetal growth and fetal growth restriction, birth trauma, increased risk of caesarean section and third degree tear. Many mothers with GDM go on to develop type 2 diabetes (T2DM) in later life. T2DM is associated with increased fat deposition in the muscle, liver and pancreas leading to insulin resistance, impaired insulin secretion and hyperglycaemia. Muscle insulin resistance and its association with raised intramyocellular lipid is one of the first detectable changes in T2DM. Low calorie dieting causes reversal of T2DM and removal of intra-organ fat. The pathophysiology of GDM is poorly understood, but fat deposition may play a similarly important role. Low calorie dieting is poorly studied and viewed with caution in pregnancy. This work explores the nature of physiological insulin resistance in pregnancy and the clinical and metabolic outcomes of reducing calorie intake to 1,200kcal/day in pregnancy affected by GDM (WELLBABE – WEight Loss Looking for Babe and mother BEtter outcomes study).

The LIPIDPREG study used magnetic resonance spectroscopy (MRS), a non-invasive technique that has not been previously used in pregnancy, to quantify intramyocellular lipid within the soleus muscle in women with normal glucose tolerance. A standardised meal test was used to calculate insulin sensitivity and secretion. Studies were done at 34 weeks gestation and 12 weeks postpartum. Eleven primiparous healthy pregnant women (age: 27-39 years, body mass index 24.0±3.1 kg/m2) and no personal or family history of diabetes underwent magnetic resonance studies to quantify intramyocellular lipid, plasma lipid fractions, and insulin sensitivity. The meal-related insulin sensitivity index was considerably lower in pregnancy (45.6±9.9 vs. 193.0±26.1; 10^{-4} dl/kg/min per pmol/l, p=0.0002). Fasting plasma triglyceride levels were elevated 3-fold during pregnancy (2.3±0.2 vs. 0.8±0.1 mmol/l, p<0.01) and the low-density density lipoprotein fraction, responsible for fatty acid delivery to muscle and other tissues, was 6-fold elevated (0.75±0.43 vs. 0.12±0.09 mmol/l; p=0.001). However, mean intramyocellular lipid concentrations of the soleus muscle were not different during pregnancy (20.0±2.3 vs. 19.1±3.2 mmol/l, p=0.64). In conclusion, the pregnancy effect on muscle insulin resistance is distinct from that underlying type 2 diabetes.

The WELLBABE study recruited women with an abnormal oral glucose tolerance test from 21 to 34 weeks (mean 27 weeks) gestation. MRS quantification of liver fat, a standardised meal test and plasma lipid profiles were performed before and after a 1,200kcal/day diet. Participants food diary and glycaemic control were reviewed on a daily basis for 4 weeks,

through the use of smartphone technology. Fourteen women, who completed the study, achieved a weight loss of 1.6±1.7 kg over the 4 week dietary period. Mean weight change was -0.4 kg/week in the study group vs +0.3 kg/week in the comparator group (p=0.002). Liver triacylglycerol level was normal but decreased following diet (3.7% [interquartile range, IQR 1.2-6.1%] vs 1.8% [IQR 0.7-3.1%], p=0.004). There was no change in insulin sensitivity or production. Insulin was required in six comparator women vs none in the study group (eight vs two required metformin). Blood glucose control was similar for both groups. The hypo-energetic diet was well accepted. Liver triacylglycerol in women with GDM was not elevated, unlike observations in non-pregnant women with a history of GDM. A 4 week hypo-energetic diet resulted in weight loss, reduced liver triacylglycerol and minimised pharmacotherapy. The underlying pathophysiology of glucose metabolism appeared unchanged.

The results of these two studies are presented in this thesis and from this work a hypothetical model of insulin resistance in pregnancy and GDM is presented. It is demonstrated that reduced calorie dietary intervention is both acceptable and feasible in pregnancy and reduced the need for medication in women with GDM. Further studies are needed in this area to unravel the true pathophysiology of GDM and to develop a reduced calorie dietary intervention that could be used in routine clinical practice.

ACKNOWLEDGEMENTS

Firstly I would like to acknowledge my supervisor, Professor Roy Taylor for all his help and support during my career, but also for encouraging me to persist with research and for his unwavering enthusiastic support throughout. I would also like to thank my other supervisors, Professor Robson and Dr Vera Araujo-Soares for their help and support during this project.

I would like to thank Dr Kieran Hollingsworth, Dr Pete Thelwell and Dr Fiona Smith for teaching me the physics of magnetic resonance and for helping me to analyse my data. I am grateful to the radiographers, Tim Hodgson, Louise Morris and Dotothy Wallace in performing the MR scans and in looking after the participants so well.

I would like to thank Alison Barnes, research dietitian, for all the work, effort and thought that she put into designing the reduced calorie diet and for supporting the women throughout.

I would like to thank the participants, recruited from the antenatal clinics of the Royal Victoria Infirmary, Newcastle upon Tyne for dedicating their time to contribute to these studies.

Finally, I am most grateful to my wife, Louise, who has given me the confidence and belief in myself to enable me to pursue this project.

This research was funded through grants from Wellbeing of Women and the North East Diabetes Trust.

DECLARATION

I conducted all the studies presented in this thesis from 2010-2015 at the Newcastle Magnetic Resonance Centre, Newcastle University with the following assistance.

Magnetic resonance examinations were carried out by the radiographers Louise Morris, Carol Smith, Tim Hodgson and Dorothy Wallace. The MR protocols were written by Dr Pete Thelwall and Dr Kieren Hollingsworth. Analysis of the ^{1}H MR spectroscopy was performed by Dr Fiona Smith. Metabolites and hormones were analysed by Mrs Annette Lane at the Diabetes Research Lab and by Mrs Louise Hughes at the Royal Victoria Infirmary. Mathematical modelling and analysis of the standardised meal test was performed by Dr Chiara Dalla Man and Professor Claudio Cobelli, Department of Information Engineering, University of Padua, Padua, Italy.

The dietary intervention package (diet guide, weekly timetable, recipe suggestions) was developed by Mrs Alison Barnes, Research Dietitian, University of Newcastle. Dietary advice and follow up was delivered by Mrs Alison Barnes and myself.

Semi-structured interviews were conducted and analysed by Dr Catherine McParlin, Research Midwife, Institute of Cellular Medicine, Newcastle University. The topic guide for interviews was developed jointly by Dr Vera Araujo-Soares (Senior Lecturer in Health Psychology, Institute of Health and Society, Newcastle University), Dr McParlin and myself.

The design and organisation of the clinical studies, recruitment of the participants, their clinical care and follow up and all other aspects of care apart from those specifically mentioned above were undertaken by myself. The composition of this thesis is my own original work. The research contained within this thesis has not previously been submitted elsewhere for the Doctorate of Medicine degree.

PUBLICATIONS FROM THIS WORK

Hodson K, Dalla Man C, Smith FE, Barnes A, McParlin C, Cobelli C, Robson SC, Araujo-Soares V, Taylor R. **Liver Triacylglycerol Content and Gestational Diabetes: Effects of Modest Calorie Restriction.** Diabetologica. 2017; 60(2):306-313

Hodson K, Dalla Man C, Smith FE, Thelwall PE, Cobelli C, Robson SC, Taylor R. **Mechanism of Insulin Resistance in Normal Pregnancy.** Horm Metab Res. 2013 Aug; 4(8):567-71

Hodson K, Robson SC, Taylor R. **Gestational Diabetes: Emerging Concepts in Pathophysiology.** Obstetric Medicine. 2010; 3: 128-132

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LIST OF ABBREVIATIONS

Acetyl CoA	Acetyl co-enzyme A
ACHOIS	Australian Carbohydrate Intolerance Study in Pregnant Women
ACS	*Acyl-co synthase*
ALT	Alanine Transaminase
AMP	Adenosine Monophosphate
ATP	Adenosine Triphosphate
AUC	Area Under Curve
BMI	Body Mass Index
BMR	Basal Metabolic Rate
cAMP	Cyclic Adenosine Monophosphate
CPT	*Carnitine-palmiyoyl Transferase*
$FADH_2$	Flavin Adenine Dinuclotide
FAS	Fatty Acid Synthase
G-6-P	Glucose-6-phosphate
GDM	Gestational Diabetes Mellitus
GKRP	*Glucokinase* Regulatory Protein
GLUT	Glucose Transporter Protein
HAPO	Hyperglycaemia and Adverse Pregnancy Outcomes
HDL	High Density Lipoprotein
HGP	Hepatic Glucose Production
HIEC	Hyperinsulinaemic-Euglycaemic Clamp
HOMA	Homeostatic Model Assessment

HOMA-%B	Homeostatic Model Assessment Beta Cell Function
HOMA-%S	Homeostatic Model Assessment Insulin Sensitivity
HOMA-IR	Homeostatic Model Assessment Insulin Resistance
hPL	Human Placental Lactogen
IGF-1	Insulin-like Growth Factor 1
IMCL	Intramyocellular Lipid
IRS-1	Insulin Receptor Substrate-1
ISI	Insulin Sensitivity Index
IV	Intravenous
IVGTT	Intravenous Glucose Tolerance Test
Kcal	Kilocalories
LDL	Low Density Lipoprotein
MFP	MyFitnessPal
MHRA	Medicines and Healthcare products Regulation Agency
MRS	Magnetic Resonance Spectroscopy
NADH	Nicotinamide Adenine Dinucleotide
NAFLD	Non-Acute Fatty Liver Disease
NEFA	Non-esterified Fatty Acids
NGT	Normal Glucose Tolerance
NMR	Nuclear Magnetic Resonance
OGTT	Oral Glucose Tolerance Test
OR	Odds Ratio
PEP	Phophoenolpyruvate
pGDM	Previous Gestational Diabetes

pGH	Placental Growth Hormone
ppm	Parts per million
PRESS	Point Resolved Spectroscopy
SAR	Specific Absorption Rate
SCBU	Special Care Baby Unit
SD	Standard Deviation
SEM	Standard Error of the Mean
SGLT	Sodium-dependent Glucose Transport Protein
Si	Insulin Sensitivity
T2DM	Type 2 diabetes mellitus
TCA	Tricarboxylic Acid
TE	Echo Time
TG	Triglyceride
TR	Repetition Time
VLDL	Very Low Density Lipoprotein
VLDL	Very Low Density Lipoprotein
WHO	World Health Organisation

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CHAPTER 1 BACKGROUND AND INTRODUCTION

1.1 Normal Pregnancy

1.1.1 Prioritizing the Fetal Environment

Pregnancy is a state of metabolic flux in which the delicate balance of metabolic homeostasis is disturbed from its status quo to one in which the needs of the fetus become paramount. The fetus needs the building blocks of life in the form of glucose, amino acids and fatty acids. To meet the potential requirements for lactation the mother needs to increase energy stores as fat.

Maternal energy requirements in pregnancy are thus increased in order to allow the placenta, membranes, amniotic fluid and fetus to grow together with increasing maternal adipose deposition requires approximately 80,000kcal (Hytten and Leitch, 1971). Human pregnancy, compared to other animal species, is marked by slow fetal growth over a long period in order to allow for the development of a complex human brain (Payne and Wheeler, 1967). Additionally, compared to other species, the human fetus is relatively small in relation to the adult. This means that on a day-to-day basis, only a small increase in energy intake is required for pregnancy to be sustained. This is advantageous in an environment where food is not always readily available as energy savings can be made to divert the small amount of energy required by the fetus (Prentice and Goldberg, 2000). It is this plasticity in maternal metabolism that allows for reproduction, even under conditions of extreme food scarcity.

Total energy expenditure for pregnancy varies according to food availability as is shown in **Figure 1**.

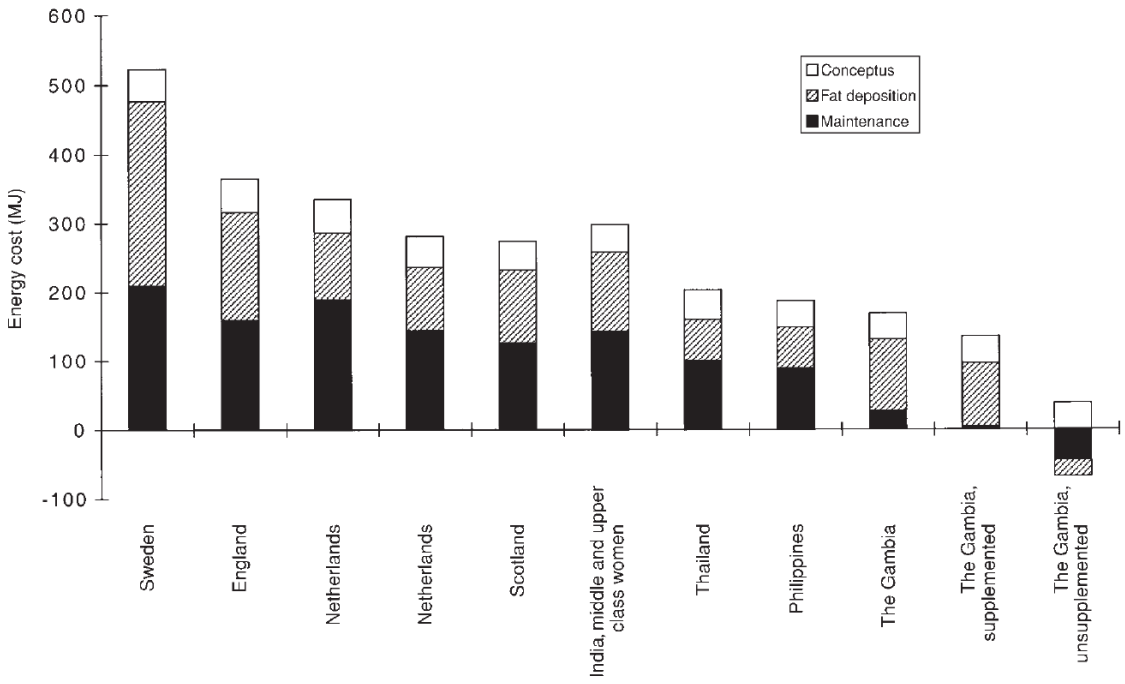

Figure 1 Total energy costs of pregnancy: conceptus (including fetal fat), fat deposition and maintenance in women from affluent and poor countries. The energy cost of the conceptus was estimated pro rata according to birth weight. The supplemented women from The Gambia received balanced protein-energy supplements (Prentice and Goldberg, 2000).

From a series of studies across different socioeconomic profiles, it has been established that the energy required to produce the fetus is constant and does not change much between high and low income groups (Prentice and Goldberg, 2000). However, there is a marked difference in maternal basal metabolic rate (BMR) in a well-nourished countries (such as the UK) compared to a countries where food is less readily available for example, The Gambia (Lawrence *et al.*, 1987; Prentice *et al.*, 1989; Prentice and Goldberg, 2000). In the UK BMR increases substantially from about 20 weeks gestation onwards. Conversely, Gambian women reduce their BMR, particularly during the rainy season when food is scarce, below pre-pregnancy levels, increasing it only slightly from around 30 weeks gestation as shown in **Figure 2** (Lawrence *et al.*, 1987).

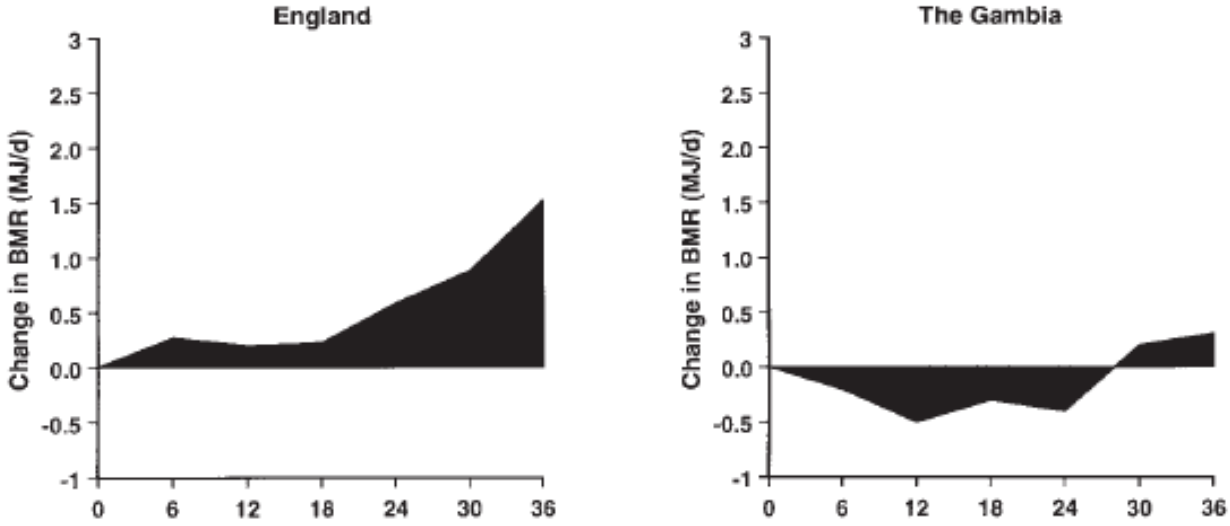

Figure 2 Basal metabolic rate during pregnancy in women from England and The Gambia (Prentice and Goldberg, 2000)

These studies show that it is possible to adapt maternal BMR in order to maintain adequate energy delivery to the fetus. This is a useful adaptation, and has been well studied in times of famine, but in developed countries we live in an environment of nutritional excess. How does maternal metabolism cope with energy saturation?

Total energy costs of pregnancy are positively correlated with pre-pregnancy fat and pregnancy weight gain, as **Figure 3** demonstrates, suggesting that there is a mechanism through which the mother can adapt her energy expenditure in pregnancy according to her nutritional status. The studies from which this data has been derived were published over 20 to 30 years ago and the average body mass index (BMI) of the women studied was 22 (Durnin *et al.*, 1987; Thongprasert *et al.*, 1987; Tuazon *et al.*, 1987; van Raaij *et al.*, 1987; Forsum *et al.*, 1988; Goldberg *et al.*, 1993). Since then, there has been a steady increase in the number of overweight and obese pregnant women (National Audit Office, 2001). The metabolic effect of this, in terms of the proportionality of energy expenditure dedicated to BMR, fat deposition and fetal weight has yet to be investigated. However, the increased frequency of gestational diabetes, fetal macrosomia and caesarean section would imply that metabolism is unable to cope with surplus energy intake in a proportion of the population.

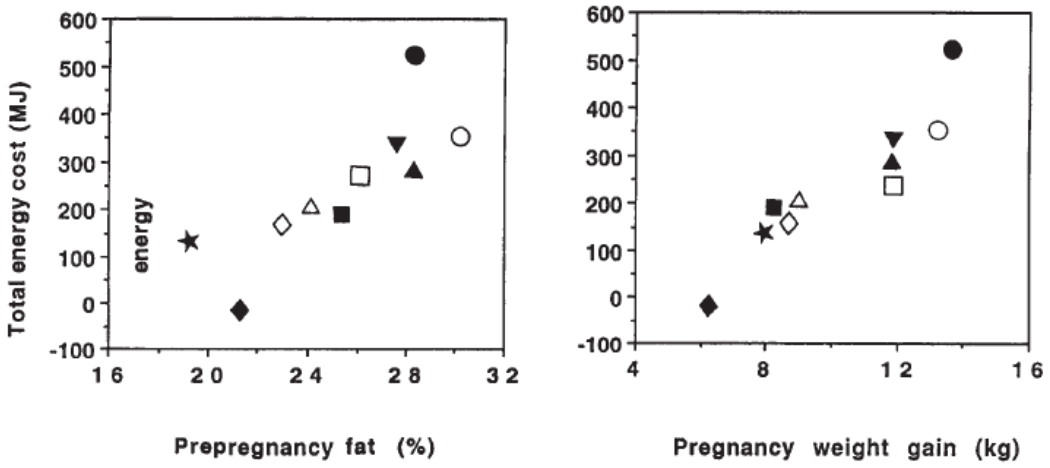

Figure 3 Intercountry correlations of the total energy cost of pregnancy with pre-pregnancy maternal fatness in women from Sweden (●), England (○), the Netherlands (▲ and ▼), Scotland (□), Thailand (△), the Philippines (■), The Gambia (★ supplemented with balanced protein-energy supplements, ◆ unsupplemented) and The Gambia (◇ different data set).
(Prentice and Goldberg, 2000)

Homeostasis is 'the physiological process by which the internal systems of the body are maintained at equilibrium despite variations in external conditions' (*Oxford Conscise Medical Dictionary*, 2010). Homeostasis is therefore responsible for diversion of energy into three components: the conceptus, maternal fat storage and additional metabolism. Understanding the hormonal control of metabolism is therefore essential to understanding the processes underlying pregnancy adaptation.

1.1.2 Overview of Metabolism

The human diet typically consists of three main food groups: carbohydrate which, in the UK, represents approximately 50% of dietary intake, protein 15% and fat 35% (Henderson *et al.*, 2003). Carbohydrates are digested and absorbed as monosaccharides. Monosaccharides are five or six carbon molecules, the most significant of which is glucose. Glucose is used by almost all tissues to produce energy and some tissues (for example the brain) are entirely glucose dependent in everyday life. The rapid decline in consciousness secondary to hypoglycaemia is a reminder that glucose is a constant requirement. Glucose is highly soluble and easily transported via the bloodstream. Its uptake into cells is regulated by glucose transporter proteins (GLUT). Glucose is readily metabolised through glycolysis (into pyruvate), releasing adenosine triphosphate (ATP) and can yield further energy through

aerobic respiration on the mitochondrial membrane through the tricarboxylic acid (TCA) cycle.

In the well-fed state surplus glucose can be stored as glycogen, a polysaccharide molecule that is stored within the cell cytoplasm. Glycogen stores are particularly abundant in the liver and muscle. Within the liver, glucose can be released from glycogen in times of glucose shortage. Muscle lacks *glucose-6-phosphatase* and so therefore cannot directly convert glycogen into glucose; "new" glucose can be manufactured from lactate (produced by the muscle) through gluconeogenesis in the liver.

Glucose is a useful energy substrate, it is easily digested and taken up into cells and yields good ATP production. However, it is quickly metabolised and glycogen reserves can decline rapidly. The rate at which glycogen stores become deplete depends on activity. On a day-to-day basis they will last approximately 24 hours, however, under endurance training e.g. marathon running they can become deplete in under two hours or less (Frayn, 2003; Rapoport, 2010). Glucose can be seen as the 'cash' of the metabolism monetary system: it is globally accepted and can be kept in a 'current' account in the form of glycogen for easy draw down later.

Fat, on the other hand, is the 'savings account' for energy storage. Fat is digested and absorbed as fatty acids and glycerol. It is then either metabolised, stored or re-packaged by the liver and transported via the bloodstream to other tissues. Fat is an efficient means of storing or mobilizing energy since lots of energy can be stored in relatively little space. Glycogen, by comparison is a large molecule stored as a colloid in water, and so is a relatively heavy and space-occupying to store. Energy from fat is obtained through beta oxidation, which typically supplies energy for most tissues in the overnight fasted state when glucose is less available.

Protein is digested and absorbed as amino acids. Amino acids are the building blocks for protein synthesis. Some amino acids can be manufactured by the liver, however there are a number of amino acids that cannot be created *de novo*, these are termed 'essential amino acids'. Amino acids that are not converted into protein cannot be stored. They are deaminated by the liver into urea, which in turn is excreted through the kidneys. The carbon backbone of the amino acid molecule can be converted into glucose through

gluconeogenesis or into ketone bodies for oxidative metabolism. Thus, in terms of energy production and storage, the two most important substrates are glucose and fat.

1.1.3 Glucose Metabolism

The digestion of carbohydrate begins in the mouth with the enzyme *amylase*. This enzyme acts to break down the bonds splitting polysaccharides into mono- and disaccharides. Pancreatic juice contains further *amylase*. Absorption of monosaccharide occurs on the brush border of the villi within the small bowel. Monosaccharides are absorbed through glucose transporters within the brush border and transported to capillaries in order to enter the bloodstream. Disaccharides (such as sucrose) are also broken down on the brush border into their monosaccharide constituents.

From the gut, blood is transferred via the hepatic portal vein to the liver where glucose can be either released into the blood stream, metabolized, converted into glycogen or other molecules (for example lipid).

Glucose is taken into cells via glucose transporter proteins present on the cellular surface membrane. There are two different type of transport protein: glucose transporter protein (GLUT) and sodium-dependent glucose transporter protein (SGLT). Different GLUT transporter proteins have varying affinity for glucose and are passive (non-energy requiring) transporters: they transport glucose along a concentration gradient. SGLT proteins require energy (in the form of ATP) to transport glucose against a concentration gradient. They are found in the epithelial cells of the gut, renal tubules and choroid plexus. There are fourteen types of GLUT protein; different tissues express differing GLUT proteins on their cell surfaces. For example, GLUT-3 is found on neurons, GLUT-1 on erythrocytes and the blood brain barrier, GLUT-4 is present on muscle and adipose tissue and GLUT-2 is present on the cell membranes of the liver, kidney and beta-cells of the pancreas. GLUT-4 can be up-regulated through hormonal influence, for example insulin will increase the number of GLUT-4 transporter proteins, thus increasing glucose uptake in the muscle and adipose tissues.

Glucose, once inside cells can be:

1. Stored as glycogen
2. Metabolised into energy (ATP)

3. Converted into other molecules (e.g. fat, amino acids, nucleic acids)

1.1.3.1 Glycogen Storage

In well-nourished individuals, glycogen forms approximately 1-2% of muscle mass and 10% of liver mass (Frayn, 2003). The purpose of glycogen in the liver is to produce glucose relatively quickly if needed (due to fasting conditions) and in the muscle its purpose is to provide glucose for rapid anaerobic metabolism during strenuous exercise.

Glycogen is synthesised by *glycogen synthase* in the cell cytoplasm. Glucose is phosphorylated to glucose 6-phosphate and then converted to glycose 1-phosphate. It is then bound to uridine diphosphate (UDP) to form UDP-glucose, see **Figure 4a**. UDP-glucose binds onto growing glycogen molecules through *glycogen synthase*. Most bonds are α(1:4) linkages, but some are α(1:6) linkages – creating the branching or tree like structure of the glycogen molecule, see **Figure 4b**. The branches increase the number of ends from which glucose can be enzyme-released.

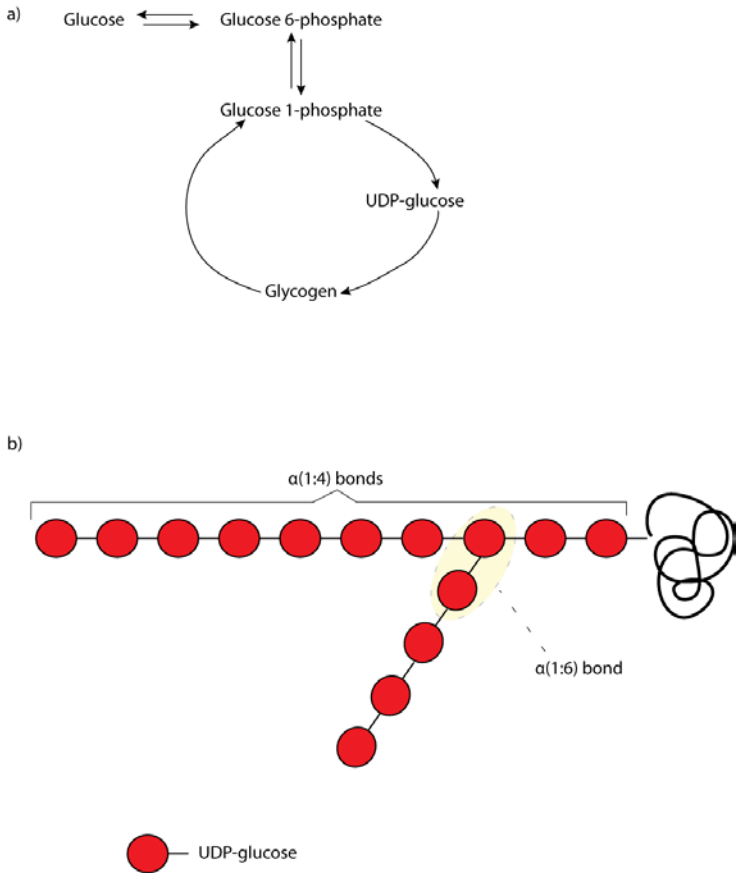

Figure 4 Glycogen synthesis – adapted from Ferrier, DR *Lippincott's Illustrated Reviews: Biochemistry*. 6th Edition p.127

To release glucose from glycogen *glycogen phosphorylase* cleaves the α(1:4) carbon bonds at the ends of the glycogen chain producing glucose 1-phosphate. Glucose 1-phoshate, in turn is converted to glucose 6-phosphate, which in muscle is directly metabolised by glycolysis (described below). In the liver, glucose 6-phosphate can be metabolised or the phosphate can removed to release glucose into the bloodstream.

1.1.3.2 Glycolysis

The metabolism of glucose to yield energy begins with glycolysis. Glycolysis describes the steps required to convert glucose into pyruvate. There are multiple steps in this process, however the three main steps are summarised in **Figure 5** below:

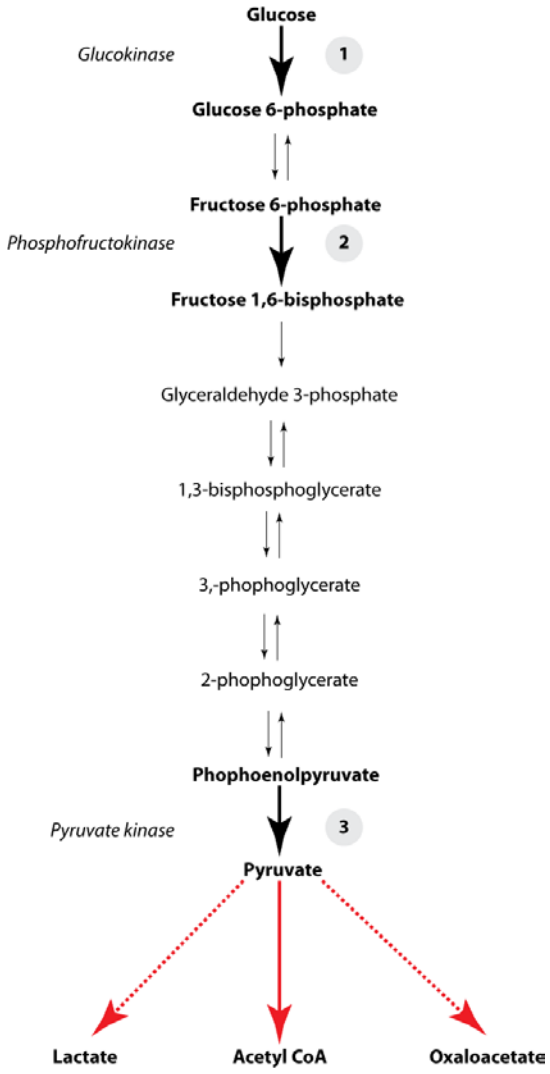

Figure 5 Glycolysis adapted from Ferrier, DR *Lippincott's Illustrated Reviews: Biochemistry*. 6th Edition p.105

The three main steps in glycolysis are:

1. Conversion of glucose into glucose 6-phosphate
2. Conversion of glucose 6-phosphate into fructose 1,6-bisphosphate
3. Conversion phophoenolpyruvate (PEP) into pyruvate

The first two steps require energy (in the form of ATP). The final step is energy yielding (producing ATP).

1. **Conversion of glucose into glucose 6-phosphate**

   Phosphorylation of glucose to form glucose-6-phosphate is the first step in glycolysis. Through the addition of a phosphate group to the glucose molecule it becomes

polarised and therefore 'trapped' within the cell cytoplasm. *Hexokinase*, an enzyme found in most tissues, is responsible for this initial step. *Hexokinase* has a high affinity for glucose and is inhibited by glucose 6-phosphate. In the liver and pancreas a different enzyme, *glucokinase,* phosphorylates glucose. *Glucokinase* has a lower affinity for glucose than *hexokinase* , this means that it requires higher glucose concentrations to work. Additionally, it has a higher rate of activity (Vmax) than *hexokinase*. Thus, in the liver, *glucokinase* operates only when the intracellular glucose concentration is high, such as after a meal, and is able to eliminate glucose quickly from the circulation. There is no rate limiting step of glucose uptake into hepatocytes, and the intracellular concentration is similar to the extracellular concentration, and hence glucokinase activity is rate limiting for glucose metabolism in the liver. *Glucokinase* is regulated by glucokinase regulatory protein (GKRP) which binds reversibly to *glucokinase* inhibiting its action by holding the enzyme in the nucleus of the cell. Fructose 6-phosphate (a product of *glucokinase*) promotes the production of GKRP, thus with normal glucose turnover *glucokinase* is inhibited. However, once glucose levels reach a threshold, *glucokinase* is released from GKRP and is able to act on (and begin to eliminate) the increased intracellular glucose concentration. *Glucokinase* acts as a glucose 'thermostat' within the liver and pancreas, switching on when glucose levels are high and switching off when low.

2. **Conversion of glucose 6-phosphate into fructose 1,6-bisphosphonate**

   Glucose 6-phosphate is converted into fructose 6-phosphate then phosphorylated to fructose 1,6-bisphosphate by *phosphofructokinase-1*. This is an irreversible reaction and is a rate limiting step in terms of glycolysis control. *Phosphofructokinase-1* is inhibited by ATP and citrate within the cell cytoplasm (i.e. glycolysis is inhibited in the presence of abundant energy within the cell), with resultant inhibition of glycolysis and promotion of glycogen storage instead. *Phosphofructokinase-1* is activated by adenosine monophosphate and fructose 2,6-bisphosphonate. Fructose 1,6-bisphosphonate is divided into two three-carbon molecules which are further metabolised into pyruvate.

3. **Conversion phophoenolpyruvate (PEP) into pyruvate**

   Pyruvate kinase converts PEP to pyruvate in an energy yielding step, producing ATP. Pyruvate can be either metabolised through the TCA cycle (see below) or reduced to lactate by *lactate dehyrdrogenase.* The conversion into lactate occurs within the cell cytoplasm in the absence of oxygen (anaerobic respiration). This commonly occurs in the muscle cells during exercise. Lactate is then transported from the muscle to the

liver where it can either be converted back into glucose or metabolised via the TCA cycle.

Glycolysis produces a net gain of two ATP molecules for each glucose molecule converted into two molecules of lactate. Under aerobic conditions glycolysis yields further ATP through the oxidation of NADH through the electron transport chain (approximately eight molecules of ATP are created per molecule of glucose converted to pyruvate).

Pyruvate can be further metabolised into acetyl CoA, a major substrate for the TCA cycle, or converted into oxaloacetate (a TCA intermediate) or in some organisms it can be converted into ethanol.

1.1.3.3 Tricarboxylic acid (TCA) cycle

The tricarboxylic acid or Krebs cycle is the final pathway for carbohydrate, fat and protein metabolism. It occurs exclusively in the mitochondria as it requires the electron transport chain within the mitochondrial membrane to oxidise, thereby releasing energy from the reduced forms of the intermediate coenzymes nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$).

Acetyl CoA is a two carbon substrate for the TCA cycle. Acetyl CoA can come from glucose, fatty acid or amino acid metabolism pathways, see **Figure 6**. The decarboxylation of pyruvate produces acetyl CoA which feeds into the TCA cycle**.**

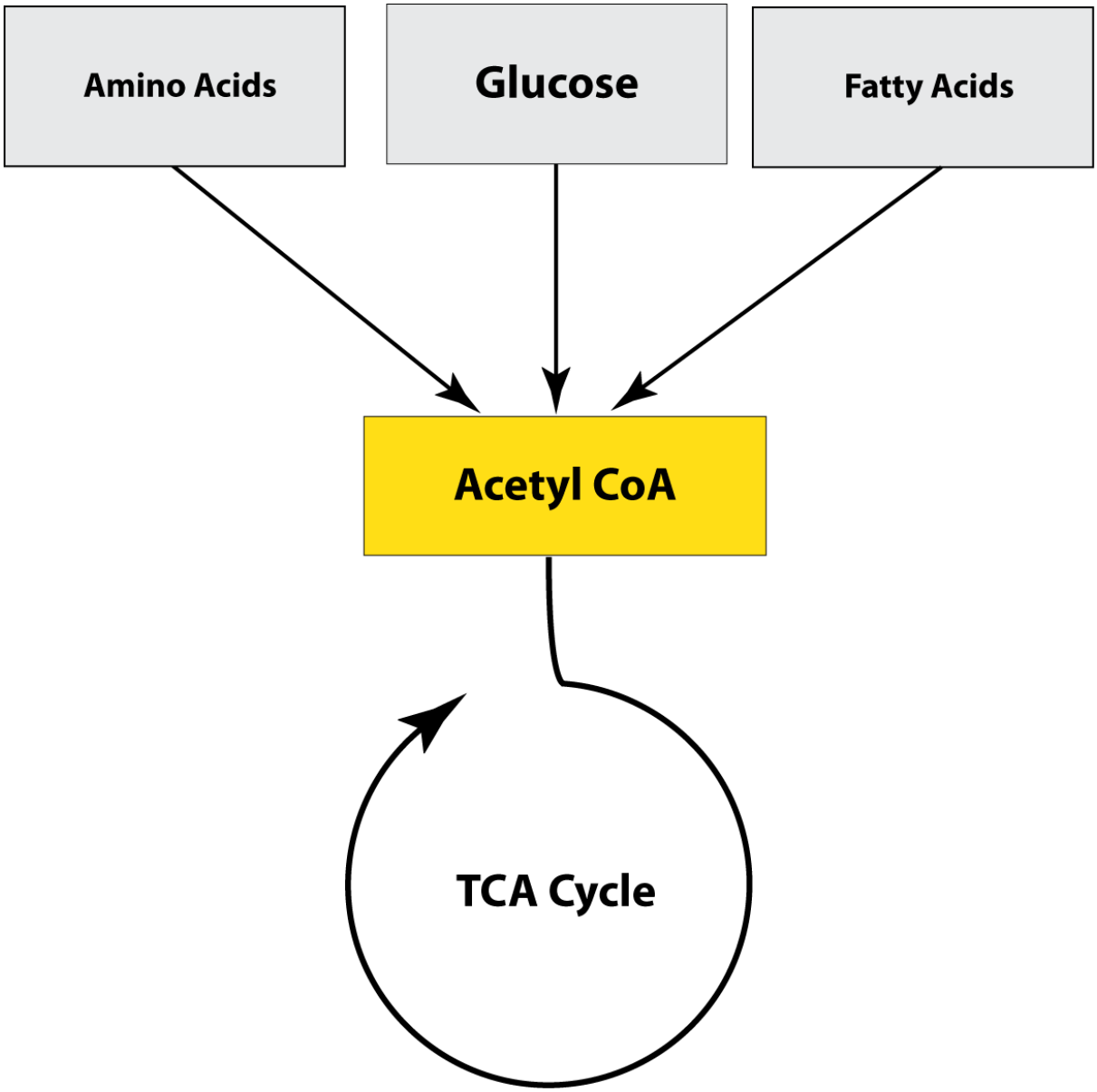

Figure 6 Acetyl CoA

Citrate synthase brings together acetyl CoA and oxaloacetate (a four carbon intermediate substrate). Citrate is then isomerized and converted into α-ketoglutarate, in the process producing carbon dioxide (CO_2) and NADH. α-ketoglutarate is metabolised to succinyl CoA, producing further CO_2 and NADH. Succinyl CoA is then sequentially converted into succinate, fumarate, malate and finally, oxaloacetate. The cycle is then complete, ready for acetyl CoA to enter the cycle again.

