First Trimester Sonographic Assessment of Maternal Abdominal Adiposity and Fatty Liver Disease and the development of Dysglycemia and Gestational Diabetes Mellitus

By

Leanne Remy De Souza

A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy

Institute of Medical Science
University of Toronto

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Doctor of Philosophy
Institute of Medical Science
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Abstract

Obesity leads to insulin resistance (IR) and is part of the pathogenesis of gestational diabetes mellitus (GDM) and subsequent type 2 diabetes mellitus (T2DM) after pregnancy. Regional abdominal adipose tissue (AT) has emerged as a critical pathogenic mediator of IR and consequent metabolic dysfunction. While the mechanistic processes that characterize the pathophysiology of visceral AT (VAT) and IR remain to be elucidated, growing evidence describes the role of pro-inflammatory adipokines, free fatty acid release and changes in adipose tissue morphology that leads to ectopic fat accumulation in organs such as the liver. Hepatic fat deposition results in asymptomatic, but consequential, non-alcoholic fatty liver disease (NAFLD). Like abdominal AT, NAFLD is typically a sub-clinical state that long predates the onset of T2DM.
Clinicians routinely screen for GDM in the second trimester, which may be too late to favourably impact maternal and fetal outcomes. Early risk detection might help prevent GDM and its deleterious effects. We conducted a series of prospective studies to measure maternal abdominal AT at the same time each participant underwent routine first-trimester ultrasound assessment of fetal nuchal translucency, to screen for trisomy 21. First, we demonstrated that elevated first-trimester maternal VAT and total adipose tissue (TAT) were each associated with IR at 16 weeks’ gestation. We also observed that TAT and subcutaneous adipose tissue (SAT) were more strongly associated with adiponectin at 16 weeks’ gestation than was body mass index (BMI). Second, with a larger cohort of pregnant women and using the same method of first-trimester sonographic assessment of AT, we found that the highest quartile of first-trimester VAT and TAT, each predicted IR at 24-28 weeks’ gestation, and was most significantly associated with a higher odds of GDM. Finally, we used first-trimester clinical ultrasound to assess for evidence of NAFLD, and found that the presence of one or more sonographic features of NAFLD was associated with dysglycemia and GDM at 24-28 weeks’ gestation. This body of work suggests that first-trimester sonographic assessment of maternal abdominal AT and NAFLD may each provide a novel way to identify in early pregnancy, those women at higher risk for developing GDM.
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Contributions

Leanne R. De Souza (author) solely prepared this thesis. All aspects of this body of work, including the planning, execution, analysis, and writing of all original research and publications was performed in whole or in part by the author. The following contributions by other individuals are formally and inclusively acknowledged:

Dr. Joel G. Ray (Primary Supervisor and Thesis Committee Member) – resources; guidance and assistance in planning, execution, and analysis of experiments as well as manuscript/thesis preparation.

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<th>Description</th>
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<tbody>
<tr>
<td>ADA</td>
<td>American Diabetes Association</td>
</tr>
<tr>
<td>ACOG</td>
<td>American Congress of Obstetricians and Gynecologists</td>
</tr>
<tr>
<td>AHA</td>
<td>American Heart Association</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine Aminotransferase</td>
</tr>
<tr>
<td>AUC</td>
<td>Area Under the Curve</td>
</tr>
<tr>
<td>AT</td>
<td>Adipose Tissue</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>CAD</td>
<td>Coronary Artery Disease</td>
</tr>
<tr>
<td>CCDS</td>
<td>Canadian Chronic Disease Surveillance System</td>
</tr>
<tr>
<td>CHD</td>
<td>Coronary Heart Disease</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence Interval</td>
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<tr>
<td>CMR</td>
<td>Cardiometabolic risk</td>
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<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular Disease</td>
</tr>
<tr>
<td>DM</td>
<td>Diabetes Mellitus</td>
</tr>
<tr>
<td>EASD</td>
<td>European Association for the Study of Diabetes</td>
</tr>
<tr>
<td>GIGT</td>
<td>Gestational Impaired Glucose Tolerance</td>
</tr>
<tr>
<td>GCT</td>
<td>Glucose Challenge Test</td>
</tr>
<tr>
<td>GDM</td>
<td>Gestational Diabetes Mellitus</td>
</tr>
<tr>
<td>Ghb</td>
<td>Glycated Hemoglobin</td>
</tr>
<tr>
<td>HDL-C</td>
<td>High-Density Lipoprotein-Cholesterol</td>
</tr>
<tr>
<td>HMW</td>
<td>High molecular weight</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>Homeostatic Model Assessment of Insulin Resistance</td>
</tr>
<tr>
<td>hsCRP</td>
<td>high sensitivity C-reactive protein</td>
</tr>
<tr>
<td>IADPSG</td>
<td>International Association of Diabetes and Pregnancy Study Group</td>
</tr>
<tr>
<td>IDF</td>
<td>International Diabetes Federation</td>
</tr>
<tr>
<td>IFG</td>
<td>Impaired Fasting Glucose</td>
</tr>
<tr>
<td>IL-6</td>
<td>Interleukin-6</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>IR</td>
<td>Insulin Resistance</td>
</tr>
<tr>
<td>ISI</td>
<td>Insulin Sensitivity Index</td>
</tr>
<tr>
<td>LDL-C</td>
<td>Low-Density Lipoprotein-Cholesterol</td>
</tr>
<tr>
<td>NAFLD</td>
<td>Non-Alcoholic Fatty Liver Disease</td>
</tr>
<tr>
<td>NCEP-ATP III</td>
<td>National Cholesterol Education Program-Adult Treatment Panel III</td>
</tr>
<tr>
<td>NDDG</td>
<td>National Diabetes Data Group</td>
</tr>
<tr>
<td>NHLBI</td>
<td>National Heart, Lung and Blood Institute</td>
</tr>
<tr>
<td>OGTT</td>
<td>Oral Glucose Tolerance Test</td>
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<tr>
<td>OR</td>
<td>Odds Ratio</td>
</tr>
<tr>
<td>RR</td>
<td>Relative Risk</td>
</tr>
<tr>
<td>SAT</td>
<td>Subcutaneous Adipose Tissue</td>
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<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>SHBG</td>
<td>Sex Hormone Binding Globulin</td>
</tr>
<tr>
<td>T2DM</td>
<td>Type 2 Diabetes Mellitus</td>
</tr>
<tr>
<td>TAT</td>
<td>Total Adipose Tissue</td>
</tr>
<tr>
<td>VAT</td>
<td>Visceral Adipose Tissue</td>
</tr>
<tr>
<td>WC</td>
<td>Waist Circumference</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>WHR</td>
<td>Waist-to-Hip Ratio</td>
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</table>
Chapter 1  General Introduction
The concept of regional adiposity, and in particular the role of visceral adipose tissue (VAT) in the pathogenesis of diabetes mellitus (DM), was first studied over 40 years ago (Vague, 1956) and was subsequently confirmed in larger observational studies reviewed by Tchernof & Despres (2013). Central abdominal obesity is consistently implicated in obesity-mediated metabolic diseases, with multiple mechanistic pathways that range from physical changes in morphology to physiological changes in adipose tissue and biochemical changes in pro-inflammatory adipokines (Trayhurn & Wood, 2005). Given the increasing prevalence of metabolic diseases such as metabolic syndrome, type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD), the role of abdominal obesity continues to receive considerable attention (Tchernof & Despres, 2013).

While growing evidence over the past two decades has highlighted the pathogenic role of abdominal obesity as a part of the complex pathophysiology leading to the progression of DM outside of pregnancy, the clinical implications and precise contribution of abdominal adiposity to insulin resistance (IR) and DM in pregnancy remain unclear. One key question concerning how adipose tissue (AT) contributes to the development of gestational diabetes mellitus (GDM) is whether it can be used to detect IR well before the onset of overt GDM. Women affected by GDM are at increased risk of T2DM and CVD in the years following pregnancy and their offspring are at increased risk of childhood obesity and DM (Leon, 1998; Boney et al., 2005; Metzger, 2007; Reece et al., 2009).

Non-alcoholic fatty liver disease (NAFLD) is also garnering marked attention as evidence suggests that asymptomatic NAFLD may be an important precursor in the development of T2DM and CVD (Lamonaco et al. 2011).
Outside of pregnancy detection of NAFLD has been shown to precede DM and those with T2DM and CVD have higher rates of NAFLD (Lamonaco et al. 2011). Recent evidence emphasizes that NAFLD may manifest sub-clinically in pregnant women during the preconception years (Castracane et al., 2012). However, whether NAFLD can be detected in early pregnancy as a precursor of IR and GDM in later pregnancy is unclear.

Maternal abdominal adiposity and NAFLD may each represent a preconception or early pregnancy risk state for GDM that might be identified during routine first-trimester prenatal sonographic screening. Studies show that even elevated glucose values that fall below the clinically prescribed thresholds for diagnosis of frank GDM, confer a greater risk of GDM and subsequent T2DM (Retnakaran et al., 2008a). It remains unknown whether a first-trimester sonographic approach to quantifying AT and NAFLD can detect even subtle abnormalities, which would prompt risk identification and allow ongoing clinical monitoring to be offered to those at risk.

1.1 Thesis Organization

This thesis is organized in a “multiple paper format”, using mainly unaltered peer-reviewed content. Chapter 2 primarily reviews the metabolic syndrome and GDM and the context of maternal obesity-mediated comorbidities, and is largely derived from a peer reviewed book chapter on the topic (De Souza et
al., 2011). The final section of Chapter 2 outlines the research aims and hypotheses governing the thesis.

Chapters 3, 4, 5 and 6 present original research addressing the research aims described in the final section of Chapter 2, each as self-contained manuscripts. Chapter 3 is a reformatted version of a paper published in the *Journal of Obstetrics and Gynaecology Canada* (JOGC), (De Souza et al., 2014). Chapter 4 is a reformatted version of a paper submitted to Diabetic Medicine (De Souza et al., 2016). Chapter 5 is a reformatted version of a paper published in *Diabetes Care* (De Souza et al., 2015). Chapter 6 is a reformatted version of a paper published in the *American Journal of Gastroenterology* (De Souza et al., 2015). The discussion sections in the aforementioned chapters are complemented by Chapter 7, which briefly summarizes the general conclusions of the thesis, as well as some opportunities for ongoing and specific future directions.
Chapter 2  The Metabolic Syndrome and GDM

This chapter is reproduced and modified in full up to and including section 2.7, for the purpose of this doctoral thesis with permission from InTech from the following:

This review focuses on the metabolic syndrome, GDM, and abdominal adiposity among pregnant women. There is a considerable body of literature that implicates abdominal AT in the pathophysiology of T2DM (Tchernof & Després, 2013) outside the context of pregnancy and is thus deliberately not discussed here. The final section outlines the aims and hypotheses of the original research within this thesis.

2.1 Introduction

The metabolic syndrome is a clustering of traditional cardiometabolic risk factors that include central obesity, dysglycemia, hypertension, hypertriglyceridemia, and reduced high-density lipoprotein (HDL) cholesterol. In recent years, its clinical utility, diagnostic criteria and underlying etiology have been the subject of continuous debate and controversy. However, it remains incontrovertible that those identified with the metabolic syndrome are at high risk for the future development of T2DM and CVD. An expanding body of evidence has also linked the metabolic syndrome with several emerging non-traditional risk factors, including markers of hepatic fat, chronic inflammation (such as C-reactive protein [CRP]), and adipocyte dysregulation (such as low circulating levels of adiponectin). Many of these features of the metabolic syndrome are also common to GDM. GDM itself has been the subject of longstanding debate and its identification signifies a high risk of developing T2DM and CVD in the future. GDM has been similarly linked to an array of non-traditional cardiometabolic risk factors, including CRP and hypoadiponectinemia. A series of studies have demonstrated that women with GDM are at risk of developing the metabolic syndrome in the years following their index pregnancy (Retnakaran et al., 2008; Retnakaran et al., 2009; ADA, 2010).
Emerging evidence shows that components of the metabolic syndrome identified in early gestation and even prior to conception can predict the subsequent development of GDM. Taken together, this body of data has raised the intriguing possibility that women who develop GDM may have an underlying latent metabolic syndrome that warrants clinical evaluation and risk factor modification. Though intricate and still incompletely understood, the gradual expansion of knowledge about interrelationships between the metabolic syndrome, GDM, and T2DM may provide us with opportunities to screen and detect metabolic dysfunction at various stages of disease progression. In this way, GDM represents an important and early “metabolic flag” for an affected mother and, perhaps, her offspring.

In the following, we explore the emerging relationship between GDM and the metabolic syndrome; we also review the definitions of each condition, their limitations and controversies, and their utility and predictive value in identifying T2DM and CVD risk. The clinical evidence for the metabolic syndrome as a precursor to the development of GDM and, in turn, T2DM is also discussed. Emerging non-traditional risk factors for both the metabolic syndrome and GDM are described, alongside evidence that suggests that the metabolic syndrome is a consequence of GDM and is a potential predictive tool to detect risk for GDM before and during early pregnancy. Finally, we consider the contention that women who develop GDM may have a latent metabolic syndrome and important emerging risk factors such as increased abdominal adiposity and fatty liver.
2.2 Metabolic syndrome

2.2.1 General classification and varying sets of diagnostic criteria

The metabolic syndrome, also referred to as the IR syndrome, was initially proposed as a model for understanding the underlying biology and risk factors for CVD. In his 1988 Banting Lecture, Gerald Reaven first described “Syndrome X” as the clustering of abnormalities related to IR (Reaven, 1988). The World Health Organization formally proposed the term “metabolic syndrome” in 1998 (DeFronzo & Ferrannini, 1991; Alberti et al., 1998) to identify those at high risk for metabolic disorders and CVD. Though the syndrome was originally intended to identify individuals at risk for CVD, it has since expanded to capture those at high risk for T2DM, with which it is thought to have a stronger association (Ford et al., 2008). Our understanding of the metabolic syndrome continues to evolve today, and is widely studied as a promising marker of cardiovascular risk. The syndrome is characterized by a clustering of central abdominal (visceral) obesity, glucose intolerance, IR, dyslipidemia and hypertension (Reaven, 1988). The presence of any one risk factor implies the existence of others, such that their concomitant occurrence collectively describes a positive dysmetabolic risk profile for CVD, or “cardiometabolic risk” (Després et al., 2008). While several organizations and authoritative bodies have proposed diagnostic criteria for the metabolic syndrome, the most cited working definitions are those of the International Diabetes Federation (IDF), the World Health Organization (WHO), and the National Cholesterol Education Program – Adult Treatment Panel III (NCEP-ATP III) (Alexander et al., 2003; WHO Expert Consultation, 2004; Alberti et al., 2005).
These authorities have translated, analyzed, and integrated information gathered from a vast, globally representative body of research to provide a set of diagnostic criteria with clinically relevant thresholds and measurements that can identify the metabolic syndrome and hence the risk of T2DM and CVD. Despite continued efforts, there are variations in its definitions, which have prompted international debate about the utility and effectiveness of the metabolic syndrome as a diagnostic tool. Table I lists the criteria and diagnostic thresholds defined by the IDF, WHO, NCEP ATP III, and, lastly, recently published harmonized criteria (discussed in section 2.2). Although the ATP III and IDF definitions differ in their diagnostic threshold criteria for the metabolic syndrome, both include the same five components: increased adiposity, hypertriglyceridemia, low levels of high-density lipoprotein cholesterol (HDL-C), hypertension and dysglycemia. The WHO definition also includes a urine albumin-to-creatinine ratio. Meeting the dichotomous cut-off points for an abnormality in three or more of the five components fulfills the requirements for diagnosis according to the ATP-III definition (Hunt et al., 2004).