Each molecule of acetyl CoA yields 12 molecules of ATP.

1.1.3.4 Glucose Production

Organs and tissues such as the brain, red blood cells and structures within the eye can only metabolise glucose. Additionally, in pregnancy the fetoplacental unit is ordinarily dependent upon a constant glucose supply. Gluconeogenesis is the biochemical mechanism through which glucose can be made *de novo* from lactate, pyruvate, glycerol and amino acids. The pathway shares many of the reversible steps of glycolysis, however there are four steps, summarised in **Figure 7**, which are specific to gluconeogenesis.

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Figure 7 Gluconeogenesis

1. Conversion of pyruvate to oxaloacetate
   In order to produce phosphenolpyruvate (PEP) from pyruvate (a three carbon molecule), pyruvate needs to be carboxylated into oxaloacetate (four carbon) by the enzyme *pyruvate kinase*. This involves the addition of carbon dioxide and the co-enzyme *biotin* is required to catalyse this step. The formation of oxaloacetate is useful, not only for gluconeogenesis, but also to replenish supplies needed for the TCA cycle.
2. Conversion of oxaloacetate into PEP
   Oxaloacetate is converted into PEP through the enzyme *PEP carboxylase*. This requires energy in the form of guanosine triphosphate and a carbon atom is removed in the form of CO_2.
3. Dephosphorylation of fructose 1,6-bis-phosphate
   Fructose 1,6-bisphosphatase removes a phosphate group from F 1,6-bisP (this bypasses the enzyme *phosphofructokinase 1* which works in the opposite direction, catalysing glycolysis). *Fructose 1,6-bisphosphatase* is inhibited by high levels of AMP. High levels of intracellular fructose 2,6-bis-phosphate (secondary to insulin) also inhibit *Fructose 1,6-bisphosphatase*, favouring glycolysis (via activation of *phosphofructokinase 1*) over gluconeogenesis.
4. Dephosphorylation of glucose 6-phosphate
   Glucose 6-phosphatase removes a phosphate group from glucose 6-phosphate, thereby releasing glucose. This enzyme bypasses the irreversible phosphorylation of glucose by *glucokinase* or *hexokinase* (glycolysis); it is found exclusively in the liver and kidney.

GLUT transporters are then responsible for moving glucose into the extracellular fluid space and finally into the blood.

The inhibition of hepatic glucose production (glycogenolysis and gluconeogenesis) by insulin is critically important in understanding the pathophysiology of type 2 diabetes (T2DM) and gestational diabetes. This will be discussed in greater detail later.

1.1.4 Fat

Fat is absorbed in the small bowel. It is digested through enzymes *gastric lipase* in the stomach, emulsified through bile salts produced by the liver and further broken down by enzymes from the pancreas. Bile salts are detergent-like molecules which convert fat into fine droplets called micelles. *Pancreatic lipase* acts on triglyceride molecules – breaking down the links between the fatty acid and glycerol. In fact, the lipase acts specifically on the 1,3 positions of the triglyceride molecule, releasing fatty acids and 2-monoacyglycerol, see **Figure 8**. Both fatty acids and 2-monoacyglycerol molecules have amphipathic properties, that is to say that they are both hydrophilic and hydrophobic. This, in conjunction with bile acids, helps to further emulsify fat. Eventually mixed micelles are formed which have a diameter 4-6nm and bring the products of triglyceride hydrolysis (fatty acids and monoacylglycerols) to the intestinal brush border.

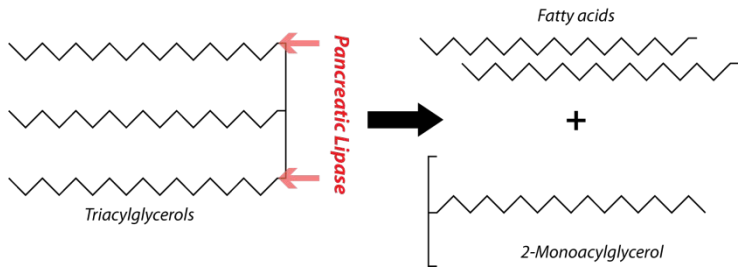

Figure 8 Conversion of triacylglycerols into fatty acids and 2-monoacylglycerol

Fatty acids are absorbed through fatty acid transporter proteins (FAT and FATP). Monoacylglycerols are either absorbed through transport proteins or cross the cellular membrane through passive diffusion. Within the enterocytes triglycerides are re-formed and packaged into chylomicrons. Chylomicrons enter the lymphatic system and flow into progressively proximal branches until they enter the systemic circulation at the thoracic duct. Some fatty acids, those with short-to-medium (12-16) carbon chains are not re-esterified and

enter the circulatory system (capillary blood) as non-esterified fatty acids (NEFA). However, most dietary fatty acids are re-packaged as chylomicrons.

Lipoprotein lipase is an extracellular enzyme bound to the capillary walls. As the chylomicrons circulate their outer lipoprotein surface binds to tissue specific receptors (for example through the apo C-II receptor). The binding of lipoprotein to receptor activates *lipoprotein lipase* which metabolises the triglyceride core of the chylomicron, converting triglyceride into its fatty acid and glycerol components. Fatty acids can be absorbed into the cell and either metabolised or stored. If they are not taken up by the cell they are bound to albumin and circulate in the blood stream until required. Glycerol is metabolised in the liver, either to produce energy or for gluconeogenesis.

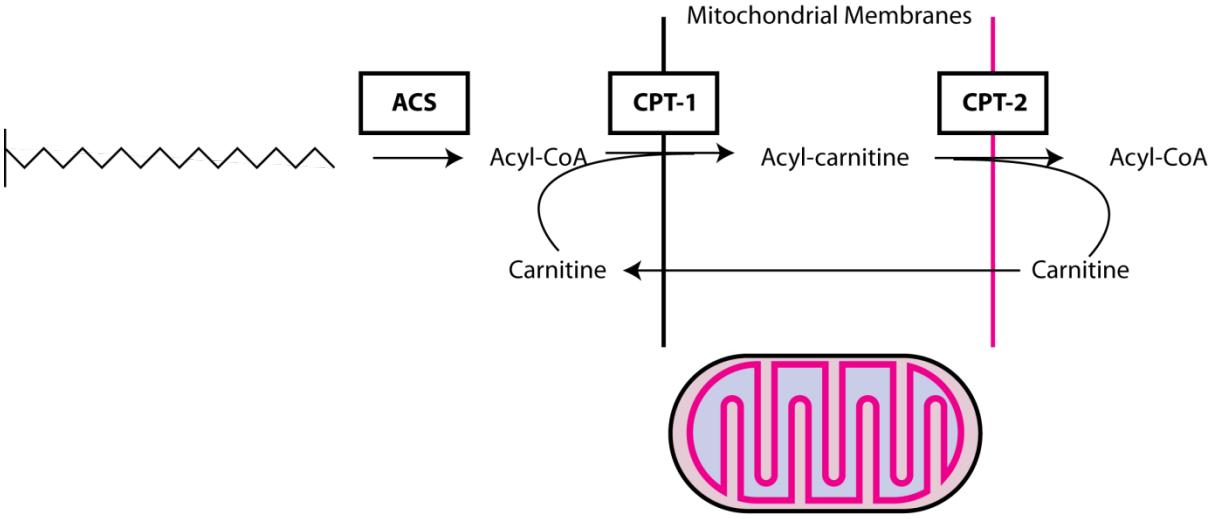

Figure 9 Transport of Fatty Acids into Mitochondria
Carnitine is a highly charged molecule and there is a specific translocase to move it across the mitochondrial membranes. A*cyl-Co synthase* (ACS); *carnitine-palmitoyl transferase* (CPT).
Adapted from Metabolic Regulation

Beta-oxidation is the process through which fatty acids are metabolised and occurs within mitochondria. On entering the cell cytoplasm the fatty acid is activated by coenzyme-A this is facilitated by *acyl-CoA synthase (ACS)* to produce acyl-CoA, as shown in **Figure 9**. In order to get the fatty acid into the mitochondria a transport protein, carnitine, is required. The acyl group is transferred to the carnitine molecule, catalysed through the enzyme *carnitine-palmitoyl transferase-1* (CPT-1). CPT-1 is an important regulatory enzyme. It is inhibited by malonyl-CoA (an intermediate molecule in *de novo* lipogenesis). This inhibitory mechanism is critical

since it stops the cyclical production and breakdown of fatty acids that could otherwise occur. Insulin inhibits the breakdown of fatty acids through increasing the intracellular concentration of malonyl-CoA and stimulating fatty acid esterification (back into triglycerides). Once inside the mitochondria, fatty acyl-CoA can be metabolised through β-oxidation. β-oxidation summarises the metabolic process through which two-carbon molecules are removed from the long fatty acid chain producing acetyl-CoA. Acetyl-CoA can then be metabolised through the TCA cycle (described above) to produce ATP. In the liver acetyl-CoA can alternatively be converted into ketone bodies (acetoacetate and 3-hydroxybutyrate). Ketone bodies are an important fuel and in most cases can be utilized instead of glucose. Ketone bodies are soluble, therefore do not require any transport proteins. Ketone bodies are produced when acetyl-CoA production exceeds the oxidative capacity of the liver. In the fasted state the liver is flooded with acetyl-CoA from β-oxidation and the breakdown of fatty acids. This inhibits *pyruvate dehydrogenase* and activates *pyruvate carboxylase* to produce oxaloacetate. The majority of oxaloacetate is used for gluconeogenesis (given that the body is in a fasted state and requires additional glucose). The excess acetyl-CoA is therefore channelled into ketone body production rather than metabolised in the TCA cycle.

1.1.4.1 Fat Synthesis and Storage

Excess carbohydrate and protein can be converted into fatty acids and glycerol to be stored as fat (predominantly in the adipose tissue). The process requires energy (in the form of ATP and reduced coenzymes NADPH) and an abundant source of acetyl CoA (obtained through glycolysis or the metabolism of amino acids). Acetyl CoA must first be converted to citrate in order for it to leave the mitochondria and enter the cell cytoplasm. *Citrate synthase* catalyses the formation of citrate from oxaloacetate and acetyl CoA. Once in the cell cytoplasm acetyl CoA is re-released by the enzyme *ATP:citrate lysase*.

Acetyl CoA is converted into malonyl-CoA, catalysed by the enzyme *acetyl-CoA carboxylase*. As previously mentioned, malonyl-CoA is an important inhibitor of the β-oxidation pathway (through inhibition of CPT1). *Acetyl-CoA carboxylase* is inhibited by long chain fatty acyl-CoA. An abundance of citrate (implying excessive free energy within the cell cytoplasm) and insulin promote the action of *acetyl-CoA carboxylase* thereby, inhibiting β-oxidation and promoting lipid storage. Excessive calorie intake over a prolonged period also up-regulates

the genetic expression of *acetyl-CoA carboxylase* enzyme, again promoting the conversion of carbohydrate into lipid.

Malonyl-CoA is a three carbon molecule. *Fatty acid synthase (FAS)* catalyses the process through which malonyl-CoA loses a carbon (as carbon dioxide) and is added as a two-carbon molecule to a growing fatty acid chain. The process is complex and involves numerous steps.

Finally, fatty acids combine with glycerol to produce triglycerides and phospholipid.

1.1.5 The glucose-fatty acid cycle

The metabolism of glucose and fatty acids are not separate entities, rather the two metabolic pathways are closely related. In 1963, Randle et al. proposed a mechanism through which fatty acids compete against glucose for mitochondrial oxidation, resulting in reduced glucose uptake and metabolism, see **Figure 10** (Randle *et al.*, 1963).

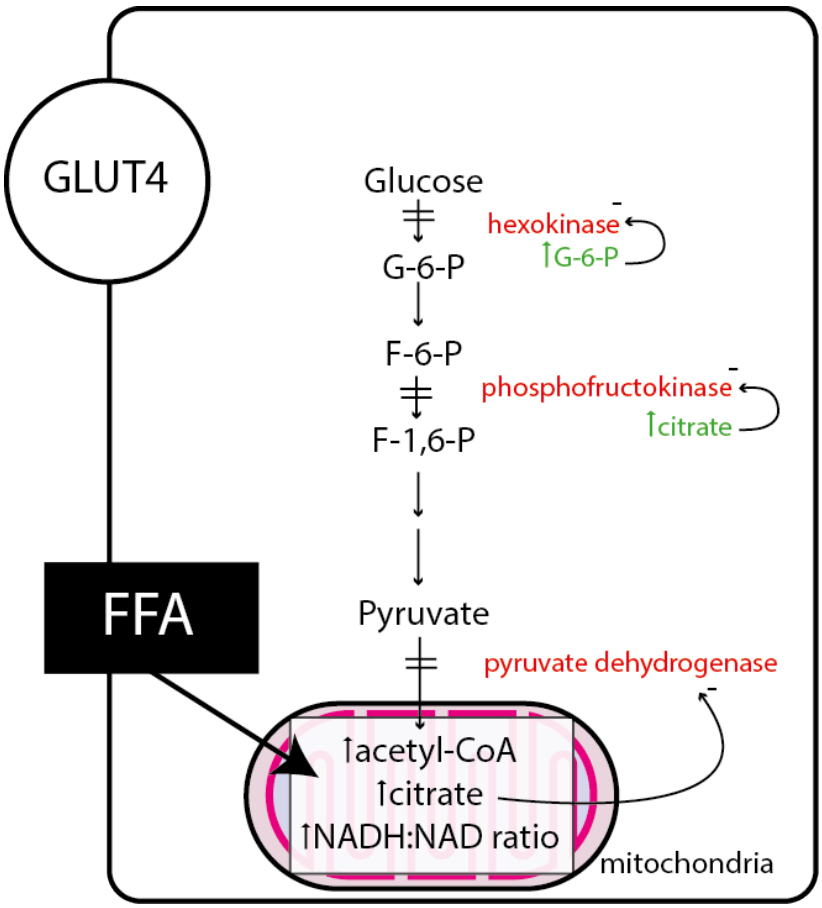

Figure 10 The Randle Hypothesis adapted from (Randle *et al.*, 1963)

Increased fatty acid oxidation leads to increased acetyl CoA and citrate formation. $NADH/NAD^+$ and ATP/ADP ratios are increased. Increased intracellular concentrations of NADH, ATP and acetyl CoA inhibit *pyruvate dehydrogenase,* thus the oxidation of pyruvate is suppressed. Increased citrate is an inhibitor of phosphofructokinase. Fructose 6-phophate and glucose 6-phosphate build up. Glucose 6-phosphate inhibits hexokinase thus glucose breakdown and oxidation is prevented. As a result, increased intracellular glucose concentrations prevents further glucose uptake by the cell.

This process occurs independent to insulin, in effect increasing fatty acid concentrations induces a state of insulin resistance, however it is not insulin signalling that is impaired, rather the presence of abundant fatty acids exerting a change in metabolic flux at the mitochondrial level.

Metabolic studies utilizing nuclear magnetic resonance (NMR) spectroscopy (a technique that allows measurement of metabolites and metabolic intermediates *in vivo*) have confirmed much of Randle's work. However, NMR evidence would suggest fatty acids directly suppress glucose transportation into muscle*,* rather than accumulation of glucose 6-phosphate and allosteric inhibition of *hexokinase* (Roden, 2004). The mechanism for this is still under investigation.

1.1.6 Summary of energy provision and substrate storage

The metabolism of glucose, fat and amino acids has been described. The common fate for all substrates is acetyl CoA. Acetyl CoA can be metabolised via the TCA cycle to produce ATP, CO_2 and water. In the liver, acetyl CoA can be converted into ketone bodies.

The fate of excess glucose has been discussed in terms of glycogen synthesis and in the conversion of glucose to fat (through polymerisation of acetyl CoA).

The regulation of these pathways, and specifically the regulation during pregnancy, is important in understanding the physiological metabolic changes that accompany pregnancy and the pathophysiological changes that occur in gestational diabetes.

1.1.7 Hormonal control of Metabolism

1.1.7.1 Insulin

Insulin is an anabolic hormone produced by the beta cell of the pancreas. The hormone acts on many tissues, but principally liver, muscle and adipose tissue via the insulin receptor present on the cell surface.

Insulin is a peptide that consists of two polypeptide chains, linked together by sulphide bridges. There are two precursor molecules to insulin: pre-proinsulin and proinsulin. Insulin is formed when the C-peptide molecule is removed within the Golgi apparatus of the beta cell. Although C-peptide has no biological function, it acts as a marker of insulin production and secretion. Insulin is stored in granules within the cell; there are several stimuli for insulin release:

1. Increased glucose concentration. The glucose concentration in the blood rises following a meal. The beta cell is adapted to be able to 'sense' glucose. The GLUT2 receptor expressed on the pancreatic cells only lets glucose into the cell beyond a certain concentration gradient. Additionally, *glucokinase*, found within the pancreatic cells, has a higher affinity for glucose than *hexokinase* enzymes found in other cells. This means that glucose is only metabolised when the glucose concentration has reached a certain threshold.
2. Amnio acid and fatty acids. Elevated amino acids, for example arginine, stimulate insulin release as do fatty acids.
3. Gastrointestinal peptides. Glucagon-like peptide-1 is produced in the cells of the intestinal wall in response to food. It acts on the beta cells to amplify the glucose-stimulated response.

Stimulation of the beta cell depends on depolarisation of its membrane, the actual release of insulin being dependent on calcium ion (Ca^{2+}) influx into the cell. Glucose within the beta cell is metabolised to produce ATP. The increase in ATP closes energy dependent potassium ion (K^{+}) transporters on the cell membrane, thus polarising the cell membrane. Ca^{2+} ions open as a result allowing Ca^{2+} into the cell. Ca^{2+} stimulates the vesicles containing insulin to merge with the cell membrane, releasing insulin into the circulation via the hepatic portal vein.

Insulin production is inhibited through low blood glucose levels. Catecholamines (adrenaline and noradrenaline) released at times of stress also inhibit insulin release and can directly counteract the effect of insulin on cell metabolism (promoting the easy availability of glucose and other metabolites for increased energy production over storage).

Insulin acts on many tissues, but principally liver, muscle and adipose tissue via the insulin receptor present on the cells of these organs. The binding of insulin to its receptor triggers multiple downstream intracellular pathways. Initially the insulin receptor is phosphorylated and activates insulin receptor substrate-1 (IRS-1), leading to:

a) glucose uptake;
b) storage of glycogen, fat and protein;
c) inhibition of glucose, amino acid and fatty acid production;

Insulin can have its effect almost immediately (for example its effect on the expression of GLUT4 and the uptake of glucose into the cell), or over hours or days (for example the up-regulation of metabolic enzymes, reflecting gene expression and transcription).

1.1.7.2 Glucagon

Glucagon is produced by the alpha cells of the pancreas. It acts to oppose the effects of insulin, chiefly to prevent hypoglycaemia. Glucagon stimulates glycogenolysis and gluconeogenesis in the liver, thus increasing the availability of glucose to the circulation. Release of glucagon is stimulated by low blood glucose levels. Elevated amino acid levels (for example following a high protein meal) also stimulate glucagon in order to counteract hypoglycaemia from too much insulin release. Adrenaline and noradrenaline, produced by the adrenal medulla in response to the central nervous system also increase glucagon production, thereby increasing glucose availability in times of stress/anxiety.

Glucagon acts to increase glucose production in the liver and to inhibit glycogen storage. Fatty acid synthesis is inhibited through deactivation of *acetyl-CoA carboxylase* and decreased malonyl CoA levels. In turn, reduced malonyl CoA levels encourage fatty acid oxidation, increasing acetyl CoA for energy or ketone body production. Glucagon facilitates the breakdown of free amino acids into three-carbon molecules for gluconeogenesis by the liver.

Glucagon binds to its cellular receptor, which in turns increases the intracellular production of cyclic adenosine monophosphate (cAMP). This then acts on protein kinases to upregulate or downregulate metabolic processes favouring glucose and substrate availability for metabolism over storage.

1.1.7.3 Other pregnancy-related hormones

Maternal storage of glucose, amino acids and fatty acids is not necessarily beneficial to the fetus as it removes the substrate's availability for fetal growth. The fetus, placenta or both induce a state of maternal insulin resistance and this inhibits maternal substrate storage. The mechanism through which this comes about is unclear, however many additional hormones are created in pregnancy, several of these affect cell metabolism and will be discussed in further detail below:

1.1.7.3.1 Cortisol

Oestrogen increases cortisol binding globulin production in the liver. As oestrogen concentrations increase during pregnancy, so too does the level of cortisol binding globulin. Proportionally more cortisol is bound in plasma and therefore not eliminated: total cortisol levels are elevated as a result. Cortisol increases two- to three-fold from 12 weeks until 26 weeks, thereafter the level plateaus until labour and delivery (Campbell *et al.*, 1987). Corticosteroids have a number of metabolic effects on tissue metabolism, namely:

a) Stimulation of fat mobilization in adipose tissue, by increased activity of *hormone sensitive lipase*.
b) Increased production of *hormone sensitive lipase.*
c) Stimulation of gluconeogenesis through activation of key enzymes (*phosphoenolpyruvate carboxykinage, glucose-6-phosphatase).*
d) Inhibition of muscle glucose uptake.
e) Increase in muscle protein breakdown.

The effects of corticosteroids serve to reduce insulin sensitivity at a cellular level. This becomes apparent, for example, in diabetic patients in whom glycaemic control is often severely disturbed following administration of corticosteroids.

1.1.7.3.2 Human Placental Lactogen (hPL)

hPL is closely related to growth hormone and prolactin in terms of its molecular structure. hPL is produced by the placenta and its concentration is directly proportional to placental mass. **Figure 11** demonstrates the steady increase in hPL until 34 weeks, after which production plateaus. hPL has affinity to both growth hormone and prolactin receptors and is important for fetal growth through enhanced protein synthesis, elevated insulin-like growth factor 1 (IGF-1) and increased glycogen synthesis (Handwerger and Freemark, 2000). hPL causes beta cell hypertrophy, increasing both basal and postprandial insulin secretion from the pancreas (Grumbach *et al.*, 1968; Martin and Friesen, 1969). hPL increases lipolysis (Grumbach *et al.*, 1966), allowing the mother to metabolise free fatty acids, thus preserving glucose, amino acids and ketone body transfer via the placenta to the fetus and contributing to pregnancy-related insulin resistance (Randle *et al.*, 1963).

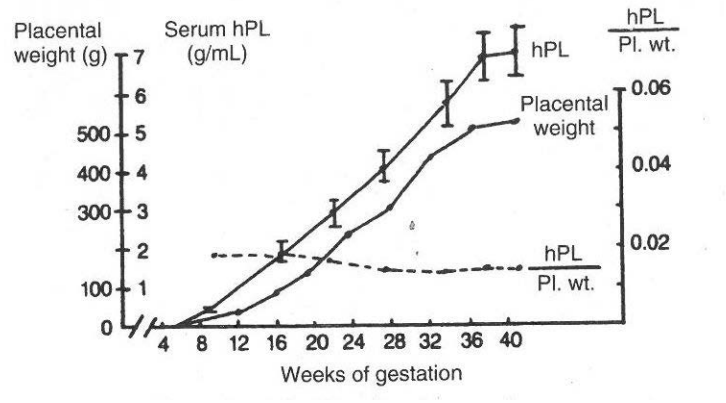

Figure 11 Placental weight (Pl. wt.) and maternal serum concentration of human placental lactogen (hPL) during pregnancy (Larsen, 2003).

1.1.7.3.3 Placental Growth Hormone (pGH)

Growth hormone (GH) is produced by the anterior pituitary gland. In pregnancy, the placenta takes over production of GH. Unlike pituitary GH, which is secreted in a pulsatile manner and is regulated by GH releasing hormone, placental GH is secreted constantly and is not regulated. The actions of GH are antagonistic to insulin; GH is typically released in response to hypoglycaemia and GH excess (acromegaly) is associated with glucose intolerance and diabetes. GH is capable of stimulating hepatic glucose production independent to insulin and glucagon control (Press *et al.*, 1984). Its mechanism of action is similar to hPL: it promotes lipogenesis, making glycerol available for gluconeogenesis,

promoting fatty acid oxidation and ketogenesis and favouring glucose release into the circulation.

1.1.8 Insulin Sensitivity and Insulin Resistance in Normal Pregnancy

Figure 12 shows the change in insulin sensitivity during pregnancy. There is a reduction in insulin sensitivity from approximately 18 weeks to 28 weeks gestation after which it plateaus until term. Following delivery, insulin sensitivity quickly returns to pre-pregnancy levels. It is unclear how insulin resistance is acquired during pregnancy. It is known that the changes in pro-insulin production, insulin binding to its receptor and insulin bound to white blood cells do not explain the insulin resistance seen (Kuhl *et al.*, 1985). Insulin resistance has been shown to be acquired through post-receptor modulation of the cell signalling pathway (Kirwan *et al.*, 2004). How this comes about is uncertain, however several factors have been postulated to play contributory roles:

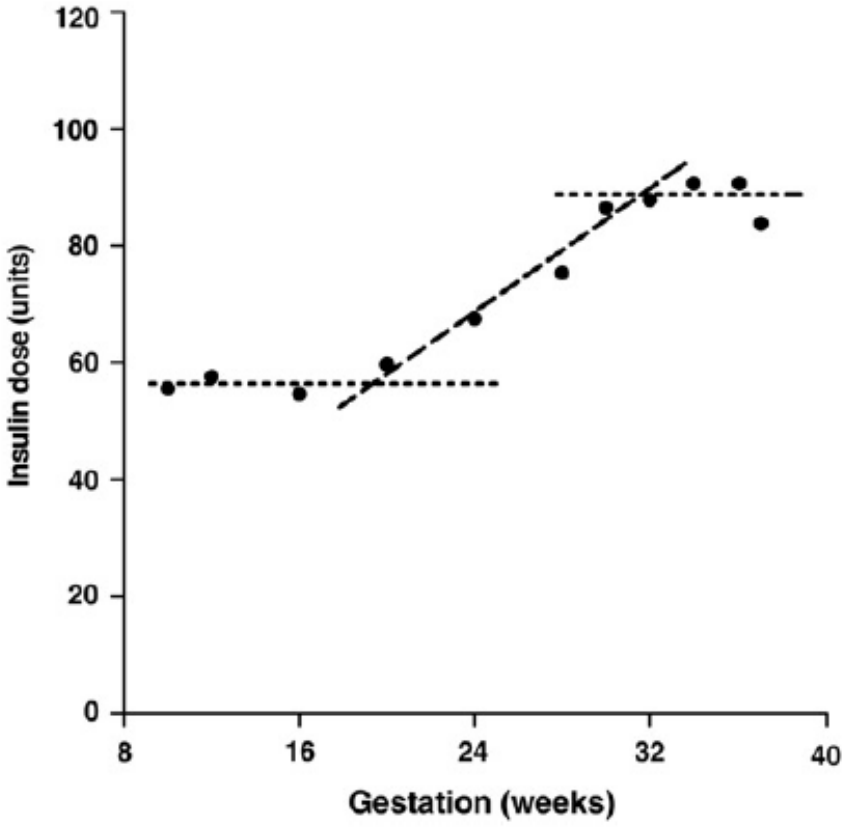

Figure 12 The time course of change in insulin sensitivity during pregnancy is reflected by the change in exogenous insulin dose to maintain glucose control in type 1 diabetes (Taylor *et al.*, 2002).

a) **Hormone signalling:** Sensitivity to insulin decreases progressively during pregnancy and swiftly returns to normal following delivery, strongly suggesting a hormonal association. Human placental lactogen, human placental growth hormone, progesterone, cortisol and prolactin are known to counteract the effects of insulin (Ryan and Enns, 1988). The relationship between reproductive hormones and insulin action is complex one. No single hormone has been identified as directly responsible for reducing insulin sensitivity. Reproductive hormone concentrations vary throughout pregnancy and it is likely that insulin sensitivity results from an interaction between many different hormones.

b) **Adipose-derived hormones:** Adiponectin is a globular protein synthesized by adipose tissue. It belongs to a group of hormones, including leptin and resistin, that control local storage and distribution of fat. Low adiponectin concentrations correlate with insulin resistant states (Ziemke and Mantzoros, 2010). Adiponectin stimulates glucose uptake in skeletal muscle and inhibits hepatic glucose production. Studies have shown that adiponectin levels decline with advancing gestation in normal pregnancy and are further reduced in gestational diabetes (Cseh *et al.*, 2004; Catalano *et al.*, 2006).

c) **Inflammation:** Normal pregnancy is a proinflammatory state. Tumour necrosis factor alpha (TNF-α), an adipo-cytokine, has been shown to promote insulin resistance through its prohibitive action on the insulin receptor. Elevation of TNF-α in pregnancy correlates with progressive insulin resistance (Kirwan *et al.*, 2002). Additionally, elevated levels of TNF-α have been found in conditions associated with hyperinsulinaemia such as obesity and T2DM (Hotamisligil and Spiegelman, 1994). However, the degree of increase of TNF-α levels is small in comparison with that seen in infection or after major trauma, and neutralization of TNF-α with monoclonal antibodies has no effect on insulin resistance in people with T2DM, implying that while TNF-α may contribute to insulin resistance, it is by no means the single causative factor (Ofei *et al.*, 1996).

Insulin resistance in pregnancy is likely to be multifactorial in origin: hormonal, subclinical inflammation and reduced adiponectin concentrations combine to modify the insulin response. The net result is a shift to lipolysis and the breakdown of fat; free fatty acids act as

a fuel for maternal metabolism (through β-oxidation). The inhibition of carbohydrate storage and decline in glucose uptake by muscle and adipose tissue, combined with promotion of glycogenolysis and gluconeogenesis in the liver favour increased blood glucose levels and fuel availability for the fetus.

1.2 Gestational Diabetes

1.2.1 A Historical Perspective

GDM is defined as 'glucose intolerance with onset or first recognition in pregnancy' (Buchanan *et al.*, 2007). Whilst this is a relatively straightforward definition, there has been, and still is, much controversy and confusion as to what constitutes 'glucose intolerance'. Prior to 1998 the thresholds used to diagnose GDM were the same as those used to diagnose diabetes in the general population (a fasting plasma glucose ≥ 7.8mmol/l and 2 hour post 75g oral glucose tolerance test ≥11.1mmol/l) (World Health Organization, 1985). Obstetric and neonatal outcomes were poor for these women, in fact they were worse than those with type 1 diabetes, possibly because hyperglycaemia went undiagnosed and untreated (Widness *et al.*, 1985; Heckbert *et al.*, 1988; Hod *et al.*, 1991). Women with blood glucose levels between normal and diabetic range were deemed to have 'impaired glucose tolerance'. Obstetric outcomes for this group were variable, with some studies showing small but increased risks, in particular: fetal macrosomia, shoulder dystocia and nerve injury (Langer *et al.*, 1987; Sermer *et al.*, 1995; Vambergue *et al.*, 2000; Jensen *et al.*, 2001; Yang *et al.*, 2002). However, the study sizes were small, outcomes were inconsistent and obesity, advanced maternal age and other medical complications were potential confounding factors. There was controversy as to whether impaired glucose tolerance was indeed pathological or whether it simply represented an exaggeration of the physiological insulin resistance of pregnancy. Nevertheless, in 1998 the WHO changed its guidelines for the diagnosis of GDM to include all women with glucose intolerance (from mildly deranged to frankly diabetic) (Alberti and Zimmet, 1998).

Studying obstetric outcome is made difficult by the fact that major adverse events are relatively rare (for example stillbirth). Large sample sizes are often required in order to establish statistical significance. Between 2000 and 2006 the Hyperglycaemia and Adverse Pregnancy Outcomes (HAPO) study recruited over 25,000 women with impaired glucose tolerance from a diverse multicultural background from 15 centres in nine countries (Metzger *et al.*, 2008). The study showed a positive correlation between higher maternal glucose and adverse perinatal outcome, although because there was no clear level of glycaemia at which outcomes became markedly worse, defining a threshold for diagnosis and treatment remained controversial (Metzger *et al.*).

At the same time as the HAPO study, two randomised controlled trials were conducted to answer the question whether there is benefit in treating GDM. The Australian Carbohydrate Intolerance Study in Pregnant Women (ACHOIS) showed that the treatment of GDM resulted in improved perinatal morbidity (Crowther *et al.*, 2005). Landon et al. demonstrated treatment reduced the incidence of large for gestational age babies with consequential reductions in shoulder dystocia and caesarean section rate (Landon *et al.*, 2009b).

Taking HAPO and the two randomised control trials together there is little doubt that GDM is associated with worsening perinatal outcome and that treatment is beneficial in reducing these risks. The question remains at what level of hyperglycaemia is it necessary to treat? And whether the effects of overzealous treatment could be harmful?

1.2.2 Impact of Gestational Diabetes

Estimates of the prevalence of GDM in the United Kingdom vary from between 1 to 24% (Farrar *et al.*, 2016). The prevalence depends on a number of factors:

a) The population studied (inner-city populations with higher proportion of black and South Asian ethnicity have higher prevalence of GDM).
b) The criteria used to diagnose GDM (the IADPSG criteria, following the HAPO study are more stringent than the WHO criteria (World Health Organization, 1985; International Association of *et al.*, 2010)).
c) Screening and testing stategy. Universal screening results in a greater number of women identified as GDM, compared to 'selective' or 'high-risk' screening strategies.

Figure 13 demonstrates the variation in prevalence of GDM from various cohort studies between 1988 and 2013 and the effect of different diagnostic thresholds. There is an increasing prevalence of GDM with maternal age and ethnicity.

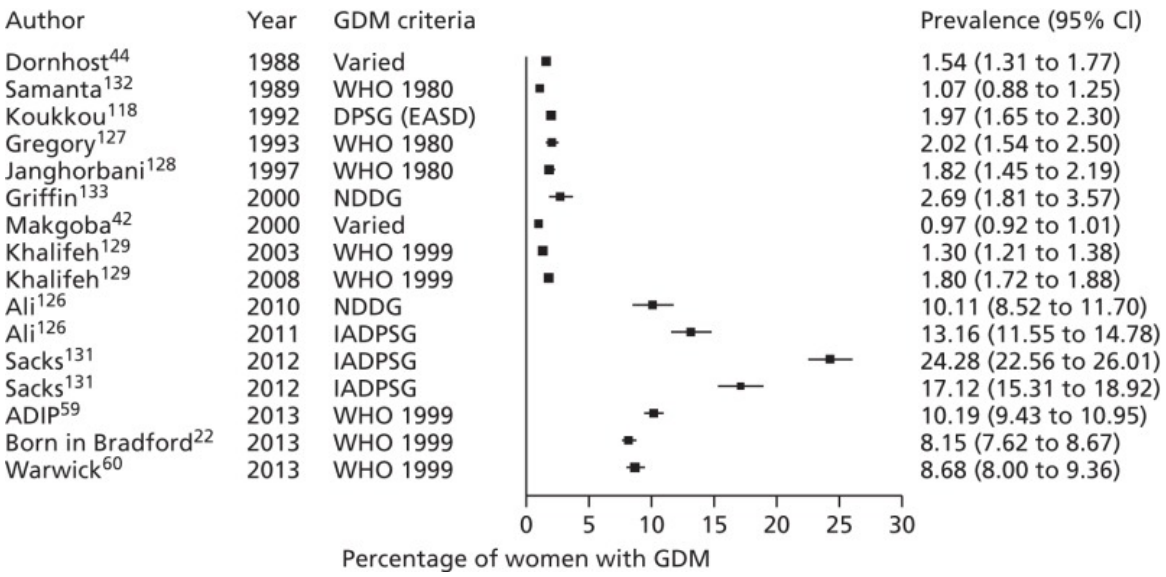

Figure 13 Prevalence of GDM by year the study was undertaken and GDM criteria used.

1.2.3 Risks to the baby

Pedersen hypothesised that elevated maternal blood glucose levels leads to fetal hyperinsulinaemia (Pedersen *et al.*, 1954). This in turn promotes storage of carbohydrate as fat leading to fetal macrosomia. The term 'macrosomia' literally means 'large body' and describes the appearance of a baby that has excessive growth both in terms of its extremities but also has organomegaly (increased liver, heart and muscle mass). Various parameters have been proposed for macrosomia including: a birth weight greater than 3500g, a birth weight between 4000-4500g, or a birth weight greater than 90th percentile for gestational age. Large for gestational age (LGA) is a birth weight greater than 90th centile or greater than 2SD (97th centile) for gestational age. This is a better descriptor than arbitrary birth weight 'cut offs' because gestational age is taken into consideration, identifying babies that are large even if delivered prematurely. The HAPO study, some 50-60 years after Pedersen's original hypothesis, clearly demonstrates the linear relationship between percentage newborn body fat, maternal glycaemia and fetal insulin, see **Figure 14** (Metzger *et al.*, 2008). Furthermore, treating high glucose levels reduces the rates of LGA by approximately 50% (Horvath *et al.*, 2010).

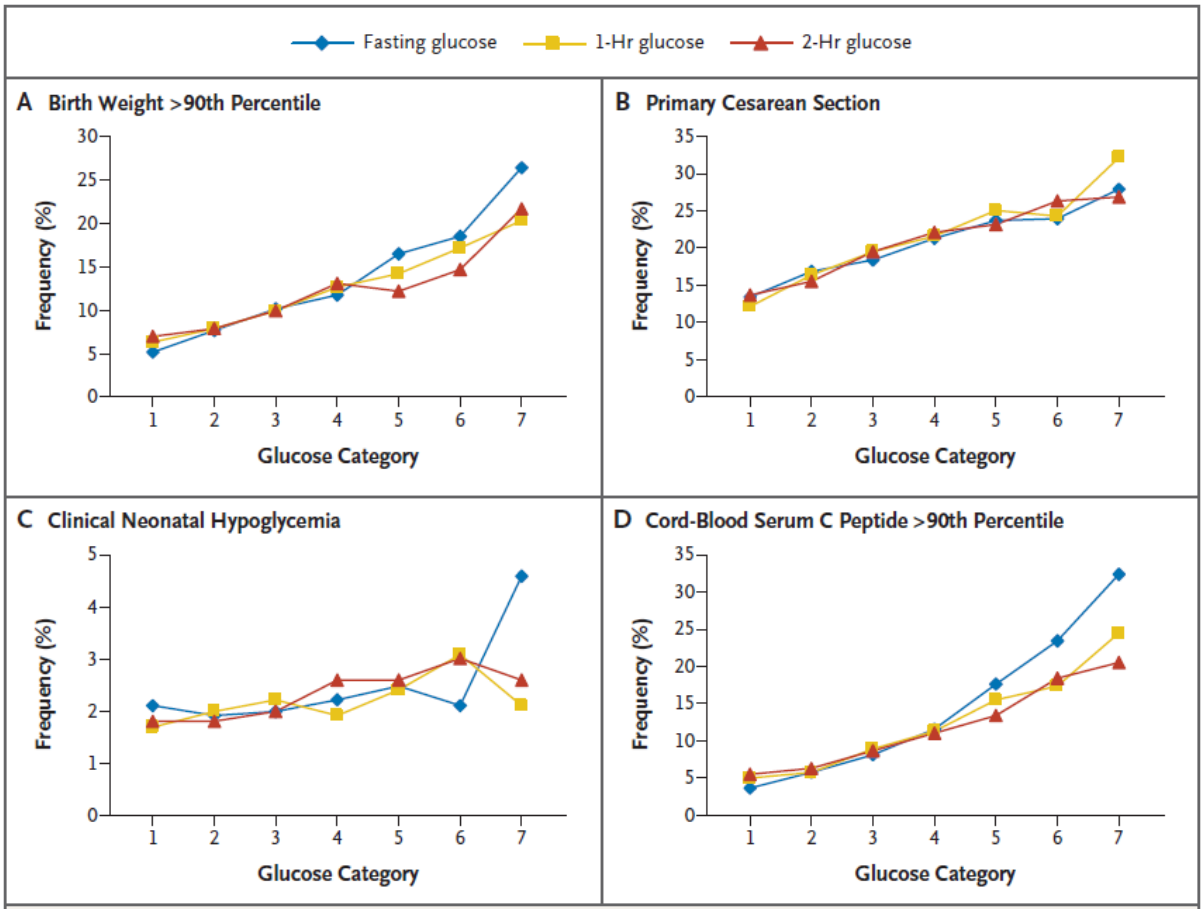

Figure 14 Frequency of outcomes according to glucose category. Adapted from HAPO (Metzger *et al.*, 2008). Fasting glucose categorised into seven groups: range 4.2 to 5.6mmol/l or more. 2-hour range 5.0 to 9.9mmol/l or more. Note substantial increase in birthweight, caesarean section, neonatal hypoglycaemia and cord C-peptide (reflective of neonatal insulin production) with increasing glucose.

The problems with LGA babies are:

1. Birth injury and shoulder dystocia. The main risk of fetal macrosomia is that of birth injury secondary to shoulder dystocia (where, during delivery, the anterior fetal shoulder becomes stuck as it descends through the maternal pelvis). This can result in two complications: neonatal hypoxia (and consequently hypoxic brain injury) and bone/nerve/brachial plexus injuries. The risk of birth injury is highest for infants with birth weight between 4500 and 4999g and > 5000g, (odds ratio (OR) 2.4(2.2-2.5) and 3.5 (3.0-4.2), respectively) (Zhang *et al.*, 2008).
2. Stillbirth/neonatal death. The risk of stillbirth increases by approximately 3-fold for birth weights between 4500-4999g and 13-fold for birth weights greater than 5000g

(Zhang *et al.*, 2008). It should be noted that the absolute risk for stillbirth is still low even in the greater than 5000g group (0.67% vs background risk of 0.05%).

3. Respiratory distress. Macrosomic babies are at a 2- to 4-fold increased risk of respiratory distress following delivery compared to normal controls (Zhang *et al.*, 2008). Babies born to mothers with diabetes are at greater risk of respiratory distress due to the negative effect of hyperinsulinaemia on surfactant production (Piper *et al.*, 1998).
4. Hypoglycaemia. Hypoglycaemia following delivery is not uncommon (affecting between 6-7% of macrosomic infants compared to a background risk of 1.6% (Weissmann-Brenner *et al.*, 2012)). It occurs as the constant glucose supply from the placenta is no longer present following cord clamping. Fetal insulin is unopposed and hypoglycaemia results. Although transient, this often requiring intravenous glucose and admission to a neonatal unit for a 24-48 hour observation period.

1.2.3.1 Risks to the Mother

Risks to the mother from gestational diabetes can be divided into risks during pregnancy, risks during delivery and long term health risks.

Risks during the pregnancy include an increased risk of pre-eclampsia and spontaneous pre-term labour (Sermer *et al.*, 1995; Hedderson *et al.*, 2003; Metzger *et al.*, 2008). Risks associated with delivery are primarily related to delivery of a large for gestational age baby including: increased risk of instrumental or caesarean delivery, anal sphincter tears, shoulder dystocia and postpartum haemorrhage (Sermer *et al.*, 1995; Jolly *et al.*, 2003; Metzger *et al.*, 2008; Baghestan *et al.*, 2010).

The long-term risk to the mother is well documented. There is a 7 to 12-fold increase risk of developing T2DM in later life; depending on the length of follow up and the population studied, the cumulative risk may be as high as 70% (Kim *et al.*, 2002; Bellamy *et al.*, 2009). There is some evidence to suggest that pregnancy itself accelerates the development of T2DM in susceptible women (a Latin American population with previous GDM) (Peters *et al.*, 1996). Even if women revert to normal glucose control following delivery, changes in lipid profile, blood pressure, micro and macrovascular changes adversely modify their cardiovascular risk profile increasing the likelihood of ischaemic heart disease and stroke in later life (Meyers-Seifer and Vohr, 1996; Hu *et al.*, 1998).

1.2.4 Similarities between gestational and T2DM

Gestational diabetes and T2DM share many features. Risk factors for the development of T2DM (ethnicity, family history, body mass index) are very similar to the risk factors for GDM. The hallmark of both conditions is progressive insulin resistance, accompanied by an inability of the pancreatic beta cells to up-regulate insulin secretion, leading to hyperglycaemia. Finally, many women with GDM will progress to T2DM in later life.

It is reasonable to conclude that the pathological processes underlying GDM and T2DM are similar, if not the same. The use of magnetic resonance to study T2DM *in vivo* has revolutionised our understanding of its pathophysiology and reversibility. A brief summary of the progress made in T2DM is summarised below.

1.2.5 Advances in understanding of type 2 diabetes

1.2.5.1 Muscle Insulin Resistance

One of the earliest detectable changes in T2DM is a decline in peripheral insulin sensitivity. Magnetic resonance spectctroscopy (MRS) studies have demonstrated reduced levels of glucose 6-phospate (G-6-P), secondary to defects in glucose transport and uptake, resulting in a deficit of muscle glycogen (Rothman *et al.*, 1992; Cline *et al.*, 1999). There is a strong correlation between intramyocellular lipid (IMCL) and muscle insulin sensitivity in non-diabetic subjects (Jacob *et al.*, 1999; Krssak *et al.*, 1999), with elevated levels of IMCL observed in those with reduced insulin sensitivity. Non-diabetic offspring from patients with T2DM (chosen as they are high risk for developing T2DM and are therefore suitable for investigating early metabolic changes) have shown that IMCL can be reduced by approximately 30% following a 1,200kcal diet for 9 weeks (Petersen *et al.*, 2012). This results in an improvement in insulin sensitivity of 60% that is independent of changes in other inflammatory and metabolic markers (tumour necrosis factor-α, interleukin 6, adiponectin, C-reactive protein, acylcarnitines and branched chained amino-acids). This suggests that the improvement in muscle insulin sensitivity is due to reduction in IMCL. This is an important finding since it demonstrates improvement in peripheral insulin sensitivity with modest weight loss (of around 5kg or 6% of body mass) in subjects with normal BMI (average BMI 24.2 ± 0.6 kg/m^2 at the beginning of the study and 22.8 ± 0.5 kg/m^2 at the end) who are at risk of diabetes, but do not have diabetes yet.

These findings lead to three important questions:

1. Where does the excess IMCL come from?
2. How does excess IMCL impair insulin sensitivity?
3. Do women with GDM have higher levels of IMCL?

Excess IMCL comes from an imbalance between fatty acid delivery and fatty acid oxidation. During periods of calorie excess the liver synthesises and exports triglycerides in the form of very light density lipoprotein (VLDL). This is delivered to the muscle and the triglycerides are broken down by *lipoprotein lipase* into fatty acids and 2-monoacylglycerol. Myocyte accumulation of long chain fatty acyl-CoA (a precursor of triglyceride) occurs if the fatty acids are not oxidised. The regulation of IMCL is not fully understood. Whilst calorie excess provides an abundance of circulating lipid metabolites, not everyone exposed to these are capable of storing them. Factors leading to greater IMCL accumulation are: male gender, older age, ethnicity (particularly South Asian) or a family history of T2DM. Genetic susceptibility must therefore play a role in this process. However, regardless of age, gender or genetics the consistent observation that negative calorie balance has the potential to reverse lipid accumulation, restoring insulin sensitivity, suggests calorie excess is the common and modifiable factor.

The mechanism through which elevated IMCL may induce insulin resistance in muscle is not fully understood and still the subject of on-going studies. Three mechanisms have been proposed: impairment of insulin signalling, reduced mitochondria (both in terms of a reduction in mitochondrial number and function) and modified metabolic flux secondary to excess fatty acid metabolism. In rodents, the build-up of diacylglycerol and ceramides activate PKCθ, thereby increasing insulin receptor substrate serine-1101 phosphorylation, decreasing insulin-stimulated IRS-1 tyrosine phosphorylation, ultimately resulting in reduced glucose uptake **Figure 15** (Petersen *et al.*, 2012). Human studies have been less consistent, with some studies supporting similar findings to rodents, and others not (Itani *et al.*, 2002; Hoeg *et al.*, 2011). Reduced density of mitochondria has been noted in muscle of insulin resistant offspring of T2DM patients. Whether the reduction in mitochondria is a cause or effect of elevated IMCL is unclear. However, following dietary intervention, mitochondria number remained similar, despite reduction in IMCL and improvements in insulin resistance were noted. This suggests that it is mitochondrial function, rather than number, that contributes to insulin resistance (Petersen *et al.*, 2012). The abundance of fatty acids within the cell cytoplasm (reflected in IMCL concentrations), compete against glucose for metabolism. High levels of acetyl-CoA saturate the TCA cycle leading to a build-up of metabolic intermediates (citrate, glucose-6-phosphate). Hence glucose breakdown and oxidation is inhibited and glucose uptake decreased in favour of

fatty acid metabolism. Insulin resistance results as the pathway through which insulin exerts its effects are effectively blocked. Lower mitochondrial numbers and impaired cell signalling further complicate an already saturated metabolic pathway.

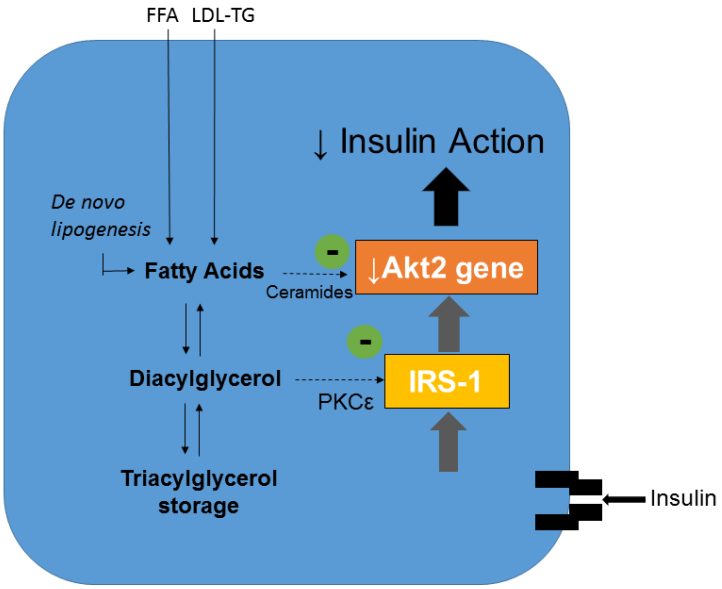

Figure 15 Mechanism of interaction between excess amounts of fatty acids, diacylglycerol and ceramide and insulin action within the hepatocyte (Taylor, 2013). Fatty acids are converted into ceramides which have a direct inhibitory effect on Akt2 gene expression, reducing downstream insulin signalling. DAG has an inhibitory effect on IRS-1 via PKCε, thus further reducing the effect of insulin signalling.

At the time of commencing this work, no studies of IMCL or intrahepatic lipid had been conducted *during* pregnancy. However, Kautzky-Willer et al., in the postpartum period (4-6 months following delivery) demonstrated that women with previous gestational diabetes (pGDM) had a 35% reduction in insulin sensitivity and a 55% higher concentration of IMCL in soleus and tibialis-anterior muscles compared to matched controls with normal glucose tolerance (Kautzky-Willer *et al.*, 2003). This study supports the concept that insulin resistance in gestational diabetes is likely to be secondary to excess intra-organ lipid deposition, as is the case for T2DM.

1.2.5.2 Liver Insulin resistance

An essential function of liver metabolism is the production of glucose from glycogen or through gluconeogenesis during the fasting state. Fasting plasma glucose levels depend entirely on hepatic glucose production. This is tightly regulated by insulin which switches off hepatic glucose production through cell signalling pathways.

Storage of liver fat occurs when energy intake is greater than energy expenditure and there is good evidence that over-feeding leads to increase in hepatic fat content (Sevastianova *et al.*, 2012). Higher insulin levels within the hepatic portal vein (in response to both excess substrate intake and increased peripheral insulin resistance) in subjects with T2DM leads to increased rates of *de novo* lipogenesis and fatty acid production (due the need to convert of excess carbohydrate into fat) (Petersen *et al.*, 2012){Schwarz, 2003 #203}. New triacylglycerol in the liver is mainly diverted into export (as VLDL) or fat storage (since fatty acid oxidation in the mitochondria is inhibited by mayonyl coA, a by-product of *de novo* lipogenesis). Excess fatty acids within the hepatocyte are converted into mono-, di- or tri-acylglycerol. Diacylglycerol activates protein kinase C epsilon type within the insulin receptor, thereby inhibiting activation signalling from the insulin receptor to IRS-1. Thus, under conditions of chronic energy excess, DAG further inhibits insulin action. Additionally, excess free fatty acids within the hepatocyte increase ceramide production. Ceramides have an inhibitory action on Akt2 expression (an important gene which codes for critical enzymes within the insulin pathway), thereby further lessening the effect of insulin signalling {Pagadala, 2012 #204}. The net result is that increased hepatocyte lipid has a negative impact on insulin signalling as summarised in **Figure 15**. A fundamental feature of T2DM is the inability of insulin to switch off hepatic glucose production. Several factors contribute to this including increased supply of glucose precursors to the liver through excess calorie intake (glycerol, fatty acids, amino acids), increased liver fat and impaired insulin signalling in the hepatocyte (Lin and Accili, 2011). Hepatic insulin resistance typically occurs later on in the disease process (the earlier changes are of muscle insulin resistance). In combination with the pancreas' inability to secrete enough insulin, hyperglycaemia and hence T2DM results.

There is a clear association between intrahepatic lipid concentrations and hepatic insulin resistance and accumulating evidence to suggest that this may be causal (Savage *et al.*, 2007; Sattar and Gill, 2014). The study of patients with T2DM who have lost significant amounts of weight, either through diet or surgical means, have shown a substantial reduction in liver fat concentration within days of reduced calorie intake, **Figure 16**. Hepatic insulin resistance

and the ability of insulin to suppress hepatic glucose production is improved, resulting in normalisation of fasting glucose levels. After four weeks of calorie restriction, liver fat and hepatic insulin sensitivity levels are normal (in fact, the latter is supra-normal, but this is secondary to hypocalorific dieting) (Lim *et al.*, 2011b). The relationship between hepatic lipid levels, improvement of hepatic insulin sensitivity and normalisation of fasting plasma glucose over time following calorie restriction is remarkable and suggests a likely causal relationship between hepatic lipid and insulin resistance (Taylor, 2008).

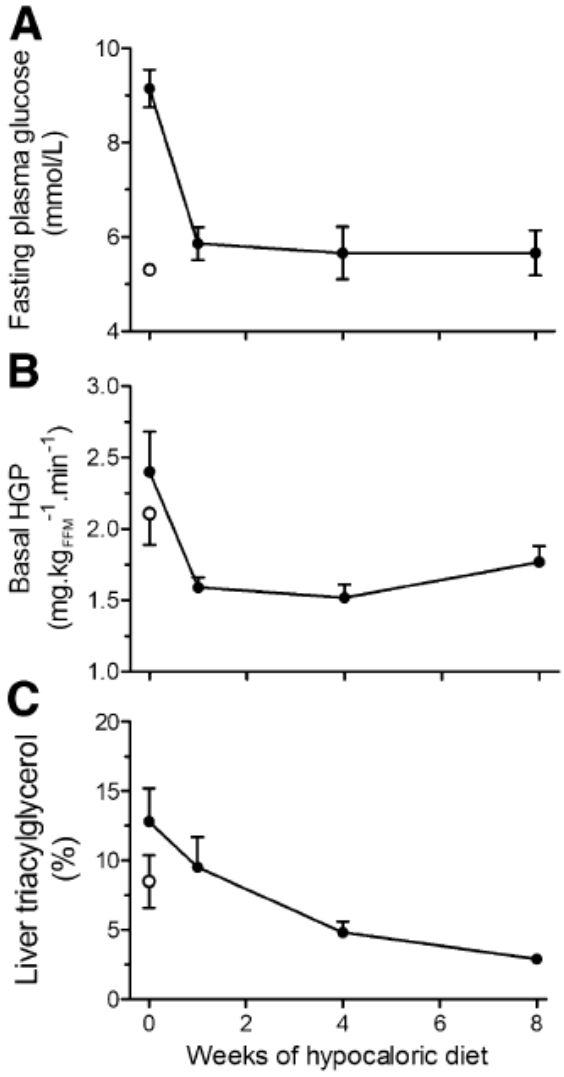

Figure 16 Effect of a very low calorie diet in T2DM on fasting plasma glucose level (A), basal hepatic glucose production (HGP) (B), and hepatic triacylglycerol content (C). For comparison, data for a matched non-diabetic control group are shown as open circles. Figure taken from Taylor R (Taylor, 2013) (Reproduced with permission from Lim et al (Lim *et al.*, 2011b)).

Akin to intramyocellular fat, the accumulation of intrahepatic fat results from an increased calorie intake over a protracted period. It is interesting to note that insulin is responsible for causing fat deposition within the liver. Insulin within the portal circulation effects this change, promoting lipogenesis and lipid storage. Patients with type 1 diabetes do not have elevated liver fat levels, and paradoxically they may have fat levels lower than the general population (Perseghin *et al.*, 2005). This is because exogenous insulin enters the systemic, rather than the hepatic portal, circulation, whereas endogenously secreted insulin produces around 3-fold higher levels in the portal compared with systemic circulation. Similarly, intrahepatic fat levels decline in those patients with T2DM who require subcutaneous insulin (Juurinen *et al.*, 2007). Reducing insulin levels within the hepatic portal circulation, either through diet, exercise, subcutaneous insulin or hyperglycaemic medication decreases liver fat content. Clearly hyperinsulinaemia plays a critical role in liver fat accumulation.

The mechanism through which fat accumulation in the liver results in impaired insulin suppression of hepatic glucose production is unclear. It is postulated that triglyceride metabolites (ceramides and diacyl glycerol) interact with the cell signalling pathway, specifically, the interaction of the insulin receptor with the insulin receptor substrate. Diacylglycerol activates protein kinase C epsilon type which inhibits the activation of the insulin receptor substrate (Taylor, 2013). This is a fundamental step in insulin action. Ceramides cause sequestration of Akt2 by and activation of gluconeogenesis enzymes. In combination, the net effect of insulin binding to its hepatocellular receptor is diminished.

1.2.5.3 Pancreatic Dysfunction

The pancreas, when initially exposed to hyperglycaemia, increases insulin production through increased beta cell mass and function. With progressive insulin resistance, the metabolic demands on the pancreas increase. Eventually, the pancreas can no longer cope and begins to fail. Quite why this happens is unexplained, but the process is thought to be driven through apoptosis, possibly related to toxic metabolic intermediates of the lipid pathway. At diagnosis of T2DM, beta cell function is typically 50% that of normal and autopsy studies have shown that the beta cell mass is 40 to 60% that of non-diabetic controls (Butler *et al.*, 2003).

The pancreas is a difficult organ to study. Not only is it located deep within the abdomen, making biopsies difficult, but it is also relatively difficult to image due to its consistency and similarity in appearance to bowel. Additionally, beta cells make up only 1-2% of the pancreas

and are scattered throughout the organ. It is therefore difficult to differentiate between processes affecting the pancreas as to those specifically affecting the beta cells. None the less, there is accumulating evidence from magnetic resonance studies that fat may contribute to beta cell dysfunction not only is there more fat within the parenchyma of the pancreas in T2DM compared to normal, but also an improvement in beta cell function follows removal of fat from the pancreas through low calorie dieting or bariatric surgery (Lim *et al.*, 2011b; Gaborit *et al.*, 2015).

GDM is different to T2DM in that the insulin resistance occurs in a much more acute time frame (over a period of about 28 weeks). It is possible that the increase in insulin resistance "unleashes" the beta cell deficit that has slowly been accumulating over the years prior to pregnancy. Alternatively, there could be an acute elevation in pancreatic fat (secondary to the hypertriglyceridaemia associated with pregnancy). Imaging studies of the pancreas are required to establish the precise pancreatic deficit specific to gestational diabetes.

1.2.5.4 Time course to type 2 diabetes

Tabak et al prospectively studied the onset of T2DM in a group of civil servants (Tabak *et al.*, 2009). Through serial oral glucose tolerance tests, insulin sensitivity and beta cell function were mapped over a 15 year time frame, comparing those who develop diabetes to those who do not. Insulin sensitivity was lower in the diabetic group compared to the controls, but that the rate of decline in insulin sensitivity was similar in both groups until 5 years prior to the diagnosis of T2DM when the diabetic group had an increased rate of decline. At this point, there was a noticeable difference in both fasting and 2-hour post prandial glucose concentrations between those who went on to develop T2DM and controls. At 2 years prior to diagnosis, the difference in glucose concentrations is marked; **Figure 17 A and B**. This observation is consistent with a gradual increase in muscle insulin resistance; followed by a later change in hepatic insulin resistance and loss of hepatic glucose production inhibition, at which point insulin sensitivity rapidly declines and hyperglycaemia prevails. The changes seen in beta cell function at four-years prior to diagnosis: an initial increase in insulin production followed by a rapid decline (**Figure 17C**) is consistent with the notion that the pancreas tries to cope with increasing hyperglycaemic demands (through up-regulation of insulin and increased beta cell mass); but ultimately fails.

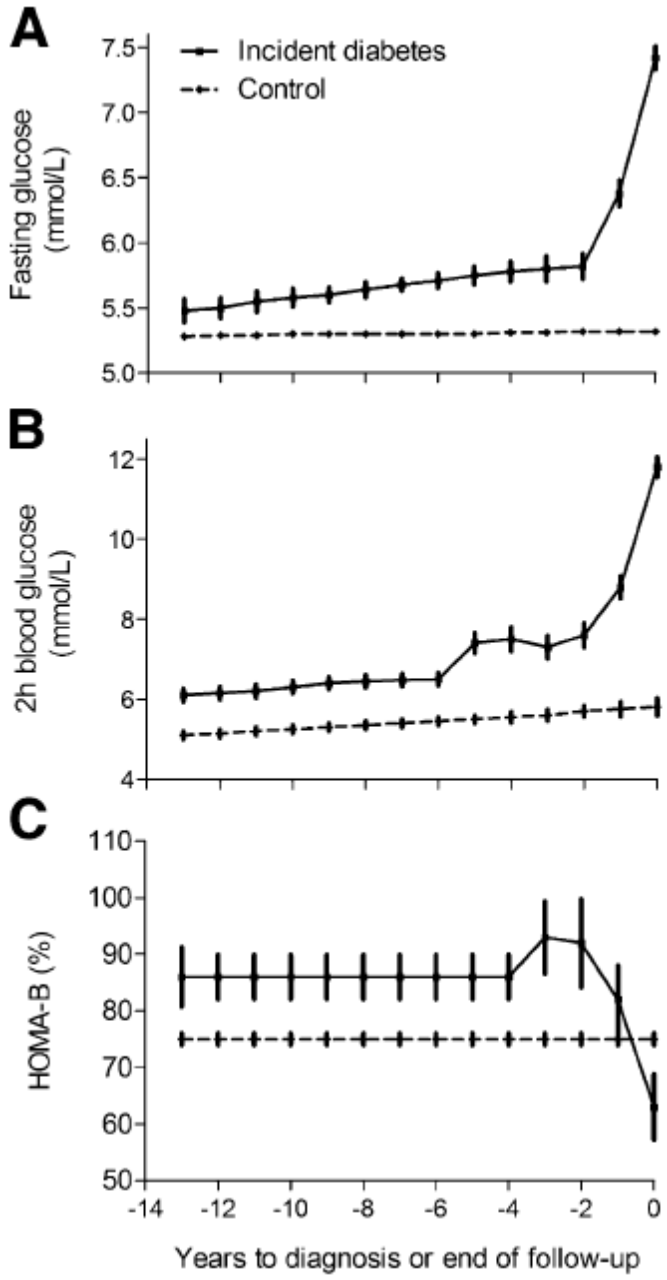

Figure 17 Change in fasting plasma glucose (A), 2h post-oral glucose tolerance test (B) and homeostasis model assessment (HOMA-B) insulin secretion (C) during 16 year follow up in the Whitehall II study. Figure taken from Taylor R (Taylor, 2013)(redrawn with permission from Tabak et al. (Tabak *et al.*, 2009))

The development of T2DM can therefore be explained considering muscle, liver and pancreas. Excessive calorie intake leads to IMCL accumulation and increased peripheral insulin resistance. As a result, more insulin is secreted into the hepatic portal circulation. Together with on-going excessive calorie intake, this results in fat storage within the liver.

Increased insulin resistance leading to increased insulin secretion and increased hepatic liver fat deposition continues as a cycle which ultimately fails, as the pancreas cannot keep up with demand.

1.2.6 Pathophysiology of Gestational Diabetes

Understanding of GDM has been principally derived from the postnatal study of women who had GDM during their pregnancies. Whilst this may reflect the underlying changes that pre-dispose to GDM there are a number of issues with retrospective studies:

1. It assumes that the metabolic disturbance pre-dates the pregnancy and that pregnancy *per se* does not cause the changes in metabolism.
2. The metabolic effects of pregnancy, a state of significant metabolic adaptation, are unknown.
3. Lactation may influence the observed changes (depending on time postpartum).

Despite these limitations, women who have had previous GDM (pGDM) have impaired insulin sensitivity and similarly elevated visceral fat deposits, similar to those observed in T2DM. To date, three magnetic resonance observational studies have been undertaken in women with pGDM:

(Kautzky-Willer *et al.*, 2003) recruited 39 women with pGDM and 22 control subjects between four and six months postpartum. The pGDM group had a 35% reduction in insulin sensitivity compared to controls and a 40-55% increase in intramyocellular lipid content (adjusted for BMI).

(Prikoszovich *et al.*, 2011) recruited 23 women with pGDM and 8 control subjects studied four to five years following delivery. The women with pGDM had a 36% increase in fat mass and a 12% decrease in insulin sensitivity (2.5-fold increase in liver fat 3.7 ± 3.5% in women with pGDM vs 1.5 ± 0.9% in controls; $p<0.05$). Intramyocellular lipid concentrations were similar (0.73 ± 0.32% vs 0.69 ± 0.5%; $p=0.08$) in pGDM vs controls respectively. However, there was a difference in IMCL concentrations in those with insulin resistance compared those who had normal insulin sensitivity (0.9 ± 0.3% vs 0.54 ± 0.32%; $p<0.003$).

(Forbes *et al.*, 2013) studied 36 women with pGDM seven years following pregnancy. Compared to controls, women with pGDM had a 2.4-fold increased liver fat concentration.

pGDM was also associated with diminished insulin secretion and a three-fold increase in VLDL apo B pool size.

These studies support the theory that GDM and T2DM may share similar aeitiological mechanisms: namely an impairment in insulin sensitivity and secretion and increased intramyocellular and intrahepatic fat stores. Whether these changes occur during pregnancy and similarly, whether they are responsible for causing GDM is unknown.

1.2.7 Dietary Intervention for the Prevention of Gestational Diabetes

Lifestyle intervention, through healthy diet and exercise, is the cornerstone of treatment for GDM. Metformin and insulin can be added if glycaemic control is not maintained through diet. National Institute of Clinical Excellence (NICE) dietary advice consists of advising women to "eat a healthy diet, to emphasise that foods with a low glycaemic index should replace those with a high glycaemic index, to take regular exercise (for example walking for 30 minutes after a meal) and to refer the woman to a dietitian" (National Institute of Clinical Excellence (NICE), 2015).

The Cochrane Collaboration have recently evaluated the benefit of both diet and exercise in *preventing* GDM (rather than for treatment) (Bain *et al.*, 2015). Their conclusion was that the is "no clear difference in the risk of developing GDM for women receiving a combined diet and exercise intervention compared with women receiving no intervention". Taking this conclusion at face value, it could be concluded that diet and exercise are of no benefit in GDM. However, this is contrary to studies in the 1990s that showed dietary intervention both cured GDM and furthermore was not detrimental to fetal wellbeing (in fact rates of fetal macrosomia were reduced through diet). Additionally, dietary intervention has been shown to successfully reverse T2DM. A closer inspection of the studies included in the Cochrane meta-analysis reveals that the level of 'dietary intervention' was markedly different between the trials and there was no consistency with regards to recommended energy intake. Fundamentally, in order to lose weight, energy intake must be less than expenditure. It is difficult to achieve a negative energy balance through exercise alone, particularly during pregnancy (Poston *et al.*, 2013). Therefore, the only way is to reduce calorie intake. Many studies included in the Cochrane meta-analysis neither stated the recommended calorie goal nor described whether women were advised to 'calorie count'. Some studies made calorie intake recommendations based on the woman's weight (eg Korpi-Hyovalti – 30kcal/kg). However, given an average weight of 70kg, the recommended calorie intake would be

2100kcal which is not substantially different to the recommended NICE calorie goal of 2200kcal/day (National Institute of clinical Excellence (NICE), 2010). It is no surprise then that the outcome of the meta-analysis is negative, since in most studies included within the analysis, calorie intake is either not stated or had been inadequately reduced. Reducing calorie intake during pregnancy is traditionally met with apprehension from women, their families and healthcare providers. There is a misconception that women should be 'eating for two' when in reality the fetus requires only a small additional calorie intake (200kcal/day in the third trimester). Only two trials included in the meta-analysis recommend a more substantial reduction in energy intake. Petrella 2013, recommended a reduction to 1700kcal for overweight and 1800kcal for obese women. Although this trial was small (33 women in the intervention group vs 28 controls) it was one of the only trials included in the review that showed a reduction in GDM through intervention (GDM was reduced from 57.1% in the control group to 23% in the intervention arm, p=0.01). None of the other trials showed reduction in GDM rates, although several showed a modest reduction in maternal gestational weight gain and rates of fetal macrosomia. However, given the fact that calorie intake in the intervention arm of most studies was minimal, these findings are perhaps not surprising. Phelan used a "Fit for Delivery" intervention programme that included dietary advice to reduce calorie intake to 20kcal/kg (equivalent to 1,500kcal per day for a 75kg woman). The study reported no difference in rates of GDM, but showed that Institute of Medicine guidelines (IOM) regarding weight gain were better adhered to with the intervention than without. Perhaps the relatively modest benefits of this study are related to the low-intensity nature of the intervention: the calorie intake of the women was neither recorded nor estimated.

1.2.8 Dietary Intervention for the Treatment of Gestational Diabetes

In the late 1980s and early 1990s several metabolic studies established that reduced calorie intake was successful for the treatment of gestational diabetes.

Knopp performed basic metabolic tests in obese women with GDM (Knopp *et al.*, 1991). Women were admitted for two weeks. During the first week they were given a diet consisting of 2400kcal/day. Insulin secretion and sensitivity were measured on day 7. During the second week women were randomised to either continue on 2400kcal/day or to reduce calorie intake to 1200kcal/day. In the calorie restricted group fasting plasma glucose fell by 17% (from 5.9 to 4.9mmol/l) compared to a 4% fall in the controls (5.4 to 5.2mmol/l). This fall in fasting glucose of 1mmol/l is clinically significant and represents a substantial

reduction in risk (Metzger *et al.*, 2008). Similarly, there was an improvement in mean plasma glucose over a 24 hour period from 6.8 to 5.4 mmol/l in the restricted group with no change in the control arm. Insulin secretion was reduced by 45% in the restricted group, reflecting a reduction in insulin requirements secondary to reduced glucose/carbohydrate intake.

Maresh studied 20 women with GDM, randomising them into diet or insulin treatment (Maresh *et al.*, 1985). The insulin group were advised to take their calorie intake to 2100kcal per day (1800kcal if obese) and insulin titrated according to their glycaemic control. The dietary group were advised to reduce calorie intake to 1800kcal per day (1500kcal if obese). The subjects were admitted along with 10 non-diabetic pregnant controls for 24 hour metabolic profile glucose sampling. The women were re-studied four weeks following intervention. Before treatment women with GDM had higher plasma glucose and 3-hydroxybutyrate levels (reflecting diabetes). Glycaemic control on diet alone was comparable to non-diabetic controls (although tended towards the upper limit of normal). The diet group lost a mean weight of 0.7kg/week compared to those on insulin who gained on average 1.5kg/week and non-diabetic controls who gained 1.1kg/week. As to be expected, 3-hydroxybutyrate levels were increased in women who dieted compared to those on insulin. Neonatal outcomes were good for all babies. This study showed that glycaemic control is achievable through dietary intervention alone with less weight gain acquired during pregnancy compared to insulin.

1.2.9 Ketones

One of the concerns about hypocalorific dieting during pregnancy is that ketone production may be detrimental to fetal development. There is no doubt that ketogenesis in the context of diabetic ketoacidosis (DKA) can be harmful to the fetus and commonly leads to intrauterine death if untreated. However, DKA represents not only ketosis but also a dramatic change in biochemical homeostasis (electrolyte abnormalities and acidaemia) which are more likely to cause fetal death than ketones *per se*. Ketosis is a physiological biochemical response to glucose depletion allowing glucose-dependent organs such as the brain to function in the fasted state. Ketosis during hypocalorific dieting represents an entirely different situation to DKA.

Nevertheless, the possibility that ketones may be harmful to fetal development has been investigated. The most influential paper by Rizzo in 1991 investigated the effect of ketones (plasma and urinary) in a population of 223 women (35 had normal glucose tolerance, 99 had

GDM and 89 had diabetes that pre-dated the pregnancy) (Rizzo *et al.*, 1991). They found an inverse correlation between maternal plasma β-hydroxybutyrate and plasma free fatty acid concentrations in the third trimester of pregnancy and the offspring's intelligence at two (Mental-development index) and three to five years (Stanford-Binet test). The correlations were independent to the effects of patient group (pre-gestational diabetes, GDM or normal glucose tolerance) and were independent to neonatal complications including prematurity, acidaemia and hypoglycaemia. It should be noted that the confidence intervals for the correlations are wide and that the r-values are relatively low (although statistically significant) implying a relatively weak correlation. Not only this, but the fact that the absolute difference in terms of IQ points between children born to mothers with high ketone levels versus low ketone levels is not stated therefore, it is not possible to make an assessment on whether the difference has any clinical significance. Furthermore, there was no correlation between urinary acetonuria and IQ which is contrary to the hypothesis. The findings of this study have not been replicated by others (Coetzee *et al.*, 1980; Naeye and Chez, 1981; Jovanovic *et al.*, 1998).

Studies of women in areas of famine have shown that calorie restriction results in lighter babies with less subcutaneous fat and that growth retardation typically only occurs with calorie intake of less than 600kcal/day. Dutch army recruits born during the Dutch potato famine had no impairment of intellectual function compared with recruits not born during the famine (Stein and Susser, 1975).

In summary, there are no substantial data to show that ketones, in the context of dieting during pregnancy, have any adverse effect on fetal and long term outcome for the child.

1.3 Use Of Magnetic Resonance Spectroscopy in Metabolism

1.3.1 History of Nuclear Magnetic Resonance Spectroscopy

Although the use of magnetic resonance spectroscopy (MRS) to quantify metabolic processes *in* vivo is a relatively recent innovation, the concept of nuclear magnetic resonance spectroscopy dates back to 1945, soon after the end of World War II. In 1952 Nobel Prizes were awarded to Edward Purcell (Harvard) and Felix Bloch (Stanford) for the "development of new methods for nuclear magnetic precision measurements and discoveries in connection therewith". These researchers discovered that it is possible to detect a signal (a voltage in a coil) when a sample is placed within a magnetic field and irradiated with radiofrequency energy of a certain frequency: the "resonant" or Larmor frequency. The signal is produced by the interaction of the sample nuclei with the magnetic field (McRobbie *et al.*, 2003). In 1971, some 25 years later, the use of this principle to demonstrate contrast between healthy tissue and disease was discovered by Raymond Damadian and magnetic resonance *imaging,* as we know it today, was born.

1.3.2 Physics of NMR

Angular momentum is the measure of the amount of rotation an object has taking into account its mass, shape and speed. Examples of angular momentum in practice include the gyroscope (which stands up when spinning due to the downwards angular momentum force exerted through the 'spike') or the stability of a bicycle as it travels forwards (and relative instability as it slows down). Atoms with magnetic properties (for example: ^{1}H, ^{13}C, ^{31}P) are aligned according to their angular momentum. They can be thought of as mini-bar magnets with a north and south pole. Under normal circumstances their alignment is related to the weak magnetic force of the earth (and therefore their orientation is random). When the nuclei are placed in a strong magnetic field they become aligned in one of two orientations (**Figure 18**):

1. **α-orientation, low energy state:** the nuclei are arranged along the external magnetic field – i.e. north pole of the nucleus is aligned to the south pole of the magnet.
2. **β-orientation, high energy state:** the nuclei are arranged against the external magnetic field – i.e. north pole of the nucleus is aligned to the north pole of the magnet.