Though all definitions include an obesity criterion, the IDF definition provides ethnicity-specific values for diagnosing abdominal obesity (Reaven, 2009). Importantly, the IDF definition requires the presence of increased waist circumference (WC) as a necessary prerequisite along with any two of the other criteria. Elevated triglycerides and/or low HDL-C must fall within the prescribed range or can be applied if a person is being treated specifically for the lipid abnormality. In addition, the defining criteria consider those with T2DM, an elevated WC and at least one other risk factor as having the metabolic syndrome. The WHO requires the presence of diabetes, impaired fasting glucose (IFG), impaired glucose tolerance (IGT) or IR, and at least two other criteria. Among the three definitions, the IDF and ATP III are more commonly cited in the literature. The prevalence of the metabolic syndrome in
U.S. adults is estimated to be approximately 22% to 34% using the ATP III definition and 39% when the IDF criteria are applied (Ford, 2005).

Table 2.1: Various diagnostic criteria for the metabolic syndrome

<table>
<thead>
<tr>
<th></th>
<th>NCEP-ATP III</th>
<th>IDF</th>
<th>WHO</th>
<th>Harmonized IDF/AHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central obesity</td>
<td>WC &gt; 102 cm- (M)</td>
<td>WC &gt; 94 cm- White (M)</td>
<td>WHR: &gt; 0.90- (M)</td>
<td>Same as IDF cut points for non-Europeans &amp; either IDF or AHA criteria for Europeans</td>
</tr>
<tr>
<td></td>
<td>WC &gt; 88 cm- (F)</td>
<td>WC &gt; 80 cm- White (F)</td>
<td>&gt; 0.85- (F)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>WC &gt; 90cm- Asian (M)</td>
<td>WC &gt; 80 cm- Asian (F)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elevated triglycerides</td>
<td>≥ 1.7 mmol/L</td>
<td>&gt; 1.7 mmol/L</td>
<td>&gt; 1.7 mmol/L</td>
<td></td>
</tr>
<tr>
<td>Reduced HDL-C</td>
<td>&lt; 1.0 mmol/L- (M)</td>
<td>&lt; 1.03 mmol/L- (M)</td>
<td>&lt; 0.9 mmol/L- (M)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt; 1.3 mmol/L- (F)</td>
<td>&lt; 1.29 mmol/L- (F)</td>
<td>&lt; 1.0 mmol/L- (F)</td>
<td></td>
</tr>
<tr>
<td>Elevated blood pressure</td>
<td>≥ 130/85 mm Hg</td>
<td>≥ 130/85 mm Hg</td>
<td>≥ 140/90 mm Hg</td>
<td>Systolic ≥ 130 and/or diastolic &gt; 85 mmHg</td>
</tr>
<tr>
<td>Fasting hyperglycemia</td>
<td>≥ 6.1 mmol/L</td>
<td>&gt; 5.6 mmol/L or diabetes or IGT</td>
<td>Diabetes, IFG, IGT</td>
<td>≥ 5.6 mmol/L</td>
</tr>
<tr>
<td>Urine albumin: creatinine ratio</td>
<td>-</td>
<td>-</td>
<td>≥ 3.4 mg/mmol</td>
<td>-</td>
</tr>
</tbody>
</table>

2.2.2 Controversy regarding the metabolic syndrome

The debate surrounding the metabolic syndrome stems from disagreement about its definition and diagnostic criteria, alongside questions related to its pathogenesis, origins and applicability across populations. However, despite this ongoing debate, central obesity and IR have been widely postulated (Lann & LeRoith, 2007) as comprising the metabolic syndrome's fundamental basis.

Categorically, the syndrome is influenced by the complex genetic, hormonal and nutritional origins of its individual component risk factors.
Discrepancies among the commonly used NCEP-ATP III, IDF and WHO definitions of the metabolic syndrome have contributed substantially to this debate. For example, ATP III and WHO differ in their criteria for blood pressure (130/85 and 140/90 mm Hg, respectively), and neither definition provides specific guidance on how to implement these diagnostic thresholds (i.e. whether to use abnormal systolic versus diastolic or both; whether to obtain measures in a particular body position; or whether to calculate an averaged measure). Recently, the American Heart Association/National Heart, Lung and Blood Institute (AHA/NHLBI) and IDF attempted to resolve such discrepancies with new harmonized criteria. These criteria, shown in Table I, include (i) clarification of the blood pressure measurement to specify elevated levels of systolic and/or diastolic pressure and (ii) elimination of abdominal obesity as a mandatory prerequisite, such that the presence of any three of the five criteria is sufficient for diagnosis of the metabolic syndrome (Alberti et al., 2010). The NCEP and IDF definitions differ in their criteria for increased fasting glucose and central obesity (using WC) (NCEP, 2001; Alberti et al., 2006). While obesity is measured by WC according to the ATP III and IDF definitions, waist-to-hip (WHR) ratio is used in the WHO definition. Furthermore, the urine albumin-to-creatinine ratio is a criterion in the WHO definition, but is not found in the ATP III and IDF definitions, while several risk factors associated with IR are not considered in any of the definitions, including physical inactivity, family history, sex and age (Kahn et al., 2005).

Further complicating the controversy is the practical observation that, despite its centrality to the metabolic syndrome, contrasting evidence suggests that many overweight or obese individuals may, by any guideline, have normal
metabolic profiles (Wildman et al., 2008), and not be prone to future development of the metabolic syndrome. Similarly, among those who display markers of the metabolic syndrome, not all are obese (Bruce & Hanson, 2010). Some lean individuals are insulin resistant and exhibit increased cardiometabolic risk. In a study of otherwise healthy obese individuals and insulin resistant lean individuals with a family history of T2DM, obesity was associated with higher IR and diastolic blood pressure, but conveyed no difference in other metabolic markers. In addition, within each BMI category, IR independently predicted the metabolic syndrome, while WC did not. Only when age was combined with WC (but not BMI) did obesity independently predict the metabolic syndrome and, even so, WC was less predictive of IR at higher WC values (Utzschneider et al., 2010). The authors concluded that insulin sensitivity is a stronger predictor of the metabolic syndrome than obesity, and is better than WC at identifying obese individuals with an otherwise healthy metabolic profile. They also recommended employing metabolic testing among lean individuals with a first-degree relative with T2DM (Utzschneider et al., 2010). Nevertheless, even when weight is considered, cut points used to define obesity are not universally agreed on and may vary by ethnicity (Després et al., 2008).

It is clear that the use of different definitions of the metabolic syndrome has impeded our ability to compare findings across research studies. In addition, there is the question of whether the diagnostic criteria are too restrictive, missing those at highest risk or, conversely, are too broad, resulting in an overestimation of the prevalence of the metabolic syndrome. Considering its inherently chronic and progressive nature, it is reasonable to infer that indicators of dysmetabolism, especially in younger adults, underestimate consequences and prediction of T2DM and CVD. Indeed, manifestation may even occur at different time points in the disease trajectory, such that risk factor assessment necessitates systematic evaluation across a spectrum of sub-diagnostic and diagnostic ranges standardized for age.
Another criticism of the current definitional status of metabolic syndrome is whether its value extends beyond that of its individual components. This criticism highlights both the redundancy of its classification as a “syndrome” and the inadvertent undermining of the importance of the individual components. Diagnosis of the metabolic syndrome, by any definition, has been studied in relation to the predictive value of the individual criteria. The Framingham study (Wilson et al., 2005) demonstrated no substantial increase in risk associated with clusters of three of the five metabolic syndrome criteria compared with clusters of only two traits. In contrast, data from the Third National Health and Nutrition Examination Survey (Ninomiya et al., 2004) indicated that each of the five components of metabolic syndrome was an independent predictor of CVD. These studies illustrate the controversy over whether a diagnosis of the metabolic syndrome provides more useful information about CVD risk than any of its individual components (Reaven, 2009). Furthermore, by the current definitions, it is unclear whether any one risk factor is more predictive than another, in the form of a weighted hierarchy.

To address these criticisms, the American Diabetes Association (ADA) and European Association for the Study of Diabetes (EASD) issued a joint statement in 2005 about the clinical utility of the metabolic syndrome; they recommended that clinicians evaluate and treat discrete risk factors, without diagnosing the metabolic syndrome, per se (Kahn et al., 2005). Specifically, rather than solely relying on diagnosis of the metabolic syndrome, identification of one or more of its component features should prompt investigation for the presence of the other features. For the latter, one may also consider specific emerging risk factors not included in the existing definition, as outlined below.
2.3 The metabolic syndrome and the identification of future risk of T2DM and CVD

The IDF recommends screening for features of the metabolic syndrome in those with T2DM (Alberti et al., 2005; Alberti et al., 2006). While current recommendations are subject to criticism and controversy, they nevertheless provide a practical basis for determining management strategies. Individuals with the metabolic syndrome have a fivefold higher risk of developing T2DM (Alberti et al., 2010). Similarly, in a study from the UK that examined the prognostic impact of the metabolic syndrome in T2DM, the investigators modified the ATP III definition to include BMI instead of WC, and found that the metabolic syndrome further predicted CVD incidence five years after the diagnosis of T2DM (Guzder et al., 2006).

Showing that dysglycemia predicts the metabolic syndrome necessarily identifies a predictive potential for T2DM as well. This is so given that the metabolic syndrome—and especially glucose intolerance—is central to the development of T2DM. In the GENFIEV study, metabolic syndrome prevalence was 42% in those with IFG, 34% in IGT, and 74% in IFG and IGT (Bianchi et al., 2010). In addition, the prevalence of IR was higher in those with the metabolic syndrome than in its absence. Hypertriglyceridemia (OR 3.38; 95% CI 2.29 to 4.99), abdominal obesity (3.26; 95% CI 2.18 to 4.89), hyperglycemia (3.02; 95% CI 1.80 to 5.07) and hypertension (1.69; 95% CI 1.12 to 2.55) were all associated with IR. These findings suggest that the prevalence of the metabolic syndrome is high in individuals with dysglycemia, and is generally associated with IR (Bianchi et al., 2010).

Dysglycemia and IR are highly predictive of T2DM. Long-term glycemic excursions will identify those at high risk for the metabolic syndrome and T2DM. In their exploratory study, Giuffrida et al. (2010) investigated the relation between glycated hemoglobin (GHb), an indicator of long-term glycemic control, and the metabolic syndrome with T2DM. Each 1% increase in GHb
was associated with a greater risk of the metabolic syndrome (OR 1.31, 95% CI 1.18 to 1.45), demonstrating a strong relation between chronic hyperglycemia and the metabolic syndrome (Giuffrida et al., 2010).

Aboriginal Canadians have a fivefold higher risk of T2DM compared with non-Aboriginals. Among the former, the metabolic syndrome can be readily identified using available clinical measures and, thus, may be a useful clinical tool (Ley et al., 2009; Reaven, 2009). In a prospective study, Ley and colleagues (2009) found that the 10-year cumulative incidence of T2DM in the Aboriginal Canadian population was 17.5%, with an age-dependent gradient ranging from 10.5% among those aged 10-19 years, to 43.3% among those aged 40-49 years. The authors reported that, at baseline, the metabolic syndrome had a low positive predictive value for future diabetes; however, the syndrome predicted incident diabetes to the same degree as IGT, while its high negative predictive value identified disease-free individuals at follow up (Ley et al., 2009).

In addition to identifying those at risk of T2DM, the metabolic syndrome independently predicts risk of CVD. In the joint statement from the ADA and the EASD, the authors emphasized the practical use of the metabolic syndrome, focusing on its predictive value for CVD (Kahn et al., 2005). A meta-analysis of a series of European trials reported that the metabolic syndrome raises the hazard ratio for CVD in women from 0.6 to 2.8 (Hu et al., 2004). Moreover, patients with the metabolic syndrome are at twice the risk of developing CVD over a 5-10 year period than those without the syndrome (Alberti et al., 2010). Several population studies have described an increased cardiovascular risk in the presence of the metabolic syndrome (Isomaa et al., 2001; Alexander et al., 2003; Hunt et al., 2004; Ford, 2004, 2005; McNeill et al., 2005). Alexander and colleagues (2003) used the ATP III criteria to assess the prevalence of coronary heart disease (CHD) among patients with the metabolic syndrome. They reported that those without the metabolic syndrome, regardless of
diabetes status, had a low CHD prevalence (less than 10%), while those with diabetes but not the metabolic syndrome were also not at increased risk of CHD (Alexander et al., 2003). Otherwise, the metabolic syndrome was a significant predictor of CHD (OR 2.07, 95% CI 1.66 to 2.59) and conferred a risk beyond that of diabetes alone (Alexander et al., 2003).

In the San Antonio Heart Study, presence of the metabolic syndrome at baseline was a significant predictor of cardiovascular mortality over a mean follow-up of 13 years (Hunt et al., 2004). Similarly, using the WHO definition, Isomaa and colleagues (2001) found that the risk for CHD and stroke increased threefold in those with the metabolic syndrome (P < 0.001) as did cardiovascular mortality (P < 0.001). In a study of individuals without diabetes or CVD at baseline, the ATP III-defined metabolic syndrome had an adjusted hazard ratio (HR) of CHD of 1.46 (95% CI 1.23 to 1.74) for men and 2.05 (95% CI 1.59 to 2.64) for women (McNeill et al., 2005).