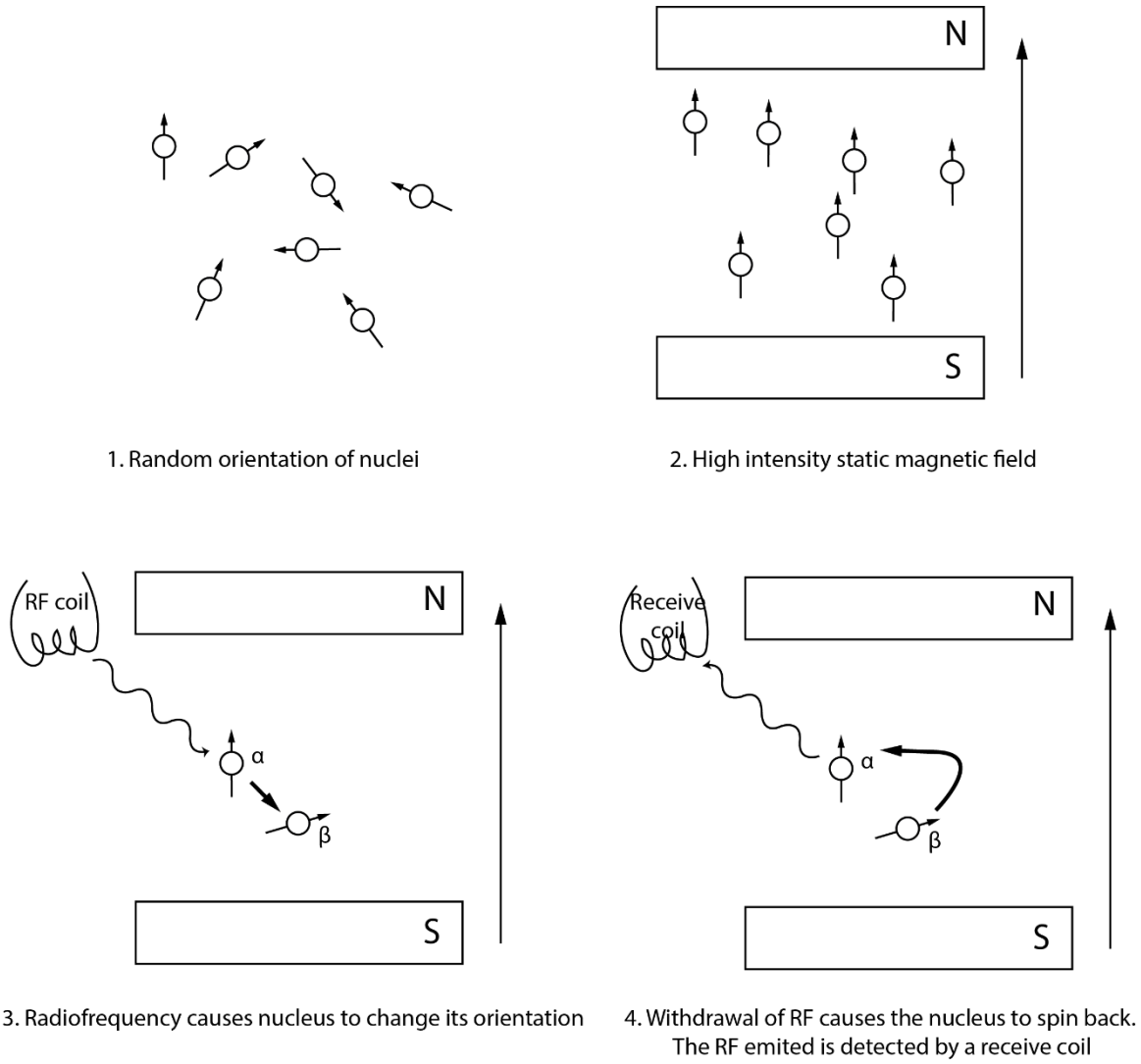

Figure 18 Principles of NMR spectroscopy

It is worth noting that nuclei are constantly 'flipping' between the alpha and beta orientations, but on average when a strong static magnetic field is applied most nuclei will be in the alpha orientation at any given moment in time.

Radio waves, at a specific frequency, are absorbed by the nucleus causing it to change orientation and change from a low to high energy state. This frequency is termed the Larmor frequency. When the radiofrequency stops the nuclei flip back emitting energy as a fluctuating magnetic field. This can be detected through a coil, in turn producing an electric current or signal.

1.3.3 Fourier Transformation

The Fourier transformation is a mathematical function which converts signals in time (or spacial) domain into a frequency domain. Fourier transformation is used in NMR to convert the different radio frequencies detected from various nuclei and compounds into a series of frequencies with different amplitudes (figure xx). Each frequency is characteristic of a certain nucleus or compound and the area under the peak corresponds to the concentration of that nucleus/compound.

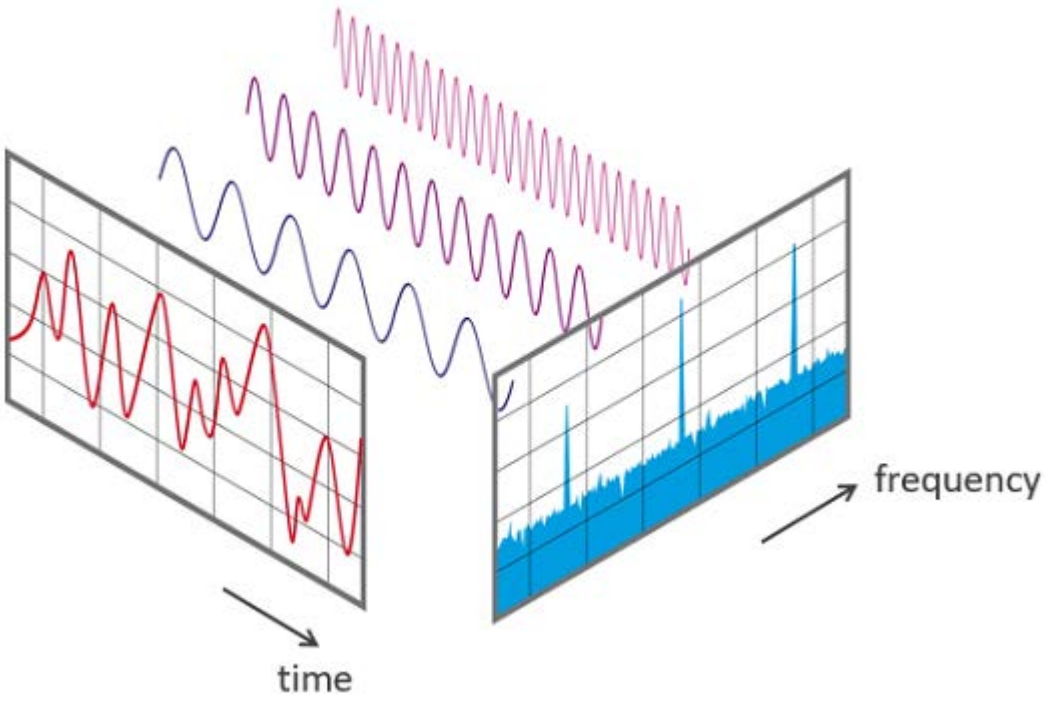

Figure 19 Fourier Transformation: conversion of a signal in the time domain into its constituent frequencies and amplitudes. Over the time period in the diagram above, three different signals are converted into three separate frequencies and amplitude (as shown by the three peaks in the frequency domain graph) (Wikipedia contributors, 2018)

The frequency of the peak is plotted on the x-axis and the amplitude of the peak on the y-axis. Different external magnetic fields have different strengths, measured in Tesla. The stronger the external magnetic field, the greater the frequency required to excite protons. For example, the frequency needed at 1.5T to excite ^{1}H protons is 64MHz, at 3T the frequency is 128MHz. Spectra cannot be compared from different scanners if expressed in frequency units, therefore parts per million (ppm) are used.

Every magnetically active nucleus within a molecule will form a peak provided it is stimulated by the range of radiofrequencies applied. The peak varies slightly according to the structure in which the nucleus is contained. The bonds joining atoms together as molecules are surrounded by electrons. The electron configuration depends upon the type of bond. These surrounding electrons distort the static magnetic field, reducing it slightly by varying degrees. The reduction in magnetic field strength changes the resonant frequency slightly and hence

the peak is 'seen' at a slightly different frequency. In this manner it is possible to identify different chemicals and compounds (because the hydrogen atoms within the compound of interest resonate at a different frequency to say, hydrogen atoms in fat or water).

1.3.4 Safety of Magnetic Resonance in Pregnancy

Magnetic resonance imaging (MRI) has been used extensively in pregnancy and there are no reports of significant adverse events. MRI is now a standard investigation for certain suspected fetal anomalies such as brain or skeletal malformations. A 3−year follow−up of twenty children examined in−utero with magnetic resonance during the second or third trimester showed no unexpected outcomes (Baker *et al.*, 1994). Other studies with longer follow−up periods have had similar findings (Kok *et al.*, 2004). Recent research using magnetic resonance spectroscopy to measure neurotransmitter levels in the developing fetal brain of women with normal pregnancies raised no safety concerns (Girard *et al.*, 2006).

For this research project a Philips 3 Tesla Achieva scanner was used which has a stronger static magnetic field than the majority of scanners used for routine clinical diagnosis. This higher magnetic field strength gives improved data quality, which allows us to perform scans to measure muscle and hepatic lipid content that would not be possible at lower magnetic field strengths. Although widely used in clinical diagnosis and research, 3 Tesla scanners have not been used extensively in pregnancy. Theoretical risks to the fetus from MR studies are noise and energy/heat deposition.

1.3.5 Noise

Acoustic noise is generated from the MR scanner when current is passed through the gradient coils. The outward force generated within the coil when current is switched on creates a loud clicking sound. Sound levels of up to 126 to 131dB have been recorded using 3T MRI (Hattori *et al.*, 2007). Exposure to loud noise over a period of time can result in long term hearing loss. Whilst hearing loss from MR is unlikely due to the relatively short duration of scanning, ear protection and modification of the scan protocol to minimise noise; the impact of noise to the developing fetal cochlear is a theoretical concern.

The fetus is relatively protected from noise due to the cushioning effect of maternal tissues and amniotic fluid. Reeves et al. followed the neonatal hearing test results of 103 neonates exposed to MR in the second and third trimester and found no evidence of substantial hearing impairment (Reeves *et al.*, 2010).

Our studies were specifically designed to minimise noise. Spectroscopy techniques are the least noisy scans and the scanning protocol was modified to reduce noise further. Sound levels were checked during phantom scanning and the maximum noise recorded was around 80-85dB, the equivalent of travelling on an underground rail network. At this noise level there is no perceived affect to the fetus.

1.3.6 Specific Absorption Rate (SAR)

Radiofrequency fields used in MR can induce tissue heating, potentially leading to burns and tissue damage. Specific absorption rate (SAR) is a measure of the rate at which energy is absorbed by the body and is expressed in watts per kilogram (W/kg). In most cases, heat acquired during MR scanning is dissipated quickly through dilatation of local blood vessels, increased blood flow and removal of heat through the skin.

In theory it may be possible to increase fetal temperature and this could potentially have adverse (teratogenic) effects, particularly in the first trimester. However, in practice, the SAR from magnetic spectroscopy is minimal, the field of interest well away from the fetus and the uteroplacental unit effectively regulates fetal temperature.

MR scan protocols in this project adhere to Medicines and Healthcare products Regulation Agency (MHRA) recommendations: minimising duration of radiofrequency exposure and utilizing 'normal operation level' as recommended by ICNIRP 2004 (International Commission on Non-Ionising Radiation (ICNIRP), 2004).

1.3.7 Advantages

The main advantage of MRS is the fact that it is non-invasive and does not involve ionising radiation. This is of great importance during pregnancy where the developing fetus is sensitive to the effects of radiation. It also means that tissues can be analysed for their metabolic content without the need for invasive biopsies. Biopsies are not only painful and potentially harmful to the patient, but the process of extracting and analysing biopsy material may be inaccurate, due to degradation of metabolites. Through MRS it is possible to accurately study metabolic processes *in vivo* using a safe, non-invasive technique.

1.3.8 Disadvantages

The main disadvantage of MRS is the relative insensitivity of the method. The human body is approximately 70% water and thus relatively large signals can be obtained from the ^{1}H

nucleus. Other nuclei, such as ^{31}P and ^{13}C are at much lower concentrations and have a lower sensitivity for NMR detection than ^{1}H. These substances are therefore harder to quantify with MRS, indeed it may not be possible if the concentration of molecules containing these atoms are very low. The stronger the static magnetic field strength, the more nuclei will align with the magnetic field and a larger signal can be generated. Hence, greater field strength enables detection of molecules in lower concentration.

Although MRS does not involve ionising radiation, the large magnetic field can be potentially harmful, mainly from any ferromagnetic object that may be forcefully drawn to the magnet and become a missile. Magnetic fields may also interfere with pacemakers, implantable defibrillators and other medical devices, these are contraindications to MR scanning.

Most MR techniques are sensitive to motion. This can be a particular problem when studying, for example the heart. However, for the purposes of the methods involved in these studies, it was possible to eliminate movement by asking the patient to stay still or to hold their breath, for example when studying the liver.

1.3.9 Magnetic Resonance methods used in the studies

In both studies, subjects were transported to the Newcastle Magnetic Resonance Centre by taxi. They then underwent screening to ensure that there was no contraindication to MR scanning. MRS studies were performed using a Philips 3 Tesla Achieva scanner (Philips Medical Systems, Best, The Netherlands). Participants were scanned in the left lateral position (to relieve pressure on the inferior vena cava). A description of the MR protocol for each study follows below.

1.3.9.1 Intramyocellular Lipid Concentration

A pair of receive-only surface coils (Philips Flex-M coils) were placed around the right calf and the scanner bed positioned so that the calf was in the centre of the scanner. Scout images of the calf were acquired to guide identification of the volume of interest within the soleus muscle.

PRESS (Point RESolved Spectroscopy) was the technique used to obtain spectral data. This involves using frequencies in different orientations (90°-180°-180°) so that the atoms spin in the yx-plane, the xz-plane and the xy-plane. The spin echo received back from these three orthogonal planes allows acquisition of the signal from a voxel.

In order to remove the signal from water (which may otherwise 'drown out' spectra from other metabolites), spectra were acquired with and without water proton suppression.

Spectroscopy data was analysed using jMRUI software (van den Boogaart *et al.*, 1996). Signal amplitudes from intra- and extra-myocellular lipids were separated by peak fitting, and quantified by comparison to the water proton signal from non-water suppressed spectra.

1.3.9.2 Intrahepatic Lipid Concentration

Studies were performed using a Philips 3 Tesla Achieva whole body scanner using a Philips multi-channel flex coil for ^{1}H imaging and spectroscopy. To avoid pressure on the inferior vena cava subjects were positioned with a left pelvic tilt. Scout images of the maternal abdomen were acquired and used to guide identification of the volume of interest within the liver.

1H spectroscopy comprised acquisition of PRESS-localised spectra at six echo times (TR = 2.8 s, TE = 36, 50, 75, 100, 125, and 150 ms, spectral width = 2 kHz, 2k data points) from a 3 x 3 x 3 cm voxel positioned in the liver to avoid large vessels.

Spectra were processed using the Java-based magnetic resonance user interface (jMRUI version 3.0) (Naressi *et al.*, 2001a; Naressi *et al.*, 2001b) using the AMARES non-linear least square fitting algorithm to determine peak areas (Longo *et al.*, 1995). Resonances of water at 4.7 ppm and the CH_2 methylene peak at 1.3 ppm in ^{1}H spectra were quantified. The mean T2 was determined for each peak by fitting a mono-exponential to the data. Signal amplitude at an effective echo time of zero was determined, and these amplitudes used to obtain the liver triglyceride fraction value. The upper limit of normal for a US population of mixed gender, multi-ethnic subjects between 30-65yrs of age has been defined as 5.5% (Szczepaniak *et al.*, 2005).

Liver triglyceride content was assessed at baseline and just after the 4 week hypocaloric diet. Subjects continued on the diet until the second assessment.

1.3.9.3 Validation

MRS methods to determine intramyocellular and intrahepatic lipid have been validated in both human and animal studies (Boesch *et al.*, 1997; Szczepaniak *et al.*, 1999; van Werven *et al.*, 2009).

Boesch et al showed that IMCL can be determined in tibialis anterior. For inter-individual reproducibility, seven subjects (31.4 +/- 6.6 years, 3 female) were compared and each subject measured in triplicate. For intra-individual variability, one subject (24 years, female) was studied on five different days, one week apart and also in triplicate on each day. Inter-individual co-efficient of variance was 6.7% and intra-individual co-efficient of variance was 6.1%. The crucial point for quantification of IMCL in human muscle is the separation of the methyl and methylene signals of extra- (EMCL) and intramyocellular lipid. This is better achieved with a stronger static magnetic field. Boesch used a 1.5 Tesla magnet, whereas this project used a 3 Tesla magnet and hence is likely to be more precise.

The reproducibility of hepatic triglyceride content using ^{1}H-MRS at 3T has been established (van Werven *et al.*, 2009). 24 subjects were assessed at baseline and after four weeks. The data was analysed for a subgroup (n=8) of subjects with fatty liver disease (as defined as hepatic triglyceride content greater than 5.6%). Each subject was studied twice on the same day to determine 'within day' reproducibility. The data are summarised in Table 1 below.

	CV	RC	ICC
Between weeks (n=24)	9.5%	1.3%	0.998
Fatty liver (n=8)	4.1%	1.3%	0.997
Within day	4.5%	0.4%	0.999

Table 1 Summary of Reproducibility Statistics. CV = coefficient of variation; RC= repeatability coefficient; ICC=intraclass correlation coefficient
Reproduced from (van Werven *et al.*, 2009)

1.4 Measuring Insulin Sensitivity and Production

Insulin sensitivity is the ability of the body to react to insulin. A person with high insulin sensitivity will require less insulin in order to lower blood glucose than those with low insulin sensitivity. Insulin sensitivity can be measured by a variety of tests.

1.4.1 Homeostatic Model Assessment (HOMA)

The homeostatic model assessment (HOMA) estimates beta cell function (%B) and insulin sensitivity (%S) as a percentage of the normal reference population. HOMA is calculated on fasting plasma insulin and glucose measurements. The calculation is based on the assumption that fasting hyperglycaemia results from a combination of insulin sensitivity and beta cell deficit and that these variables change to a greater or lesser degree. HOMA uses computer mathematical modelling to calculate an array of potential fasting plasma glucose and insulin concentrations at any given degree of insulin resistance and beta-cell deficit. The model can then be used to estimate insulin resistance (HOMA-IR), which is the reciprocal of insulin sensitivity (HOMA-%S), and beta cell function (HOMA-%B). The values HOMA-%S and HOMA-%B are given as percentages compared to the reference population.

HOMA-IR correlates well with the euglycaemic-hyperinsulinaemic clamp and the hyperglycaemic clamp. HOMA-%B correlates with measures of insulin production (for example the hyperglycaemic clamp and the frequently sampled intravenous glucose tolerance test). The main advantage of HOMA is that it is cheap and simple to perform as it relies on a single fasting blood sample and does not require infusions of glucose or insulin. It is useful for epidemiological studies, particularly as large populations can be screened quickly and effectively. However, due to the fact that it tests fasting conditions only, it is not sensitive at detecting postprandial abnormalities of glycaemic control. The main limitation of HOMA is that it reflects hepatic rather than peripheral insulin sensitivity and, as has been demonstrated through clamp studies, the two can be very different.

1.4.2 Oral Glucose Tolerance Test (OGTT)

The oral glucose tolerance test (OGTT) is a dynamic test of insulin sensitivity, most commonly used to screen for diabetes (in the general population) and gestational diabetes (in pregnancy) (Alberti and Zimmet, 1998). The test consists of a fasting plasma glucose (at time 0) followed by ingestion of 75g of glucose (commonly given as a drink, such as Lucozade). A second blood sample is taken 120 minutes after glucose ingestion.

Although traditionally only glucose is measured at 0 and 120 minutes the test can be adapted to measure insulin and glucose at various time points following glucose ingestion (Matsuda and DeFronzo, 1999). It is then possible to calculate an insulin sensitivity index (ISI) which has been validated against the euglycaemic-hyperinsulinaemic clamp. The advantage of ISI is that it tests both hepatic and peripheral insulin sensitivity and for that reason is more comprehensive than HOMA. In addition the ratio of change in insulin to change in glucose concentrations over the first 30 minutes of the test can be used to calculate insulin secretion (Haffner *et al.*, 1995).

The OGTT has limitations both for assessment of glycaemic and insulin sensitivity indices. Different rates of gastric emptying mean that the results can be influenced by glucose absorption rather than glucose handling. There is a large variation in gastric emptying not only within the population, but within the same individual making the repeatability of the test unreliable. It should also be noted that although insulin sensitivity can be calculated through various methods based on the OGTT, few of these have been validated against the gold standard of a euglycaemic-hyperinsulinaemic clamp.

1.4.3 Intravenous Glucose Tolerance Test (IVGTT)

The intravenous glucose tolerance test (IVGTT) is similar to the oral glucose tolerance test in that a bolus of glucose is administered, although this is given IV rather than orally. This eliminates the effects of gastrointestinal factors which may affect the absorption and therefore the appearance of glucose to the bloodstream. The test requires two intravenous lines, one to administer glucose (and sometimes insulin/arginine) and another to draw samples (Bergman *et al.*, 1979). Blood samples are taken at various time intervals following bolus glucose administration to track insulin secretion and the disappearance of glucose from the circulation. The minimal model computes insulin sensitivity (Si) based on the disappearance of glucose per unit of insulin over time.

Advantages of the IVGTT are that it is reliable and reproducible (more so than the OGTT). It has been validated against the euglycaemic-hyperinsulinaemic clamp (Beard *et al.*, 1986; Bergman *et al.*, 1987), although is more straightforward and less expensive and demanding to administer. It is possible to study insulin resistance and insulin secretion independently (which is not possible through the OGTT). The disadvantages of the IVGTT are that it still involves invasive intravenous catheter, is expensive and requires multiple blood draws.

Additionally, artificially increasing glucose concentrations to high levels in the context of pregnancy may have some (albeit likely minor) effect on the fetus.

1.4.4 Hyperinsulinaemic-euglycaemic Clamp (HIEC)

The hyperinsulinaemic-euglycaemic clamp (HIEC) remains the gold standard for assessing insulin sensitivity and was first described by DeFronzo et al in 1979 (DeFronzo *et al.*, 1979). The test is a direct assessment (in that it involves infusing insulin at a constant rate). The aim is to increase plasma insulin concentrations to greater than 100μU/ml over the basal level and to maintain these elevated insulin concentrations over a 2-4 hour period. 20% glucose is infused at a variable rate and is titrated to maintain glucose levels between 5.0-5.5mmol/L. Once steady state (as defined by a period of greater than 30 minutes of the clamp during which the coefficients for variation in blood glucose, plasma insulin and glucose infusion are less than 5%) has been reached then the rate of glucose infusion is equivalent to peripheral glucose disposal (assuming complete suppression of hepatic glucose production). Individuals who are insulin sensitive will require greater rates of glucose infusion compared to those who are insulin resistant. Insulin sensitivity (S_I) is the expression of glucose clearance per unit change in plasma insulin concentration:

$$Si = \frac{M}{G \times \Delta I}$$

M = glucose disposal rate

G = steady state blood glucose concentration

ΔI = difference between fasting and steady state insulin concentrations

The euglycaemic-hyperinsulinaemic clamp test measures whole body glucose disposal under steady state conditions from which insulin sensitivity can be accurately calculated. Additionally radioisotopes allow the tracing of endogenous glucose production and disappearance from which hepatic and peripheral insulin resistance can be determined. The test is reproducible and comparable between subjects.

Disadvantages of the HIEC are that it assumes complete suppression of hepatic glucose production. Particularly in individuals with impaired glucose tolerance, this may not be true. The HIEC is time consuming and labour intensive and requires an experienced doctor to

manage technical complications that may arise from the test. Additionally, the test is performed in supra-physiological conditions (for example the peripheral concentration of insulin in the clamp is much higher than the portal concentration). S_i is only measured in the steady state and neither the pulsitility of normal insulin release nor the postprandial modification of insulin action are factored in the clamp test, as they would in physiological conditions of the postprandial state.

Whilst the test can be performed in pregnancy, it is restrictive and does not allow the pregnant woman to move about. Additionally, one is keen to avoid hypoglycaemia and invasive tests during pregnancy.

1.4.5 Standardised Meal Test

An oral glucose tolerance test or meal test is a method of determining insulin resistance and insulin secretion under normal physiological conditions. However, in contrast to the intravenous glucose tests, the rate of glucose appearance (R_a) into plasma has to be estimated, **Figure 20**. This is done through a mathematical model (the minimal model) which estimates glucose disposal according to two differential equations. The first equation describes glucose kinetics assuming a single compartment. The second equation describes the effect of insulin on glucose levels in a compartment outside plasma. The difference between measured glucose and predicted glucose represents insulin sensitivity. This model has been validated against the euglycaemic clamp (Dalla Man *et al.*, 2002). Whilst this model has not been validated in pregnancy, there is no concern that the model may be inaccurate. Oral glucose uptake and appearance are generally slower during pregnancy due to delayed stomach emptying and transit time. The model does not assume that glucose appearance is a standardised constant, rather the model derives glucose appearance from multiple sampling of plasma glucose, insulin and c-peptide over a two-to-three hour time period following oral glucose intake. Separate analysis of three systems: the oral glucose minimal model, the c-peptide minimal model and the insulin minimal model mean that insulin sensitivity, beta cell responsiveness and hepatic excretion are calculated independently to each other, minimising the likelihood that pregnancy will affect the modelling exercise (Cobelli *et al.*, 2014).

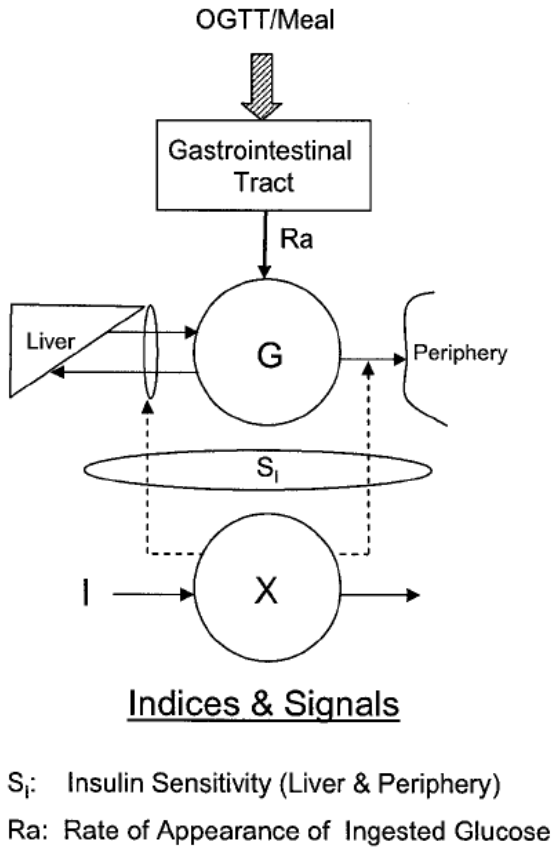

Figure 20 Glucose Minimal Model Glucose with its key indexes: insulin sensitivity (Si) and rate of appearance of ingested glucose (Ra); I, plasma insulin concentration; X, insulin action.

Figure reproduced from (Dalla Man *et al.*, 2005)

The c-peptide minimal model estimates insulin secretion, **Figure 21**. This includes a dynamic component which relates to secretion of promptly releasable insulin and is proportional to the rate in increase in glucose concentration through a constant (Φ_d). A static component relates to new insulin formation to a releasable pool (Φ_s). The c-peptide model has been validated against HEIC and fasting IVGTT (Basu *et al.*, 2003; Steil *et al.*, 2004).

Dalla Man and Cobelli have combined the minimal model and c peptide models to produce a meal test protocol that estimates both insulin sensitivity and beta-cell response to a standardized meal test (Dalla Man *et al.*, 2005). The advantage of this protocol is that it can be completed over two-hours with only seven blood samples (for glucose, insulin and c-peptide) required. This reduced protocol has excellent correlation with results from the longer 5-hour OGTT or 7-hour meal tests.

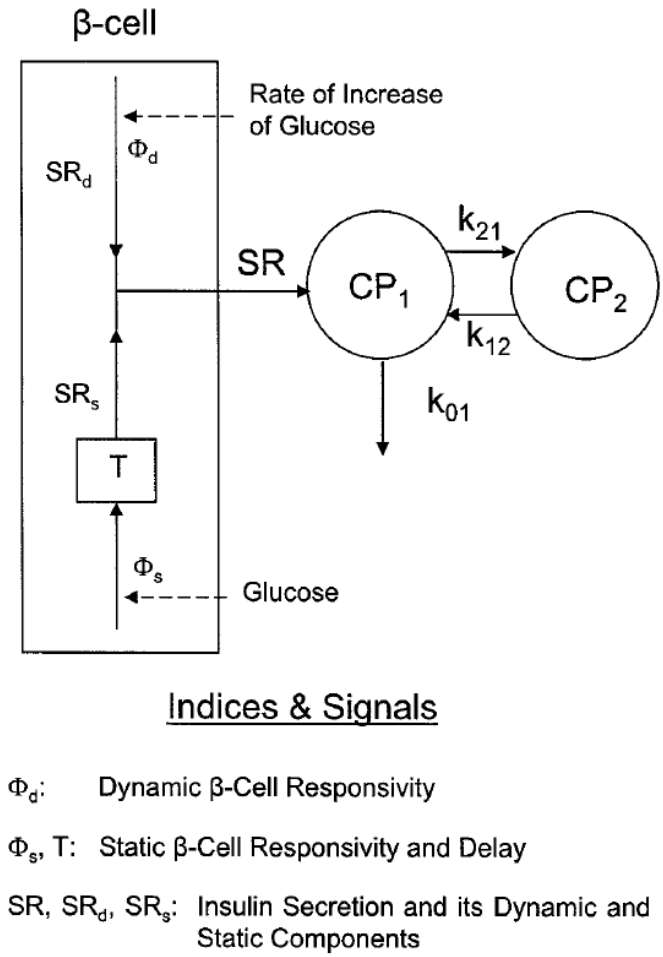

Figure 21 C-peptide Oral Minimal Model with its key indexes and signals: dynamic (Φ_d) and static (Φ_s) β-cell responsivity, delay of provision of new insulin (T), and insulin secretion (SR) with its dynamic (SR_d) and static (SR_s) components. Reproduced from (Dalla Man *et al.*, 2005)

The advantages of the standardised meal test are that it is a physiological test, reflective of everyday metabolism in comparison to HOMA (which represents the fasted state) and HIEC tests which are done under non-physiological parameters. The test is easy to perform and does not require a medically qualified practitioner. The results are reproducible and have been validated against the HIEC (Dalla Man *et al.*, 2005). Disadvantages to the test are that it is relatively time consuming (between 2-3 hours in duration), participants have to be fasted for the test and have to consume a relatively large breakfast. Additionally, mathematical modelling is required in order to obtain the results. For the purposes of the LIPIDPREG and WELLBABE studies, this was done by Chiara Della Man, University of Padua, Italy.

1.5 Hypothesis, aims and objectives

1.5.1 Hypothesis

- The physiological insulin resistance of pregnancy is secondary to accumulation of intramyocellular lipid, and this is exaggerated in women who develop gestational diabetes (GDM).
- Women with GDM have higher concentrations of liver fat and muscle fat than pregnant women without GDM.
- Calorie restriction to 1,200kcal/day reduces liver fat concentration and improves insulin sensitivity in women with GDM.
- Calorie restriction is acceptable, safe and possible in pregnant women with GDM.

1.5.2 Aims and Objectives

- To develop magnetic resonance techniques to study maternal muscle and liver fat concentration during pregnancy and the postpartum period.
- To study the relationship between intramyocellular lipid concentration and insulin sensitivity in pregnancy and post-partum.
- To determine the effects of an energy restricted diet on maternal liver fat metabolism.
- To study the feasibility and acceptability of dietary intervention in pregnancy.

CHAPTER 2 METHODS

2.1 LIPIDPREG study: Muscle Lipid Metabolism in Normal Pregnancy

2.1.1 Research Subjects

Healthy pregnant women were recruited from the antenatal classes at the Royal Victoria Infirmary, Newcastle upon Tyne Hospitals NHS Foundation Trust. Exclusion criteria included a past history of diabetes or gestational diabetes, family history of diabetes or gestational diabetes, family ethnic origin with a high prevalence of diabetes, current steroid medication or a contraindication to magnetic resonance imaging such as pacemaker, ferromagnetic implant or fragments or claustrophobia.

The study was discussed with the women during the class in an informal manner and a patient information sheet (Appendix A) was given to those women who expressed an interest in participating. The women were given the opportunity to discuss the study with the researcher (KH) through telephone or email correspondence.

2.1.2 Anthropometry

Body weight was measured to the nearest 0.1kg with the subject in normal clothing (shoes removed) on digital scale (Seca Ltd., Birmingham, UK). Height was measured to the nearest 0.5cm using a stadiometer (Seca Ltd., Birmingham, UK). Body mass index was calculated from weight and height (BMI= weight (in kg) divided by height (in meters) squared).

2.1.3 Intravenous cannulation and blood sampling

An 18 gauge intravenous cannula (Vasofix; B. Braun Medical Ltd., Sheffield, UK) was inserted in a distal forearm vein (typically in the hand). Anaesthetic cream was available if required. Baseline blood samples (fasting) were taken for: urea & electrolytes, liver function tests, HbA1c, fasting glucose, insulin, c-peptide, non-esterified fatty acids (NEFA) and full blood count. The cannula was flushed with normal saline between blood draws to ensure patency. Consequently, the first 3ml of each blood draw was discarded prior to obtaining a sample in a fresh syringe for analysis.

During the meal test the hand was warmed using microwavable hand warming packs in order to obtain arterialised blood samples. This method of arterialisation has not been validated in pregnancy. These were replaced at 30 minute intervals throughout the study.

Blood glucose samples were immediately analysed at the bedside using a calibrated YSI glucose analyser. Blood samples for insulin, c-peptide and NEFA were put onto ice. Following collection of all the samples, they were spun down in a centrifuge and the plasma separated before being frozen at -40ºC and stored at the Newcastle Magnetic Resonance Centre. Batched samples were then taken to the Diabetes Research Lab for analysis. Other blood samples were taken to the laboratory at the Royal Victoria Infirmary for analysis.

2.1.4 Metabolites and Hormone Assays

Plasma glucose levels were measured by the glucose oxidase method (YSI glucose analyser; Yellow Springs Inc., Ohio, USA) (CV for measurement [control (10 mmol/l)]: 2.8%). Plasma insulin and C-peptide levels were both measured using ELISA kits (DAKO; Ely, Cambridgeshire, UK) (CV for measurement [insulin range 400-500 pmol/l]: 5.5% and [C-peptide range 1.20-2.00 nmol/l]: 7.1%). Plasma NEFA concentration was measured on a Roche Cobas centrifugal analyser using an enzymatic colorimetric Wako kit (Wako Chemicals, Neuss, Germany) (CV for measurement [range 1.02-1.25 μmol/l]: 3.2%). HbA_{1c} was measured by Biorad HPLC (TOSOH Corporation, Tokyo, Japan).

2.1.5 Magnetic Resonance Spectroscopy

MRS studies were performed using a Philips 3 Tesla Achieva scanner. Participants were scanned supine with a left lateral pelvic tilt (to relieve pressure on the inferior vena cava). A pair of receive-only surface coils (Philips Flex-M coils) were placed around the right calf and the scanner bed positioned so that the calf was in the centre of the scanner. Scout images of the calf were acquired to guide identification of the volume of interest within the soleus muscle.

A volume-localised ^{1}H spectrum was acquired from this volume of interest (PRESS localisation, TR=4sec, TE=35msec, 64 repetitions, total acquisition time=4 minutes). Spectra were acquired with and without suppression of signal from water protons. Spectroscopy data was analysed using jMRUI software (van den Boogaart *et al.*, 1996). Signal amplitudes from intra- and extra-myocellular lipids were separated by peak fitting, and quantified by comparison to the water proton signal from non-water suppressed spectra.

2.1.6 Standardised Meal Test

The participant was given a standardized breakfast comprising two Weetabix, 200ml semi-skimmed milk, 200ml orange juice, a white bread roll, 20g jam and 10g margarine this provided 575kcal (72% carbohydrate, 15% protein, 13% fat). They were asked to eat the breakfast as quickly as they could. Timing of samples commenced from the time that eating began. Blood samples for glucose, insulin and C-peptide were taken via an intravenous cannula at 10, 20, 30, 60, 90 and 120 minutes after the meal (Dalla Man *et al.*, 2002; Dalla Man *et al.*, 2005).

2.1.7 Calculations

Fasting insulin sensitivity was calculated from plasma insulin and glucose using the HOMA index (Matthews *et al.*, 1985).

Meal test data was sent to collaborators Dalla Man and Cobelli, University of Padua, Italy for calculation of insulin sensitivity and beta-cell responsivity indices. The oral glucose minimal model was used to calculate insulin sensitivity (S_I) during the meal test, which measures the overall effect of insulin to stimulate glucose disposal and inhibit glucose production (Dalla Man *et al.*, 2002). Beta-cell responsivity indexes were estimated using the oral C-peptide minimal model (Breda *et al.*, 2001; Dalla Man *et al.*, 2005), incorporating age-associated changes in C-peptide kinetics (Van Cauter *et al.*, 1992). Φ_{total} describes the insulin response to a given increment in glucose and is a composite of the dynamic component ($\Phi_{dynamic}$), representing release of immediately available insulin, and the static component (Φ_{static}), representing production of new insulin into a releasable pool. Disposition indices (DI) were calculated by multiplying Φ_{total}, $\Phi_{dynamic}$ and Φ_{static}, by S_I .

2.1.8 Obstetric and neonatal outcomes

Pregnancy outcome data was collected for each participant. This included:

- Gestation at delivery
- Mode of delivery
- Complications during pregnancy/labour
- Breastfeeding
- Neonatal birth weight
- Neonatal complications (admission to Special Care Baby Unit, SCBU)

2.2 WELLBABE Study: the effect of dietary intervention on liver fat metabolism in women with gestational diabetes

2.2.1 Research subjects

Between January and August 2015, 16 women with a singleton pregnancy and greater than 26 weeks gestation were recruited from the antenatal clinic at the Royal Victoria Infirmary, Newcastle upon Tyne following a diagnosis of GDM based on a positive 75g oral glucose tolerance test (fasting glucose greater than or equal to 5.5mmol/l, 2-hour glucose greater or equal to 7.8mmol/l) (World Health Organization, 1985). Women were seen in the clinic by KH following the diagnosis of GDM to discuss the management of diabetes, but also to discuss the study. Women were given a patient information sheet (Appendix C) and given the researcher's contact details in order to obtain further information if needed.

In order to compare the study group to routine care, each participant was matched (by age, BMI, parity and ethnicity) to two women from the Royal Victoria Infirmary maternity database who underwent standard antenatal care for GDM.

2.2.2 Anthropometry

Subjects were weighed at each visit to the Newcastle Magnetic Resonance Centre – using the same equipment as in the LIPIDPREG study described above. In particular, weight loss or gain during the four-week diet period and in the postpartum period was recorded.

Comparator subjects had been weighed in the antenatal clinic at the Royal Victoria Infirmary. These weights were obtained from the case notes.

2.2.3 Fetal Growth

Fetal growth was measured by ultrasound (Voluson E8, General Electric Company, USA) at 28, 32 and 36 weeks gestation by a trained obstetric ultrasonographer. The Hadlock formula (Hadlock *et al.*, 1985) was used to calculate fetal weight through abdominal circumference and femur length measurements (Chitty *et al.*, 1994).

2.2.4 Magnetic Resonance Spectroscopy (MRS)

Studies were performed using a Philips 3 Tesla Achieva whole body scanner (Philips Medical Systems, Best, The Netherlands) using a Philips multi-channel flex coil for ^{1}H imaging and spectroscopy. To avoid pressure on the inferior vena cava subjects were positioned with a

left pelvic tilt. Scout images of the maternal abdomen were acquired and used to guide identification of the volume of interest within the liver.

^{1}H spectroscopy comprised acquisition of PRESS-localised spectra at six echo times (TR = 2.8 s, TE = 36, 50, 75, 100, 125, and 150 ms, spectral width = 2 kHz, 2k data points) from a 3 x 3 x 3 cm voxel positioned in the liver to avoid large vessels.

Spectra were processed using the Java-based magnetic resonance user interface (jMRUI version 3.0) (Naressi *et al.*, 2001a; Naressi *et al.*, 2001b) using the AMARES non-linear least square fitting algorithm to determine peak areas (Longo *et al.*, 1995). Resonances of water at 4.7 ppm and the CH_2 methylene peak at 1.3 ppm in ^{1}H spectra were quantified. The mean T2 was determined for each peak by fitting a mono-exponential to the data. Signal amplitude at an effective echo time of zero was determined, and these amplitudes used to obtain the liver triglyceride fraction value. The upper limit of normal for a US population of mixed gender, multi-ethnic subjects between 30-65yrs of age has been defined as 5.5% (Szczepaniak *et al.*, 2005).

Liver triglyceride content was assessed at baseline, just after the 4 week hypocaloric diet and postnatally (between 12 and 28 weeks postpartum in a subset of the study group). Subjects continued on the diet until the second assessment.

2.2.5 Meal test

A standardised meal test followed MR scanning using the same protocol as the first study (described above), with the addition of a further blood sample at 180 minutes (for glucose, insulin and c-peptide).

Standardised meal testing was performed before commencing the diet (visit 1) and after four-weeks of dietary intervention (visit 2).

2.2.6 Dietary Review

Participants were given an individualised 1,200kcal/day diet plan. This is described in detail in Chapter 4.

2.2.7 Home blood glucose monitoring/Dietary compliance

Women were supplied with a Bayer Contour meter as part of routine care. They were asked to measure glucose levels daily before breakfast (fasting) and one-hour after their main meal.

Data were relayed to KH daily either through MyFitnessPal or by telephone. The need for metformin and/or insulin was assessed as part of routine management.

Participants were encouraged to maintain the 1,200kcal/day diet through regular contact with the research team. In most cases this was through MyFitnessPal and/or text messaging. Each participant was telephoned on a weekly basis during the dietary period to check whether there were any problems or issues.

2.2.8 Semi Structured Interview

Semi-structured interviews were conducted by an independent research midwife (CMcP) experienced in qualitative methodology. An interview schedule was developed using the Theory Domain Framework (Michie *et al.*, 2005) to explore motivation to engage in the diet, beliefs about consequences, emotions (e.g. fears) amongst other domains.

2.3 Statistics

Data were analysed in SPSS V21.0. Continuous variables are expressed as mean ± standard error of the mean. Continuous data were compared using the two-tailed, paired Student t-test and ANOVA when there were multiple groups. Categorical data were compared using Wilcoxon signed−rank test. Significance was set at <0.05.

Normality of data was assessed using the Shapiro-Wilk test. In both studies: glucose, insulin, C peptide, total cholesterol, HOMA, Si, NEFA, maternal weight and height, and neonatal birth weight were normally distributed. Liver fat and disposition index were not normally distributed.

2.4 Power Calculation

Sample size was planned for each study to maximize the likelihood of demonstrating a statistically significant and true difference in the primary outcome parameter.

In the LIPIDPREG study a power calculation was not possible on the primary outcome measure of intramyocellular lipid concentration because, due to the novel nature of the technique in pregnancy, there were no studies on which to base an estimate of the perceived difference. We based the sample size on previous magnetic resonance metabolic studies in non-pregnant subjects that had similar aims.

For the WELLBABE study, a power calculation was undertaken centered on expected change in liver triglyceride. This was based on a previous study from our institution in subjects with T2DM as there is no published data on liver triglyceride in human pregnancy (Lim *et al.*, 2011b). In our previous study liver triacylglycerol fell from 12.8±2.4% to 4.8±4.2% over a 4 week period. Given that the degree of energy restriction in the present study was 60% less than in the previous study, and that fall in liver triacylglycerol is proportionate to reduction in energy intake, we assumed that the reduction in liver triacylglycerol would be 60% of that previously reported. Thirteen participants would be required to demonstrate an absolute fall in liver triacylglycerol of 4.8% (assuming SD of 4.2 and baseline level of 12%) with 95% power at the 5% significance level.

CHAPTER 3 INVESTIGATING INTRAMYOCELLULAR TRIGLYCERIDE AND INSULIN RESISTANCE IN NORMAL PREGNANCY

3.1 Background

Normal pregnancy is associated with progressive insulin resistance, although the mechanisms underlying this remain unclear (Catalano *et al.*, 1991). In T2DM and obesity, insulin resistance is closely linked to accumulation of triglycerides in muscle (Krssak *et al.*, 1999; Ravikumar *et al.*, 2005). Insulin resistance within muscle is one of the earliest detectable changes in T2DM and this deficit is found in high prevalence in the offspring of patients with T2DM (Warram *et al.*, 1990). Pregnancy is associated with increased plasma triglyceride concentrations, particularly low-density lipoprotein (LDL-TG), which is the lipid fraction responsible for delivery of triglyceride to muscle and other tissues Kousta *et al.*, 2003. Previous studies have shown that intramyocellular lipid (IMCL) concentrations are between 66-88% higher (depending upon which calf muscle is studied) in women who had gestational diabetes during their pregnancy, compared to normal controls (Kautzky-Willer *et al.*, 2003; Kousta *et al.*, 2003).

It was hypothesised that the physiological insulin resistance of pregnancy is secondary to accumulation of IMCL, and that this is exaggerated in women who go on to develop gestational diabetes. No studies to date have investigated IMCL during pregnancy.

3.2 Research design and methods

3.2.1 Study Population

Women were recruited from antenatal classes at the Royal Victoria Infirmary, Newcastle upon Tyne NHS Foundation Trust at approximately 28 weeks gestation. Exclusion criteria included a past history of diabetes or gestational diabetes, family history of diabetes or gestational diabetes, family ethnic origin with a high prevalence of diabetes, current steroid medication or a contraindication to magnetic resonance imaging such as pacemaker, ferromagnetic implant or fragments or claustrophobia. All women gave written, informed consent prior to participation in the study (Appendix A). The study protocol and documentation was reviewed by Newcastle upon Tyne and North Tyneside Local Research Ethics Committee 2 (09/H0907/16).

3.2.2 Schedule of metabolic testing

Each subject was studied at 34 weeks gestation and again at 12 weeks postpartum (Visit 1 & 2, **Figure 22**). Prior to each visit, participants were advised to avoid vigorous exercise and were fasted from midnight. They were advised to drink water only on the morning of the study. A taxi was arranged to bring the participants to the research centre.

On arrival participants were weighed (as described in Chapter 2) and an intravenous cannula was inserted in the distal forearm. Baseline bloods were taken for glucose, insulin, C-peptide, full blood count, urea & electrolytes, liver function tests, HbA_{1C} and lipid profile. In order to quantify IMCL, the participant then underwent a MRS scan of the soleus leg muscle (Chapter 2). Following the scan, a standardised meal test was performed. The participants remained in the department for the duration of the meal test (2 hours) during which timed blood samples were taken at 10, 20, 30, 60 and 120 minutes following consumption of the supplied breakfast. The cannulated hand was warmed using microwavable hand-warmers in order to arterialise the sample. Glucose was analysed on-site using a YSI analyser. Insulin and C-peptide samples were taken in a plain tube, allowed to clot and spun down. Serum was decanted from the spun sample and frozen at -40○C for later analysis at the Diabetes Research Lab, Newcastle University. Participants were given feedback regarding their glucose profile during the meal test.

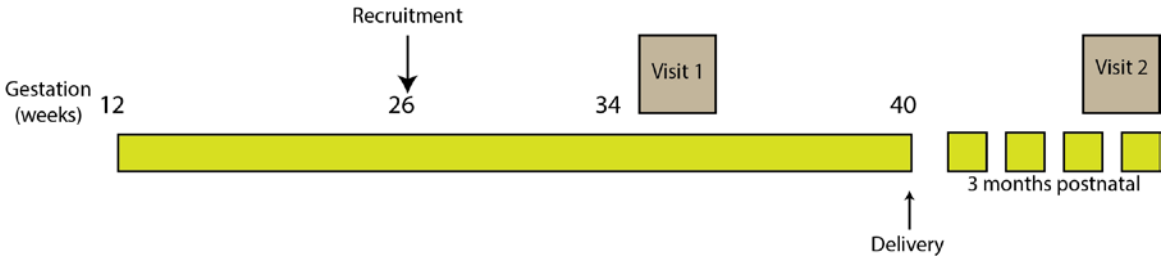

Figure 22 Schedule of visits for investigating muscle metabolism in normal pregnancy

3.2.3 Participant Demographics

Eleven primiparous women (mean age: 31±3 years, mean BMI: 22±4) underwent MRS and standard meal testing at 34 (range 33-35) weeks gestation and 12 weeks postpartum **(Table 2)**. One woman was excluded as her MRS data was technically unsatisfactory. All women had singleton pregnancies, delivered at term and mean birth weight was 3.29kg.

	Age (years)	Weight (kg)	Height (cm)	BMI	Parity	Breast or Bottle Feeding	Mode of Delivery	Birth weight (kg)
1	32	63	170	22	0	Bottle	Instrumental	3.30
2	27	62	168	22	0	Breast	Normal	3.54
3	29	56	157	22	0	Breast	Instrumental	3.25
4	32	71	165	26	0	Breast	Caesarean	3.54
5	32	60	172	20	0	Breast	Instrumental	2.92
6	29	75	168	26	0	Breast	Normal	3.80
7	34	72	178	22	0	Bottle	Normal	2.94
8	39	62	171	21	0	Breast	Caesarean	3.42
9	31	69	170	23	0	Bottle	Caesarean	2.76
10	31	84	165	30	0	Breast	Instrumental	3.45
11	30	72	158	26	0	Breast	Normal	3.28

Table 2 Participant Characteristics

3.3 RESULTS

3.3.1 Plasma glucose

Fasting plasma glucose was lower in pregnancy compared to the post partum (3.8 ± 0.1 vs. 4.0 ± 0.1 mmol/l; p=0.16). Although, in this study, it was not statistically significant, this observation has previously been described by others. Two hour post-meal plasma glucose was higher during pregnancy (5.9 ± 1.0 vs. 5.0 ± 0.7 mmol/l ; p<0.01) as was area under curve (AUC) glucose (585 ± 22 vs 496 ± 26 mmol/l, p=0.05; **Figure 23**).C

3.3.2 Insulin and C-peptide

During pregnancy substantially higher plasma insulin levels were required to achieve glucose control, and at 30 minutes post-meal, plasma insulin levels were almost two-fold higher;

510 ± 63 vs 273 ± 30 pmol/l for pregnant and postpartum groups respectively (p=0.01; **Figure 23**).

Plasma insulin levels peaked at 30 minutes during pregnancy and at 60 minutes postpartum. Again, this may reflect the delayed appearance of glucose in the circulation due to delayed gastric emptying in pregnancy. There was a 2.7-fold increase in total insulin production during the standardized meal tests (area under curve 57336 ± 4890 vs 20922 ± 2245 pmol/l; p=0.0001).

C-peptide concentrations followed a similar profile to insulin secretion in both the pregnant and non-pregnant state. It should be noted that C-peptide is excreted by the kidneys, whilst insulin is excreted through the liver, this may explain the slightly higher C-peptide to insulin levels in the postpartum state (insulin more rapidly metabolised than C-peptide excreted).

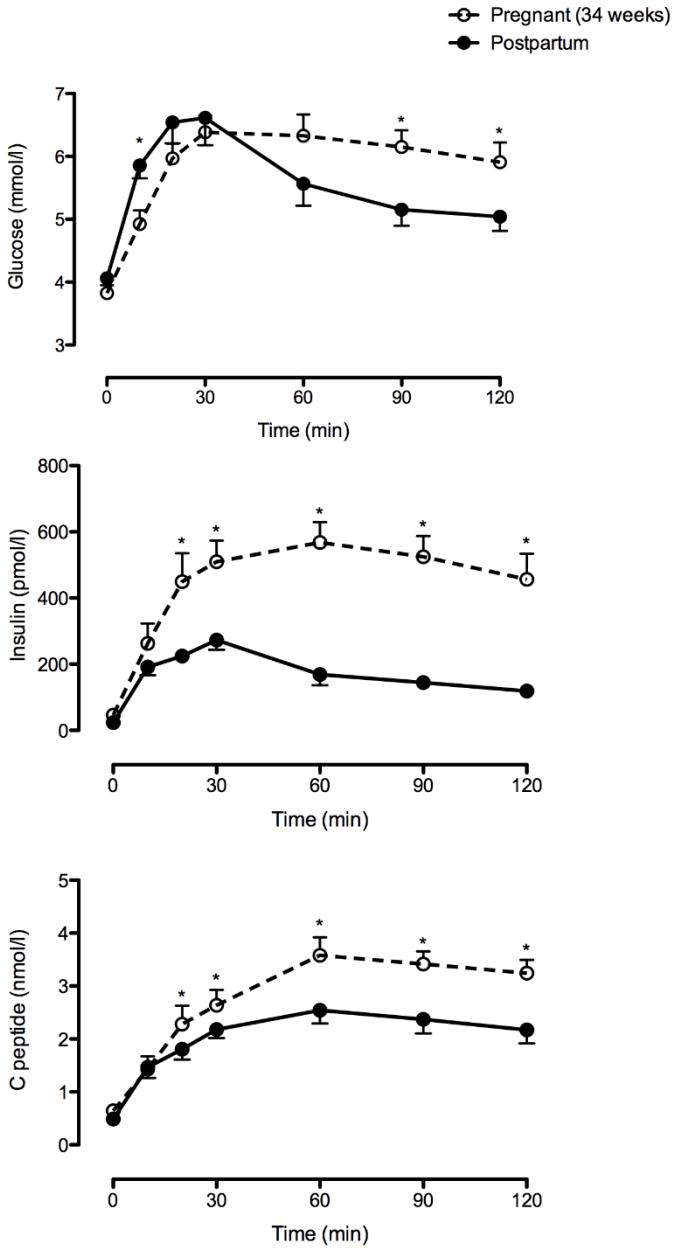

Figure 23 Mean +/- SEM plasma glucose, insulin and C-peptide concentration following a standardised meal test during pregnancy (open circles, dashed line) and postpartum (solid circles, solid line); (SEM – standard error of the mean). Student's T-test: *p>0.05 Area under curve (AUC) glucose pregnant vs postpartum (718 vs 673 respectively), AUC insulin (9531 vs 3488 respectively), AUC C-peptide (351 vs 258 respectively).

3.3.3 Insulin sensitivity

Insulin resistance in the fasting state, as measured by HOMA was almost two-fold higher in pregnancy (1.3 ± 0.6 vs 0.7 ± 0.3, p=0.009; **Figure 24**). After the meal, the difference in insulin sensitivity was even more marked. Calculated insulin sensitivity (S_I) was approximately four-fold lower in pregnancy compared to postpartum (45.6 ± 9.9 vs 193.0± 26.1; 10^4 dl/kg/min per pmol/l, p=0.0002). As the beta-cell response to a given increment in glucose (Φ_{total}) did not change in pregnancy compared to postpartum (90.3 ± 8.2 vs 111.6 ± 21.8; 10^{-9}/min, p=0.26), the total disposition index (DI_{total}) increased during the transition from pregnancy to postpartum (7301 ± 1990 vs 39682 ± 10657; 10^{-4}dl/kg/min per pmol/l, p=0.01).

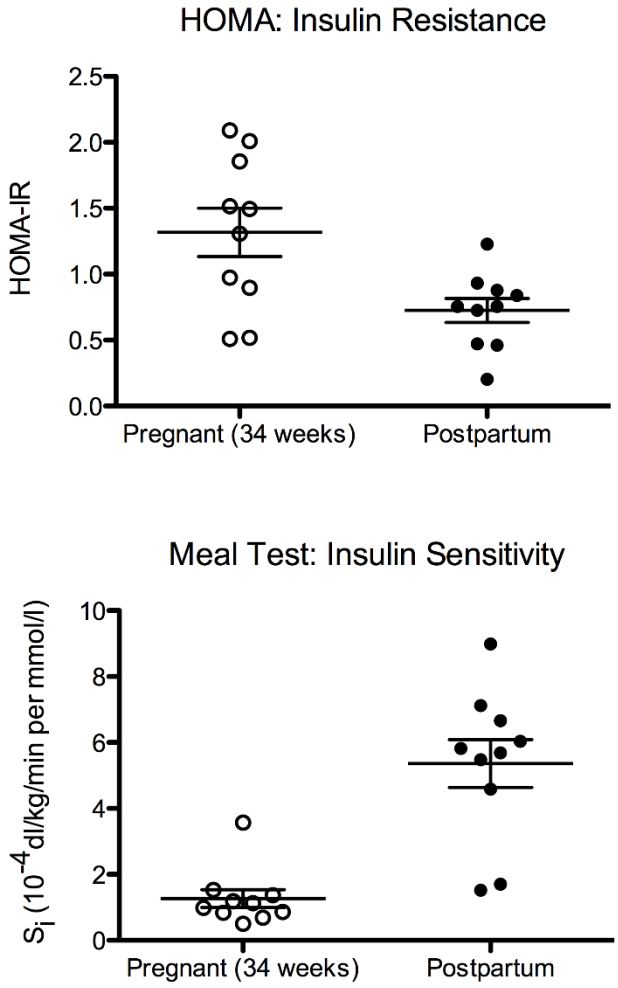

Figure 24 Homeostatic Model of Insulin Resistance during pregnancy and postpartum (top graph). Insulin sensitivity index (Si) as measured by a standardised meal test (bottom graph). Error bars are mean±SEM. Student's T test, p=0.009 for HOMA and p=0.0002 for Si

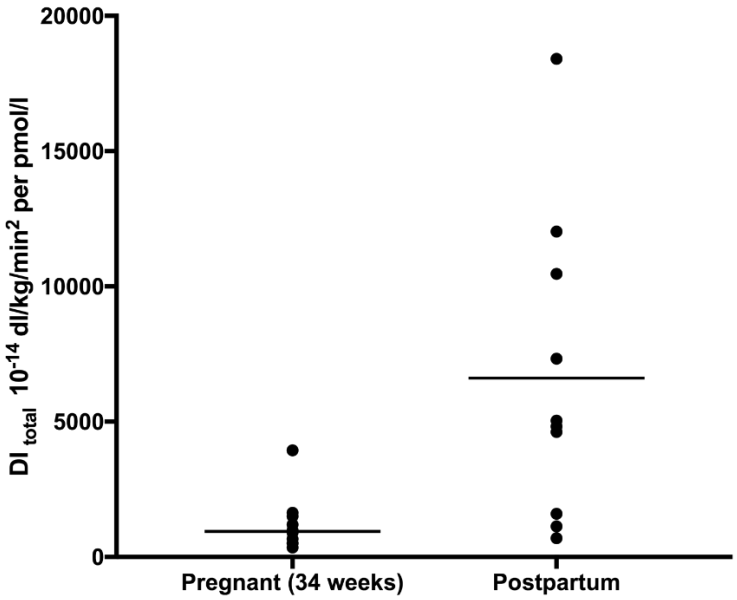

Figure 25 Disposition index (DI) at 34 weeks pregnant and postpartum. Bar indicates median DI. Wilcoxon signed rank test; p=0.024

3.3.4 Intramyocellular lipid

Figure 26 shows the individual data on IMCL concentration for each subject. The mean IMCL concentration of the soleus muscle was normal during pregnancy and did not significantly change postpartum (20.0±2.3mmol/l vs 19.1±3.2mmol/l, p=0.64).

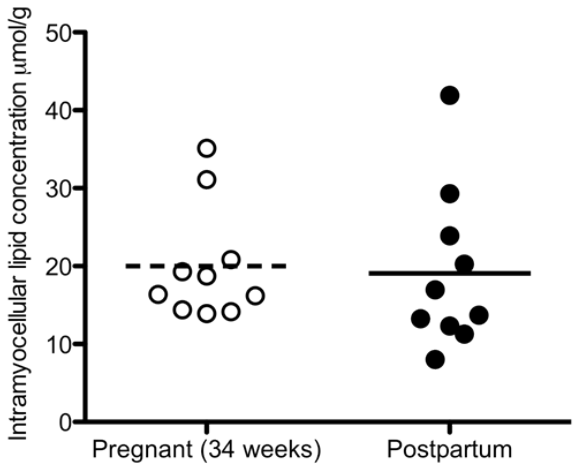

Figure 26 Intramyocellular lipid concentrations during pregnancy (○) and postpartum (●). Line indicates mean lipid concentration during pregnancy and postpartum. Student's T test p=0.64

3.3.5 Lipid profile

Fasting plasma triglyceride levels were elevated three-fold during pregnancy (2.3 ± 0.2 vs 0.8 ± 0.1 mmol/l, p<0.01). However, LDL-TG, responsible for fatty acid delivery to muscle and other tissues, was six-fold elevated (0.75 ± 0.43 vs. 0.12 ± 0.09 mmol/l; p=0.001) (**Figure 27**). Fasting plasma NEFA concentrations were similar during pregnancy compared to postpartum (0.40 ± 0.03 vs 0.41 ± 0.03 mmol/l, p=0.91). There was a modest increase in plasma total cholesterol, including low-density lipoprotein and very low-density lipoprotein components during pregnancy (**Table 3**).

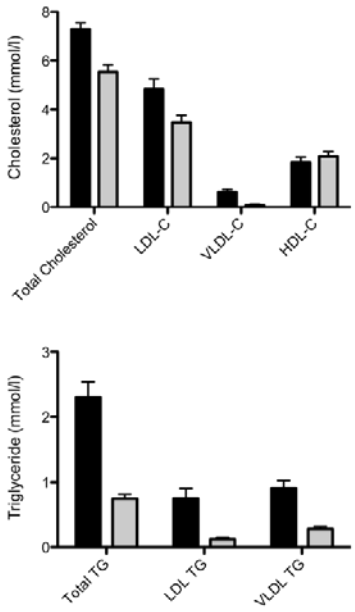

Figure 27 Mean ± SEM plasma cholesterol and triglyceride lipoproteins in healthy women during pregnancy (black bars) and in the postpartum period (grey bars)

	1st MR (34 weeks pregnant)	2nd MR (12 weeks postpartum)	*p*-value
Fasting Glucose (mmol/l)	3.8 ± 0.1	4.1 ± 0.1	0.14
Fasting Insulin (pmol/l)	46.2 ± 6.0	24.0 ± 2.6	<0.01
HOMA	1.3 ± 0.2	0.7 ± 0.1	<0.01
Total Cholesterol (mmol/l)	7.3 ± 0.2	5.5 ± 0.3	<0.01
HDL-C (mmol/l)	1.8 ± 0.2	2.1 ± 0.2	0.4
LDL-C (mmol/l)	4.8 ± 0.4	3.5 ± 0.3	0.02
VLDL-C (mmol/l)	0.6 ± 0.1	0.1 ± 0.06	<0.01
Total Triglyceride (mmol/l)	2.3 ± 0.2	0.8 ± 0.06	<0.01
LDL-TG (mmol/l)	0.8 ± 0.1	0.1 ± 0.03	<0.01
VLDL-TG (mmol/l)	0.9 ± 0.1	0.3 ± 0.03	<0.01
FFA (mmol/l)	0.4 ± 0.03	0.4 ± 0.03	0.90
HbA_{1C} (%)	5.3 ± 0.2	5.4 ± 0.3	0.18
Insulin Sensitivity Index (S_I) (10^{-4} dl/kg/min per pmol/l)	1.27 ± 0.27	5.36 ± 0.73	<0.01
Muscle triglyceride (mmol/l)	20.0 ± 2.3	19.1 ± 3.2	0.64

Table 3 Baseline Indices (mean ± SEM), Insulin Sensitivity from the Standardised Meal Test and Muscle Triglyceride on Magnetic Resonance Spectroscopy (HOMA: homeostatic model assessment, NEFA: Non-esterified fatty acids, HDL: high density lipoprotein, LDL: low density lipoprotein, VLDL: Very low density lipoprotein, C: cholesterol, TG: Triglyceride)

3.4 Discussion

This study shows that pregnancy does not appear to affect IMCL concentrations even though there is a four-fold increase in insulin resistance during pregnancy compared to postpartum. A three-fold increase in plasma triglycerides was observed as was a small increase in postprandial glucose concentrations, consistent with the insulin resistant state. In addition, the feasibility of MRS at 3 Tesla during pregnancy was demonstrated with the technique well tolerated by all the participants.

These results differ from observations in other insulin resistant states, in particular T2DM where IMCL levels are often elevated and mark the beginning of the pathophysiological process (Perseghin *et al.*, 1999; Virkamaki *et al.*, 2001; Ravikumar *et al.*, 2005). Intracellular fatty acids are metabolised within the cell to intermediaries, especially diacylglycerol and ceramides, which directly cause insulin resistance through preventing translocation of the GLUT4 glucose channel thereby preventing glucose uptake (Roden, 2004). This is the first step in the uptake and metabolism of postprandial glucose, as described in detail in Chapter 1. The present study indicates that there must be alternative pathways of inducing insulin resistance in pregnancy resulting in equally profound changes. Kirwan et al studied the reversal of insulin resistance in a similar group of normal, non-obese pregnant women, only using muscle biopsy as opposed to MRS (Kirwan *et al.*, 2004). Insulin sensitivity improved by 74% in the postpartum period and this was accompanied by a 42% increase in insulin receptor expression, a 69% increase in IRS-1 protein and a reduction in the p85a alpha regulatory subunit of phosphatidylinositol 3-kinase. These changes allow a greater response to insulin-receptor binding through expression of downstream signalling mechanisms and increased numbers of insulin receptors. Pregnancy regulation of cellular mechanisms and gene expression is thus likely to be hormonal in origin (possibly a combination of corticosteroid and placental e.g. human placental lactogen).

This study investigated the relationship between insulin resistance and IMCL deposition in a group of normoglycaemic, non-obese pregnant women. Although no change in IMCL was noted in this group, evidence from postpartum studies in women with prior GDM would suggest that elevated muscle lipid may contribute to the 'additional' insulin resistance seen in these women during pregnancy (Kautzky-Willer *et al.*, 2003). A longitudinal study comparing muscle fat and measuring insulin resistance in women with and without GDM is necessary to test this hypothesis further.

This study utilised noninvasive techniques (MRS) under normal conditions (meal test) to evaluate everyday metabolic physiology. It is one of the first studies to utilize 3 Tesla magnetic resonance in pregnancy in a research setting. The procedure was well tolerated by all the women and no adverse fetal effects were noted. Scanning protocols were modified to reduce noise and energy deposition. MRS is superior to muscle biopsy as it is noninvasive and less susceptible to contamination and processing artefact (Szczepaniak *et al.*, 1999; Howald *et al.*, 2002).

Meal testing was utilized in this and the subsequent study, and was performed following an overnight fast. This was well accepted by the participants and ketone levels were not unduly high as a result. The breakfast itself was substantial (575kcal) and was not always fully eaten. In order to be able to compare the results from paired samples, the amount of food eaten was noted and repeated on subsequent testing. The standardised meal test is a dynamic test of insulin secretion and glucose uptake in the context of physiological conditions (i.e. it reflects day-to-day parameters of glucose uptake, exposure and disposal). By contrast, HOMA is a non-dynamic estimation of insulin resistance under fasting conditions whilst clamp tests operate under extreme conditions of hyperinsulinaemia or hyperglycaemia. The meal test was advantageous for pregnant women as it is relatively non-invasive (requiring an intravenous cannula, but not drug infusions), avoids hypoglycaemia and allows freedom of movement. However, the study was relatively prolonged (between 2 and 3 hours duration) and this may have an impact on the design of future studies. A quicker assessment of beta cell function (e.g. OGTT or HOMA) and insulin sensitivity may make participation easier.

Limitations of the study must be considered. A small sample size was necessary to make detailed magnetic resonance possible and to perform more extensive metabolic testing than would be feasible with a larger sample size. Secondly, the population studied (white British, non-obese, nulliparous) is not representative of the general pregnant population. Strict inclusion criteria were used in order to obtain as homogeneous a sample as possible to avoid confounding factors such as body mass index and ethnicity. Finally, MRS was limited to the soleus muscle. Although some studies have shown relationships between insulin sensitivity and IMCL in tibialis anterior only (Kautzky-Willer *et al.*, 2003), IMCL in the soleus is most widely used as an indicative muscle and has been shown to reflect whole body insulin sensitivity (Krssak *et al.*, 1999). To scan more than one muscle would have prolonged scanning time, which was not felt reasonable given women were in the third trimester of pregnancy.

In conclusion, this study demonstrates that the insulin resistance of healthy pregnancy does not appear to be associated with IMCL deposition, in contrast to other insulin resistant states. The insulin resistance of muscle that characterises late pregnancy must be caused by a distinct, gestation-mediated mechanism.

CHAPTER 4 LIVER TRIGLYCERIDE CONTENT AND GESTATIONAL DIABETES: EFFECTS OF MODERATE CALORIE RESTRICTION

4.1 Background

GDM has long been recognised to be an early manifestation of T2DM, with many shared pathogenic features (Prentki and Nolan, 2006). It has been demonstrated that individuals with T2DM can durably be returned to non-diabetic glucose control by substantial weight loss and that this depends initially upon a reduction in liver triglyceride content (Taylor, 2013; Steven *et al.*, 2016b). However, information on liver triglyceride content in GDM is lacking even in animal models.

In T2DM, both liver triglyceride and fasting plasma glucose are normalised within 7 days of a substantial reduction in calorie intake (Lim *et al.*, 2011a). Over a period of weeks, a more moderate reduction to 1200kcal/day decreases liver triglyceride content and plasma glucose (Petersen *et al.*, 2005). High levels of liver triglyceride are known to be present years before the diagnosis of T2DM (Shibata *et al.*, 2007) and women with previous GDM have been shown to have markedly elevated liver triglyceride levels (Tiikkainen *et al.*, 2002; Forbes *et al.*, 2011). As normal pregnancy is associated with a greater than two-fold increase in plasma triglyceride levels (Barrett *et al.*, 2014) a physiological rise in liver triglyceride would be expected during pregnancy as these parameters are usually closely associated (Hwang *et al.*, 2007). This may be exaggerated in pregnancies complicated by GDM.

Food restriction in pregnancy as a possible way to improve adverse metabolic factors understandably raises concerns. Many concerns are unfounded (Dornhorst *et al.*, 1991), and conversely both obesity and gestational diabetes are known to confer substantial risks (Crowther *et al.*, 2005; Metzger *et al.*, 2008). Meta-analysis has shown that weight loss in pregnancy in otherwise healthy women reduces not only the incidence of GDM but also that of pre-eclampsia, gestational hypertension, and preterm birth, with no effect on fetal growth (Thangaratinam *et al.*, 2012). However, weight loss during pregnancy is not recommended in current guidelines (National Institute of Clinical Excellence, 2008). Further, there is no objective information on how specific advice to decrease calorie intake would be accepted by women with GDM.

Whilst a hypocaloric dietary intervention may prove to be an effective treatment for GDM, the key question is whether it can be delivered and be successful in a clinical setting. Akin to any intervention that involves behavioural change, careful analysis of the current behaviour and the possible facilitators and barriers to change are essential prior to developing any interventional package (Medical Research Council, 2008).

The primary aims of this study were therefore to define both the extent of liver triglyceride accumulation during pregnancy in women diagnosed with GDM and the effect upon this of modest calorie restriction. The metabolic effects and acceptability of calorie restriction in GDM were also examined.

4.2 Methods

4.2.1 Study Population

Between January and August 2015, 16 women with a singleton pregnancy and greater than 26 weeks gestation were recruited from the antenatal clinic at the Royal Victoria Infirmary, Newcastle upon Tyne following a positive 75g plasma oral glucose tolerance test (fasting glucose greater than or equal to 5.5mmol/l, 2-hour glucose greater or equal to 7.8mmol/l) (World Health Organization, 1985). Women undergoing a OGTT had been screened according to NICE guideline: BMI above 30kg/m^2, previous baby weighing 4.5kg or more, previous GDM, first-degree relative with diabetes, ethnic origin with a high prevalence of diabetes (National Institute of Clinical Excellence, 2008). Women with multiple pregnancy or any contraindication to MRI (ferromagnetic implant/claustrophobia/ abdominal circumference >102cm) were excluded.

To compare weight change and pregnancy outcomes each subject was matched with two comparators with gestational diabetes on the basis of parity, age, ethnic origin and body mass index. The comparators were identified from the Caldicott approved hospital maternity database.

The WELLBABE (**WE**ight **L**oss **L**ooking for **Ba**by and mother's **Be**tter Outcomes) study was approved by the Newcastle and North Tyneside Ethics Committee (14/NE/1085) and all women gave written informed consent. The study was registered with the ISRCTN Registry (17505466).

4.2.2 Schedule of metabolic testing

Women were invited to participate at the first clinic visit after diagnosis of GDM. A MRS scan and a standardised meal test was measured before and after the 4-week hypocaloric diet. Fetal growth scans, measuring abdominal circumference as described by Chitty (Chitty *et al.*, 1994), were conducted at 28, 32 and 36 weeks gestation and data on weight and home blood glucose monitoring were collected. In the light of the data obtained during the study, further ethical permission was obtained to carry out post-partum liver triglyceride measurements and fasting blood tests between 12 and 28 weeks after delivery.

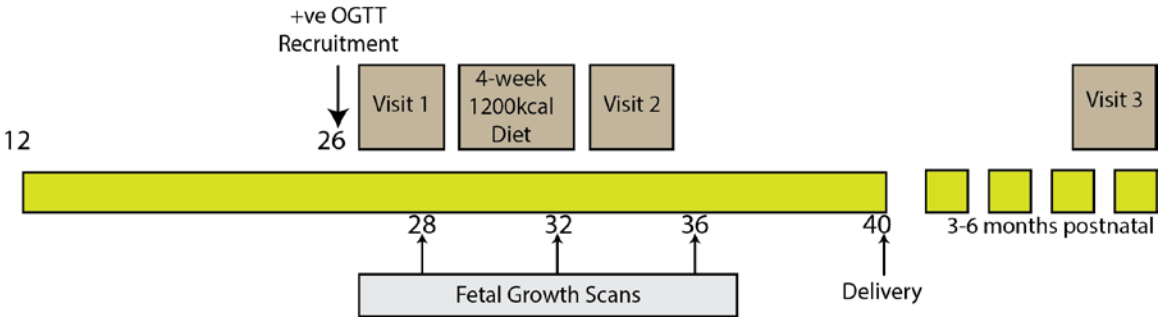

Figure 28 WELLBABE schedule. Pregnancy is represented by the yellow line between 12 and 40 weeks. Women were recruited at 26 weeks following a positive oral glucose tolerance test (OGTT). Metabolic testing (MR liver, standardised meal test) occurred at visit 1. Women underwent a 4 week 1,200kcal diet before repeat metabolic testing at visit 2. Women had further metabolic testing (MR liver and HOMA) at 3-6months postpartum (visit 3). (OGTT – oral glucose tolerance test).

4.2.3 Dietary Intervention

The 1200kcal/day diet (50% carbohydrate, 25% protein, 25% fat) was designed to limit calorie intake whilst ensuring nutritional adequacy (food portion plan plus a calcium-containing pregnancy multivitamin) during pregnancy. Specific advice about the diet plan was provided in a face-to-face consultation, delivered by trained team members, after following the standardised meal test at visit 1. A consultation checklist was used to ensure all aspects of the dietary intervention were addressed. KH or AB (dietitian) outlined the rationale for the diet, and participant's individual motivation, facilitators and barriers to the implementation of the dietary changes were explored. The diet portion plan was fully explained and a supporting booklet provided (Appendix C) along with a sample 7 day meal plan and suggested recipes. Each participant's usual intake was reviewed and a plan agreed to modify this in order to match the 1200kcal portion plan. Where necessary the portion plan could be

modified at this stage to reflect individual food preferences. To facilitate portion control and reduce participant burden a portion cup marked to measure appropriate portions of breakfast cereals, rice and pasta was provided. Additionally, to aid timely feedback, MyFitness Pal™ (MFP), a smartphone application, was used to record dietary intake. Women consented to sharing their dietary and glycaemic control data with the research team (KH and AB) so that their progress could be monitored daily and support and advice given via MFP messaging or via telephone call accordingly (initiated on request by participants or based on the assessment of the MFP data by the study team). Following completion of the diet KH provided dietary advice and a revised portion plan for the remainder of the pregnancy term. In most cases the women were advised to avoid weight gain and continue on ~1500kcal/day, although this was individualised according to weight loss and glycaemic control.

4.2.4 Qualitatative Study

An interview schedule (Appendix E) was constructed using the domains of the Theory Domain Framework (Michie *et al.*, 2005). This was done in collaboration with Catherine McParlin (Research Midwife) and Dr Vera Araujo-Soares (Senior Lecturer in Health Psychology, Institute of Health and Society, Newcastle University).

Semi-structured interviews were completed between one and four weeks following the dietary intervention period (in most cases whilst the women were still pregnant). Catherine McParlin conducted the interview and with participant consent, interviews were recorded and transcribed. The content was then analysed using Nvivo software (QSR International, 2014).

4.3 Results

4.3.1 Patient Demographics

Sixteen women were recruited. Two women dropped out (during weeks 1 and 2) citing pressure of time and social circumstances. One subject was unable to undergo magnetic resonance studies (claustrophobia) but underwent all other aspects of the protocol. In order to compare the clinical effect of the hypocaloric diet with that of standard management, matched comparators with GDM were identified from the maternity database (n=28; complete data available on 26) (**Table 4).** Subjects were matched for age, height, parity and had similar hyperglycaemia on the diagnostic 28 week oral glucose tolerance test.