Ford and colleagues (2004) showed a linear association between the ATP III-based metabolic syndrome and CVD-related mortality as well as all-cause mortality. A meta-analysis of worldwide data from studies published between 1998 and 2005 showed pooled relative risks (RR) of 1.27 (95% CI 0.90 to 1.78) for all-cause mortality, 1.65 (95% CI 1.38 to 1.99) for CVD and 2.99 (95% CI 1.96 to 4.57) for T2DM using the ATP III-defined metabolic syndrome; in the studies that used the exact WHO definition, the pooled RRs were 1.37 (95% CI 1.09 to 1.74) for all-cause mortality and 1.93 (95% CI 1.39 to 2.67) for CVD (Ford, 2005). Thus, there is considerable evidence for the predictive value of the metabolic syndrome for identifying risk of T2DM and CVD.
2.3.1 The metabolic syndrome and emerging non-traditional risk factors

As the components of the metabolic syndrome continue to be better understood, the syndrome appears to be a promising diagnostic and screening tool. Recent studies have identified chronic low-grade inflammation as a systemic consequence of obesity that is related to both metabolic and vascular disease. For example, the inflammatory nature of atherosclerosis prompted the study of inflammatory proteins, such as CRP, as potential predictors of CVD. Indeed, epidemiological studies have shown the independent relation between CRP and coronary artery disease (CAD) (Ridker, 1997, 1998).

Nakano et al. (2010) investigated the clinical significance of LDL and CRP in CAD risk (Nakano et al., 2010). Among those without the metabolic syndrome, high CRP was not associated with a higher risk of CAD; however, those with both high CRP and the metabolic syndrome experienced a doubling of their risk of CAD (Ridker et al., 2003; Sattar et al., 2003). Despite uncertainty regarding the utility of adding CRP to the metabolic syndrome definition, Ridker et al. (2004) has advocated the further investigation of its potential as a predictive tool and the possibility of it becoming a meaningful addition to criteria for the metabolic syndrome.

Previous studies have also examined the use of AT biomarkers, including adiponectin, in predicting CVD risk. Adiponectin is an adipocyte-derived polypeptide—an adipokine—that is inversely associated with obesity, IR and T2DM.

As a protective adipokine, it inhibits gluconeogenesis and suppresses lipogenesis. Low levels of adiponectin result in reduced fatty acid oxidation and increased fat accumulation in the liver. AT plays a central role in the metabolic syndrome, and low adiponectin levels are associated with the metabolic
syndrome. In addition, the strong association between hypoadiponectinemia and CVD risk implicates adiponectin in the disease trajectory. Compared with lean controls, patients with the metabolic syndrome and T2DM have lower circulating levels of total and high molecular weight (HMW) adiponectin, and higher levels of leptin and interleukin-6 (IL-6). Decreased total and HMW adiponectin and increased levels of leptin and IL-6 are characteristic of patients with the metabolic syndrome and T2DM (Lee et al., 2009). There may also be a link between low adiponectin and non-alcoholic fatty liver disease (Matsubara et al., 2004).

Hepatic dysregulation is characterized by IR-related steatosis and oxidative stress (Kim & Younossi, 2008). NAFLD—ranging from simple steatosis (fatty infiltration) to inflammatory steatohepatitis (NASH), to long-term injury (fibrosis)—is a strong indicator of insulin resistance in non-pregnant adults (Youssef & McCullough, 2002). The process of NAFLD development is in itself an extension of IR that reduces free fatty esterification and triglyceride storage in AT, subsequently resulting in the deposition of free fatty acids in non-adipose tissues, especially the liver (Utzschneider & Kahn, 2006). Hence, NAFLD is considered to be the principal liver manifestation of the metabolic syndrome (Kim & Younossi, 2008), since it requires the presence of IR and is closely associated with T2DM (Targher et al., 2007). In a recent study of adults with newly diagnosed T2DM, there was significant interplay between T2DM and liver injury, likely explained by NAFLD (Porepa et al., 2010). NAFLD may also be detected with the novel serum marker, Fetuin-A, an endogenous inhibitor of insulin receptor tyrosine-kinase, and a recognized “hepatokine”. Elevated plasma Fetuin-A levels positively predict the incidence of T2DM independent of other established risk factors (Ix et al., 2008; Stefan et al., 2008). In a study of 330 adults at risk for T2DM, liver fat was the strongest predictor of prediabetes (Kantartzis et al., 2010). Among studied biochemical measures, serum Fetuin-A was a more significant predictor of fasting hyperglycemia than serum adiponectin (Kantartzis et al., 2010). In addition,
individual liver enzymes such as alanine aminotransferase have varying positive associations with the components of the metabolic syndrome (Zhang et al., 2010).

Hyperuricemia is prevalent among those with the metabolic syndrome; however its clinical utility remains controversial. Nonetheless, it appears to be a predictor of the metabolic syndrome. One hypothesis is that enhanced IR due to fatty acid synthesis in the liver may be linked to additional purine synthesis, thereby accelerating production of uric acid. Since IR is considered an underlying mechanism connecting visceral obesity and the metabolic syndrome (Matsuura et al., 1998), it follows that IR is related to elevated serum uric acid levels in those with the metabolic syndrome (Borges et al., 2010). While a sex-dependent association between hyperuricemia and the metabolic syndrome is apparent, there is no current evidence for its association with sex hormones.

Sex hormone binding globulin (SHBG) is a liver-derived glycoprotein regulated by insulin, which inhibits its production in hepatocytes. Low serum SHBG levels are associated with increased IR and hyperinsulinemia. In a recent review, Brand et al. (2011) examined 52 observational studies and found that, for both sexes, the metabolic syndrome was associated with low levels of SHBG.
In addition to traditional measures for GDM, these emerging non-traditional risk factors are the same as those described for GDM (Figure 2.2). Accordingly, they raise the question of whether the metabolic syndrome relates to GDM.
2.4 Gestational Diabetes Mellitus (GDM)

2.4.1 General definition and varying sets of diagnostic criteria

GDM, defined as glucose intolerance of varying severity with first onset in late pregnancy, bears many of the same risk factors as T2DM, including older maternal age, a family history of T2DM, non-White ethnicity, obesity, sedentary lifestyle and previous GDM (Dornhorst et al., 1992; Ray et al., 2001; Hedderson & Ferrera, 2008; Hillier et al., 2008a). (Table 2.2). GDM has both short-term and long-term consequences for a mother and her child (Table 2.3).

Table 2.2: Maternal risk factors for GDM

<table>
<thead>
<tr>
<th>Maternal risk factor (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family history of T2DM in a first-degree relative (Hillier et al., 2008a)</td>
</tr>
<tr>
<td>Elevated pre-pregnancy/early pregnancy weight and/or excessive weight gain in pregnancy</td>
</tr>
<tr>
<td>(Hillier et al., 2008b; Ray et al., 2001)</td>
</tr>
<tr>
<td>Non-White ethnicity (e.g., Hispanic, First Nations, South Asian, East Asian or Pacific Islander</td>
</tr>
<tr>
<td>and African Americans) (ACOG, 2010; Dornhorst et al., 1992)</td>
</tr>
<tr>
<td>Older maternal age (ACOG, 2010)</td>
</tr>
<tr>
<td>Previous diagnosis of GDM, IGT or IFG in pregnancy (Landon, 2010)</td>
</tr>
<tr>
<td>Previous delivery of a macrosomic infant (ACOG, 2010)</td>
</tr>
<tr>
<td>Polycystic ovary syndrome (ACOG, 2010)</td>
</tr>
<tr>
<td>Hypertension (Hedderson &amp; Ferrara, 2008)</td>
</tr>
</tbody>
</table>
Table 2.3: Potential sequelae of GDM for mother & child

<table>
<thead>
<tr>
<th>Individual affected</th>
<th>Timing</th>
<th>Potential sequelae (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetus/infant</td>
<td>Short-term</td>
<td>Macrosomia &amp; large for gestational age birthweight (ADA, 2010) associated with shoulder dystocia (Athukorala et al., 2007) &amp; neonatal musculoskeletal &amp; brachial plexus injury (Christoffersson &amp; Rydhstroem, 2002)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Neonatal hypoglycem ia (ADA, 2010)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Respiratory Distress Syndrome (ADA, 2010)</td>
</tr>
<tr>
<td>Fetus/infant</td>
<td>Long-term</td>
<td>Higher risk of childhood obesity (Hillier et al., 2008a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Higher risk of T2DM and hypertension (Leon, 1998; van Hoorn et al., 2002; Boney et al., 2005)</td>
</tr>
<tr>
<td>Mother</td>
<td>Short-term</td>
<td>Pregnancy-induced hypertension (Joffe et al., 1998)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Caesarean delivery (Naylor et al., 1996)</td>
</tr>
<tr>
<td>Mother</td>
<td>Long-term</td>
<td>T2DM (Feig et al., 2008; Retnakaran et al., 2008)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CVD (Retnakaran et al., 2009)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GDM in a subsequent pregnancy (Metzger, 2007; ADA, 2010)</td>
</tr>
</tbody>
</table>

Acute maternal effects include pregnancy-induced hypertension and higher risk of Caesarian section; long-term consequences include increased risks of T2DM and CVD. Neonatal complications include fetal macrosomia and the associated risk of shoulder dystocia (Athukorala et al., 2007), which in turn can lead to neonatal musculoskeletal and brachial plexus injury (Christoffersson & Rydhstroem, 2002). Long-term sequelae are childhood obesity, the metabolic syndrome, and higher risk of T2DM and hypertension (Joffe et al., 1998; Leon, 1998; Boney et al., 2005; Athukorala et al., 2007; Metzger, 2007; Reece et al., 2009).
Though practice varies, many countries recommend that all pregnant women be screened at 24 to 28 weeks’ gestation with a 1-hour 50-g glucose challenge test (GCT), followed by a confirmatory 2-hour 75-g, or 3-hour 100-g oral glucose tolerance test (OGTT). While a strategy of selectively screening only women at high risk of GDM may improve the true positive detection rates, some women without classical risk factors for GDM will be missed accordingly. Table 2.4 outlines some commonly used diagnostic criteria for GDM.

Table 2.4: Diagnostic criteria for GDM according to commonly used definitions

<table>
<thead>
<tr>
<th></th>
<th>NDDG (National Diabetes Data Group)</th>
<th>ADA 2003</th>
<th>WHO 1999</th>
<th>IADPSG 2010 (Harmonized)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnostic OGTT</td>
<td>3-h 100-g</td>
<td>3-h 100-g</td>
<td>2-h 75-g</td>
<td>2-h 75-g</td>
</tr>
<tr>
<td>OGGT-Fasting</td>
<td>5.8 mmol/L</td>
<td>5.3 mmol/L</td>
<td>7.0 mmol/L</td>
<td>5.1 mmol/L</td>
</tr>
<tr>
<td>OGGT-1-h</td>
<td>10.5 mmol/L</td>
<td>10.0 mmol/L</td>
<td>-</td>
<td>10.0 mmol/L</td>
</tr>
<tr>
<td>OGGT-2-h</td>
<td>9.2 mmol/L</td>
<td>8.6 mmol/L</td>
<td>7.8 mmol/L</td>
<td>8.5 mmol/L</td>
</tr>
<tr>
<td>OGGT-3-h</td>
<td>8.0 mmol/L</td>
<td>7.8 mmol/L</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Abnormal values needed for diagnosis</td>
<td>&gt; 2</td>
<td>&gt; 2</td>
<td>&gt; 1</td>
<td>&gt; 1</td>
</tr>
</tbody>
</table>

Despite variations in diagnostic criteria, the utility of these definitions for predicting clinical outcomes has been demonstrated. As an example, while the ADA and WHO diagnostic criteria for GDM differ slightly (International Association of Diabetes and Pregnancy Study Group [IADPSG], 2010), the antepartum 2-hour 75g OGTT predicts adverse pregnancy outcomes based on both criteria. The ADA criteria resulted in an increased risk of macrosomia (RR 1.29, 95% CI 0.73 to 2.18), preeclampsia (RR 2.28, 95% CI 1.22 to 4.16) and perinatal death (RR 3.10, 95% CI 1.42 to 6.47) (Schmidt et al., 2001), and similar results were observed using the WHO criteria.
Some speculate that the restrictive diagnostic criteria for GDM may overlook the risks faced by women with lesser degrees of dysglycemia (Vambergue et al., 2000; Ferrara et al., 2007). Others assert that lack of international uniformity and agreement of diagnostic thresholds for GDM limits their utility within clinical settings (Metzger & Coustan, 1998). For example, the UK guidelines recommend that only high-risk groups be screened (IADPSG, 2010). In Canada, screening for GDM is routinely done, but not in a universal manner (Wen et al., 2000). Furthermore, current guidelines do not account for the variable risk attributed to ethnicity, for which there are considerable differences in the prevalence of GDM. In a study of ethnicity and postpartum metabolism in women with prior GDM, South Asian Indian women had higher serum triglycerides and lower HDL-C levels, while African-Caribbean women had higher WC, blood pressure, and insulin levels (Savitz et al., 2008).

### 2.4.2 Controversy regarding GDM

As with the metabolic syndrome, GDM has also been the subject of controversy, especially surrounding the timing of screening, the choice of diagnostic test, and the defining thresholds on these tests for its identification. Existing guidelines used to identify GDM, and hence the high risk of T2DM following pregnancy, were initially adapted from criteria that were applied to the non-pregnant population; they were not designed to identify those at risk for adverse perinatal outcomes (IADPSG, 2010). Extensive research has led to modifications of the definition (Cutchie et al., 2006) following the original publication of the criteria (O’Sullivan & Mahan, 1964). Of note, these original criteria were based on the identification of those women at risk of developing diabetes in the years after the index pregnancy (O’Sullivan & Mahan, 1964).

The clinical justification for screening for GDM currently focuses on the prevention of fetal macrosomia and associated obstetrical complications.
(Retnakaran et al., 2009c). Notably, this focus has resulted in a single set of diagnostic criteria used to identify women at risk for two different adverse outcomes (Retnakaran et al., 2009c), which effectively leads to the assumption that a diagnosis of GDM optimally identifies the risks of both macrosomia and postpartum prediabetes/diabetes. In a study designed to test this assumption, subjects representing the full spectrum of antepartum glucose tolerance underwent a 3-hour OGTT, and the results showed that only fasting glucose emerged as a significant predictor for delivery of a large-for-gestational-age (LGA) infant, with an OR of 2.0 (95% CI 1.20 to 3.34) per 1 mmol/L incremental increase (Retnakaran et al., 2009c).

However, all three post-load measures were significant predictors of postpartum prediabetes/diabetes (1-h glucose: OR 1.37, 95% CI 1.17 to 1.61; 2-h glucose: OR 1.55, 95% CI 1.32 to 1.83; 3-h glucose: OR 1.30, 95% CI 1.10 to 1.53). Thus, fasting glucose values may better predict LGA risk, but post-load values better predict postpartum glucose intolerance (Retnakaran et al., 2009c). Clearly, an additional challenge to the GDM diagnostic definition includes how the results are applied.

The prevailing consensus within the existing framework for diagnosing GDM is that hyperglycemia, including levels below those for overt diabetes, is associated with the adverse pregnancy outcomes common to GDM. In addition, most agree that screening for GDM at 24-28 weeks’ gestation identifies individuals in whom effective management can reduce glycemic excursions and minimize adverse perinatal outcomes. It remains to be determined, however, whether these current strategies can effectively reduce long-term risks of the metabolic syndrome, T2DM and CVD in affected women (Nolan, 2011).

Indeed, women who do not meet the prescribed thresholds for GDM may incur glucose-mediated fetal macrosomia (Sermer et al., 1995; Mello et al., 1997; Rudge et al., 2000; Scholl et al., 2001), and may be at risk for T2DM and CVD
The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) examined the risks associated with glucose values below traditional thresholds used to diagnose GDM. The study findings were translated by the IADPSG, in order to harmonize the existing diagnostic criteria (Table 2.4). The practical implications of these revised criteria include the universal adoption of a 2-hour 75-g OGTT. In doing so, these recommendations may identify an increased number of women at lower risk for complications. How the IADPSG recommendations will impact the risk of long-term development of metabolic diseases remains unknown at this time (IADPSG, 2010).

2.4.3 GDM and the Identification of future risk of T2DM and CVD

It is estimated that worldwide 20-60% of women with a history of GDM will eventually develop T2DM. Indeed, the relation between GDM and T2DM is well described, and the two conditions share a similar pathophysiology characterized by IR of peripheral tissues and insufficient secretion of insulin by the pancreatic beta cells to compensate for this resistance (Buchanan, 2001; Buchanan & Xiang, 2005; Retnakaran et al., 2010a). Pregnancy itself has been described as a “stress test” for T2DM and CVD (Reece et al., 2009). It necessarily involves a state of severe acquired IR that is comparable to that of a non-pregnant person with T2DM (Bergman, 1989).

Furthermore, adult offspring with prediabetes, born to women with previous GDM, display a 6.8-times higher risk of T2DM (Clausen et al., 2008), demonstrating the cyclical nature of diabetes (Damm, 2009) and the compounding effects of dysmetabolism in pregnancy.
Since O’Sullivan’s early research illustrating high rates of IGT in the years following GDM (O’Sullivan, 1991), many studies have investigated the phenomena of elevated risk of T2DM attributed to previous GDM. In their systematic review and meta-analysis to quantify the risk of T2DM following GDM, Bellamy and colleagues (2009) found that women with GDM had an increased risk of developing T2DM compared with those women who had normoglycemic pregnancies (RR 7.43, 95% CI 4.79 to 11.51. Epidemiological evidence shows that, for all populations and ethnic groups, GDM increases the risk of T2DM (Ben-Haroush et al., 2004). While the shared risk factors between GDM and T2DM imply a common etiology, the salient message is that women with a history of GDM represent a highly vulnerable group for the development of T2DM. This is readily evident in the 2% prevalence of T2DM following GDM as early as six weeks postpartum, with reported rates of 50-60% at 5-10 years postpartum, and 70% by 28 years postpartum (Kim et al, 2002; Lauenborg et al., 2004).

Furthermore, at the population level, the health significance of GDM is apparent in the number of individuals with diabetes preceded by GDM. In their meta-analysis of follow-up studies of women with previous GDM, Cheung and Byth (2003) calculated the population-attributable risk percent (PAR%) for the proportion of cases of T2DM associated with prior GDM. The PAR% ranged from 10-31% (Cheung & Byth, 2003). These data suggest that up to one third of parous women with T2DM have a history of GDM.

In addition to identifying women at risk for T2DM, GDM also has implications for future risk of CVD. Indeed, women with a history of GDM are at risk for subclinical atherosclerosis (Tarim et al., 2006). Studies also show an increased prevalence of cardiovascular risk factors in women with previous GDM (Verma et al., 2002; Lauenborg et al., 2005; Carr et al., 2006). Shah and colleagues (2008) used large population-based administrative databases to examine the CVD risk in women with a history of GDM. They found that 11.5 years after
delivery, the HR for CVD in women with GDM was 1.71 (95% CI 1.08 to 2.69) (Shah et al., 2008). Moreover, even mild glucose intolerance in pregnancy is associated with an increased risk of CVD. Compared with normoglycemic women who did not receive an OGTT, those who had an abnormal GCT followed by an OGTT that was not diagnostic of GDM still had an increased risk of CVD within 12 years of the index pregnancy (Retnakaran & Shah, 2009b).

Though not causally related, GDM is likely a stronger marker of a higher risk for developing cardiometabolic dysfunction after an affected pregnancy. In a longitudinal study comprising 12-18 years of follow up, 45% of women with previous GDM went on to develop hypertension compared with only 4% in the control group (Mestman, 1972). Another study demonstrated significantly higher rates of dyslipidemia, hypertension and mortality 26 years after GDM (O’Sullivan, 1991). Further exacerbating the burden of disease is a family history of T2DM, which adds to the elevated risk associated with GDM. Carr and colleagues (2006) quantified the increased risk of CVD in women with GDM and a family history of T2DM, compared with women without such history (OR 1.85, 95% CI 1.21 to 2.82) (Carr et al., 2006). It is generally recommended that women with GDM undergo a postpartum OGTT to detect ongoing dysglycemia. If lower thresholds for GDM are adopted, then more women are likely to be screened for T2DM postpartum. One hopes that this will offer a preventive opportunity that would otherwise be missed in these women.

2.4.4 GDM and emerging non-traditional risk factors

CVD is described as an inflammatory disease, with analogous findings in diabetes and obesity (Stern, 1995). Studies have also demonstrated the presence of inflammation in GDM, with high concentrations of serum CRP associated with GDM, but which are attenuated by further adjustment for BMI
(Wolf et al., 2003; Winzer et al., 2004). In a cross-sectional study examining the role of maternal obesity in the association between CRP and GDM, pre-pregnancy BMI emerged as the most important determinant of serum CRP concentration, independent of GDM (Retnakaran et al., 2003). It thus emerges that obesity may mediate a systemic inflammatory response that underlies the relation between CRP and GDM. Similar to its potential as a risk factor for the metabolic syndrome, adiponectin is also a promising marker of GDM.

Compared with unaffected women, those with GDM have lower levels in pregnancy of both total and HMW adiponectin (Retnakaran et al., 2004; Retnakaran et al., 2007). These lower levels of total and HMW adiponectin are associated with both IR and pancreatic beta-cell dysfunction (Retnakaran et al., 2005; Retnakaran et al., 2007). Hypoadiponectinemia in pregnancy independently predicts postpartum metabolic dysfunction, including fasting glycemia, IR and beta-cell dysfunction (Retnakaran et al., 2010d). Thus, hypoadiponectinemia may play a role in the development of T2DM in women with a history of GDM.

Another novel marker potentially associated with GDM is the presence of a fatty liver. In non-pregnant women with previous GDM who underwent an magnetic resonance imaging (MRI) of the liver, those with high liver fat had elevated fasting serum triglyceride and insulin concentrations, and lower whole-body insulin sensitivity than those with low liver fat on MRI (Tiikkainen et al., 2002). Given that NAFLD is common in T2DM, Forbes and colleagues (2011) investigated the prevalence and risk for NAFLD among European women with previous GDM. The prevalence of NAFLD was much higher in women with previous GDM (38%, 95% CI 28 to 47) than in those without GDM (17%, 95% CI 10 to 24) (Forbes et al., 2011).

Limited evidence exists for the association between uric acid and GDM, although its predictive value in T2DM makes it a promising candidate for
studies of GDM. High uric acid levels have been detected in women with GDM (Seghieri et al., 2003). SHBG (Smirnakis et al., 2007) is another biochemical marker of much interest. Bartha et al. (2000) compared serum SHBG levels between women with and without GDM, and found that SHBG levels were lower in the GDM group. Similarly, SHBG, in addition to adiponectin, was shown to be lower in women with GDM than unaffected controls (Nanda et al., 2011). Even when measured in early pregnancy, first-trimester SHBG levels were lower among women who went on to develop GDM compared with their peers (187 nmol/L vs. 233 nmol/L) (Thadhani et al., 2003).

In addition to traditional measures for GDM, these emerging risk factors are the same as those described for the metabolic syndrome arising outside of pregnancy (Figure 2.1 and Figure 2.2). Accordingly, they raise the question of whether the metabolic syndrome relates to GDM.
2.5 Risk of the metabolic syndrome and its sequelae following GDM

2.5.1 Development of the metabolic syndrome after GDM

Evidence that the metabolic syndrome both precedes and follows GDM suggests an increased lifetime risk of T2DM in women with prior GDM. In addition to chronic beta-cell dysfunction, women with GDM have chronic IR that is apparent after delivery. Indeed, in the decade after pregnancy, many
women with previous GDM exhibit features of the metabolic syndrome. Considering the shared risk factors of the metabolic syndrome and T2DM, and the similarities between GDM and T2DM, it is not surprising that GDM is likewise associated with the metabolic syndrome. Akinci and colleagues (2010) collected antepartum characteristics of women who developed the metabolic syndrome in their later years. Pre-pregnancy obesity, weight gain, and OGTT fasting glucose levels each predicted the development of the metabolic syndrome (using the NCEP and IDF definitions). Moreover, even a fasting glucose concentration above 5.5 mmol/L at the antepartum OGTT was an independent predictor of the metabolic syndrome (Akinci et al., 2010). Indeed, many studies have demonstrated an increased prevalence of features of the metabolic syndrome following GDM.

Egeland and Meltzer (2010) investigated the effects of GDM on future risk of metabolic and cardiovascular abnormalities. The prevalence of glucose intolerance at 15 years’ follow-up was 44.4% among women with prior GDM versus only 13.1% in those without GDM. WC at 15-year follow-up was the strongest predictor of this difference (Egeland & Meltzer, 2010). Similarly, in a 2002 U.S. study, the prevalence of the metabolic syndrome was 27.2%, 11 years after pregnancy in women with previous GDM, compared with only 8.2% in unaffected controls (Verma et al., 2002). Lauenborg et al. (2005) estimated the risk of metabolic syndrome in a Danish cohort of women 9.8 years after delivery. Women with previous GDM had a threefold higher risk of metabolic syndrome compared with non-GDM controls (Lauenborg et al., 2005). A similar study in Europe reported prevalence of the metabolic syndrome of 21% and 4.6%, respectively, 8.5 years postpartum (Bo et al., 2004b). Indeed, prior gestational hyperglycemia in the absence of fulfilling the overt criteria for GDM results in a future risk of the metabolic syndrome 2-4 times that of those with normoglycemia in pregnancy. This risk is 10-times higher in women with concomitant pre-pregnancy obesity (Bo et al., 2004a). Thus, even mild gestational hyperglycemia predicts the metabolic syndrome (Bo et al., 2004b),
and the metabolic syndrome is increasingly more likely to develop over time following the index pregnancy (Bo et al., 2006). These studies highlight the chronic nature of the metabolic dysfunction associated with GDM. Furthermore, they raise the possibility that a diagnosis of GDM may indicate the presence of an underlying latent metabolic syndrome (Retnakaran et al., 2010b).

### 2.5.2 Development of the metabolic syndrome in the early postpartum after the diagnosis of GDM

GDM has been implicated as an early expression of the metabolic syndrome (Haffner & Taegtmeyer, 2003).

Indeed, it was recently reported that both GDM (OR 2.05, 95% CI 1.07 to 3.94) and the milder state of GIGT (OR 2.16, 95% CI 1.05 to 4.42) independently predict postpartum metabolic syndrome by three months postpartum, even after adjustment for covariates (Retnakaran et al., 2010d).

By three months postpartum, women with GDM and GIGT also exhibit non-traditional risk factors associated with the metabolic syndrome, including low levels of adiponectin and increased serum CRP (Retnakaran et al., 2010c). While many of the metabolic disturbances of pregnancy resolve after delivery, growing evidence supports the concept that pregnancy provides an opportunity to observe a pronounced expression of an otherwise subclinical metabolic disorder. Such metabolic disturbances, which include the metabolic syndrome component disorders, may indeed be apparent prior to the diagnosis of GDM.
2.6 Prediction of GDM by the metabolic syndrome components and associated risk factors

2.6.1 Prediction of GDM by the metabolic syndrome components in early pregnancy

It is quite likely that the metabolic syndrome exists prior to the development of GDM. Indeed, it has even been proposed that GDM is itself a component of the metabolic syndrome (Clark et al., 1997). In their study, Clark et al. (1997) showed that, at the time of their antepartum OGTT, women with GDM expressed markers of the metabolic syndrome, including low serum HDL cholesterol and higher fasting plasma insulin, triglycerides, free fatty acids, and elevated pre-pregnancy BMI. These common features of the metabolic syndrome were each individually predictive of GDM and persisted after adjustment for differences in BMI (Clark et al., 1997).

In addition to conventional measures of the metabolic syndrome, several non-traditional biomarkers have emerged as possible predictors of GDM. As discussed earlier, low adiponectin is a risk factor for T2DM and an emerging risk factor for the metabolic syndrome and GDM. Using a prospective nested case-control study design, Williams et al. (2004) determined whether first-trimester hypoadiponectinemia predicts GDM. They found that 73% of those with GDM had a low adiponectin level compared with 33% of controls (adjusted OR 4.60, 95% CI 1.80 to 11.60). Similarly, Lain and colleagues (2008) found that women with low adiponectin concentrations in the first trimester were much more likely to be diagnosed with GDM (OR 10.2, 95% CI 1.3 to 78.7).
In choosing an optimal early serum marker to predict GDM, Smirnakis and colleagues (2007) compared SHBG, high-sensitivity CRP, and HOMA-IR in the late first trimester and early second trimester of pregnancy. Serum SHBG was lower, and serum CRP higher, in women who went on to develop GDM, who also had elevated HOMA-IR in the second trimester. After multivariate analysis, SHBG emerged as the best predictor of GDM (Smirnakis et al., 2007). Alternatively, Wolf et al. (2003) found that the risk of developing GDM was higher in women in the upper versus lower tertiles of first-trimester CRP, after adjusting for confounders (OR 3.6, 95% CI 1.2 to 11.4). Importantly, the association was attenuated when BMI was included in the analysis (OR 1.5, 95% CI 0.40 to 5.5) (Wolf et al., 2003), suggesting that obesity confounds the relation between inflammation and GDM.