	Participants	Controls	*p*
N	14	26	-
Age (years)	32.2±4.3	30.9±4.4	0.409
Ethnicity – White British	14	26	NS
Weight (kilograms)	93.1±13.9	82.5±20.8	0.119
Height (centimetres)	1.64±0.06	1.64±0.05	0.943
Body Mass Index	34.6±5.0	31.0±8.2	0.206
Nulliparous (n)	8 (57%)	15 (58%)	NS
FH of GDM/T2DM (n)	12 (86%)	N/A	-
OGTT – 0 min (mmol/l)	5.0±0.8	5.2±0.9	0.997
OGTT – 120 min (mmol/l)	8.5±0.6	9.0±1.8	0.881

Table 4 WELLBABE Study Participant Characteristics

4.3.2 Weight change

During the hypocaloric diet, subjects lost a mean of 0.4 ± 0.1 kg (n=14) per week during the intervention compared to a weight gain of 0.3 ± 0.1 kg (n=26) per week in the comparator group (p=0.002). Total weight loss amongst subjects was 1.6 ± 0.4 kg compared with 1.2±0.3 kg weight gain in comparators. Six subjects lost more than 2 kg (2.1 - 5.6 kg), five lost between 0.3-1.2 kg and three subjects put on weight (0.2 - 0.5 kg).

4.3.3 Dietary compliance, weight loss and blood glucose control

Dietary data from MyFitness Pal, blood glucose control, weight loss and treatment are documented in **Table 5** below. Food diary completion rates varied from 11-100%. Average reported calorie intake was 1137±30 kcal/day. Participants with 100% food diary compliance had greater weight loss compared to those with lower compliance (3.0±0.75 kg vs. 0.9±0.4 kg; p=0.05). Two participants required metformin for high blood glucose readings (participants 2 and 3). Despite participant 3 having lost 1.2 kg and beingn compliant with her food diary, her fasting glucose readings remained elevated and for this reason she required metformin treatment.

Participant number	Average fasting glucose (mmol/L)	Average postprandial glucose (mmol/L)	No. of readings outside range	Average kcal/day	% diary complete	Weight loss (kg)	Treatment
1	4.9	7.2	7	1023	100	3.5	
2	6.0	6.7	27	1172	11	0.3	Metformin
3	5.5	5.7	15	1170	100	1.2	Metformin
4	5.1	6.9	7	1208	100	5.6	
5	5.0	6.2	9	1145	94	3.3	
6	5.1	7.3	12	1254	90	-0.4	
7	5.0	8.6	11	1210	85	-0.5	
8	4.1	5.5	1	917	61	2.5	
9	4.4	5.5	1	964	96	1.1	
10	4.4	6.3	4	1066	100	2.6	
11	4.8	6.5	1	1280	100	2.1	
12	5.5	7.1	7	1047	46	-0.2	
13	4.4	5.9	2	1237	93	1.1	
14	4.0	6.4	0	1221	61	1.2	

Table 5 Summary of blood glucose, dietary compliance, weight loss and treatment during the 1,200 kcal/day dietary period of the WELLBABE study

4.3.4 Liver Triglyceride

Before dietary intervention, at gestational age ranging from 26 to 34 weeks, median liver triglyceride was 3.7% (IQR 1.2 - 6.1%). After four weeks of dietary intervention the median liver triglyceride decreased by 51% to 1.8% (IQR 0.7-3.1%; p=0.021, Wilcoxon Signed Rank). Individual data are shown in **Figure 29**. One women had very high pre-diet liver triglyceride levels (>20%); repeat analysis excluding this individual did not change the statistical significance (3.4% (IQR 1.1-4.1%) pre-diet, 1.4% (IQR 0.7-2.9%) post-diet; p=0.006). This woman had obstetric cholestasis which explains the high liver triglyceride

levels, she also had deranged liver function tests (ALT 62 and 78 before and after dietary intervention respectively).

Measurement of liver triglyceride was repeated between 12 and 28 weeks postpartum; median levels were similar to pre-diet (pre-diet 2.8±1.1%, postpartum 3.2±1.4%, p=0.48).

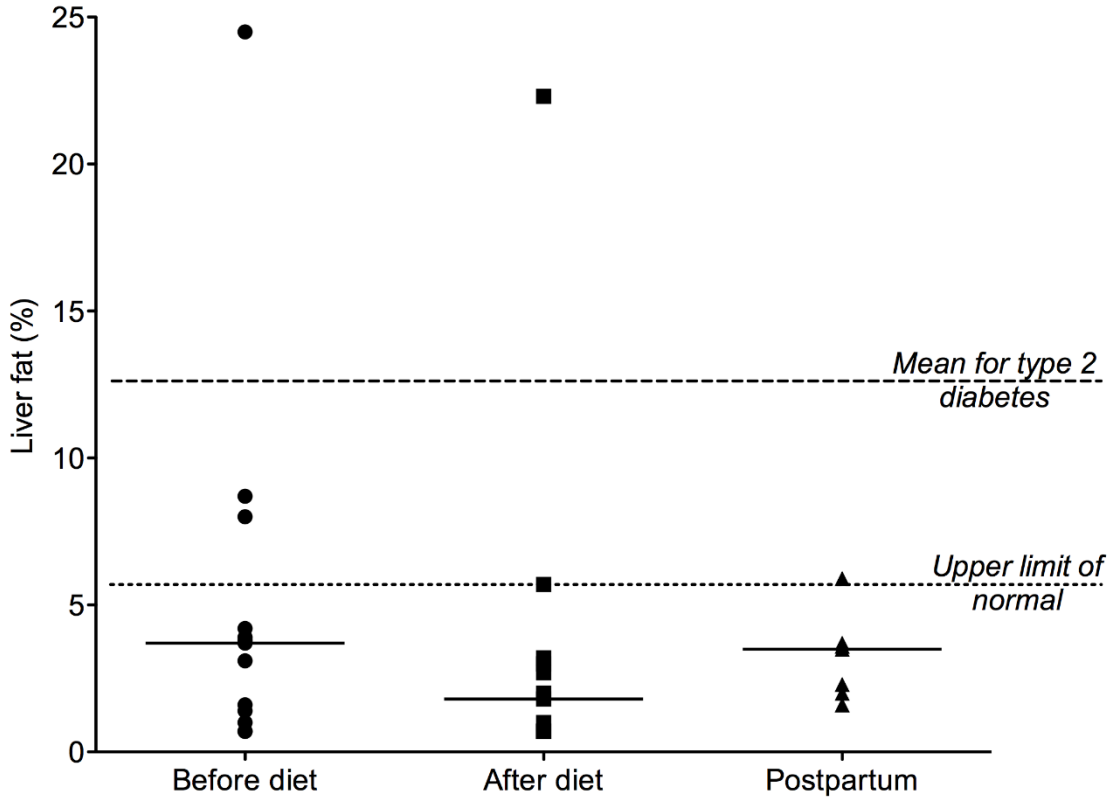

Figure 29 Percentage liver fat before (circles) and after (squares) dietary intervention and postpartum (triangles). The bar shows the median change in percentage liver fat. The upper limit of normal range and mean percentage fat content in T2DM are shown. Difference between before and after diet (Wilcoxon Signed Rank) p=0.021. Difference between pre-diet and postpartum p=0.48**.**

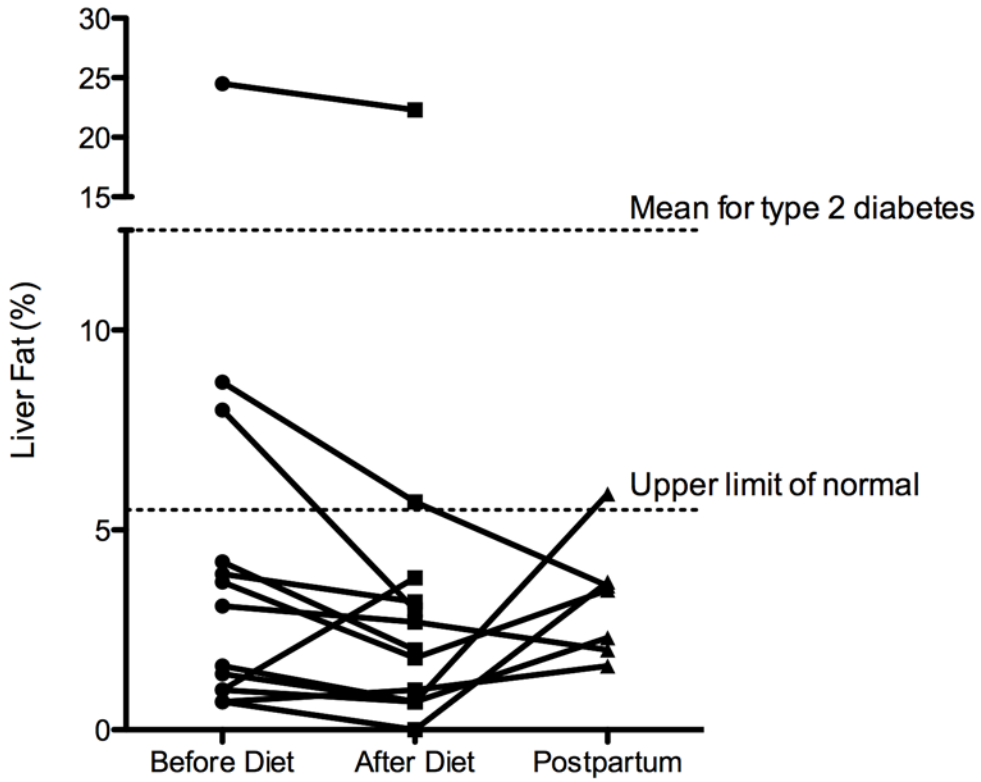

Figure 30 Liver fat percentage before and after a 1,200kcal dietary intervention and postpartum. Lines link the same subject before diet, after diet and postpartum.

4.3.5 Standardised meal test

Fasting plasma glucose remained unchanged after the dietary period (4.3 ± 0.2 mmol/l vs 4.3 ± 0.1 mmol/l, p=0.49). HOMA2, a reflection of insulin resistance in the fasted state, was similar before and after dietary intervention (1.3 ± 0.1 vs 1.4 ± 0.1, p=0.47), however HOMA2 was lower postnatally 1.1 ± 0.1 ($p<0.01$). The postprandial glucose concentration curve was similar before and after dietary intervention (**Figure 32**). Before intervention, glucose concentrations peaked at 60 mins 8.1 ± 0.3mmol/l before the diet and 8.4 ± 0.4mmol/l after diet. There was no statistically significant difference between fasting insulin and C peptide before and after the diet (insulin: 38.4 ± 5.2 vs. 48.3 ± 6.2pmol/l, p=0.12; C peptide: 0.61 ± 0.05 vs. 0.65 ± 0.05nmol/l, p=0.36). Acute insulin secretion did not change during the standard meal test after the diet (Φ_{total} 62.1 ± 4.9 vs 58.4 ± 3.4; $x10^{-9}$/min, p=0.51). Insulin concentrations peaked at 60 mins (before diet 493 ± 41 pmol/l vs after diet 495 ± 63 pmol/l). C peptide levels peaked at 90 minutes before diet 3.0 ± 0.2 nmol/l and at 120mins after the diet 3.7 ± 0.2 nmol/l. There was no change in post-meal insulin sensitivity (S_I) before and after diet (9.9 ± 1.1 vs 9.3 ± 1.4 $x10^{-4}$dl/kg/min per uU/ml, p=0.54).

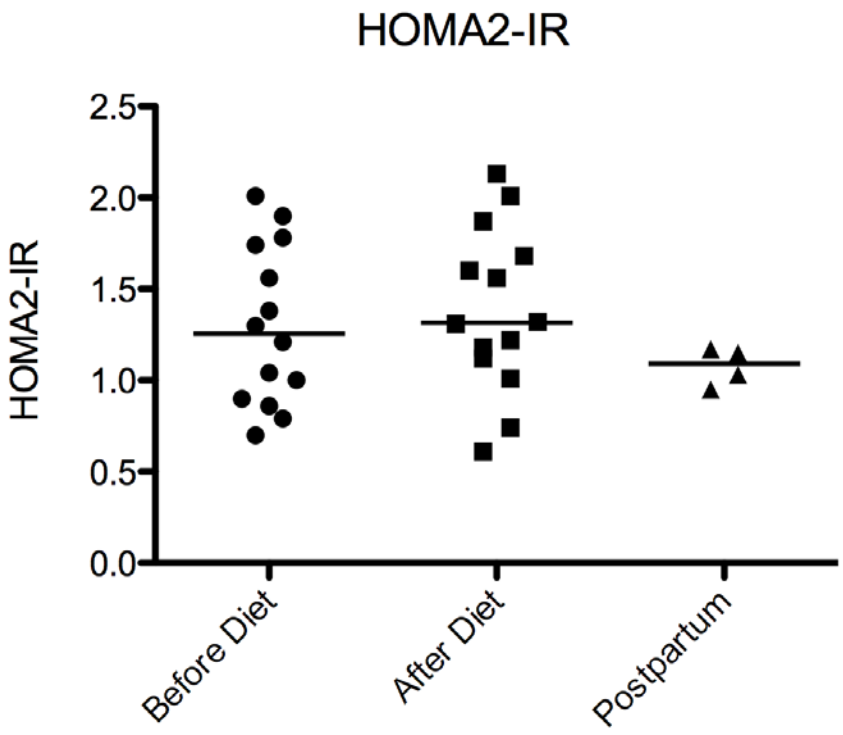

Figure 31 Homeostatic model of insulin resistance 2 demonstrating insulin resistance before and after diet. Bars represent mean values. Student's T-test; difference between before and after diet (p=0.47); difference between before diet and postpartum (p=0.1).

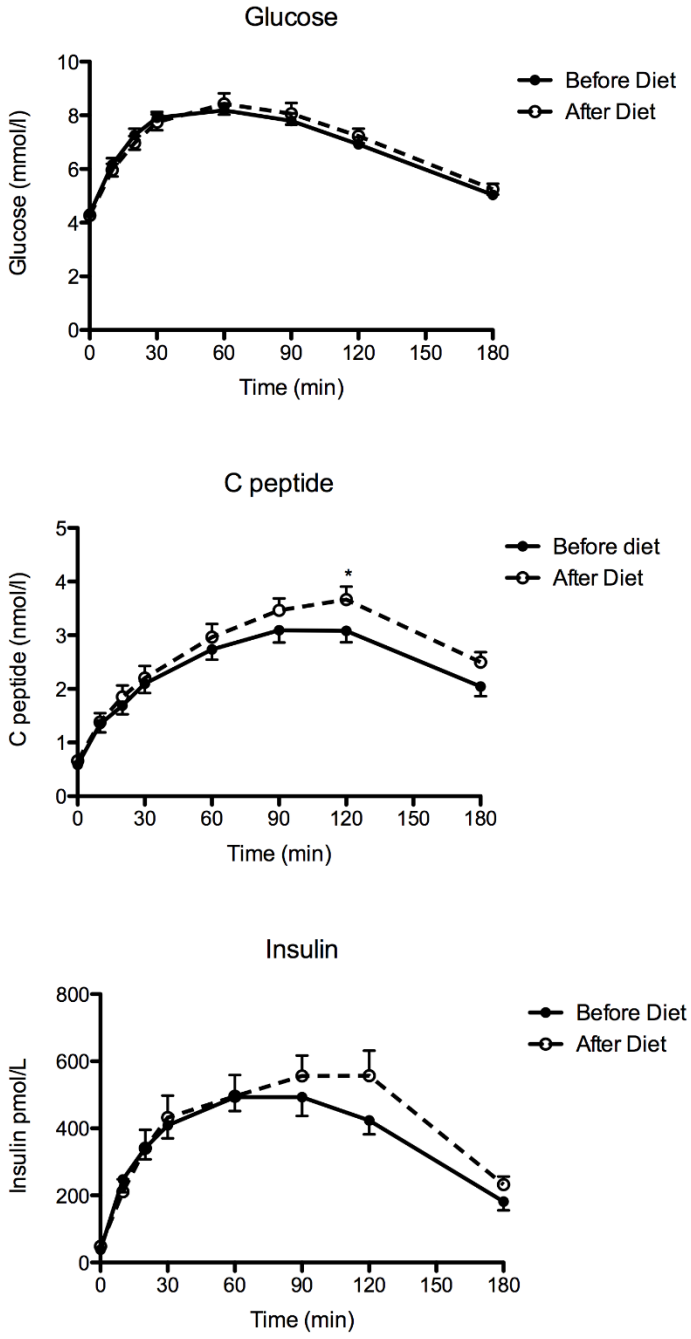

Figure 32 Glucose, insulin and C peptide curves following a standardised meal test. All points non-significant except *p<0.05 (Student's T test).

4.3.6 Lipid Profile

Lipid profiles before and after the diet and during the postnatal period are shown in **Figure 33.** The raised plasma triglyceride of pregnancy was not changed by the hypocaloric diet. Similarly, there was no difference in high density lipoprotein (HDL) and non-HDL cholesterol levels before and after the diet during pregnancy. Mean triglyceride and cholesterol levels fell after delivery ($p<0.02$ for both).

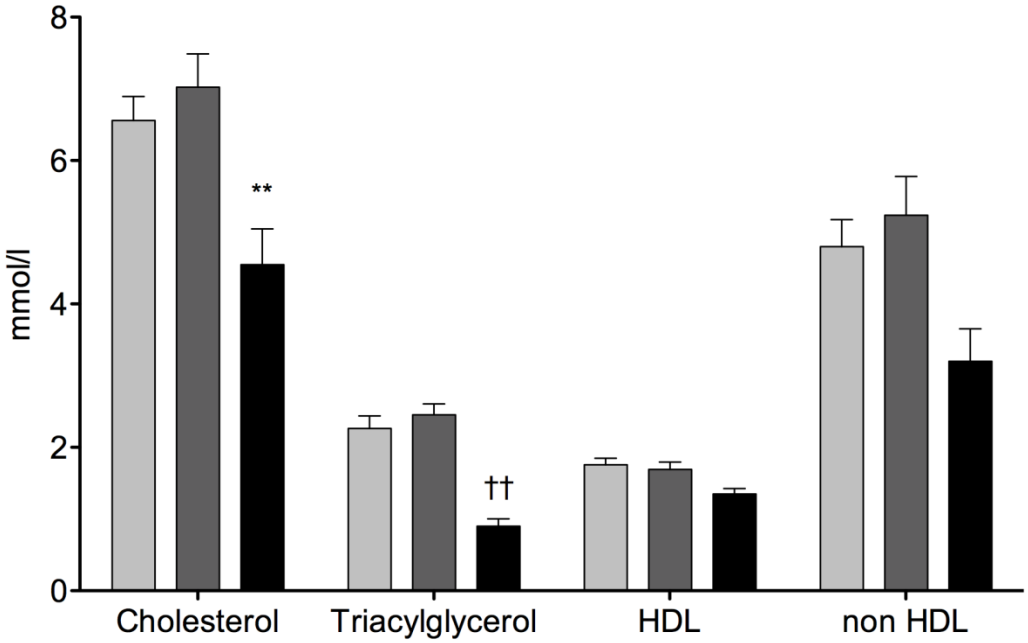

Figure 33 Lipid profile before (light grey bars), after (dark grey bars) and postpartum (black bars). ** and † †indicate $p<0.01$ pre- and post-diet vs postpartum (Student's T test).

4.3.7 Glucose control

None of the hypocaloric diet group required insulin therapy compared with 6 of the 26 women in the comparator arm. Two of the diet group required metformin therapy (weight loss 0.3kg and 1.2kg) compared with a total of 8 of the comparators (**Table 6**), six women requiring both metformin and insulin in the comparator population.

Despite the pharmacotherapy, home blood glucose monitoring during the 4 week intervention period showed identical mean levels for diet and comparator groups (fasting

4.9 ± 0.6 vs. 4.9 ± 1.0; post-prandial 6.6 ± 0.8 vs. 6.6 ± 0.9 mmol/l). Mean HbA_{1C} did not change (5.2 ± 0.1% [34 ± 1.0mmol/mol] vs 5.3 ± 1.1% [34 ± 1.3mmol/mol], p=0.89).

4.3.8 Maternal and Fetal Outcomes

All of the dietary intervention group women expressed positive thoughts about the experience of decreasing calorie intake during pregnancy. There was no difference between mode of delivery between subjects and comparators. No women had shoulder dystocia or third degree tear. No difference in the rate of increase in fetal abdominal circumference was observed between diet and comparator groups; **Figure 34**. There was also no difference in the rate of fetal abdominal growth between subjects with greater than 2kg weight loss (11.3 ± 0.4 mm/wk), subjects with less than 2kg weight loss (10.4 ± 0.2 mm/wk) and comparators (10.9 ± 0.2 mm/wk). One subject and four comparators had a fetus with an abdominal circumference greater than 97th centile after 34 weeks of pregnancy. There was no difference in birthweight between subjects and comparators; **Table 6**. One baby in the study group was admitted to SCBU for chylothorax, detected at 36 weeks gestation. No babies had neonatal hypoglycaemia.

	Age	Weight (kg)	Height (cm)	BMI	Wt loss (kg)	Parity	MOD	Birth weight (kg)	Treatment
1	34	94	162	36	2.8	2	ElLSCS	3.355	
2	25	94.7	156	39	0.3	1	ElLSCS	3.100	Metformin
3	31	94.7	165	35	1.2	0	EmLSCS	3.330	Metformin
4	37	109.7	172	37	5.6	2	SVD	3.740	
5	31	112.6	168	40	3.3	0	EmLSCS	2.970	
6	32	114.1	166	41	+0.4	0	SVD	3.570	
7	24	82.5	155	34	+0.5	0	SVD	3.570	
8	24	105.6	169.5	37	2.5	0	Forceps	3.660	
9	30	76	168	27	1.1	1	SVD	2.920	
10	39	81.6	168	29	2.6	1	SVD	3240	
11	34	77.1	154	33	2.1	0	SVD	3.500	
12	34	96.8	171	33	+0.2	1	SVD	3.600	
13	36	72.3	155.5	30	1.1	0	ElLSCS	3.290	
14	31	91.3	162.5	35	1.2	0	Forceps	3.595	

Table 6 Maternal and fetal outcomes from dietary intervention study

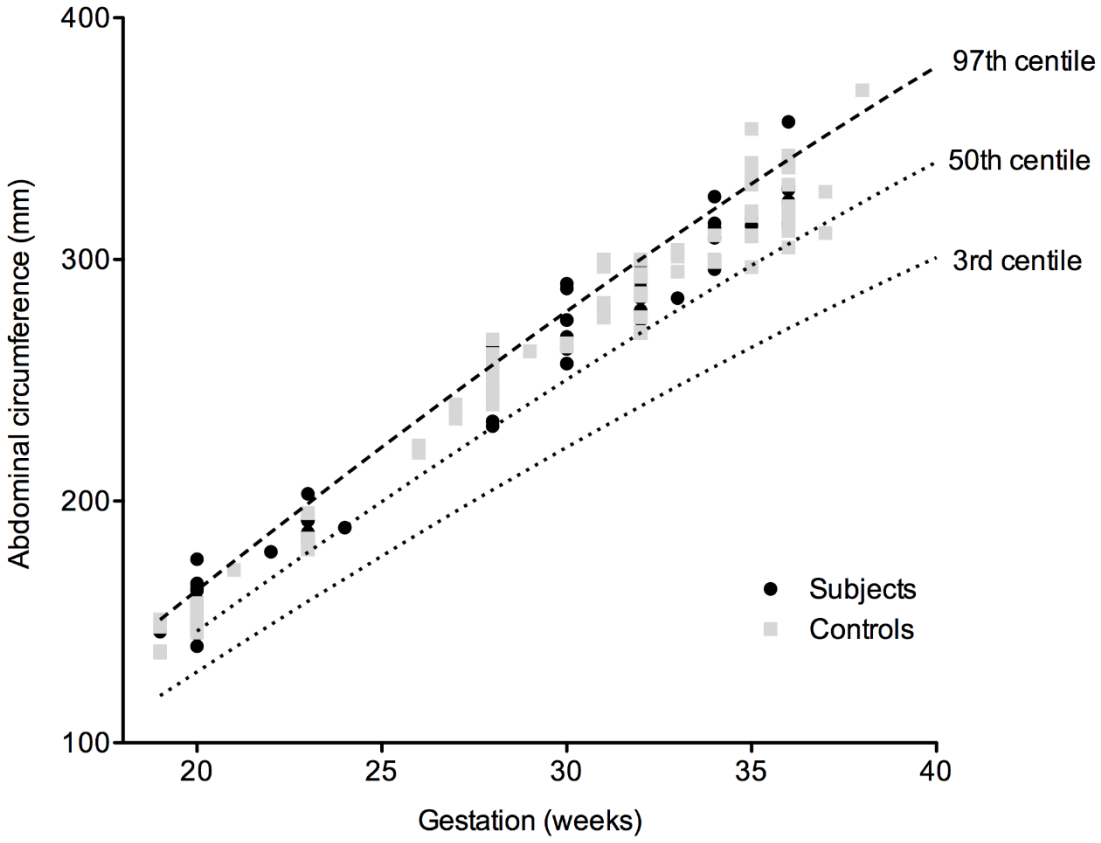

Figure 34 Fetal abdominal circumference

4.3.9 Qualitative Study

Dietary intervention was well accepted by the participants, many of whom felt the diet was something they "had to do" for the health of their baby.

"I just thought right this is what I've got to do and even, I think it's more of an incentive because I was doing it for the health of my baby, I wasn't just doing it for me."

"I had to do it for, for my baby. You know. So I think that helped a lot as well"

They had no concerns about calorie restriction causing harm, all anxieties surrounded the possible impact of GDM on the fetus.

"when KH was talking about the effects of gestational diabetes on the baby, I was like, do you know what, if I can reduce the effects of that and stop that from happening I would much rather do a study like this to prevent it".

Women felt a responsibility to try to reduce risks. Other motivating factors included medication avoidance, controlling weight, and cutting down future T2D risk. Being monitored and wanting to provide reliable results also provided incentives.

"I've got diabetes, you know, it's a bit disappointing and then as soon as I got the plan and I got into it and I got used to like the food and the portions and things like that I was just like oh yeah I've lost another three pounds this week you know, I felt, I felt great".

The women felt well supported by the research team. Social support from family and friends was also important; involvement in the diet by partners was especially helpful.

"taking part in the study getting the extra scans, getting the extra blood tests, having the phone calls all the time, them checking up on us, so that, that's been a good thing, so. I would, if someone said to us would you do it again, I would say yes, I would. Though it was tough I would still probably do it again".

Women reported having more energy, better sleep and feeling fitter. They were glad they had participated and reported learning valuable lessons that they would continue to use.

"I actually felt quite positive about it. I felt I was doing a really good job and that it was just helping my baby, so I was really pleased. I didn't feel down at all".

In conclusion, women with GDM recruited into the WELLBABE study were willing and able to reduce calorie intake if provided with adequate information, support, monitoring and reassurance.

4.4 Discussion

This study shows that GDM is not characterised by supra-normal liver triglyceride levels. Dietary intervention did bring about weight loss of 0.4 kg/week, in comparison to weight gain of 0.3 kg/week in controls and was associated with a halving of liver triglyceride content. Surprisingly, there was no change in insulin sensitivity following dietary intervention, nor was there any significant change in insulin secretion. Glycaemic control on

the diet was similar to the control group despite less medication and no insulin therapy to maintain glucose within the range 4-8 mmol/l.

The observation of normal liver triglyceride levels was unanticipated from review of the literature. Previous studies have demonstrated that non-pregnant women with a prior history of GDM have elevated liver triglycerides (Tiikkainen *et al.*, 2002; Forbes *et al.*, 2013) and a greater risk of non-alcoholic liver disease in later life (Ajmera *et al.*, 2016). Given that excess intrahepatic triglyceride is an important underlying factor in the development of T2DM, with average levels of 12 ± 2.4% (Shibata *et al.*, 2007; Taylor, 2013), it was anticipated that increased fat would be observed in women with newly diagnosed GDM. Furthermore, raised liver triglycerides are associated with elevated plasma triglycerides in T2DM, and plasma triglyceride levels are increased in GDM pregnancy (Forbes *et al.*, 2013). Abnormal lipid metabolism appears to have a key role in GDM (Barrett *et al.*, 2014).

The present data are the first *in vivo* magnetic resonance liver triglyceride readings to be reported in human pregnancy. The observation that liver triglyceride is apparently normal in the majority of participants raises the possibility that liver triglyceride may in fact decrease in pregnancy, despite the well-recognised increase in plasma triglyceride. Levels that are otherwise normal in the non-pregnant state may be associated with GDM. It is interesting to note that liver triglyceride, but not plasma triglyceride, decreased following dietary intervention. This is likely to reflect the physiological increase in plasma triglyceride after the first trimester and change in nutritional status is unlikely to change this requirement. It is possible that liver fat levels fall reciprocally with elevation in plasma triglyceride. This is consistent with the observed association between liver steatosis (as determined by liver ultrasound) in the first trimester and subsequent risk of GDM (De Souza *et al.*, 2016) and the association between raised alanine transaminase (ALT) and GDM (Yarrington *et al.*, 2016). In contrast to this study, De Souza et al showed an association between increased liver fat and GDM. However, women were scanned at an early gestation between 11 and 14 weeks, before the onset of either raised plasma triglycerides or significant insulin resistance. We scanned later in pregnancy when liver fat may have been mobilised as plasma triglyceride. This is further substantiated by the fact that liver triglyceride fell after dietary intervention, but plasma triglyceride remained the same. The present study did not observe any marked change in liver triglyceride after pregnancy, although it is not known how long it may take for liver triglyceride levels to return to pre-pregnant levels. This would need to be evaluated in a further study.

A criticism of the study may be that the level of dysglycaemia was relatively low, although the baseline tests were necessarily carried out several days after diagnosis and it was not feasible to withhold advice to decrease sugar consumption, which could have decreased baseline levels. The study population does not represent the wider GDM population given the predominantly Caucasian population of the North East of England and the selection criteria for nulliparous women. Age and BMI were similar to those of participants in other larger studies (Crowther *et al.*, 2005; Landon *et al.*, 2009a). HbA1c levels at the time of diagnosis of GDM are not expected to be elevated due to the relatively recent rise in blood glucose levels. Indeed, HbA1c levels at diagnosis in the study group were the same as those seen in the Newcastle GDM database (5.4 ± 0.1% [36 ± 0.1 mmol/mol] vs 5.4 ± 0.1% [36 ± 0.4 mmol/mol]).

The time course of return of normal first phase insulin secretion for people with T2DM during a very low calorie diet has been defined (Lim *et al.*, 2011a). Even at a lower intake of 2.5-3.3 MJ (600-800 kcal)/day, eight weeks was required for normal insulin secretion to be restored, and at four weeks the improvement was modest. The present study necessarily used a less severely restricted diet of 5 MJ (1200 kcal)/day, and as a first step this was advised for four weeks only. The lack of change in insulin secretion following the test meal is therefore not unexpected. Further work is required to establish whether the insulin secretory abnormality in GDM (Saisho *et al.*, 2010), being of short duration, is more readily reversed than that of T2DM.

Dietary weight loss during pregnancy is viewed with caution by many obstetricians, even though obesity is a major risk factor for macrosomia and associated adverse outcomes. The benefits of minimising weight gain during pregnancy in the present era of steady weight gain during adult life were first reported several years ago (Bain *et al.*, 2015). This is especially relevant in GDM (Garner *et al.*, 1997; Crowther *et al.*, 2005; Landon *et al.*, 2009a). A clear decrease in calorie intake has been achieved on a whole clinic basis by Asbjornsdottir and colleagues who achieved decrease in median weight gain during pregnancy from 12.1 to 3.7kg (Asbjornsdottir *et al.*, 2013). This was associated with decrease in large for gestational age infants (39% to 12%) and perinatal morbidity (71 to 35%). At the time when GDM is diagnosed there is likely to be increased motivation to decrease calorie intake. All 14 women who completed the study reported that they were comfortable with the explanation of likely benefit for their baby. The present study is unique in demonstrating effectiveness and acceptability of advising modest weight loss at the time of diagnosis of GDM. It lays the

foundation for a prospective randomised study of dietary weight loss from the time of diagnosis of GDM.

The limitations of this study must be considered. Although small numbers of women were studied, their characteristics were similar to that of other larger studies of GDM. The study was large enough to demonstrate a statistically significant difference in weight loss/gain between intervention and comparator groups. As women were advised of the diagnosis of GDM and the aims of the study at a clinic visit several days before the baseline measurements for the study, the baseline necessarily reflects an initial dietary intervention. It was noted that mean fasting glucose fell from 5.0 to 4.3 mmol/l in the short time period between OGTT and baseline measurements. Avoidance of insulin therapy is associated with major benefit in simplifying peri-partum obstetric management as well as minimising weight gain, personal inconvenience and use of healthcare resources. Even so, most women were in the lower range of plasma glucose for diagnosis of GDM and it will be important to study women with higher presenting blood glucose levels.

The present study defines an important question of liver triglyceride physiology in normal and GDM pregnancy, and provides data to inform design of further studies. Additionally, there is a need for a prospective randomised therapeutic study of dietary weight loss from the time of diagnosis of GDM.

CHAPTER 5 COMPARISON BETWEEN NORMAL PREGNANCY AND GDM

As the meal test component of the LIPIDPREG and WELLBABE studies use the same methodology, it is possible to compare the glycaemic profile of women with GDM and those with normal glucose tolerance (NGT). It is important to acknowledge that there are differences between the two groups beyond the presence or absence of GDM (such as BMI and differences in gestation between the two studies) and so the first group of women are not a true control group for the second. However, there are interesting observations that can be made when the two groups are compared and these are worthy of further discussion.

5.1 Glucose metabolism

The postprandial glucose curve is shown in **Figure 35**. Fasting blood glucose was higher in women with GDM compared to the NGT group (4.3±0.1 vs 3.8±0.1mmol/l; p=0.03). The non-pregnant group have the lowest postprandial glucose concentration curve. After 30 minutes plasma glucose concentrations are, on average, 1.5mmol/l higher in the GDM compared to the normal pregnant group (p<0.02 for all values beyond 30 minutes).

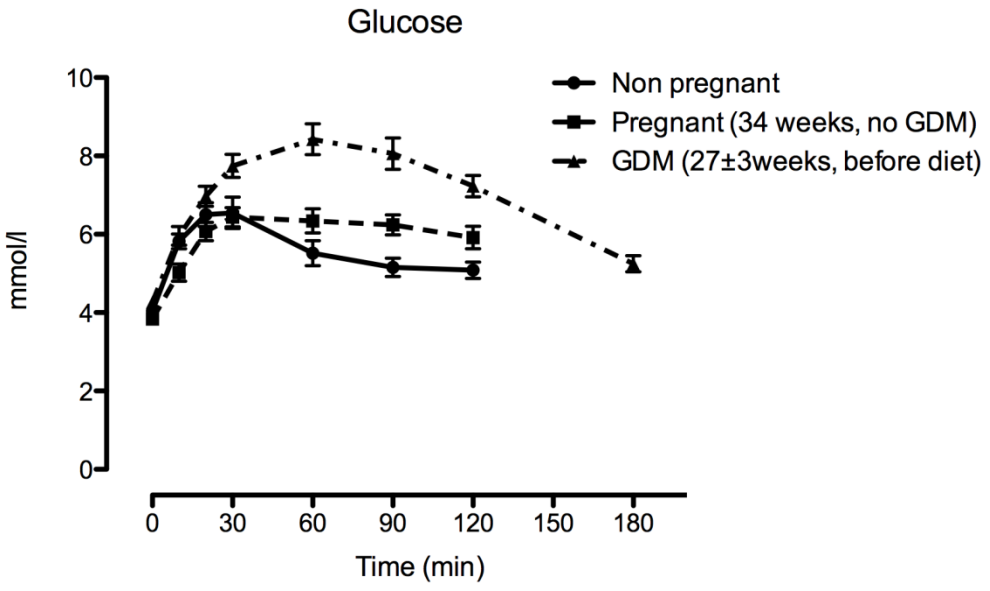

Figure 35 Postprandial glucose curve following standardized meal test. Non-pregnant vs NGT vs GDM.

5.2 Insulin Resistance

5.2.1 Homeostatic Model of Insulin Resistance (HOMA)

There was a significant difference between fasting insulin resistance (HOMA-IR) in the non-pregnant and the NGT and GDM groups (**Figure 36**). There was no difference in HOMA-IR between the GDM and the NGT groups (1.3 ± 0.2 vs 1.1 ± 0.2; p=0.8).

5.2.2 Insulin Sensitivity (Standardised meal test)

There was a four-fold increase in insulin sensitivity in the non-pregnant state. The GDM group at 27 weeks gestation, had comparable insulin sensitivity to the NGT group at 34 weeks gestation (1.3±0.3 vs 1.6±0.18; p=0.25) (**Figure 36**).

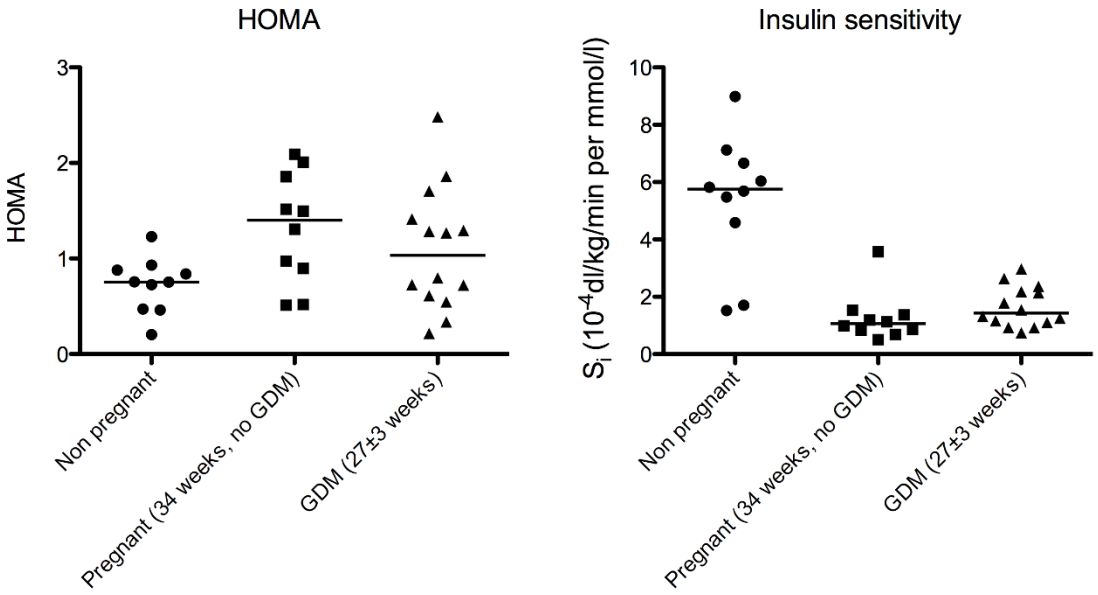

Figure 36 Differences in HOMA (left graph) and Si (right graph) between non-pregnant women, pregnant women (34 weeks with no GDM) and women with GDM (at approximately 27 weeks). Bar represents mean values.