Qiu and colleagues (2004) found that, even after adjusting for maternal pre-pregnancy BMI and other confounders, women with CRP in the highest versus lowest tertiles experienced a 3.5-times increased risk of GDM (95% CI 1.2 to 9.8).

Moreover, even lean women had an OR for GDM of 3.7 (95% CI 1.6 to 8.7), suggesting that the association between elevated CRP and GDM may not solely depend on the presence of maternal obesity (Qiu et al., 2004). However, Savvidou and colleagues (2010) evaluated various first-trimester conventional and novel biomarkers, including adiponectin and CRP, and found only a low HDL-C and a high tissue plasminogen activator were significant independent predictors of GDM. Laughon et al (2009) reported that a first-trimester concentration of uric acid in the highest quartile had an OR for GDM of 3.25 (95% CI 1.35 to 7.83) after adjusting for BMI and age.

Together, these emerging risk factors present an opportunity for early detection of GDM and, possibly, the identification of an effective tool for the long-term
prevention of the metabolic syndrome.

It is likely that components of the metabolic syndrome exist before and after GDM. Similar to T2DM, where persons with IGT and IFG are at significant risk of T2DM, so too may be the case for the metabolic syndrome in early pregnancy. Ray and colleagues (2010) coined the term “gestational prediabetes” to describe the absence of diabetes before pregnancy, and the presence of a blood glucose level (or a related marker) in early pregnancy that is higher than normal, but not yet high enough to meet the diagnostic criteria for GDM (Ray et al., 2010). Given the promising findings of using emerging biomarkers to detect dysmetabolism in early pregnancy and predict GDM, the next step is to identify a robust biomarker that can be assayed at a low cost in early pregnancy. Since they are chronic in nature, metabolic abnormalities likely precede pregnancy, which means that they should be detectable in early pregnancy as well.

2.6.2 Prediction of GDM by the metabolic syndrome components prior to pregnancy

A modest body of literature exists related to the existence of the metabolic syndrome prior to the detection of GDM. Gunderson and colleagues (2009) examined pre-pregnancy cardiometabolic risk factors and the risk of GDM in subsequent pregnancies. They found that metabolic impairment often predated the onset of GDM, and that 27% of overweight women with one or more cardiometabolic risk factor developed GDM (Gunderson et al., 2009). Normoglycemia with at least one metabolic risk factor (i.e. low plasma HDL-C and/or hyperinsulinemia) was present before pregnancy in 34% of those who developed GDM; among overweight women, the presence of any cardiometabolic feature was associated with an almost 4-times higher risk of GDM.
Hedderson and Ferrara (2008) measured blood pressure before and in early pregnancy, and found that women with mild hypertension in early pregnancy had a small increased risk of GDM (OR 1.56, 95% CI 1.16 to 2.10). Those with frank hypertension had a twofold-increased risk of GDM (OR 2.04, 95% CI 1.14 to 3.65) compared with normotensive women, even after adjusting for confounders. These findings were paralleled by mild (OR 1.44, 95% CI 0.95 to 2.19) and frank (OR 2.01, 95% CI 1.01 to 3.99) hypertension detected before pregnancy (Hedderson & Ferrara, 2008).

2.7 Summary of the Metabolic Syndrome and GDM

We have reviewed the parallels and associations between the metabolic syndrome and GDM. Both conditions have relied on differing diagnostic criteria and a history marked by controversies about how they are best defined and their clinical utility. Both conditions can identify patients at increased future risk of T2DM and CVD. Furthermore, both conditions have been associated with a similar set of emerging non-traditional risk factors. Consistent with these parallels, women with GDM are at increased risk of the metabolic syndrome both early postpartum and in the years thereafter.

The metabolic syndrome and its associated risk factors may precede the diagnosis of GDM, both in early gestation and even prior to pregnancy. Taken together, these data suggest that GDM may represent a transient “unmasking” of a latent metabolic syndrome, which may extend in both directions through (i) the pre-gravid state and early pregnancy, and (ii) the early and late postpartum period. Figure 2.3 illustrates this lifetime continuum that may link the metabolic syndrome, GDM, T2DM, and CVD. The chronic nature of the features of the
metabolic syndrome suggests that what we know about the temporal relation between the metabolic syndrome (MetS), GDM, T2DM, and CVD is limited.

Figure 2.3: Theoretical framework and conceptual model for latent metabolic syndrome preceding GDM (De Souza et al., 2011)

Relevance

Early screening using GCT or OGTT is clinically restricted to those individuals at high risk for GDM, and evidence shows that over 40% of GDM cases would therefore be missed as they are left undiagnosed (Kaaja & Ronnemaa, 2009).
The global burden of diabetes has been estimated at more than 285 million individuals with an expected increase to 439 million by 2030 (Shaw et al., 2010). The prevalence of obesity and related metabolic dysfunction worldwide is a vivid demonstration of the undiscriminating potential of cardio-metabolic diseases across ethnicities and age groups.

In this context, the emerging relation between the metabolic syndrome and GDM may offer the opportunity for early detection of at-risk individuals, long before the manifestation of overt disease. Ideally, this opportunity may lead to new strategies using novel modifiable risk markers of maternal obesity that ultimately lead to disease prevention. As such, the emerging relation between the metabolic syndrome and GDM represents an important area of research that may hold both clinical and public health implications.

2.8 Pathophysiology of Central Abdominal Adipose Tissue

2.8.1 Heterogeneity of obesity and adipose tissue distribution

Obesity is a critical public health issue worldwide as it continues to escalate beyond the once occidental world (Finucane et al., 2011). While obesity is physiologically defined by the accumulation of excess body fat, the universal estimate of whole body obesity remains the BMI, calculated as weight in kilograms divided by height in meters squared (Keys et al., 1972). The BMI follows a J-shaped relation to morbidity and mortality risk, wherein a very low BMI is associated with increased mortality even after considering underlying morbidity including chronic conditions such as cancer (Whitlock et al., 2009; Berrington de Gonzalez et al., 2010) and a high BMI is associated with a
progressive increase in the risk of the metabolic comorbidities of T2DM and CVD (Wormser et al., 2011).

Despite its widespread utility in estimating secular trends in obesity-mediated diseases, BMI is not without its limitations. Overweight and obesity as defined by BMI confers a high risk of comorbid conditions compared with the normal-weight condition, yet epidemiological studies highlight discordant findings in metabolically healthy individuals (Wildman et al., 2008). BMI assumes an even distribution of adipose tissue and does not distinguish muscle tissue (Després et al., 2001). Inconsistent results have been reported for cardiovascular outcomes in healthy individuals, with some studies demonstrating a linear relation between BMI and cardiovascular risk and others showing no significant association (Wannamethee et al., 1998; Wilson et al., 2002).

The limitation of BMI has been highlighted in studies of individuals who are metabolically obese, normal-weight and who typically exhibit normal BMIs, yet suffer from metabolic complications related to obesity (St-Onge, Janssen, Heymsfield, et al., 2004). In contrast, metabolically healthy obese individuals who typically experience BMIs above 30 kg/m² do not experience insulin resistance or dyslipidemia (Karelis et al., 2004).

A major limitation in studies that exclusively measure BMI is that they omit regional body fat distribution data and, in particular, central abdominal adipose tissue measures.

2.8.2 Biology and etiology of central AT

Whole body AT is comprised of both subcutaneous and internal or visceral AT (VAT). Subcutaneous AT (SAT) is the layer found between the skin and the
aponeuroses and fasciae of the muscles and includes mammary tissue (Shen et al., 2003). The SAT depot contains two morphologically distinct compartments separated by a fascial plane (superficial fascia): the superficial layer, evenly distributed under the abdominal epidermis and the deeper SAT layer, located beneath the superficial AT layer (Kelley et al., 2000). Internal AT includes intrathoracic (pericardial AT) and intra-abdominopelvic AT (intra and extraperitoneal AT). The intraperitoneal adipose layer contains two major components, the greater omentum and mesentery (Shen et al., 2003).

Methodological imaging studies of the abdomen focus on the abdominopelvic region, with the abdominal muscle wall designating the anatomical boundary; therefore, VAT measures have indiscriminately included omental, mesenteric as well as extraperitoneal AT. It is important to note that only intraperitoneal mesenteric and omental AT are drained by the portal vein, because this mechanism is thought to be the pathological link between VAT and inflammation and dysmetabolism (Shen et al., 2003; Tchernof & Després, 2013), (Figure 2.4).
VAT and SAT also display relevant morphological and pathophysiological differences, for example, fat lobules of SAT are organized in a regular fashion, whereas those of the deep subcutaneous layer and internal compartments, especially the greater omentum, are large, irregular, and less-organized (Shen et al., 2003).

The size of each fat compartment adds to the morphological complexity of AT. Adipocyte compartment size arises from the integration of both size and total number of lipid-laden adipocytes (Tchernof and Després, 2013).
In their review of the pathophysiology of visceral obesity, Tchernof and Després (2013) propose a model wherein excess visceral adiposity may be a marker of dysfunctional SAT that is deficient in expanding through hyperplasia during energy surplus and therefore is ineffective as an energy buffer, which results in a lipid “spillover” and accumulation—ectopic fat deposition—of lipids in other viscera with harmful cardiometabolic consequences (Figure 2.5).

Figure 2.5: Chronic energy surplus model of impaired SAT expansion via hyperplasia (Després, 2012).

Caloric overload may result in hyperplasia or hypertrophy, the latter resulting in inflamed AT and a systemic inflammation and lipid spillover that results in ectopic fat deposition, IR, and a deteriorated cardiometabolic risk profile (CMR) (Després, 2012).
Adipocyte hypertrophy, especially during weight gain, is another potential causal factor in the development of IR (Simas & Corvera, 2014). The mechanistic pathways involve a limited capacity of hypertrophied adipocytes to store additional fat, resulting in ectopic fatty acid oxidation, lipotoxicity and inflammation in multiple tissues such as the liver (Pickup, 2004; Lumeng & Saltiel, 2011). Enlarged adipocytes secrete pro-inflammatory factors that trigger macrophage infiltration, resulting in AT damage that minimizes adipocyte storage capacity (Simas & Corvera, 2014).

Alternatively, increased AT mass can also beneficially be generated through hyperplasia (Simas & Corvera, 2014). It has been demonstrated that hyperplasia in VAT and SAT is correlated with decreased risk of lipid, glucose and insulin abnormalities (Hoffstedt et al., 2010). SAT has been suggested to be inherently able to undergo hyperplasia, such that expansion of this depot is associated with decreased odds of IR by 48% while VAT expansion increases odds of IR by 80% (McLaughlin et al., 2011).

2.8.3 Methodological challenges to measuring central adiposity

Anatomical and morphological differences in central AT impose methodological limitations that cannot be overcome by current imaging techniques such as computed tomography (CT) scanning or MRI, resulting in some underestimation of the theoretical association between the amount of fat in each compartment and metabolic alterations (Tchernof and Després, 2013).

VAT samples are relatively difficult to obtain in humans as only surgical procedures permit its accurate measurement, however even surgery-based
studies have focused on omental AT and failed to distinguish superficial from deep SAT layers and should therefore be interpreted with caution (Tchernof and Després, 2013).

CT scanning is the gold standard for quantification of body fat distribution; however, its use is limited due to high costs, radiation exposure, and impractical application in large-scale epidemiological research (Bartha et al., 2007). MRI does not involve radiation, but high costs preclude its use in large-scale research (Bartha et al., 2007). Moreover, to date, studies employing MRI have considerable variability in their identification and definition of a low-risk comparator group, largely owing to measures being obtained at different abdominal cross-sectional areas (Williams et al., 1996; Nicklas et al., 2003; Després and Lemieux, 2006).

Ultrasound has been validated against CT scanning as an accepted approach to quantifying abdominal fat distribution (Bartha et al., 2007) and is less costly, with negligible radiation. Ultrasound is also highly accessible in clinical settings and especially practical where CT scanning or MRI is prohibitive, as with pregnancy.

WHR and WC have been studied as surrogate estimates of VAT. Cut-off values for both WHR and WC have been proposed for men and women (Bjorntorp, 1985; Pouliot et al., 1994). However, it has been established that WHR cannot reliably predict VAT and may underestimate VAT reduction during central and peripheral weight loss (Pouliot et al., 1994; Després et al., 2001). Similarly, a limitation of WC is that it cannot distinguish between SAT and VAT (Lemieux et al., 2007), nor can it selectively account for abdominal versus truncal adiposity.
Given that anthropometric estimates of central abdominal obesity have the advantage of quickly achieving a crude estimate of visceral adiposity, Lemieux and colleagues (2007) used fasting triglyceride concentrations and WC to define the “hypertriglyceridemic waist,” as a simple phenotypic tool to predict excess visceral adiposity in clinical settings. Nonetheless, the authors affirm that this algorithm does not replace the direct measure of visceral adiposity, but rather serves as a simple screening tool that should be further investigated (Lemieux et al., 2007; Tchernof and Després, 2013). A similar index, lipid accumulation product (LAP), may eventually demonstrate clinical utility if further investigated (Kahn, 2006).

2.8.4 Biology and etiology of NAFLD

NAFLD defines a histological disease spectrum that ranges from simple fatty liver, to nonalcoholic steatohepatitis (NASH) to fibrosis and cirrhosis (Targher et al., 2007). Usually asymptomatic, simple fatty liver remains benign in most affected people; however in obesity-mediated disease, even a slight degree of hepatic fat is relevant to the management of metabolic dysfunction and disease progression. The gold standard for measuring NAFLD is liver biopsy, which is high-risk, invasive, costly, and includes sampling errors due to inter-observer variations and inconsistent distribution throughout the liver (Yeh & Brunt, 2007).

NAFLD is considered the liver’s manifestation of the metabolic syndrome and is associated with T2DM, IR and hyperglycemia (Targher et al., 2007), as well as being the most common liver disease worldwide. Prevalence rates that range between 2% to 80% have been reported in North and South America, Europe, Australia and Asia (Alisi et al., 2011).
The etiopathogenesis of NAFLD might involve various proposed mechanistic pathways, including the “multiple hits” theory and the portal theory. The former suggests that hepatic AT accumulation and IR lead to simple steatosis as part of a “primary hit” and the resulting vulnerable liver is susceptible to a “secondary hit” that may give rise to NASH. These secondary hits include pro-inflammatory cytokines, oxidative stress, and mitochondrial dysfunction from AT (Figure 2.6). According to the portal theory, excess free fatty acids liberated from visceral AT enter into the portal vein and deposit on the liver (Item & Konrad, 2012). On the other hand, those with dysfunctional SAT compartments also experience ectopic AT deposition to organs such as the liver (the ectopic fat hypothesis) (Gallagher et al., 2009).

Figure 2.6: The multi-hit hypothesis for the pathogenesis of NAFLD (Edmison & McCullough, 2007).

According to the model, the first hit is caused by dyslipidemia or free fatty acid (FFA) accumulation in the liver, which leads to steatosis. Inflammation, oxidative stress, and abdominal apoptotic mechanisms comprise the second
hit, leading to the progression of NASH and eventually resulting in NAFLD (Edmison & McCullough, 2007).

An alternative theory, the gut-liver axis hypothesis, describes the role of bacterial endotoxins of intestinal origin that initiate an immune response and cascading necro-inflammatory lesions that progress from steatosis to NASH and severe fibrosis (Baffy, 2009). In genetically predisposed populations, the thrifty geneotype hypothesis posits that whole body IR, once protective, now results in the inappropriate accumulation of AT in muscle and liver tissues and eventual ectopic fat deposition (Kraegen & Cooney, 2008). Regardless of the prescribed theory, IR is central to the development of NAFLD.

Nutritional factors that lead to fatty liver include obesity and excess nutritional intake, while endocrine factors include DM and IR. Obesity is the main non-alcoholic cause of fatty liver and the degree of steatosis correlates with increase in body weight in both adults and children (Sherlock & Dooley, 1997). Moreover, NASH is observed mainly in obesity and most frequently in middle-aged women with DM (Stefan, 2008). Importantly, metabolic phenotype is heritable, which may lead to the generational transfer of NAFLD via epigenetic changes to the fetal liver, a process exacerbated by the recent evidence of transmission of microbiota from a mother to her infant, impacting on energy retention and immune function and leading to a predisposition to NAFLD as reviewed in Brumbaugh & Friedman (2014).
2.8.5 Central adiposity and fatty liver in pregnancy

Liver fat content in obese women is strongly associated with IR more than any other measures of body composition, which is consistent with impaired capacity of AT to sequester fat in those who develop GDM (Tiikkainen et al., 2002). Differences in plasma fatty acid profiles between women with and without GDM suggest similar alterations of fatty acid metabolism in GDM compared with T2DM (Chen et al., 2010; Bowers et al., 2012).

Differential risk of hyperplasia in adipose tissue depots is apparent in those with GDM; sonographic evaluation of SAT and VAT mass in the first trimester identifies an increased risk of abnormal glucose intolerance in women above the upper quartile of VAT depth but not in those with elevated SAT depth (McLaughlin et al., 2011).

During gestation, this differential risk profile may vary according to which compartment the weight gain occurs and whether this AT expansion is by hyperplasia or hypertrophy. For example, a woman with gestational weight gain occurring in the SAT compartment by hyperplasia may be at a much lower risk of GDM than a woman whose VAT expands through hypertrophy—even if net fat accumulation is equal (Simas & Corvera, 2014).

To date, only a few small studies have investigated ultrasound-measured central adiposity and NAFLD in pregnancy.
2.8.7 Study population and setting

The prevalence of diabetes varies across Canada. In 2008-09, after accounting for differences in age distribution among the provinces and territories, Ontario emerged as having one of the highest prevalence rates of diagnosed diabetes, while Nunavut, Alberta, and Quebec ranked lowest (Figure 2.7). Diabetes remains one of the most common chronic diseases in Canada with close to 2.4 million Canadians aged one year and older living with diagnosed diabetes (either type 1 or type 2), according to the Canadian Chronic Disease Surveillance System (CCDSS).

![Age-standardized Prevalence (%)](image)

**Figure 2.7:** Age-standardized† prevalence of diagnosed diabetes among individuals aged one year and older, by province/territory, Canada, 2008/09

† Age-standardized to the 1991 Canadian population. (Public Health Agency of Canada, 2011)

The province of Ontario is home to the majority of immigrants to Canada. Many of these immigrants are from populations at higher risk of T2DM,
including those immigrating from South Asia, Africa, and Latin America. Ontario has the highest proportion of Chinese, East Asians, and Southeast Asians compared with any other province (Public Health Agency of Canada, 2011). These populations all have a higher risk of developing diabetes and related complications (Public Health Agency of Canada, 2011). Given that Ontario also has high rates of overweight and obesity compared with other provinces, it is an important population in which to conduct research on obesity-mediated diseases.

The city of Toronto is the most populous city in Canada, with inhabitants that make up the largest degree of ethnic diversity in the entire country.

![Figure 2.8: The city of Toronto, Ontario, Canada with geographic boundaries denoting regions in the greater Toronto area (GTA)](image)

It is estimated that one in ten deaths in Canadian adults was attributable to diabetes in 2008-09 (Public Health Agency of Canada, 2011). People with
diabetes are over three times more likely to be hospitalized with CVD than the general population, which imparts a significant health care burden. Additionally, diabetes complications are associated with premature death. Diabetes rates are up to three to five times higher in First Nations (CDA, 2013).

The rates of diabetes continue to surpass global estimates and, given the chronic nature and pathogenesis of obesity-mediated morbidity, the full extent of diabetes has yet to be realized (Fox et al., 2007). However, providing accurate estimates of the global burden of diabetes in terms of morbidity and mortality poses several challenges, including the lack of valid and timely data in some countries, as well as the variability of diagnostic criteria used worldwide. The International Diabetes Federation estimated that the global age-standardized prevalence of diabetes among adults aged 20 to 79 years was 6.4% in 2010, representing 285 million people worldwide (IDF, 2009; Shaw et al., 2010). Compared with the prevalence of diabetes in European, American, and Oceania countries included in this study, the rate for Canada was the third-highest. Compared with the rate of 9.2% in Canada, rates were much lower in all African countries, as well as in most Asian and Latin American/Caribbean countries.

Between 1998-99 and 2008-09, statistical analyses identified an overall increase in incidence rates of diagnosed diabetes, with certain age groups contributing to this overall increase over time, namely children aged 1 to 19 and working-age adults aged 30 to 49 (Public Health Agency of Canada, 2011). This includes women of reproductive age. Those who develop GDM have a sevenfold increased risk of developing T2DM; in fact, a third of women with T2DM have a history of GDM (Cheung & Byth, 2003; Bellamy et al., 2009). In Toronto, the rates of diabetes continue to climb and have an ethnic-specific gradient increase in vulnerable populations (Figure 2.9) (Statistics Canada, 2006).
Figure 2.9: Diabetes rates in Toronto, Ontario, Canada (Statistics Canada, 2006) showing ethnic enclaves.

2.9 Thesis Aims and Hypotheses
There are currently no prevention approaches to GDM. GDM is routinely screened for at 24 to 28 weeks' gestation. As described in the preceding review, the diagnostic criteria and timing of diagnosis for GDM continues to be debated worldwide. Early detection of GDM remains laudable as traditional risk factors have less of a role in identifying those at high risk for GDM when in our modern era many women begin pregnancy with these traditional risk factors.

The overall goal of this thesis is to study new methods for the early detection of GDM. Its primary aim is to examine the association between first-trimester ultrasound measurement of abdominal AT and the continuum of dysglycemia and IR in early- and mid-gestation, as well as the outcome of GDM in mid-pregnancy. A co-primary aim is to explore the association between first-trimester maternal sonographic signs of NAFLD and the development of dysglycemia and GDM in mid-pregnancy. These two ultrasound measurements were studied within a large, outpatient urban clinical setting at the same time in the first trimester that women were currently having their routine ultrasonographic assessment of fetal nuchal translucency test for trisomy 21.

The content of this research has implications for the potential development of prevention strategies in early pregnancy to mitigate the deleterious effects of IR, impaired glucose homeostasis, and the eventual onset of GDM, a critical risk factor for T2DM and CVD, especially in high-risk patients.

The specific hypotheses tested to address these aims are the following:
1) Higher first-trimester abdominal AT measured by ultrasonography at 11-14 weeks' gestation is associated with greater IR and lower insulin sensitivity at 16-22 weeks' gestation well before traditional screening and diagnosis of GDM at 24-28 weeks gestation (Chapter 3).

2) Higher first-trimester abdominal AT measured by ultrasonography at 11-14 weeks' gestation is associated with low serum adiponectin concentration at 16-22 weeks' gestation (Chapter 4).

3) Higher first-trimester abdominal AT measured by ultrasonography at 11-14 weeks' gestation is associated with the later development of impaired glucose homeostasis, greater IR, and a higher odds of GDM at 24 to 28 weeks' gestation (Chapter 5).

4) Presence of maternal fatty liver, measured by ultrasonography at 11-14 weeks’ gestation is associated with the later development of impaired glucose homeostasis, IR, and a higher odds of GDM at 24 to 28 weeks’ gestation (Chapter 6).
Chapter 3  Abdominal Adiposity and IR in Early Gestation

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3.0 Abstract

Objective: High pre-pregnancy BMI is a known risk factor for GDM, although the contribution of abdominal adiposity to IR in pregnancy is not well-understood. The objective of this study was to assess the association between abdominal adiposity in early pregnancy and IR.

Research Design and Methods: A prospective cohort study design was followed in 79 pregnant women. VAT depth was measured by ultrasonography at 11-14 weeks’ gestation, at the time of routine fetal nuchal translucency assessment for trisomy 21. A 2-hour 75-g oral glucose tolerance test was subsequently completed at 16-22 weeks’ gestation and IR was estimated by the homeostatic model assessment for insulin resistance (HOMA-IR) as well as by the Insulin Sensitivity Index (ISI).

Results: Upon adjusting for maternal age, parity, ethnicity and pre-pregnancy BMI, VAT depth explained 42% of the variance in HOMA-IR, which was slightly better than the variance in the multivariable model examining HOMA-IR and pre-pregnancy BMI (40%). For ISI, the model variance values were 36% and 32%, respectively.

Conclusions: Measurement of maternal AT depth at the time of routine first-trimester ultrasonography may provide some additional information about maternal IR, in addition to pre-pregnancy BMI.
3.1 Introduction

Approximately 4% to 8% of pregnancies in Canada are affected by GDM (Dyck et al., 2002). Maternal obesity is associated with a higher risk of GDM (Solomon et al., 1997) and adverse pregnancy outcomes (Kim et al., 2002; Ehrenberg et al., 2004; Surkan et al., 2004). Moreover, GDM and T2DM share common risk factors (Ehrenberg et al., 2004), including pre-pregnancy obesity and non-Caucasian ethnicity (Kim et al., 2002). It is understood that women with a history of GDM are at increased risk of T2DM (Kim et al., 2002; Retnakaran et al., 2008a); however, those with even subtle abnormalities of glucose homeostasis in pregnancy are also at higher risk of developing glucose impairment and T2DM (Retnakaran et al., 2008a).

Current guidelines recommend screening for GDM at 24 to 28 weeks’ gestation with a 1-hour glucose challenge test (GCT), followed by a confirmatory 2-hour oral glucose tolerance test (OGTT), or simply screening with the 2-hour OGTT alone (CDA, 2013). However, initiating dietary or pharmacological therapy after 24 to 28 weeks’ gestation may be too late to favorably impact fetal growth or placental integrity. First-trimester assessment of IR using HOMA-IR or the ISI appears to correlate well with later development of GDM (Kirwan et al., 2002; Ozcimen et al., 2008). Other first-trimester biomarkers have also been shown to predict the future onset of GDM, albeit with limited consistency (McElduff et al., 2005; Retnakaran et al., 2005; Lain et al., 2006; McLaughlan et al., 2006; Maghbooli et al., 2007; Smirnakis et al., 2007; Georgiou et al., 2008; Weerakiet et al., 2008).

Central obesity, measured in early pregnancy may be another method to predict the onset of GDM. In a pilot study, we showed that ultrasound-measured VAT depth above the upper quartile in early pregnancy was associated with a positive GCT in later pregnancy (adjusted OR 16.9, 95% CI 1.5 to 194.6) (Martin et al., 2009), independent of BMI.
Whether central AT depth is associated with IR or dysglycemia in early pregnancy remains unknown. Accordingly, we investigated the relation between first-trimester measurement of central AT depth and IR in early pregnancy.

3.2 Research Design and Methods

We conducted a prospective cohort study at a general obstetrics outpatient clinic at St. Michael's Hospital in Toronto, Ontario, Canada. The study was approved by the Research Ethics Board of St. Michael’s Hospital, and participants provided written informed consent. Women aged 18 years and older were eligible for study entry if they had a viable singleton pregnancy at 11-14 weeks' gestation. We excluded those who had known pre-pregnancy T2DM or a prior pregnancy affected by GDM.

At 11-14 weeks’ gestation, the abdominal AT compartments were distinguished and measured by a trained ultrasound technician at the time of a routine fetal ultrasound to test for trisomy 21. The technique described by Armellini et al. (1990) was used to measure abdominal SAT and VAT using a Philips IU22 ultrasound machine and a 5-2 or 9 MHz probe. Participants were scanned by one of four experienced sonographers who performed three measurements of the SAT and VAT depths. Each rater was masked to the others’ assessment, and the measurements were recorded on separate data collection sheets. SAT depth was measured from the SAT layer to the outer border of the rectus abdominus muscle at the level of the linea alba and the umbilicus. VAT depth was measured from the inner border of the rectus abdominus muscle, at the level of the linea alba, to the anterior wall of the abdominal aorta (Figure 3.1). TAT depth was measured from the SAT layer to the anterior wall of the abdominal aorta. Depth and zoom settings were
standardized, such that the aorta was at the bottom of the screen and the vertebral bodies were just visible.

The performance characteristics of this technique include an inter-observer reliability of 0.79 (95% CI 0.69 to 0.88) for SAT and 0.87 (95% CI 0.82 to 0.93) for VAT (Martin et al., 2009).

Figure 3.1: Anatomical measurement points in the assessment of visceral (peritoneal) adiposity using transabdominal ultrasonography (Martin et al, 2009)

A brief questionnaire was completed by each participant immediately following the 11-14-week ultrasound. Therein, we collected information about age, ethnicity (Caucasian, Black, South Asian, East Asian or Other), self-reported pre-gestational height and weight, and family history of T2DM in a
first-degree relative. Current weight was measured in person using a calibrated scale during the routine ultrasound appointment.