5.3 Insulin secretion

Plasma insulin levels were lowest in the non-pregnant group, where insulin concentrations peaked at 30 minutes. In both pregnant groups (GDM and NGT), insulin peaked later at 60 minutes. Insulin secretion was higher in the NGT group compared to the GDM group and this was statistically significant at 30 and 60 minutes ($p<0.05$)(**Figure 37**). Total insulin secretion during the meal test was (area under curve): non-pregnant 23,752, pregnant with NGT 65,409 and GDM 50,196. Women with GDM had a 23% reduction in insulin secretion during the meal test compared to pregnant women with NGT.

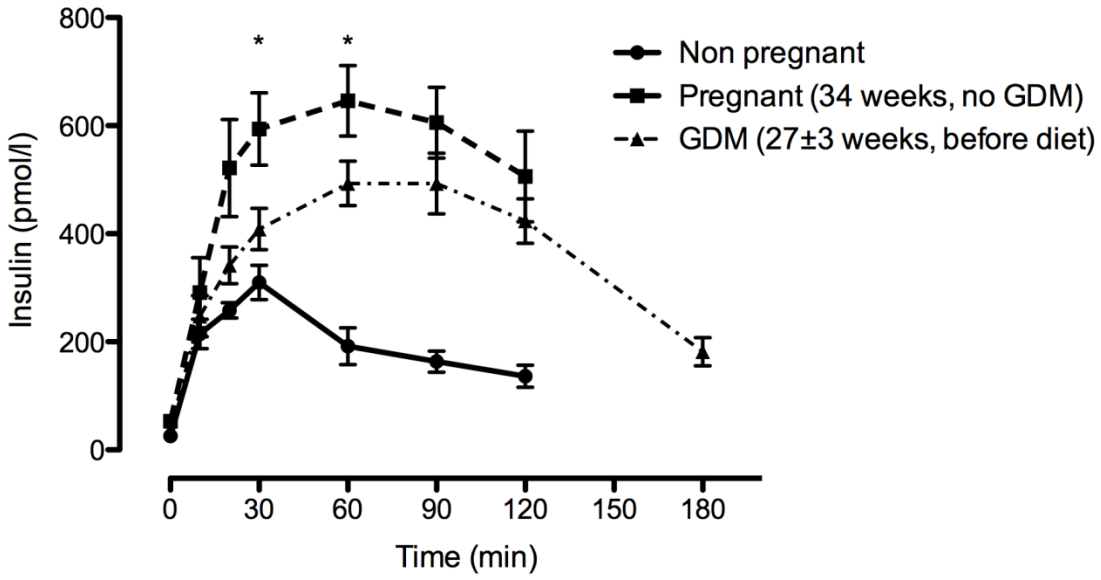

Figure 37 Insulin secretion curve following a standardised meal. Non-pregnant vs. NGT vs GDM.

Following dietary intervention the area under the insulin secretion curve increased, but was not significantly greater (approximately 8%) this is particularly noticeable from 90 minutes onwards (**Figure 38** – grey line).

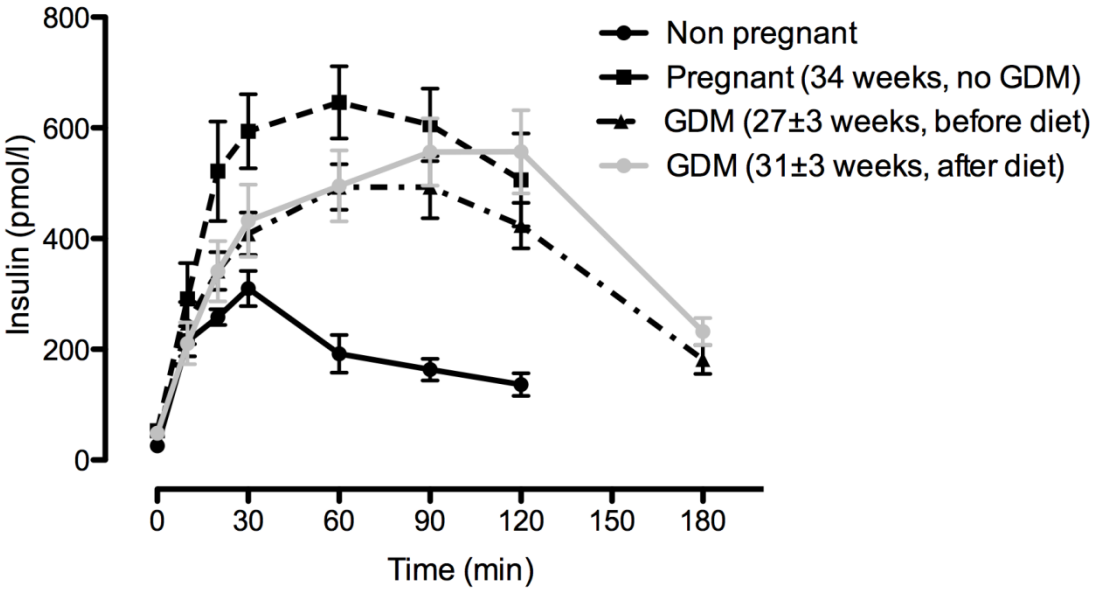

Figure 38 Insulin secretion curve following a standardised meal. Non-pregnant vs. NGT vs. GDM (before diet) vs. GDM (after diet – light grey line).

5.4 Discussion

At the earlier gestation of 27 weeks, blood glucose levels in women with GDM were approximately 1.5mmol/L higher than those with NGT at 34 weeks. Despite higher blood glucose levels, measures of insulin resistance between GDM and NGT women were similar. However, women with NGT had higher insulin secretion than those with GDM and this may explain the difference in blood glucose levels between the two groups.

Although it might be anticipated that women with GDM would be more insulin resistant than those with NGT, in actual fact there appeared to be no difference in insulin sensitivity between GDM and NGT groups; despite higher levels of blood glucose in the GDM group during the meal test. Although previous studies have shown that women with GDM have greater insulin resistance compared to matched controls with NGT, there is evidence that the difference may not be as great as perhaps anticipated. Catalano performed clamp studies in two groups of BMI matched women (lean and obese) with and without GDM (Catalano *et*

al., 1993; Catalano *et al.*, 1999). During pre-pregnancy and early pregnancy, insulin sensitivity varied considerably between NGT and GDM groups (**Figure 39**). However, by late pregnancy the difference was less marked and in the lean population there was hardly any difference in insulin sensitivity at all (**Figure 39**, **blue arrow**).

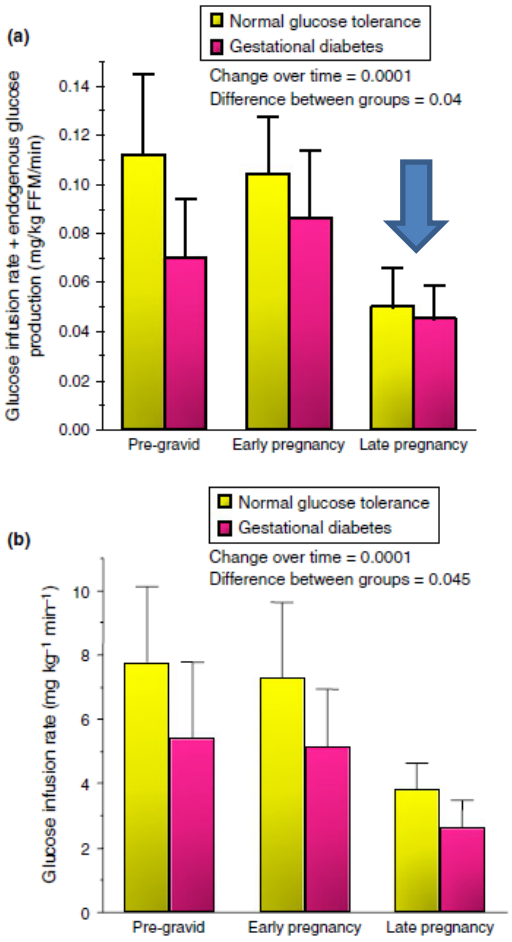

Figure 39 Longitudinal changes in peripheral insulin sensitivity in (a)lean women and (b)obese women as indicated by infusion of glucose required to maintain euglycaemia (90 mg/dl) + endogenous glucose production during insulin infusion (mean ± SD). Blue arrow shows the difference in insulin sensitivity in late gestation.
Reproduced from (Catalano, 2014)

In the WELLBABE study, women with GDM secreted 23% less insulin during the meal test compared to women with NGT in the LIPIDPREG study. The first phase insulin response (the insulin response from fasted to 10mins following food ingestion) were similar for both groups (insulin increased by approximately 200pmol/l). However, second phase insulin release was relatively impaired in GDM compared to NGT groups. This corresponds with

Catalano's work, who observed that first phase insulin responses in both NGT and GDM groups were similar, however his observation was of greater insulin secretion in GDM although this was still inadequate to meet the demands of insulin resistance imposed (Catalano *et al.*, 1999).

In the WELLBABE study the second phase insulin response was dampened in comparison to women with NGT in the LIPIDPREG study. Although this may reflect the difference in gestation between the two groups, it may also be a genuine observation. GDM has been described as a 'relative beta-cell deficit' (Buchanan, 2001). Women with GDM are unable mount the same insulin response to a given reduction in insulin sensitivity as women with NGT, as summarised in **Figure 40.** This is in keeping with the observation from the WELLBABE study where a fall in insulin sensitivity was accompanied by a seemingly inadequate rise in insulin secretion.

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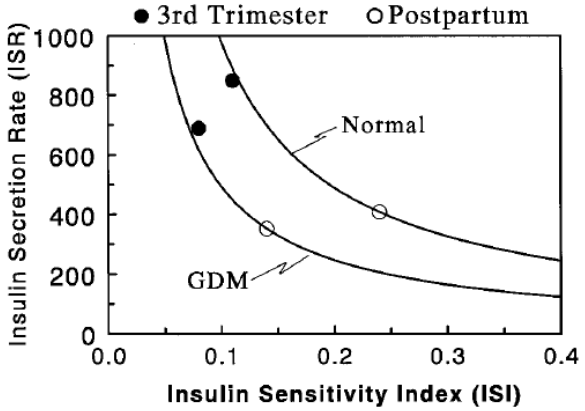

Figure 40 Beta-cell response to insulin resistance in pregnant women with normal glucose tolerance and GDM. As insulin sensitivity falls in pregnancy (open circle to black circle), insulin secretion increases proportionally. In GDM the beta cell response is less than NGT (lower curve) for any given change in insulin resistance (Buchanan, 2001).

T2DM only occurs when beta function becomes impaired (Ferrannini *et al.*, 2004; Cali *et al.*, 2009; Tabak *et al.*, 2009). Increased fat storage within the pancreas and the resultant effect of chronic excessive fatty acids on the beta cells inhibits the insulin response to glucose (Carpentier *et al.*, 2003; Kashyap *et al.*, 2003). Previous work from Newcastle University has shown that low calorie dieting and substantial weight loss results in mobilisation of fat from

the pancreas and resolution of first-phase insulin secretion to normal (Lim *et al.*, 2011b; Steven *et al.*, 2016a). In the WELLBABE study, dietary intervention resulted in a small improvement in insulin secretion in women with GDM. Given that the dietary intervention was less extreme (1,200kcal as opposed to 800kcal/day for the Lim et al study) and for a shorter time period (4 weeks vs. 8 weeks) any improvement in insulin secretion is likely to be less dramatic and may not have been seen with the small number of participants included in the WELLBABE study. Further studies are needed to examine the pancreas in pregnancy. Specifically, these studies should determine pancreatic function and the relationship between its size and composition (fat content) in normal pregnancy and GDM.

CHAPTER 6 DISCUSSION

6.1 Introduction

These studies take forward knowledge of the physiology of insulin resistance in normal pregnancy and introduce novel data on intramyocellular lipid and hepatic fat deposition. There was a four-fold decrease in insulin sensitivity during normal pregnancy and a two-fold increase in serum triglycerides. However, this was not secondary to changes in intramyocellular lipid concentrations. In other insulin resistance states changes in muscle fat stores with decreased muscle uptake of glucose is the first detectable change to occur (Warram *et al.*, 1990). This effect was not observed in normal pregnancy and leads to the conclusion that the insulin resistance of pregnancy is mediated through a different mechanism. It appears likely that hormonal factors are causative.

In GDM, insulin sensitivity was reduced as expected, however median liver fat stores were within the normal (non-pregnant) range. Given the similarities between GDM and T2DM it was expected that liver fat levels would be elevated as they are in T2DM (mean liver fat content is approximately 13% (Lim *et al.*, 2011b; Steven *et al.*, 2016b)). It is interesting to note that, despite third trimester liver fat levels within the normal range, hypocaloric dieting was associated with a further reduction in liver fat. The lack of control group prevents distinction between the effect of dietary intervention and the effect of progressive gestation on liver fat. Although the diet had little effect in terms of glucose disposal, insulin sensitivity and insulin secretion, dietary intervention did result in a reduced need for metformin and/or insulin treatment when compared to a 'standard antenatal care' group who had standard dietary intervention (one consultation with an NHS dietitian and a leaflet). This is an interesting observation and requires to be followed up in larger randomised controlled trials of hypocaloric dieting after diagnosis of GDM.

Both studies are novel in their use of 3 Tesla magnetic resonance techniques to study metabolism *in vivo* during pregnancy. These studies were well tolerated by the participants and no adverse effects were reported.

Calorie restriction during pregnancy is considered controversial. For this reason, dieting to the level of 1,200 kcal/day or below has not previously been extensively studied, most

studies employ either a very modest reduction in calorie intake or are very general in terms of simply recommending 'healthy eating' and/or exercise. Our dietary intervention was more engaging than most, with full explanation of the reasons for the hypocaloric diet and employing smartphone technology and social media to maintain enthusiasm and adherence to the diet. Clearly, the delivery of health/diet plans is a key feature to their success.

From this work we gain better understanding of the practicalities of studying metabolism in pregnancy, new insights into the metabolism of normal and GDM pregnancy, a better understanding of the delivery of calorie reduction in pregnancy and experience in delivering calorie restriction/dietary intervention to pregnant women with GDM.

6.2 Studying Metabolism During Pregnancy

6.2.1 Recruitment

Pregnant women are notoriously under-represented in research (Baylis, 2010). This is often through fear of doing harm to the baby, or that it is somehow 'wrong' to use an experimental research design on pregnant women. Yet this approach means that as a society we lack good, effective, evidence-based treatments for diseases such as gestational diabetes that affect a significant proportion of women and cause a substantial amount of maternal and neonatal morbidity.

This research project has been planned with the pregnant woman at the focus. Both studies were carefully designed in order to make recruitment and participation in the study as easy as possible. For example, in the recruitment phase of both studies, KH spoke with women directly. For the LIPIDPREG study, this was done during antenatal classes and in the dietary study this was done at first clinic visit following the diagnosis of GDM. Meeting a member of the research team personally meant that the women could gain better and more detailed information about the study and also gave them an opportunity to build a relationship with the researcher from an early stage, helping to build trust and confidence.

As a result, recruiting for the LIPIDPREG study was easier than anticipated and occurred over approximately four cycles of antenatal classes. Recruitment into the WELLBABE study was harder, but still achievable within an eight-month period. Approximately one in four women approached for WELLBABE were recruited. Reasons for non-participation included a perceived difficulty in maintaining 1,200kcal diet, time pressures and family/work

commitments. Interestingly, fear that dieting or magnetic resonance may harm the baby was not cited as a reason for non-participation. Both studies involved at least two morning sessions at the Newcastle Magnetic Resonance Centre, which is a relatively substantial time commitment. Once recruited, drop-out rates from the studies were low (no-one dropped out of the muscle study and only two women dropped out of the dietary study) reflecting the good relationship between the researcher and the participant.

If hypocaloric dieting is confirmed as an effective management for GDM both the provision of information and the way in which it is delivered will be critical to its success as an intervention. The benefits of weight loss in pregnancy have been established to include: a reduction in the incidence of GDM, pre-eclampsia, gestational hypertension and pre-term birth with no effect on fetal growth (Thangaratinam *et al.*, 2012). A change in public health message is required in order to promote weight loss and dietary intervention during pregnancy and to educate women that it is both safe and beneficial to them and their baby.

6.2.2 Magnetic Resonance

Prior to the LIPIDPREG study, published in 2013, there were very few studies utilizing magnetic resonance at 3 Tesla. Almost all magnetic resonance for clinical and research purposes was reported at 1.5 Tesla. Whilst there is no known biological effect of scanning, even up to very high magnetic field strengths (Schenck, 2000; Shellock and Crues, 2004), using 3 Tesla in pregnancy was novel. Scan protocols were developed and modified by the magnetic resonance physicists at the Newcastle Magnetic Resonance Centre. Women were scanned at a slight pelvic tilt (to offset the weight of the gravid uterus, enabling venous return) and to minimise noise. Care was taken to avoid local power deposition (SAR) which could theoretically cause a rise in temperature. The radiographers were sympathetic to the need for extra reassurance and time to complete the magnetic resonance studies.

Magnetic resonance was well tolerated by the participants, as indicated by the low drop-out rates and positive comments received from the participants following the scans. Only one subject developed claustrophobia in the scanner and could not complete the study. All babies were born in good condition without any evident congenital abnormality, except one baby born with a chylothorax. It is unlikely that this was related to magnetic resonance since the baby had an increased nuchal translucency at 11 weeks gestation and prior to magnetic resonance scanning. The increased nuchal translucency (with normal karyotype) reflects

abnormal lymphatic drainage at an early stage which became more apparent as the pregnancy progressed. All babies passed their neonatal hearing tests.

These two studies, whilst small in size, add to the previously absent literature of the safety and acceptability of 3 Tesla magnetic resonance in pregnancy.

6.2.3 Dietary Intervention

The 1,200kcal/day diet required careful explanation both at the recruitment stage and during delivery of the intervention. The ability of the body to mobilise fat and carbohydrate in order to prioritise fuel delivery to the fetus was explained. It was explained that, despite being low in calories, the diet would contain all the micronutrients that are essential for pregnancy and the developing fetus. Women were encouraged to discuss the diet with their partner and family, since it is known that peer-support can have a significant effect on dietary compliance (Gruber, 2008; Balantekin *et al.*, 2014; Pratt *et al.*, 2015). Several women brought their partners along to the study and in two cases the partners were also overweight and participated in the 1,200kcal/day diet. These women lost the most amount of weight.

"My husband was with us and we both instantly said that we both wanted to do it and I said I was more than happy to try it if it helped me and if the research helped other people then I was more than happy to do it."

The input from an experienced dietitian was crucial to the development of the diet and to give women the reassurance that the diet was safe. Through putting a sample diet through software (WinDiets version 8, Aberdeen UK), the macro and micronutrient breakdown of a 'typical' 1,200kcal diet was analysed to ensure that it was nutritionally balanced and contained all the necessary trace elements (Appendix D). As a result, the diet was modified slightly for pregnancy to increase calcium intake through a greater number of dairy food portions.

Regular communication through MyFitnessPal, text messaging and telephone calls were an essential component of the dietary intervention. This received positive feedback from participants during the semi-structured interviews.

"Taking part in the study getting the extra scans, getting the extra blood tests, having the phone calls all the time, them checking up on us, so that, that's been a good thing, so. I would, if someone said to us would you do it again, I would say yes, I would. Though it was tough I would still probably do it again".

The dietary plan was successful in most participants. Only two put on weight and in both cases this was less than 0.5kg. Given that weight gain at this stage in pregnancy is 0.5kg/week (Butte *et al.*, 2003), even this small amount of weight actually represents a net weight loss, compared with the average gain, of 1.5kg over the four-week dietary period. Those women most engaged in inputting their food diaries into MyFitnessPal lost the most weight. In randomised controlled trials, the use of smartphone apps has been associated with better dietary compliance, higher physical activity levels and greater weight loss (Coughlin *et al.*, 2015).

A criticism of the study is that the level of dietary input maybe unachievable in routine NHS care. However, it could also be argued that the current dietary input for women with GDM is inadequate and has little effect on changing eating behaviour. It is possible that the disappointing results from other dietary studies reviewed by the Cochrane Collaboration (Bain *et al.*, 2015) are as a result of inadequate dietary intervention and an inability to engage women into the dietary process. Most dietary interventions included in this review concentrated on dietary composition rather than reduction in energy intake. Additionally many studies have tried to combine diet and exercise. The UK Pregnancies Better Eating and Activity Trial (UPBEAT) is a recent phase two pilot study to investigate the application of diet and exercise intervention to obese pregnant women (Poston *et al.*, 2013). Specifically this exploratory trial was to determine whether these interventions achieved a change in dietary and exercise behaviour. Dietary intervention consisted of a recommendation to change diet to low-GI and to reduce saturated fat; however, energy intake was not discussed. The intervention was delivered by health trainers over an eight week period. 24 hour food recall was used to assess diet: energy intake decreased from 1,850kcal (controls) to 1600kcal (intervention; p=0.016) and there was a substantial decrease in GI and saturated fat intake. Physical activity did not increase. Disappointingly, despite a reported reduction in calorie intake there was no difference in maternal weight gain, gestational diabetes or large for gestational age babies. The WELLBABE study demonstrated that maternal weight loss was achievable through a more stringent calorie goal of 1,200kcal/day, with calorie intake being the focus of the intervention. Dietary compliance was assessed through food diaries (via MyFitness Pal) and weight loss, again this is more time-consuming for participants than a 24-hour food recall, but is perhaps associated with better accuracy and provides instantaneous feedback. Keeping a food diary has been shown to be an effective means to weight loss, with more data input into the diary being associated with greater weight loss (Hollis *et al.*, 2008). It is surprising that there was no benefit in the dietary intervention with regard to maternal

weight gain and gestational diabetes in the UPBEAT trial. UPBEAT did show that dietary intervention was more achievable in pregnancy than exercise, which was not adopted by the participants. Other studies have confirmed the difficulties in achieving physical exercise in pregnancy. The FitFor2 study, a randomised controlled trial of exercise training programme in pregnancy, showed no benefit of exercise on blood glucose, insulin sensitivity and birthweight in pregnant women who were overweight and at risk of gestational diabetes compared to controls (Oostdam *et al.*, 2012). Studies outside of pregnancy confirm that weight loss through diet is easier to achieve (Janssen *et al.*, 2002; Yoshimura *et al.*, 2014). Although the combination of weight loss and exercise is beneficial outside of pregnancy (Miller *et al.*, 2013), focusing on intensive dietary intervention for those motivated to lose weight *during* pregnancy may be a better use of resources.

6.3 Metabolism in Normal Pregnancy & GDM

6.3.1 Muscle fat

"Pregnancy is an insulin resistant state", however the mechanism through which insulin resistance is acquired is not fully understood. We hypothesised that, because gestational diabetes shares many features with T2DM and because gestational diabetes appears to be an extension of the physiological insulin resistance of pregnancy (Metzger *et al.*, 2008), the mechanism through which insulin resistance is acquired in pregnancy would be the same as in T2DM. One of the earliest detectable changes in T2DM is an alteration in muscle insulin resistance (Warram *et al.*, 1990) and observational studies would suggested that increased muscle fat deposition is contributory to this process (Jacob *et al.*, 1999). Indeed, on reversing the process through a 1,200kcal diet, muscle lipid is seen to decrease at the same time as insulin sensitivity improves (Petersen *et al.*, 2012). Yet, results from the LIPIDPREG study suggest that intramyocellular lipid concentrations does not change in pregnancy. Other studies have demonstrated increased intramyocellular lipid in women with previous GDM and on-going insulin resistance (Kautzky-Willer *et al.*, 2003; Prikoszovich *et al.*, 2011). A different mechanism must account for the physiological decrease in insulin sensitivity in pregnancy, it is most likely that this is hormonal in origin given the rapid improvement in insulin sensitivity following delivery and the abruption of the placental circulation. This is still to be established.

6.3.2 Liver fat

An inability to suppress hepatic glucose production is a feature of progression from impaired glucose tolerance to T2DM (Weyer *et al.*, 1999). Pregnancy is associated with an increase in basal endogenous glucose production of approximately 30% by late pregnancy in both lean and obese women (Catalano *et al.*, 1993). Insulin suppression of hepatic glucose production was reduced (80%) in women with gestational diabetes compared to normal glucose tolerance (95%) in late pregnancy. Metabolic studies in participants with T2DM have shown that hepatic insulin resistance is related to intrahepatic fat content. Greater levels of liver fat contribute to greater insulin resistance and failure of insulin to suppress hepatic glucose production (Ravikumar *et al.*, 2005; Perseghin *et al.*, 2006; Gastaldelli *et al.*, 2007). Again, reversal of the liver fat process promotes suppression of hepatic glucose production (Lim *et al.*, 2011b; Steven *et al.*, 2016a).

In type 2 diabetes liver fat averages approximately 13% at diagnosis (Lim *et al.*, 2011b; Steven *et al.*, 2016b). The WELLBABE study showed that liver fat was not elevated in pregnancy affected by gestational diabetes. This is remarkable given that the population were obese and had insulin resistance, two factors that are known to be associated with elevated liver fat levels outside of pregnancy (Caiazzo *et al.*, 2014). Studies in postnatal women with prior gestational diabetes have shown elevation of liver fat (Forbes *et al.*, 2011; Forbes *et al.*, 2013) and that higher levels of liver fat are associated with increased insulin resistance (Tiikkainen *et al.*, 2002) and increased risk of type 2 diabetes in later life (Bozkurt *et al.*, 2012). Closer evaluation of the studies in which liver fat was determined by MR, however, reveals that there is a diverse distribution of liver fat within the prior GDM population. Tiikainen et al studied 27 obese non-diabetic women with prior gestational diabetes (pGDM) with magnetic resonance spectroscopy of the liver to measure intrahepatic liver fat. Liver fat ranged from between 1.5 to 23%. Forbes et al studied 110 women with pGDM and 113 control women with NGT. Both groups had similar BMI. The pGDM group had a higher prevalence of non-acute fatty liver disease (NAFLD) 37% compared to controls 17% as determined by ultrasound. Forbes went on to study a subgroup (n=36) with MR spectroscopy to find, again a wide range of liver fat levels ranging between 0.11 to 24.3%, the NGT without NAFLD had very low liver fat (median 0.22% IQR 0.11-1.23) as compared to the pGDM group without NAFLD (median 0.53% IQR 0.15-2.11). However, despite a significant proportional difference, actual liver fat levels are low in both groups. Prikoszovich used MR spectroscopy to determine liver fat in 23 glucose tolerant non-obese women with pGDM against 8

controls with NGT. Liver fat was elevated in those with pGDM although again, the actual levels are not high: pGDM 3.7 ± 3.5 vs. 1.5 ± 0.9% ($p<0.05$).

The liver fat levels in these studies are comparable to the findings from the WELLBABE study. Certainly the wide variance of liver fat in both normal and pGDM are to be considered in informing a power calculation for any further studies of liver fat in pregnancy. The longitudinal effect of pregnancy on liver fat levels is unknown, neither human nor animal models having examined this specific issue. It is known that plasma triglyceride more than doubles in pregnancy and it could be speculated that the increase in VLDL-TG originates from mobilization of fat from the liver and adipose tissue. Mobilisation of fat stores may also account for the physiological reduction in liver enzymes typically seen in the third trimester of pregnancy (perinatology.com, 2016). ALT rise is typically seen in patients with NAFLD, particularly those with insulin resistance and its elevation is an indirect marker of liver fat (Maximos *et al.*, 2015). As liver fat decreases with diet, so too does ALT (Lim *et al.*, 2011b; Copaci *et al.*, 2015; Steven *et al.*, 2016b). It can be hypothesised that prioritisation of triglyceride to the fetus is achieved in pregnancy through mobilisation of fat stores in the liver, in turn explaining the observed reduction in ALT by the third trimester (**Figure 41** shows a conceptual representation of the hypothesised changes). The effect of GDM within this process is uncertain, but both elevated liver fat (De Souza *et al.*, 2016) and elevated ALT (Yarrington *et al.*, 2016) in the first trimester are associated with an increased risk of GDM in later pregnancy. A longitudinal study is now needed to explore the relationship between liver fat, plasma lipids, liver enzymes and hepatic insulin resistance in normal and GDM pregnancy.

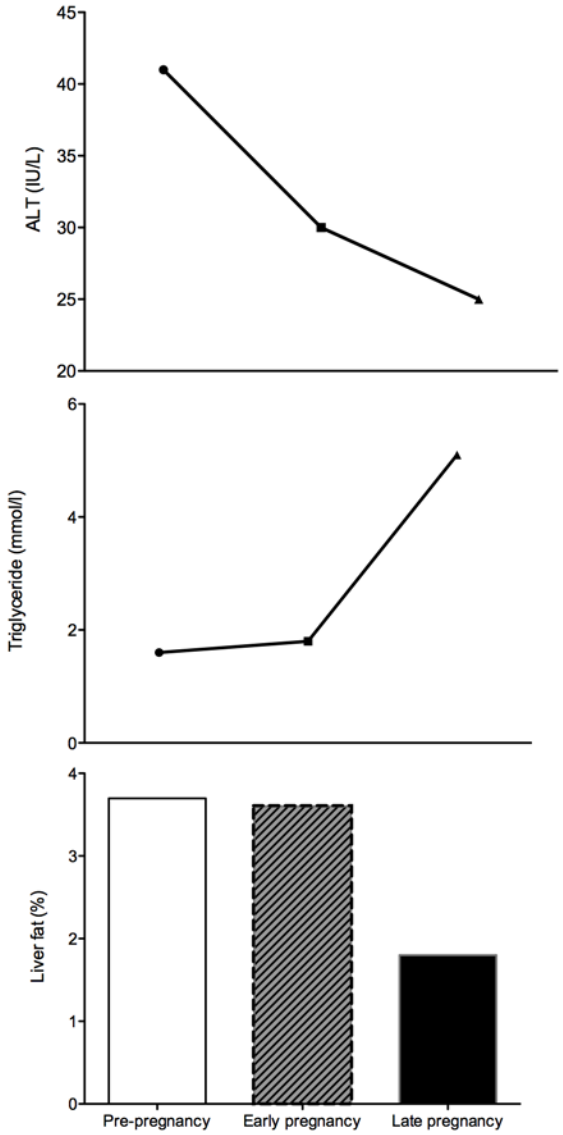

Figure 41 Postulated change in liver fat during pregnancy in relation to changes in plasma ALT and triglyceride. ALT and triglyceride values taken from (perinatology.com, 2016). Liver fat values are taken from the WELLBABE study (postpartum and pre-diet values used for pre-pregnancy and late pregnancy respectively), bar with dotted outline represents estimated (yet to be established) change in liver fat.

6.3.3 Pancreatic fat

To date, much of the focus of GDM research has been on insulin resistance, although Buchannan recognised the concept of a 'beta-cell deficit relative to the degree of insulin resistance' (Buchanan, 2001). He observed that, although there was a difference in insulin sensitivity between women with GDM and those with NGT, this difference was small during the third trimester of pregnancy. Insulin sensitivity in the LIPIDPREG and WELLBABE studies were very similar, despite there being an obvious difference in blood glucose during the meal test. Hyperglycaemia, the hallmark of GDM, arises when insulin secretion cannot overcome the degree of insulin resistance, clearly reflecting a problem with beta-cell function.

Type 2 diabetes is often viewed as an inevitably progressive condition with worsening of beta cell function and mass over time (Turner *et al.*, 1999; Butler *et al.*, 2003). Excessive pancreatic fat is thought to play a pivotal role in the pathophysiology of beta cell deficit (Lim *et al.*, 2011b; Taylor, 2013). Whether the pancreatic defect seen in GDM is also associated with excess pancreatic fat is unstudied. However, women with GDM at five year follow up had a 4.8-fold increased risk of having prediabetes. The main contributing factor was impaired beta cell dysfunction over insulin resistance/sensitivity and worsening beta cell function correlated with increased visceral fat (measured with DEXA) (Lekva *et al.*, 2015). Interestingly, the rapid deterioration of both insulin sensitivity and beta cell function seen in women with prior GDM (as compared to women with normal glucose tolerance) is not explained by differences in adiposity, rather the existing fat and/or beta-cell robustness potentially play a more important role (Xiang *et al.*, 2013).

Pancreatic MR studies are relatively new in the field of type 2 diabetes, as such no MR studies have been done in pregnancy. The relationship between pancreatic function and GDM has not been extensively studied, although the presence of a rapid decline in beta cell function has been demonstrated in Japanese women, just prior to developing GDM (Saisho *et al.*, 2013). There is clearly a need to define pancreatic morphology, composition and function in normal pregnancy and GDM using best possible methodology for the quantification of pancreatic fat (Al-Mrabeh *et al.*, 2017).

6.3.4 Effects of GDM on the fetus and potential for magnetic resonance spectroscopy

Gestational diabetes not only affects the growth of the fetus in utero, predisposing to either fetal macrosomia or intrauterine growth restriction, but also has an impact on the future child and adolescent metabolic profile. The EPOCH study (Exploring Perinatal Outcomes amongst Children) demonstrated higher BMI, greater waist circumference, increased subcutaneous abdominal fat and increased skin fold thickness in 6- to 13-year olds born to mothers with GDM after adjusting for socioeconomic and other potential confounding factors (Crume *et al.*, 2011b). There are strong links with GDM and offspring obesity and metabolic syndrome (Pettitt *et al.*, 1993; Crume *et al.*, 2011a). Epigenetics describes the heritable changes in gene expression that occur without changes in the DNA. It is postulated that the abnormal metabolic uterine environment affects fetal development through epigenetic mechanisms (for example DNA methylation and histone modification). Target organs include the liver, pancreas, adipose tissue and muscle (McMillen and Robinson, 2005). This leads to transgeneration transmission; a vicious cycle of mothers with GDM giving birth to offspring who are pre-disposed to metabolic syndrome, leading to a new generation of mothers with GDM. Whether it is possible to break this cycle through treating GDM is still under investigation. Interestingly, following up the children born from the ACHOIS study (Crowther *et al.*, 2005) suggests that although treatment reduced macrosomia at birth, it did not result in a change in BMI at age 4- to 5-years old (Gillman *et al.*, 2010). However, further research is necessary and likely to take many years to collate.

In view of the fact that derangement in the maternal metabolic profile has such an impact, both in the fetus and in the longer term metabolic health of the child and future adult, it would be interesting to examine fetal metabolism using MR techniques. In particular to define the physiology of in utero exposure to hyperglycaemia in relation to development of the liver, pancreas, adipose tissue and muscle. Magnetic resonance spectroscopy has been used in utero in relation to neurological development and neurotransmitter concentrations, however its widespread use is limited. There are several technical problems in scanning the fetus that need to be overcome. These include fetal movement, the relatively small fetal size, the difficulty in obtaining good spectra due to signal to noise ratio and the potential distance the tissue of interest is away from the receive coil. Despite these difficulties, it has been possible to obtain spectra from the fetal liver, heart and brain (Fenton *et al.*, 2001). Fetal MRS is still at an early stage in its use and development, but is clearly an important avenue of exploration for future work.

6.4 Future Work

This project has successfully provided novel MR data on some of the changes in maternal liver and muscle fat that occur during pregnancy and GDM. Ideally such a project would have taken a more thorough and systematic approach to understanding, firstly the physiology of normal pregnancy and then secondly to use the same techniques to unravel the pathophysiology of GDM. However, the project has been constrained by difficulty in obtaining funding, the main obstacle being the concern by grant committee members over whether women would be willing to reduce calorie intake during pregnancy. In order to move forwards step-by-step small charitable funds were secured to fund the WELLBABE proof of concept study. Now that the acceptability and feasibility of reducing calorie intake during pregnancy has been established, funding can be sought for a much larger project.

One of the first studies to be done was to try to unravel the cause of insulin resistance in normal, non-diabetic pregnancy. From the LIPIDPREG study it has been established that, unlike other insulin resistant states, muscle lipid deposition is not the cause. The fact that insulin resistance is reversed almost immediately following delivery implicates a pregnancy or hormonal factor (Taylor and Davison, 2007). A simple study would be to measure various hormone concentrations (e.g. hPL) in pregnant women in the third trimester and then to take timed samples every few hours following delivery. Through measuring hormone profiles in the immediate postpartum period and comparing the levels to those during pregnancy it should be possible to determine the cause of insulin resistance.

The next step would be to determine the physiology of normal pregnancy in women with normal glucose tolerance in relation to muscle, liver and pancreatic fat and insulin sensitivity and secretion and glycaemic control and compare this to women with GDM. A longitudinal study would be most appropriate in order to determine the changes that occur in early, mid and late gestation and to compare this to the postpartum state. Establishing the pre-pregnant state would be ideal, however, this is difficult to achieve as it would involve scanning an unknown quantity of healthy women, some of whom may not become pregnant, or may become pregnant out with the time scales of the study. Studying women in the postpartum period is arguably equivalent to the 'pre-pregnant' state. Depending on resources, studies could be made at various different times postnatally to see the effect of 'pregnancy reversal' on organ fat deposition. Additionally, the effect of breastfeeding and lactation on fat deposition could be studied.

In order to compare women with GDM against those with NGT four groups would need to be recruited:

1. NGT, normal BMI
2. NGT, overweight/obese BMI
3. GDM, normal BMI
4. GDM, overweight/obese BMI

It is important to recruit women with GDM and normal BMI since they represent approximately 25% of the GDM population in Newcastle (KH audit data from Royal Victoria Infirmary, 2011) and obesity may act as a confounding factor in terms of organ fat deposition. Since the variance of organ fat in pregnancy is unknown (although we can speculate from WELLBABE and other studies that the variance of liver fat is likely to be wide), a rule-of-thumb would be to recruit at least 35 participants into each group (Lancaster *et al.*, 2004). This would provide important background data in order to be able to perform a power calculation for the subsequent intervention study.

Fetal MRS of the liver and muscle could be developed in collaboration with the MR physicists at the Newcastle Magnetic Resonance centre. Studies could be done, perhaps in a subgroup of the participants of the longitudinal study described above. This would serve to obtain some initial data and to develop the technique further.