At 16-22 weeks’ gestation, at the time of routine blood sampling for integrated prenatal screening, each participant completed a 2-hour 75g OGTT following an overnight fast. In addition to serum glucose, we measured the fasting 1-hour and 2-hour serum insulin concentration. The HOMA-IR and ISI composite at 0, 60 and 120 minutes were calculated as previously described (Ozcimen et al., 2008; DeFronzo & Matsuda, 2010).

HOMA-IR assesses IR using fasting glucose and insulin according to the formula:

\[
\text{HOMA-IR} = \frac{\text{Fasting plasma insulin} \times \text{fasting plasma glucose}}{22.5}.
\]

ISI assesses insulin sensitivity using fasting and postprandial plasma glucose and insulin according to the formula:

\[
\text{ISI} = \frac{10,000}{\sqrt{\left(\text{fast glucose} \times \text{fast insulin}\right) \times \left(\text{mean glucose @ OGTT} \times \text{mean insulin @ OGTT}\right)}}
\]

Insulin concentration was measured in pmol/L, so the data were converted to µU/mL to calculate HOMA-IR. Glucose concentration was measured in mmol/L, so the data were converted to mg/dL to calculate ISI.

\[3.2.1\text{ Data analysis}\]

The ISI was positively skewed, thus data were log-transformed. Univariate and multivariable linear regression analyses were used to quantify the
association between VAT depth at 11-14 weeks’ gestation (continuous, in cm) and i) HOMA-IR and ii) ISI, each at 16-22 weeks’ gestation, with the latter derived using the 2-hour 75-g OGTT.

Multivariable models adjusted for maternal age (continuous in years), parity, ethnicity (Caucasian versus Non-Caucasian), family history, and pre-pregnancy BMI (continuous in kg/m²). All variables were included a priori. Variance inflation factors were under 2.5, indicating minimal multi-collinearity among measures of BMI and adiposity. Residual plots suggested adequate goodness-of-fit in the models.

The $r^2$ in each model was used to express the variability explained by each of the linear regression models. A Forest plot was created to provide a visual representation comparing the adjusted $r^2$ (95% CI) for each measure of maternal obesity (i.e. pre-pregnancy BMI, BMI at 11-14 weeks’ gestation, and SAT, VAT and TAT depth at 11-14 weeks’ gestation) and its association with HOMA-IR and ISI at 16-22 weeks’ gestation. In the models that assessed BMI, we did not adjust for AT depth, given that the aim was to assess whether central AT depth better explains the association between obesity and IR than BMI alone.

Statistical analyses were performed using SAS (version 9.1.3; SAS Institute, Cary, NC).
3.3 Results

There were 79 women recruited, at a mean (SD) age of 31.9 (4.9) years (Figure 3.2).

- Women aged 18+ years
- Singleton pregnancy
- No previous diabetes mellitus or chronic condition
- Underwent a first-trimester ultrasound for nuchal translucency

\[(n = 110)\]

Excluded 27 women for the following reasons:
- Transferred to another hospital during study \((n = 5)\)
- Declined OGTT \((n = 22)\)

\[n = 83\]

- Incomplete OGTT \((n = 4)\)

\[\text{Final cohort} \quad n=79\]

**Figure 3.2: Flow chart of participant inclusion**

Pre-pregnancy BMI ranged from 16.6 to 48.8 kg/m\(^2\). VAT depth ranged from 1.4 to 9.1 cm, with a mean of 3.9 (1.6) cm. A total of 13 women developed GDM (Table 3.1).
Table 3.1: Maternal characteristics and both exposure and outcome variables for all 79 study participants

<table>
<thead>
<tr>
<th>Maternal Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD) age at time of enrolment, years</td>
<td>31.9 (4.9)</td>
</tr>
<tr>
<td>Maternal Caucasian ethnicity</td>
<td></td>
</tr>
<tr>
<td>Median (IQR) number of prior live births</td>
<td>0 (0-1)</td>
</tr>
<tr>
<td>No. prior live births</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>51 (64.6)</td>
</tr>
<tr>
<td>≥ 1</td>
<td>26 (32.9)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>4 (5.1)</td>
</tr>
<tr>
<td>No. exceeding cut off for 50-g GCT at 24-28 weeks’ gestation**</td>
<td>16 (21.1)</td>
</tr>
<tr>
<td>No. who developed GDM following the confirmatory 2-hour 75g OGTT**</td>
<td>13 (17.1)</td>
</tr>
<tr>
<td>Mean (SD) pre-pregnancy weight, kg</td>
<td>64.9 (16.8)</td>
</tr>
<tr>
<td>Mean (SD) height, cm</td>
<td>163 (7.3)</td>
</tr>
<tr>
<td>Mean (SD) pre-pregnancy BMI, kg/m²</td>
<td>24.3 (5.6)</td>
</tr>
<tr>
<td>Mean (SD) BMI at 11-14 weeks’ gestation, kg/m²</td>
<td>25.5 (5.5)</td>
</tr>
<tr>
<td>Mean (SD) net weight gain (between pre-pregnancy and 11-14 weeks’ gestation), kg</td>
<td>3.4 (3.4)</td>
</tr>
<tr>
<td>Mean (SD) net change in BMI, kg/m²</td>
<td>1.3 (1.3)</td>
</tr>
<tr>
<td>Mean (SD) VA) depth, cm</td>
<td>3.9 (1.6)</td>
</tr>
<tr>
<td>Mean (SD) fasting serum glucose concentration at 16-22 weeks’ gestation, mmol/L</td>
<td>4.3 (0.58)</td>
</tr>
<tr>
<td>Mean (SD) 1-hour serum glucose concentration at 16-22 weeks’ gestation, mmol/L</td>
<td>6.4 (1.8)</td>
</tr>
<tr>
<td>Mean (SD) 2-hour serum glucose concentration at 16-22 weeks’ gestation, mmol/L</td>
<td>5.5 (1.4)</td>
</tr>
<tr>
<td>Mean (SD) fasting serum insulin concentration at 16-22 weeks’ gestation, pmol/L</td>
<td>42.0 (24.9)</td>
</tr>
<tr>
<td>Mean (SD) 1-hour serum insulin concentration at 16-22 weeks’ gestation, pmol/L</td>
<td>433.3 (302.3)</td>
</tr>
<tr>
<td>Mean (SD) 2-hour serum insulin concentration at 16-22 weeks’ gestation, pmol/L</td>
<td>341.1 (311.3)</td>
</tr>
<tr>
<td>Mean (SD) homeostatic model assessment of insulin resistance (HOMA-IR) at 16-22 weeks’ gestation</td>
<td>1.4 (0.09)</td>
</tr>
<tr>
<td>Mean (SD) Insulin Sensitivity Index (ISI) at 16-22 weeks’ gestation”</td>
<td>10.7 (7.9)</td>
</tr>
</tbody>
</table>

*All data are presented as a number (%) unless otherwise indicated.

**Available for 76 out of 79 participants

Abbreviations: Interquartile range (IQR), Standard deviation (SD)
The $r^2$ for the adjusted models examining the association between VAT depth and measured HOMA-IR or ISI were slightly higher than the $r^2$ for the model of the relation between pre-pregnancy BMI and measured HOMA-IR or ISI (Table 3.2, Figure 3.3). The $r^2$ in the models of the relation between TAT depth and HOMA-IR or ISI were each higher than those for SAT depth and VAT depth (Figure 3.3).

Table 3.2: Maternal VAT depth at 11-14 weeks’ gestation versus pre-pregnancy BMI and the association of each with (i) HOMA-IR and (ii) ISI at 0 to 120 minutes, each measured at 16-22 weeks’ gestation

<table>
<thead>
<tr>
<th>Measure of IR at 16-22 weeks’ gestation</th>
<th>Model</th>
<th>Variable(s) included in the model</th>
<th>VAT Depth (cm) at 11-14 weeks’ gestation</th>
<th>Pre-pregnancy BMI (kg/m²)</th>
<th>Beta coefficient (95% CI) for the change in HOMA-IR or ISI per 1-cm increase in VAT depth</th>
<th>$r^2$ (95% CI) value for the model</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOMA-IR</td>
<td>Model 1A</td>
<td>VAT depth</td>
<td>0.18 (0.09 to 0.28)</td>
<td>0.16 (0.04 to 0.30)</td>
<td>Pre-pregnancy BMI</td>
<td>0.08 (0.04 to 0.10)</td>
</tr>
<tr>
<td></td>
<td>Model 1B</td>
<td>VAT depth, maternal age, parity, ethnicity and pre-pregnancy BMI</td>
<td>0.09 (-0.01 to 0.19)</td>
<td>0.42 (0.21 to 0.53)</td>
<td>Pre-pregnancy BMI, maternal age, parity and ethnicity</td>
<td>0.07 (0.05 to 0.10)</td>
</tr>
<tr>
<td>ISI at 0, 60 and 120 minutes</td>
<td>Model 2A</td>
<td>VAT depth</td>
<td>-0.13 (-0.22 to -0.04)</td>
<td>0.11 (0.11 to 0.25)</td>
<td>Pre-pregnancy BMI</td>
<td>-0.06 (-0.08 to -0.03)</td>
</tr>
<tr>
<td></td>
<td>Model 2B</td>
<td>VAT depth, maternal age, parity, ethnicity and pre-pregnancy BMI</td>
<td>-0.09 (-0.18 to -0.00)</td>
<td>0.36 (0.15 to 0.47)</td>
<td>Pre-pregnancy BMI, maternal age, parity and ethnicity</td>
<td>-0.05 (-0.08 to -0.03)</td>
</tr>
</tbody>
</table>

Measures of BMI were adjusted for maternal age, parity and ethnicity. Measures of AT depth were adjusted for maternal age, parity, ethnicity, family history and pre-pregnancy BMI.
Figure 3.3: Forest plot comparing variance (adjusted $r^2$, 95% CI) for different measures of maternal obesity in early pregnancy and their association with insulin resistance

Obesity measures included pre-pregnancy BMI, BMI at 11-14 weeks’ gestation, as well as SAT, VAT and TAT depth at 11-14 weeks’ gestation. IR was determined at 16-22 weeks’ gestation using the HOMA-IR or the ISI after a 2-hour 75-gram OGTT. Measures of BMI were adjusted for maternal age, parity and ethnicity. Measures of AT depth were adjusted for maternal age, parity, ethnicity, family history, and pre-pregnancy BMI.
VAT depth and pre-pregnancy BMI were associated with AUC glucose, with VAT depth demonstrating stronger associations than pre-pregnancy BMI, and VAT depth explaining more of the variance in AUC glucose (12%, 95% CI 0.020.27) compared with pre-pregnancy BMI (2%, 95% CI 0.0 to 0.12), a trend that was more pronounced after adjusting for covariates wherein VAT depth explained 20% of the variance in AUC glucose (95% CI 0.02 to 0.32) compared with the 9% explained by pre-pregnancy BMI (95% CI 0.0 to 0.20), (Table 3.3).

Table 3.3: Comparison of maternal VAT depth in early pregnancy with pre-pregnancy BMI and the association of each with AUC glucose in early pregnancy (16-22 weeks’ gestation) among 68 participants

<table>
<thead>
<tr>
<th>Measure of maternal obesity at 11-14 weeks’ gestation</th>
<th>VAT Depth (cm)</th>
<th>Pre-pregnancy BMI (kg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AUC glucose at 16-22 weeks’ gestation</strong></td>
<td>Beta coefficient (95% CI) for the change in AUC glucose per 1-cm increase in VAT depth</td>
<td>r² (95% CI) value for the model</td>
</tr>
<tr>
<td>Model 1A VAT depth</td>
<td>19.9 (7.4 to 32.3)</td>
<td>0.12 (0.02 to 0.27)</td>
</tr>
<tr>
<td>Model 1B VAT depth and maternal age, parity and ethnicity</td>
<td>20.4 (7.9 to 32.9)</td>
<td>0.20 (0.02 to 0.32)</td>
</tr>
</tbody>
</table>
3.4 Discussion

We found a positive association between first-trimester ultrasound measures of central adiposity and IR in early pregnancy. These associations were in addition to maternal age, parity, ethnicity, family history, and pre-pregnancy BMI, and were trending towards being slightly more pronounced than the association between pre-pregnancy and early pregnancy BMI with IR.

Central adiposity is a recognized risk factor for DM and CVD (Després, 1993). In recent decades, VAT has emerged as an independent predictor of T2DM and the metabolic syndrome (Pascot et al., 2001; Bartha et al., 2007). The pathology of VAT and IR remains unclear, though it appears to include the release of free fatty acids into the hepatic portal circulation (Bjorntorp et al., 1993; Suzuki et al., 1993; Arner, 1999;) alongside other metabolic mediating factors, such as low adiponectin (Retnakaran et al., 2005). Little is known about the contribution of central AT depth to IR in pregnancy; thus, we used a practical and validated ultrasound-based screening approach to quantify AT depth at the time of routine fetal nuchal translucency assessment (Armellini et al., 1990; Martin et al., 2009).

Only a few studies have investigated central AT depth and glucose homeostasis in pregnancy (Bartha et al., 2007; Martin et al., 2009; Cisneiros et al., 2013; Gur et al., 2014). Bartha et al. measured VAT at 11-14 weeks’ gestation in 30 healthy pregnant women and found a significant correlation between fasting glucose and VAT \( r = 0.37 \), but not SAT \( r = 0.09 \); VAT \( r = 0.59 \) was also better correlated to HOMA-IR than was BMI \( r = 0.42 \) (Bartha et al., 2007). Recently, Gur et al. (2014) found that VAT in early pregnancy was a stronger predictor of developing GDM than was BMI.
In our prior pilot study, we found that higher VAT depth in early pregnancy was associated with a positive GCT in later pregnancy, whereas SAT depth was not (Martin et al., 2009). What remained unknown was the degree to which these findings might be explained by IR.

Our current study suggests that ultrasound measures of central AT depth may explain slightly more variation in IR than BMI alone. While TAT and VAT depth each explained a similar degree of variance in HOMA-IR, TAT is mostly characterized by VAT. Research on SAT depth and IR outside of pregnancy emphasizes a distinction between the deep and superficial compartments of the SAT layer, describing two histologically unique tissues separated by a discrete fascial plane (Markman et al., 1987; Alexander et al., 1992; Carey et al., 1996; Misra et al., 1997; Kelley et al., 2000). The thickness of the deep SAT layer varies with obesity, and has been shown to better predict IR than the superficial SAT compartment (Alexander et al., 1992; Carey et al., 1996; Misra et al., 1997; Kelley et al., 2000). The relative contributions of VAT versus deep SAT to dysmetabolism in pregnancy should be further characterized.