Having established the physiology of fat deposition in liver, muscle and pancreas in normal pregnancy and GDM in relation to insulin sensitivity, insulin secretion and glycaemic control a randomised controlled trial (RCT) of dietary intervention versus standard antenatal care can be designed along *MRC: Developing and Evaluating Complex Interventions* guidance. The first phase of this would be a small pilot RCT to establish:

- Likely effect change in clinical outcomes (improvement in glycaemic control, need for medication, reduction in macrosomia)
- Likely effect change in physiological outcomes (organ fat deposition, insulin sensitivity/secretion)
- Success of diet (in terms of weight loss)
- Acceptability of diet and study protocol (dropout rates/compliance/etc)
- Best method to achieve dietetic input (telephone/SMS/MyFitnessPal/face-to-face)

- Ease of recruitment
- Randomisation – is it possible?
- Economic evaluation

For this intervention, it may not be possible to randomise treatment: there will be women willing to diet and those not. Since compliance with the diet is important in order to get a meaningful result a different approach may be that for each woman recruited who is willing to diet, a matched control (for approximate BMI and ethnicity) who undergoes standard antenatal care, but is unwilling to diet is also recruited. In recruiting for the WELLBABE study, there were a number of women who were willing to be studied, but declined as they felt unable to do the dietary intervention component.

The results and experience of this pilot study would inform a much larger RCT of dietary intervention for GDM. The focus of this RCT would be to improve clinical outcome (improve glycaemic control, reduce fetal macrosomia), but more intensive metabolic studies may also be done in a small subgroup.

Much has been gained from long term population cohort studies in terms of studying health and wellbeing throughout the life course (Medical Research Council, 2014). Clearly, given the transgeneration transmission hypothesis there is value in following up the children born to women included in these proposed studies. Both the longitudinal and intervention study would provide valuable information regarding the outcome of GDM pregnancy both treated and untreated with dietary intervention. A long term research strategy should include biophysical, metabolic and magnetic resonance analysis of these children as they develop into adults and parents of the future generation.

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APPENDIX A PATIENT INFORMATION SHEET

The Newcastle upon Tyne Hospitals NHS
NHS Foundation Trust

Newcastle University

Lipid Metabolism in Normal Pregnancy

A Magnetic Resonance Pilot Study

Professor R. Taylor, Professor S. Robson, Dr K. Hodson

1. Introduction

You are being invited to take part in a research study. It is important for you to understand why the research is being done and what it will involve before you decide whether to take part. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information.

2. What is the purpose of the study?

During pregnancy, some women develop high sugar levels (gestational diabetes). They can be at risk of having large babies, difficult deliveries and sometimes stillbirth. At the moment, we do not fully understand why some women develop gestational diabetes and others do not and what the best sugar level should be during pregnancy. To try to understand this better, we would like to study how the body deals with sugar during normal pregnancy, using a new imaging technique (magnetic resonance spectroscopy [MRS]) that does not involve any X rays. This will show us, through pictures, how the muscle uses sugar and fat to produce energy. In the future, we will compare these results with those from women with gestational diabetes. This may help us to improve their treatment during pregnancy and reduce their risk of problems.

3. Why have I been chosen and do I have to take part?

You have been chosen because you are healthy and your pregnancy is progressing normally. It is up to you to decide whether or not to take part. If you do decide to take part you will be asked to sign a consent form; a copy of your consent and this information sheet will be given to you to keep. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw, or a decision not to take part, will not affect the standard of the care you receive.

4. What will happen to me if I take part?

If you agree to take part, you will be invited to attend the Newcastle Magnetic Resonance Centre for two sessions. Each session will last approximately four hours. One will be when you are 34 weeks pregnant; the other session will be 12 weeks after your baby has been born. We will ask you to avoid vigorous exercise and alcohol the day before the study. You will also be asked not to eat from midnight. At each session, a drip will be sited in your hand so that we can take blood from you. This will only be done once and all blood tests will be taken from

this drip. A MRS scan of your leg muscle will be then be done. The scan will take 10-15 minutes and will involve lying on your side whilst your leg is inside the scanner (a large and sometimes noisy tube). You will be given ear protectors to wear. After the scan, you will be given breakfast which may include yoghurt, cereal, toast and fruit juice. We will try and accommodate any dietary requirements. We will take some more blood tests from the drip over the next two hours. During this time you will be encouraged to relax and can walk around if you wish. We will provide a taxi to take you (and your baby) to and from the research centre. We will also have a midwife present who will be able to help you with your baby during the second session.

5. What are the risks and benefits of taking part?

An experienced doctor will place a drip in your arm; this may be slightly uncomfortable, but it will allow us to take several blood samples without further needles. You can have local anaesthetic cream if you want. The study will involve an MRS scan. The scan can be noisy but should not cause any other discomfort. Only your leg will be scanned. Magnetic resonance is a safe imaging method that does not use X-rays. Although the specific scanner (3 Tesla) we are using for this study has not been used widely in pregnancy, this will not expose your baby to any extra risk. You will be helping us to improve our understanding of gestational diabetes. In the future, the results of this study may help prevent and treat this condition.

6. Will my taking part in this study be kept confidential?

All information collected about you during this study will be strictly confidential. It will be stored on computers approved by the NHS and University of Newcastle. If for any reason, information about you needs to leave these computers, your name and address and other identifiable information will be removed so that you cannot be recognised from it. Only the researchers and the representative of the Research and Ethics committee will have access to the data collected during the study. Your GP will be informed of your involvement in this study.

7. What will happen to the results of the research study?

It will take us about one year to complete the study. Once the study stops, no further involvement will be expected on your part. We anticipate the results will be published in a medical journal. You will not be identified in any report or publication. We may also use the results from this study for further research. If you indicate that you are interested, we can write to you with results from this study

8. What will happen if I don't want to carry on with the study?

If you withdraw from the study, we will destroy all your identifiable samples, but we will need to use the data collected up to your withdrawal.

9. What if there is a problem?

In the event that something does go wrong and you are harmed during the research and this is due to someone's negligence then you may have grounds for legal action for compensation against the Newcastle upon Tyne NHS Foundation Trust but you may have to pay your legal costs. If you have a concern about any

aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure (or Private Institution). Details can be obtained from the hospital.

10. Who is funding and organising the research and who has reviewed it?

The study is funded by Newcastle University. It is being carried out by a team of researchers at Newcastle University. The study has been approved by the Newcastle & North Tyneside Research Ethics Committee.

11. Contact for further information

If you have any questions about the study please contact: Professor R. Taylor (Tel: 0191 xxx xxxx) or Professor S. Robson (Tel: 0191 xxx xxxx)

Thank you for reading this information sheet.

The Newcastle upon Tyne Hospitals NHS
NHS Foundation Trust

Centre Number:
Study Number:
Patient Identification Number for this trial:

Lipid Metabolism in Normal Pregnancy – A Magnetic Resonance Pilot Study

Professor R. Taylor, Professor S. Robson, Dr K. Hodson

Please Initial Box

1. I confirm that I have read and understood the information sheet dated 26/01/2009 (version 2.0) for the above study and have had the opportunity to consider the information, to ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation 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. ☐

3. I understand that relevant sections of my medical notes and data collected during the study may be looked at by responsible individuals from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

4. I agree to my GP being informed of my participation in the study ☐

5. I agree to take part in the above study. ☐

_____	_____	_____
Name of patient	Signature	Date
_____	_____	_____
Name of person taking consent	Signature	Date

APPENDIX B PATIENT INFORMATION SHEET

The Newcastle upon Tyne Hospitals NHS
NHS Foundation Trust

Newcastle University

Dietary Intervention for the Treatment of Gestational Diabetes:

A Magnetic Resonance Study

Professor Roy Taylor, Professor Stephen Robson, Dr Vera Araujo-Soares,

Dr Ken Hodson

1. Introduction

You are invited to take part in a research study. It is important for you to understand why the research is being done and what it will involve before you decide whether to take part. Please take time to read the following information carefully and discuss it with others if you wish. Do ask us if there is anything that is not clear or if you would like more information

2. What is the purpose of the study?

During pregnancy, some women develop high blood sugar levels (gestational diabetes). This can increase the risk of having larger babies, more difficult deliveries and the need for baby to be looked after in the Special Care Baby Unit after birth. It is thought that gestational diabetes occurs due to excessive calorie intake over a period of time, leading to excessive storage of fat in the liver. Eating less in pregnancy (through a calorie controlled, fully balanced diet) in pregnancy has been used as treatment for gestational diabetes. The advantage of this approach is that tablets and insulin may not be necessary and the decrease in weight may be associated with better outcome for mother and baby. However, we need to understand how this affects the body and how successful it is in avoiding need for drug treatments.

In particular we need to study the effect of reduced calorie intake on the amount of fat within the liver. This is done using a magnetic scanner (similar to an MRI). This is safe during pregnancy. This will show us how the body changes over a four-week period of eating less. Additionally, we would like to find out how women with gestational diabetes feel about making such a change to food intake.

3. Why have I been chosen and do I have to take part?

You have been chosen because you have recently been diagnosed with gestational diabetes. It is up to you to decide whether or not to take part. If you do decide to take part you will be asked to sign a consent form; a copy of your consent and this information sheet will be given to you to keep. You are, of

course, still free to withdraw at any time and without giving a reason. A decision to withdraw, or a decision not to take part, will not affect the standard of the care you receive.

4. What will happen to me if I take part?

If you agree to take part, you will be invited to attend the Newcastle Magnetic Resonance Centre for three sessions. The first session will be around 28 weeks of pregnancy. It is done as soon as possible to measure your liver fat and test how your body handles food 'at baseline'. We will then do a detailed analysis of your current dietary intake and advise you about how to plan your meals to decrease calorie intake whilst maintaining all micronutrients and vitamins that your body requires during pregnancy. We will ask you to record all your food intake over a four week period using the smartphone app MyFitnessPal and can provide you with a smartphone if needed. You will be seen at two weekly intervals in the antenatal clinic and a member of the research team will be available by telephone at all times should you need help or advice during the study period. During the study period we will check baby's growth with an ultrasound scan every two weeks (instead of every four weeks as usual). After four-weeks of the diet, we will invite you back to repeat the liver scan and body metabolism tests.

The first two sessions at the Newcastle Magnetic Resonance Centre will last approximately four hours. We will ask you to avoid vigorous exercise and alcohol the day before the study. You will also be asked not to eat from midnight, although you can drink water as you wish. At each session, your weight, height, blood pressure and a dip test of your urine will be recorded. A special scan of your liver will be then be done. The scan will take 10-15 minutes and will involve lying on your side whilst you are inside the scanner (a large tube). You will be given ear protectors to wear. After the scan, a small plastic tube (a cannula) will be sited in your hand so that we can take blood samples painlessly. There is a possibility that some people may experience a little localised bruising following the insertion of a cannula; this is a completely normal reaction. You will be asked to eat a full breakfast (to include cereal, milk, bread roll, butter/spread and jam and orange juice; we will accommodate any dietary requirements). Blood tests will be taken over the next two hours. During this time you will be encouraged to relax and can walk around if you wish. We will provide a taxi to take you to and from the research centre.

You will be invited to attend the Newcastle Magnetic Resonance Centre around 12 weeks following delivery of your baby. A scan of your liver and a single blood sample will be taken and this visit lasts approximately 30 minutes.

Following the study a research midwife will contact you to invite you participate in a short interview (of approximately 45 minutes duration). This is to explore your feelings towards the diet and the impact that it has had on your day-to-day life. This can be arranged at a time that is convenient to you and can be combined with an antenatal clinic appointment. With your permission, the interview may be recorded.

5. What are the risks and benefits of taking part?

Women are often reluctant to reduce their calorie intake during pregnancy for fear that they may harm their baby. However, gestational diabetes and high blood sugar levels are known to be harmful and there is evidence that baby will be helped by eating less. There is no evidence at all that reducing calorie intake harms the baby. Overall, preventing complications through dietary means is likely to be beneficial.

During your visit to the Newcastle Magnetic Resonance Centre an experienced doctor will place the small plastic tube in your hand using local anaesthetic cream if you wish. This will allow us to take several blood samples without further needles. The study will involve a magnetic resonance scan. The scan makes a noise, but much less than a normal MRI scan. It should not cause any other discomfort. Magnetic resonance scanning is a safe test that does not use X-rays.

You will benefit from extra care and support during your pregnancy. You will get direct feedback from the study with regards to the effects of the diet on your sugar control and liver fat. You will be able to ask detailed questions about your future risk of diabetes and are likely to find out useful information. In addition, you will be helping us to improve our understanding of gestational diabetes. In the future, the results of this study may simplify and improve treatment of this condition.

6. Will my taking part in this study be kept confidential?

All information collected about you during this study will be strictly confidential. It will be stored on computers approved by the NHS and Newcastle University. If for any reason, information about you needs to leave these computers, your name and address and other identifiable information will be removed so that you cannot be recognised from it. Only the researchers and the representative of the Research and Ethics committee will have access to the data collected during the study. Your GP will be informed of your involvement in this study.

7. What will happen to the results of the research study?

It will take us about one year to complete the study. Once the study stops, no further involvement will be expected on your part. We anticipate the results will be published in a medical journal. You will not be identified in any report or publication. We may also use the results from this study for further research. If you indicate that you are interested, we can write to you with results from this study

8. What will happen if I don't want to carry on with the study?

If you withdraw from the study, we will destroy all your identifiable samples, but we will need to use the data collected up to your withdrawal.

9. What if there is a problem?

In the event that something does go wrong and you are harmed during the research and this is due to someone's negligence then you may have grounds for legal action for compensation against the Newcastle upon Tyne NHS Foundation Trust but you may have to pay your legal costs. If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their

best to answer your questions. If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure (or Private Institution). Details can be obtained from the hospital.

10. Who is funding and organising the research and who has reviewed it?

The study is funded by the North East Diabetes Trust. It is being carried out by a team of researchers at Newcastle University. The study has been approved by the Newcastle & North Tyneside Research Ethics Committee 2.

11. Contact for further information

If you have any questions about the study please contact: Professor Roy Taylor (Tel: 0191 xxx xxxx) or Professor Stephen Robson (Tel: 0191 xxx xxxx)

Thank you for reading this information sheet.

The Newcastle upon Tyne Hospitals NHS
NHS Foundation Trust

Newcastle University

Centre Number:
Study Number:
Patient Identification Number for this trial:

Dietary Intervention for the Treatment of Gestational Diabetes: A Magnetic Resonance Study

Professor Roy Taylor, Professor Steven Robson, Dr Vera Araujo-Soares, Dr Ken Hodson

Please Initial Box

1. I confirm that I have read and understood the information sheet dated 05/11/2014 (version 2.0) for the above study and have had the opportunity to consider the information, to ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation 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. ☐

3. I understand that relevant sections of my medical notes and data collected during the study may be looked at by responsible individuals from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

4. I agree to my GP being informed of my participation in the study ☐

5. I agree that my interview can be audio recorded. ☐

6. I agree to take part in the above study ☐

_____	_____	_____
Name of patient	Signature	Date
_____	_____	_____
Name of person taking consent	Signature	Date

When completed, 1 for patient; 1 for researcher site file; 1 (original) to be kept in medical notes.

WELLBABE Study
1200 Kcal Diet Plan

Gestational Diabetes and Food

Your doctor or diabetes midwife will have explained the importance of regulating your blood glucose levels when you have gestational diabetes. This can often be achieved by making dietary changes, with additional medications if required.

Most foods are a combination of protein, fat and carbohydrate in different proportions. It is the carbohydrate in foods which has the most effect on blood glucose levels. This is because carbohydrate is broken down into glucose during digestion. The glucose then enters the blood.

Carbohydrates include starchy foods such as bread, rice, potatoes, pasta and cereals (including flours), added sugars (e.g. sucrose), and also natural sugars found in fruit and milk. They do not need to be avoided completely; however it is important to eliminate foods and drinks which contain lots of added sugar:

1. Cut out sugary pop, squashes and juices. Swap to diet/zero/slimline pop and no added sugar squashes, as these do not raise blood glucose levels. Most flavoured waters are also a good choice, but check the ingredients list and labels, as a few do contain added sugar.
2. Limit fruit juice to 1 small glass (150ml) per day and have this with food. Avoid smoothies and milkshakes which contain lots of sugar.
3. Use a low calorie sweetener in place of added sugar in hot drinks and on cereals e.g. Canderel, Splenda, Hermesetas, Stevia.
4. Jelly contains lots of added sugar (31 sugar cubes in a block of jelly). Swap to sugar free jelly which contains no sugar at all. No Added Sugar Angel Delight is also available.
5. Choose tinned fruit in natural juice rather than in syrup or light syrup.
6. Buy reduced sugar jam and marmalade.
7. Diet/light fruit yogurts use sweetener instead of sugar.
8. Avoid sweets (jellies, boiled sweets, toffees etc.) and ice lollies. Sugar free mints and chewing gum are fine to have (in moderate amounts as the sweeteners used can have a laxative effect)

How else can I keep my blood glucose levels in target?

- Spread your food (especially your carbohydrates) across the day, eating little and often

- Try to be as active as you can within your limitations. A 20 minute walk after a meal can help to reduce blood glucose levels more quickly.

- Manage your weight during and after pregnancy – this is the aim of the WELLBABE study you are participating in.

How will weight loss help me and my baby?

- Weight loss helps insulin to work more efficiently to remove glucose from your blood. By improving your blood glucose levels your baby is less likely to grow bigger than expected, making delivery easier. It is also less likely that your baby will have a low blood sugar (hypo) after delivery if your blood glucose is well controlled.

- At a lower weight you are less likely to develop Type 2 Diabetes in the future, or develop gestational diabetes again in future pregnancies.

- Continuing the healthy changes you have made after pregnancy can help the whole family to eat a healthy diet and maintain a healthy weight – this is important as Type 2 Diabetes does run in families.

Before you Begin

It is helpful to think about what you hope to gain (what it would mean to you) from following the diet plan/losing weight, and also what challenges might arise during the 4 weeks of the diet.

Benefits	Difficulties
E.g. able to fit into clothes better, will be less breathless when climbing the stairs	E.g. I tend to eat when I'm stressed/bored/upset, there are always cakes/biscuits around at work, I have a birthday in 2 weeks time

For each difficulty you have identified, think about how you could manage this in a different way i.e. without resulting in eating more than planned.

Difficulty	Plan
E.g. I eat when I am stressed	Manage the stress by talking to a friend or letting it out in another way (punchbag/a good shout in the garden)

What will I eat on the 1200 kcal diet?

The diet plan consists of set quantities of foods from 6 different food groups. This will provide you with a varied and balanced low calorie diet which is designed to provide maximum nutrition for you and your baby. You will also be taking a pregnancy multivitamin while following the diet to supplement the nutrition from foods.

Food Group	Number of Portions Daily
Starchy Foods	4
Protein	2
Milk and Dairy	2
Fruit	1
Vegetables	4
Fats & Oils	2

You can distribute these foods as you wish across the day to suit your preferred eating pattern. The lists on the following pages give portion sizes for the different food groups.

Other Factors

- Drink 2 litres of sugar/calorie free fluids per day while following the diet plan. This includes water, diet fizzy pop, no added sugar squash, decaffeinated tea and coffee, herbal teas. Limit caffeinated tea/coffee to 2 mugs (4 cups) per day.
- Avoid calorie containing drinks (sugary pop and squash, smoothies, milkshakes, lattes etc.). Alcohol should be avoided as per standard advice for pregnancy.
- You will need to avoid many higher calorie snacks to remain within the 1200 calorie limit. This includes sweets, chocolate, biscuits, crisps and cakes. There are snack ideas on page 9 of this booklet.

Portion Sizes

Starchy Foods (Allowance 4 portions per day)

100kcal per portion

1 Portion:	
1 medium slice of bread or toast	30g uncooked rice, pasta or bulgur wheat
1 sandwich thin	75g cooked rice or pasta
1 mini pitta bread	4 new potatoes in skins (150g)
1 small chapatti/1 flour tortilla	2 egg-sized old potatoes (boiled or mashed)
1 ½ Weetabix	100g cooked sweet potato
1 shredded wheat	8 oven chips
1 crumpet or scotch pancake	2 crispbreads
30g porridge oats (uncooked)	3 plain crackers
25g breakfast cereal (3 tblspns)	2 small Yorkshire puddings
2 Portions:	
45g muesli (not granola)	
1 full size pitta bread	
1 bagel or English muffin	
1 medium bread bun (50g)	
1 nest of noodles	
1 medium jacket potato (200g cooked weight)	
1 individual packet (110g) couscous	

Choose these starchy foods to help keep you fuller for longer:
Wholegrain breakfast cereals, porridge oats (not instant), wholegrain/granary bread, sourdough bread, sweet potato, new potatoes in skins, basmati rice, pasta, noodles. Note: Pulses also contain slow release carbohydrate and are a good choice to fill you up without adding lots of calories.

Protein Foods – Meat, Fish, Eggs, Beans & other non-dairy sources of protein (Allowance 2 portions per day) 150kcal per portion

1 Portion:	
2 medium eggs	3 rashers grilled back bacon
1 small grilled chicken breast (100g)	2 small slices corned beef (70g)
1 fillet of baked/steamed cod (150g)	4 sandwich-sized slices roast ham (120g)
1 small grilled lamb chop or lamb steak (60g)	150g Quorn / Soya / Tofu
2 thin slices roast meat (75g)	150g cooked prawns
½ tin baked beans in tomato sauce (150g)	100g smoked salmon
1 tin tuna in brine or spring water	45g (1 fillet) smoked mackerel
½ tin salmon in brine	200g cooked/tinned kidney beans, chickpeas, lentils etc.
1 small grilled tuna/salmon steak (100g)	1 tin sardines in brine or tomato sauce
1/3 small pack lean beef mince (75g)	3 grilled fish fingers

Avoid processed meat products (burgers, sausages, pies, chicken kiev etc.) while following the diet plan.

Milk & Dairy Foods (Allowance 3 portions per day)

100kcal per portion

1 Portion:	
200ml semi skimmed milk allowance for cereal and tea/coffee (or unsweetened soya milk)	300ml skimmed milk or unsweetened rice milk
150g (small pot) diet/light yogurt or fromage frais	50g low fat cheese spread (2 matchboxes)
Small matchbox size piece of cheese (25g full fat, 35g reduced fat)	75g cottage cheese/100g reduced fat cottage cheese*
125g natural yogurt/ 160g low fat natural yogurt/190g fat free natural yogurt	

*Should be made with pasteurise milk

Vegetables (Allowance 4 portions per day)

30kcal per portion

1 PORTION:	
3 heaped tablespoons (80g) raw or cooked vegetables (fresh, frozen or tinned)	Use a wide variety of vegetables, choosing different colours to give a range of nutrients. Avoid avocados while following the diet plan. Note: potatoes and sweet potatoes count as starchy foods rather than vegetables
2 heaped tablespoons peas, sweetcorn or broad beans	
1 dessert bowl fresh salad	
200ml tomato juice	
100g (1/4 tin) tinned tomatoes	
80g tomato salsa	
8 olives	

Fruit (Allowance 1 portion per day)

60kcal per portion

1 PORTION:	
1 medium piece of fruit (e.g. apple, pear, orange etc.)	1 slice of large fruit (melon, pineapple)
1 small banana	Small handful grapes, cherries or berries
2 small fruits (e.g. plums, satsumas etc.)	3 tablespoons fruit salad (fresh or tinned in juice)
4 dried prunes or apricots	1 ½ tablespoons raisins

150ml pure unsweetened fruit juice can count as a maximum of one portion of fruit per day, however fresh/tinned/frozen whole fruits are preferable as they are higher in fibre.

Fats, Oils & Spreads (allowance 2 portions per day)

60kcal per portion

1 PORTION:	
2 tspn (10g) marg/spread	1 ½ tspn salad dressing (fat free dressing is not counted)
4 tspn (20g) light marg/spread	3 tspn soured cream (30g) or 6 tspn if reduced fat
2 tspn vegetable/olive oil (low calorie spray oil not counted)	2 tspn crème fraiche (20g) or 4 tspn if reduced fat
4 tspn salad cream or light mayo (20g)	3 tspn double cream (12g) or 8 tspn single cream (30g)
2 tspn mayonnaise (10g)	175ml gravy (made with granules, no fat added)
10g nuts e.g. 10 peanuts/almonds, 6 cashews (avoid honey roasted)	40g (4tspn) houmous or 8 tspn reduced fat houmous
10g (1 tspn) low fat peanut butter	45ml ketchup or brown sauce
1 1/2 tspn jam or marmalade (20g)	4 tspn reduced sugar jam or marmalade

Snacks

The following are snack ideas for between meals. Remember that snacks still count towards your overall calorie allowance so should be part of your meal plan, not added as extras.

- Vegetable sticks and tomato salsa dip (1 veg portion)
- 1 crumpet with 2 tspn spread (1 starchy, 1 fat portion)
- 2 crispbreads or oatcakes with 50g low fat cottage cheese (1 starchy, ½ dairy portion)
- 1 piece of fruit (1 fruit portion)
- 1 small slice of malt loaf (35g) (1 starchy portion)
- Diet/light yogurt (125 - 150g pot) (1 dairy portion)
- 1 matchbox-sized piece of cheese (chopped into cubes) and 8-10 pickles e.g. pickled onions, gherkins (1 dairy portion, 1 veg portion)
- 1 mini pitta bread or 5 breadsticks with houmous (1 starchy, 1 fat portion)
- 1 cup (25g) plain or salted Popcorn (1 starchy portion)
- 1 low calorie packet of crisps (<100kcal/bag) e.g. Quavers, Skips, French Fries etc. (1 starchy portion)
- 2 biscuits (<50kcal each) e.g. ginger biscuit, nice, malted milk, rich tea, shortcake, iced ring, small cookie, pink wafer, jaffa cake (1 starchy portion)
- Sugar free jelly (not counted)
- Low calorie hot chocolate e.g. options/highlights made with milk (reduce milk portion by 50ml) 1 dairy portion)
- 10g nuts (e.g. 10 peanuts) + 1 raisin snack box (1 fat, 1 fruit)
- 8 olives (1 protein, 1 veg)

Family Meals and Recipes

You may be cooking for others in the family, so it's helpful to be able to work out the number of portions from each food group in the whole recipe using the portion lists on pages 3-6. You can then work out how many portions are in one serving.

Here is an example:

Chilli con Carne (serves 4)

Recipe Ingredient	Food Group	Portions in recipe	Portions per person
Vegetables (1 large onion, 1 red pepper)	Vegetables	2	0.5
450g lean minced beef	Protein	6	1.5
400g tin chopped tomatoes	Vegetables	2	0.5
400g tin red kidney beans	Protein	2	0.5
600g cooked rice	Starchy foods	8	2
Not counted: 2 garlic cloves, 1 tspn chilli powder, tomato puree, black pepper			

Per serving, this meal provides 1 portion of vegetables, 2 portions of protein and 2 portions from the starchy foods group.

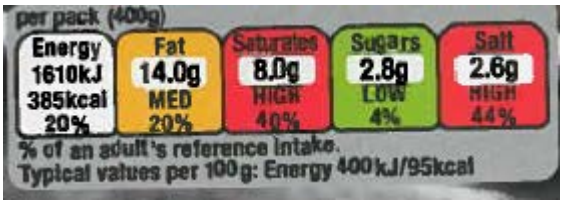
Shop-Bought Meals (Ready Meals, Sandwiches etc.)

1. Note that processed foods tend to be less filling, so try to cook your own meals wherever possible, or add extra vegetables to fill you up.
2. As a guide, choose products which contain under 400kcal per portion.
3. Look out for healthy option ranges as these tend to be lower in calories, fat and salt.

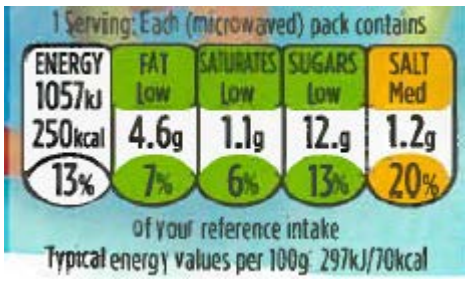
4. Compare front of pack Traffic Light labelling, and choose options with fewer reds and more greens, especially for saturated fat. This type of fat increases insulin resistance, making it more difficult for your insulin to control your blood glucose levels.

   As an example:

Meal 1 (Shepherd's Pie)



Meal 2 (Chicken & Veg Sizzler)



Both meals are under 400kcal, but meal 1 is high in saturated fat and salt (2 reds), whereas meal 2 is low in saturated fat and medium in salt content – more greens. You might choose meal 1 occasionally and meal 2 more often.

5. Work out the number of portions in the meal
   This can be done by eye (e.g. estimating the number of slices of bread and amount of filling in a sandwich), or can be worked out more accurately from the ingredients list on the pack. To do this:

   i. Find the ingredients list on the pack. Ingredients are listed in descending order, so the first ingredient makes up most of the weight of the product and the last ingredient on the list makes up the smallest amount.
   ii. The list will give you the proportions of the key ingredients. For example:

Ignore ingredients in brackets, as these just tell you what is in each of the main ingredients. For example, Mashed Potato (50%) (Potato, Butter (**Milk),** Salt, White Pepper) tells you that the mash is made up of the ingredients in brackets.

iii. Work out the weight of each of the main ingredients (ignore herbs, spices, preservatives etc.). This product weighs 400g. From the label:
Mash (starchy food) = 50% of the whole product which is 200g
Lamb (protein food) is 30% of the whole product which is about 65g
Carrot and onion (veg) make up 4% of the product. Working out 10% (40g) and halving this (20g) will give about 5% (accurate enough!) So 20g is veg.

iii. Compare these to the portion sizes on pages 5-8:
1 portion potato = 150g, so there are about 1.5 portions in the meal (200g).
1 portion lamb = 60g, so there is 1 portion in the meal.
1 portion veg = 80g, so there is only ¼ portion in the meal.

iv. Use my fitness pal for any foods not in the tables. You can work out the number of portions from the kcal (1 starchy = 100kcal etc).

Managing Hunger

You may worry that you will feel hungry while following the diet plan. The following tips will help you reduce and manage hunger:

1. **If you feel hungry, try drinking a pint of water**
2. Make sure you are eating all of your food portion allowances each day.

3. **A bowl of soup or salad before a meal helps to fill you up and is low in calories.**
4. Choose fewer refined carbohydrates (made from processed white flour and sugars) and more of the starchy foods which keep you fuller for longer – see the box on the bottom of page 3 for suggestions.
5. **Use a smaller bowl and plate. Your plate will look fuller and you will feel more satisfied after a meal.**
6. Eat slowly to give 'fullness' signals a chance to reach your brain. If you eat quickly or on the go, you won't start to feel full until 20 minutes after you have finished.
7. **Focus on what you are eating. If you are doing other things like watching TV, working or reading you won't appreciate what you have eaten and won't feel so satisfied.**
8. Hunger is often confused with cravings. If you have eaten a meal in the last 2 hours, you are unlikely to be experiencing true hunger. Try distraction techniques like going for a walk, reading or having a bath – anything that will occupy you and take your mind off food. Acknowledge that you *could* eat but you aren't going to this time. Cravings will eventually die away, so persevere and delay responding to them.
9. **If you are still hungry, try the snack ideas on page 9**
10. Remember, hunger is a positive sign that the diet plan is working!

Plan a day's intake

FOOD & DRINKS	STARCHY FOODS	PROTEIN FOODS	DAIRY	FRUIT	VEGETABLES	FATS/OILS
Breakfast						

Mid-Morning						
Lunch						
Mid Afternoon						
Evening Meal						
Evening Snacks/Supper						
(Portions in Plan) **TOTAL PORTIONS:**	**(4)**	**(2)**	**(2)**	**(1)**	**(4)**	**(2)**

WELLBABE Study Contacts:

Dr Ken Hodson

Email: kenneth.hodson@ncl.ac.uk

Alison Barnes (study dietitian)

Email: alison.barnes@ncl.ac.uk

APPENDIX D NUTRITIONAL ANALYSIS OF 1,200KCAL/DAY DIET

An example 7 days of the 1,200kcal/day diet was entered into WinDiets (see below). The calorie content, macro- and micro-nutrient content of a typical diet are summarised in the table below, together with the recommended intake for pregnancy. All participants were told to take a multivitamin in order to ensure that the micronutrient content of the diet was complete. Several vitamin tablets are available and the table gives the range of micronutrient content for the common ones that were consumed (Sanatogen Pregnancy Mum to Be, Centrum Pregnancy Care, Sanatogen Mother to Be, Seven Seas Pregnancy Multivit,Tesco Multiplus Pregnancy, Sainsburys Pregnancy Plus, Pregnacare Original).

Macronutrient	**Amount**
Energy (kcal)	1178
Fat (g)	33.6
Saturated fat (g)	11.5
Polyunsaturated fat(g)	5.6
Monosaturated fat (g)	11.2
Protein (g)	76.8
Carbohydrate (g)	151.2
Sugars (g)	50.3
Starch (g)	94.5
Non-milk Extrinsic Sugars	12.4
NSP	17.4
Water	924.9

	Amount	**Multivitamin**	**Total**	**Recommended Daily Intake**
Vitamin A (μg)	1085	0	1085	700- (<3300)
Thiamin B1 (mg)	1.43	1.2-1.4	2.63-2.83	0.48
Riboflavin (mg)	1.41	1.4-1.8	2.8-3.2	1.4
Niacin (mg)	33.3	14-19	47.3-52.3	8
Vitamin B6 (mg)	1.72	1.6-2.6	3.3-4.3	1.15
Vitamin B12 (μg)	4.22	2.5-3	6.7-7.2	1.5
Folate (3rd trimester) (μg)	250	400	650	300
Pantothenic Acid (mg)	4.2	6-8.7	10-12.9	3-7* (*No RNI)
Biotin (μg)	25.5	50-200	75-225	10-200*
Vitamin C (mg)	113.5	70-110	183-233	50
Vitamin D (μg)	1.72	5-10	6.7-11.7	10
Vitamin E (mg)	6.46	8.5-12	14.9-16.4	3.8-6.2*
Calcium (mg)	723	0-170	723-893	700
Magnesium (mg)	270	239	60-100	299-339

Phosphorus (mmol)	40.1	0-4.3	40.1-44.4	10.9
Sodium (mmol)	105.7	0	105.7	70
Potassium (mmol)	71.13	0-7.7	71.13-78.83	90
Chloride (mmol)	99.4	0	99.4	70
Iron (mg)	9.6	14-17	23-26	14.8
Zinc (mg)	9	7-15	15-23	7
Copper (mg)	0.92	1	1.92	1.2
Selenium (µg)	56	0-75	56-131	60
Manganese (mg)	2.07	0-2	2.07-4.07	1.4
Iodine (µg)	145	14-200	159-345	140

APPENDIX E INTERVIEW SCHEDULE

Exploring adherence to a reduced calorie diet in gestational diabetes

Interview Topic Guide

Interviewer`s protocol

a) Hello NAME, my name is NAME and I am a member of the research team carrying out the Dietary Intervention in GDM study. We are meeting to see what your experience of the diet is and how you are doing overall.
b) Everything we talk about is confidential and anonymous and only very few members of the team will be able to look at the content of the interviews for the purposes of analysis.
c) I will use a recorder so that I can go back to what we will have talked about, but please try to ignore the recorder if possible and make yourself comfortable, this will be a very informal conversation and we just want to hear your thoughts.
d) You can stop the interview or withdraw from participation completely at any time.
e) Feel free to interrupt me with questions if you have any. Should we start with the interview?

Interview questions

1	What were your thoughts when you were asked to cut down on the amount of food? **Prompts:** Advantages? Disadvantages? Will I be capable of doing it? Have you thought about the consequences to the baby? Have you thought about the consequences for you? (e.g. help controlling GDM; help control sugar levels; feeling better; feeling more energetic)	**Beliefs about consequences** **Beliefs about capabilities** **Knowledge**
2	What were your feelings when you were asked to cut down on the amount of food?	**Emotion**

	What are your feelings now that you have carried out this reduced calorie diet in pregnancy?	
3	Could you tell me if you had some doubts about engaging in this diet? **Prompts:** Were you concerned about: Not knowing how to do it Not having the skills/ability Consequences Failing to achieve goals?	**Knowledge** **Beliefs about capabilities** **Beliefs about consequences**
4	How difficult did you imagine it would be to eat less and lose some weight during pregnancy?	**Skills**
5	How difficult did you actually find it to eat less and lose some weight during pregnancy?	**Skills**
6	At the beginning of the diet how confident did you feel about being able to stick to it?	**Optimism**
7	Over 4 weeks have now passed since you started the reduced calorie diet (RCD). Could you please tell me about your overall experience of it so far? **Prompts:** Did you have any cravings? Were you disappointed by the reduced choice of food? Were you tempted to break the diet? Did your mood or motivation change? Did anything help or hinder you when sticking to the diet? Did you lose any weight?	**Behaviour regulation, Intentions, Emotions, Environment, context, resources**
8	Did you have any strategies that helped you to continue with the diet? **Prompts:** Any Support from friends/family/partners	**Social influence**
9	How satisfied are you with the RCD and with the outcomes you have achieved so far? **Prompts:** Have you lost any weight? Did you find it easy to follow the diet? Were you satisfied with the food you were allowed/quantity? Were your energy levels affected?	**Reinforcement, Environment, context, resources**
10	Have you noticed any week-to-week changes? **Prompts:** Were there any other rewards associated with the RCD?	**Reinforcement**
11	How much effort did you spend planning your meals on the RCD? **Prompts:** Did the RCD just become part of your usual routine?	**Memory, attention, decision process**
12	How easy did you find it to plan what you would eat?	**Skills**
13	Were there any times when you didn't stick to the RCD if so was it a conscious decision?	**Memory, attention, decision process**

	Prompts: Did you forget or was it inconvenient?	
14	Have you experienced any lapse(s) during the diet period? **If yes** Can you tell me more about specific situation/s **Prompts:** when this happened? What did it feel like after you lapsed? Has anything changed since the lapse? (for example did you put any measures in place to reduce the chance of it happening again?) **If not** What helped you successfully continue with the **Prompts:** regime/ overcome your temptations? Did you have a strategy from the start? If so would you mind describing it? How did you feel like after you resisted possible temptations? Has anything changed since then?	**Behaviour regulation, Goals, Intentions, Emotion, Social influences**
15	Whilst doing the diet did you know how to access the information you needed to meet the diet targets? **Prompts:** Were you able to get recopies, did you know how to access nutritional information?	**Skills**
16	What kind of support (if any) would you have appreciated during this phase of the RCD? **Prompts:** To help overcome the problems and temptations To access more information/ideas	**Environment, context, resources**
17	Do you feel that carrying out/taking part in the diet was an appropriate part of your life? **Prompts:** Did you feel taking part was the right thing to do? Did you feel a responsibility to try to do the diet?	**Social professional role and identity**
18	Was taking part in the diet important to you at this time? **Prompts:** Please expand on the reasons why	**Social professional role and identity**
19	Would you feel capable of continuing on this diet?	**Beliefs about capabilities**

Thank the participant!