HOMA-IR is classically represented by fasting levels of glucose and insulin, as a reflection of the balance between hepatic glucose output and IR, yet IR certainly affects postprandial glucose metabolism (Schianca et al., 2003). HOMA-IR may not optimally identify those women at high-risk for GDM but who have normal glucose tolerance in early pregnancy. During early pregnancy, many women experience some degree of hypoglycemia, and hyperinsulinemia that manifests as a relatively low HOMA-IR (Phelps et al., 1981). In one study, 83% of women with elevated 2-hour serum insulin levels at < 16 weeks’ gestation, went on to develop GDM (Bito et al., 2006). The constraints of HOMA-IR have also been well documented in young non-diabetic women with polycystic ovarian syndrome who exhibit post-prandial, but not fasting, IR (Fulghesu et al., 2005).
Given that IR in GDM and T2DM includes the loss of both hepatic and peripheral insulin sensitivity, HOMA-IR alone may not sufficiently estimate total body insulin sensitivity. Indeed, the ISI considers fasting and post-load measures during a standard 2-hour 75-g OGTT, which may overcome the limitations of using HOMA-IR in early pregnancy (Kirwan et al., 2002; Radaelli et al., 2010). The significant inverse association between VAT and ISI described herein supports this possibility.

Limitations of our study include a small sample size of only 79 participants, not all of whom underwent assessment of IR, which precluded separate analyses comparing HOMA-IR or ISI among those who developed GDM. We also relied on self-reported pre-pregnancy height and weight, which is susceptible to reporting bias. Strengths of this study include the use of a standardized protocol to evaluate abdominal adiposity in a multi-ethnic population at the time of a routine prenatal ultrasound, which affords practical, cost-effective clinical application if further validated.

Evidence suggests that pregnant women whose GCT is abnormal, but whose OGTT is normal, may have pre-existing beta cell dysfunction (Retnakaran et al., 2008a). Moreover, even subtle abnormalities of glucose homeostasis may be predictive of a “pre-diabetic” state in pregnancy—impaired fasting glucose and/or impaired glucose tolerance—and underlying IR before the overt onset of GDM (Ray et al., 2010). Measuring central AT depth may further describe IR severity beyond pre-pregnancy BMI alone and, thus, may offer an opportunity to focus earlier screening and prevention strategies for GDM. Women identified as having early pregnancy IR, as indicated by elevated central AT, may one day be eligible for first-trimester interventions aimed at lowering their risk of overt GDM and the perinatal complications associated with this diagnosis, though this remains to be established in future large scale prospective studies.
Chapter 4 First-trimester Maternal Abdominal Adiposity and Adiponectin in early gestation

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Segue

In the previous chapter we showed that women with elevated abdominal adiposity on ultrasound in the first trimester of pregnancy have evidence of IR later in early pregnancy at 16-22 weeks’ gestation. Whether this ultrasound measure of maternal abdominal adiposity can also describe early pregnancy indicators of other, novel markers of IR such as low adiponectin is not known.

Adiponectin is a hormone secreted exclusively from adipose tissue and is considered to be protective, with insulin-sensitizing effects. Low adiponectin is associated with both previous GDM and also T2DM in studies outside of pregnancy. Low adiponectin, therefore, reflects IR, and may be detectable in early pregnancy, given that IR is chronic in nature and likely predates conception.

In the previous chapter we showed that the early pregnancy sonographic measurement of SAT, VAT and TAT can be used to detect early pregnancy IR as early as 16 weeks’ gestation.

In this chapter we explore whether there is an association between ultrasound-measured SAT, VAT and TAT and low adiponectin in early pregnancy at 16-22 weeks, well before the conventional diagnosis of GDM at 24-28 weeks.
4.0 Abstract

Objective: Low adiponectin is associated with gestational diabetes (GDM). Whether different abdominal adiposity compartments influence adiponectin during pregnancy is unknown. We examined the association between first-trimester abdominal SAT, VAT and TAT depth and mid-pregnancy adiponectin.

Research Design and Methods: Among 50 women, SAT, VAT and TAT depth were prospectively measured by ultrasonography at 11-14 weeks’ gestation. Serum adiponectin was measured at 16-22 weeks’ gestation.

Results: After adjusting for maternal age, parity, ethnicity and family history of T2DM, neither pre-pregnancy BMI ($r^2 0.12$, 95% CI -0.03 to 0.27), BMI at 11-14 weeks ($r^2 0.14$, 95% CI -0.02 to 0.30) nor VAT ($r^2 0.16$, 95% CI 0.00 to 0.32) significantly explained variation in serum adiponectin compared with SAT ($r^2 0.23$, 95% CI 0.05 to 0.41) and TAT ($r^2 0.25$, 95% CI 0.07 to 0.43).

Conclusions: First-trimester SAT and TAT are more strongly associated with adiponectin than is BMI.
4.1 Introduction

Adiponectin is an emergent adipokine derived exclusively from adipose tissue, exhibiting a paradoxical association with adiposity and linked to the development of dysmetabolism and GDM (Shehzad et al., 2012). Adiponectin is also associated with IR and T2DM and exhibits pleiotropic actions that include improved insulin sensitivity (Shehzad et al., 2012).

The mechanistic action of adiponectin involves both insulin-sensitizing and anti-inflammatory properties (Yamauchi et al., 2001; Kim et al., 2007). While isolated amounts of adiponectin are purported to be higher in VAT than in SAT, the absolute contribution of adiponectin from SAT is higher owing to the higher mass of the subcutaneous compartment (Motoshima et al., 2002).

GDM is screened for and diagnosed in the late second trimester, yet IR likely occurs well before the diagnosis of GDM (CDA, 2013). It is well-known that adiponectin is associated with an insulin-resistant state and therefore low adiponectin is expected in those with GDM or T2DM (Shehzad et al., 2012). Whether elevated maternal abdominal adiposity can predict lower levels of this protective hormone earlier in pregnancy, well before the diagnosis of GDM, is unknown.

We recently described an association between first-trimester VAT, and TAT, but not SAT depth and first-trimester markers of insulin sensitivity (De Souza et al., 2014). Herein we examine, for the first time, whether these three different AT compartments, measured in the first trimester of pregnancy, are associated with hypoadiponectinemia in mid-pregnancy, well before the time of conventional screening and diagnosis of GDM.
4.2 Research Design and Methods

The methods have been reported previously (De Souza et al., 2014). In brief, we conducted a prospective cohort study in a general obstetrics outpatient clinic at St. Michael’s Hospital in Toronto, Ontario, Canada. Eligible participants were aged 18 years and older with a viable singleton pregnancy at 11 to 14 weeks’ gestation and without pre-pregnancy T2DM or a prior pregnancy affected by GDM.

As previously described, abdominal AT compartments were distinguished and measured by a trained ultrasound technician at the time of a routine fetal ultrasound between 11 to 14 weeks’ gestation (Martin et al., 2009). SAT depth was measured from the SAT layer to the outer border of the rectus abdominus muscle at the level of the linea alba and the umbilicus. VAT depth was measured from the inner border of the rectus abdominus muscle, at the level of the linea alba, to the anterior wall of the abdominal aorta. TAT depth was measured from the SAT layer to the anterior wall of the abdominal aorta. Measurements were taken using a Phillips IU22 ultrasound system (Phillips Electronics NV, Eindhoven, The Netherlands) with either a 5-2 MHz or 9 MHz probe. Depth and zoom settings were standardized, such that the aorta was at the bottom of the screen and the vertebral bodies were just visible. The performance characteristics of this technique include an inter-observer reliability of 0.79 (95% CI 0.69 to 0.88) for SAT and 0.87 (95% CI 0.82 to 0.93) for VAT (Martin et al., 2009).
Information about maternal age, ethnicity (Caucasian, Black, South Asian, East Asian, or other), self-reported pre-gestational height and weight, and a family history of T2DM in first-degree relatives was collected. At 16 to 22 weeks’ gestation, each participant completed a 2-hour 75g OGTT following an overnight fast.

Venous blood samples for laboratory measurement of fasting serum adiponectin concentration were measured by enzyme-linked immunoassay (ELISA) (Human adiponectin Cat. # EZHADP-61K EMD Millipore, St. Charles, MO).

4.2.1 Statistical Analyses

The natural logarithmic transformation of adiponectin was used, as the data were positively skewed. Data were analyzed from a larger prospective study (De Souza et al., 2014). Univariate analyses quantified the variation ($r^2$) in serum log adiponectin explained by the continuous measure of pre-pregnancy BMI, BMI at 11-14 weeks’ gestation, as well as SAT, VAT and TAT depth, each measured at 11 to 14 weeks’ gestation. Multivariable linear regression models were used to adjust for maternal age (continuous in years), parity, ethnicity (Caucasian versus non-Caucasian) and family history of T2DM (Adjusted Model #1). A separate multivariable linear regression model (Adjusted Model #2) further included pre-pregnancy BMI (continuous in kg/m²) in assessing the $r^2$ between SAT, VAT or TAT and serum log adiponectin. Residual plots suggested adequate goodness-of-fit in the models. All $P$ values were reported using a two-sided significance level of 0.05. Statistical analyses were performed using SAS version 9.1.3 (SAS Institute Inc., Cary, NC). The study was approved by the Research Ethics Board of St. Michael’s Hospital, Toronto, Ontario, Canada.
4.3 Results

Fifty adiponectin samples were available for analysis. Women were a mean (SD) age of 31.9 (4.9) years. Pre-pregnancy BMI ranged from 16.6 to 48.8 kg/m².

- Women aged 18+ years
- Singleton pregnancy
- No previous diabetes mellitus or chronic condition
- Underwent a first-trimester ultrasound for nuchal translucency

\[
\text{(n = 79)}
\]

Excluded 15 women for the following reasons:
- Transferred to another hospital during study (n = 2)
- Declined OGTT (n = 13)

\[
\text{n = 64}
\]

- Incomplete OGTT (n = 4)
- Unusable adiponectin data (n=10)

\[
\text{Final cohort}
\]

\[
\text{n=50}
\]

Figure 4.1: Flow chart of participant inclusion
The unadjusted $r^2$ value describing the variation in adiponectin was highest for TAT depth (Table 4.1). SAT depth (20%, 95% CI 2 to 38) and TAT depth (23%, 95% CI 5 to 41) each significantly explained a portion of the variance in adiponectin, while pre-pregnancy BMI, BMI at 11-14 weeks and VAT depth did not (Adjusted Model #1, Table 4.1). The overall variance in adiponectin described by SAT and TAT depth was minimally improved by adding pre-pregnancy BMI to the model (Adjusted Model #2, Table 4.1).

Table 4.1: Association between BMI, as well as first-trimester abdominal SAT, VAT and TAT at 11-14 weeks’ gestation, and serum log adiponectin measured at 16 to 22 weeks’ gestation among 50 pregnant women.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unadjusted model</th>
<th>Adjusted Model #1*</th>
<th>Adjusted Model #2†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beta coefficient (95% CI) for log adiponectin</td>
<td>$r^2$ (95% CI)</td>
<td>Beta coefficient (95% CI) for log adiponectin</td>
</tr>
<tr>
<td>Pre-pregnancy BMI (kg/m$^2$)</td>
<td>-0.01 (-0.02 to 0.004) p = 0.20</td>
<td>0.03 (-0.06 to 0.11)</td>
<td>-0.004 (-0.01 to 0.01) p = 0.36</td>
</tr>
<tr>
<td>BMI at 11-14 weeks (kg/m$^2$)</td>
<td>-0.01 (-0.02 to 0.002) p = 0.09</td>
<td>0.06 (-0.06 to 0.18)</td>
<td>-0.01 (-0.02 to 0.01) p = 0.23</td>
</tr>
<tr>
<td>SAT depth at 11-14 weeks (cm)</td>
<td>-0.06 (-0.11 to -0.02) p = 0.007</td>
<td>0.14 (-0.01 to 0.29)</td>
<td>-0.06 (-0.10 to -0.01) p = 0.03</td>
</tr>
<tr>
<td>VAT depth at 11-14 weeks (cm)</td>
<td>-0.05 (-0.09 to -0.004) p = 0.032</td>
<td>0.09 (-0.04 to 0.22)</td>
<td>-0.04 (-0.08 to 0.01) p = 0.11</td>
</tr>
<tr>
<td>TAT depth at 11-14 weeks (cm)</td>
<td>-0.04 (-0.07 to -0.002) p = 0.002</td>
<td>0.19 (0.02 to 0.36)</td>
<td>-0.04 (-0.07 to -0.01) p = 0.01</td>
</tr>
</tbody>
</table>

*Using linear regression analysis, adjusted for maternal age, parity, ethnicity and family history of diabetes mellitus.
†Using linear regression analysis, adjusted for pre-pregnancy BMI, maternal age, parity, ethnicity and family history of diabetes mellitus.
4.4 Discussion

SAT and TAT depth, measured by ultrasound in early pregnancy, each better explained the variation in serum adiponectin than did BMI or VAT. This is consistent with our previous findings of a stronger association between early pregnancy SAT and TAT depth with first-trimester stimulated markers of IR (De Souza et al., 2014). The propensity for first-trimester SAT or TAT to also predict hypoadiponectinemia in mid-pregnancy further describes a dysmetabolic predisposition for GDM and subsequent T2DM (Arita et al., 1999; Retnakaran et al., 2005).

While the inverse relation between central adiposity and adiponectin concentration is well-established (Arita et al., 1999; Coker et al., 2009), little is known about the association between regional AT distribution and adiponectin levels in pregnancy. VAT is purported to be diabetogenic and pathogenically linked to IR (Coker et al., 2009). However, recent research has also described the morphological and functional variability of the SAT compartment, wherein the deep SAT layer bears similar physiologic function to VAT and a parallel association with IR (Kelley et al., 2000; Coker et al., 2009). Depot-specific adiponectin gene expression has been shown to vary in the SAT or VAT compartments in response to insulin-stimulated conditions (Hernandez-Morante et al., 2008). TAT may more closely represent the pathophysiology of central adiposity in generating IR; regional adipose tissue depots could be physiologically different during pregnancy.

Low serum adiponectin predicts postpartum IR and beta-cell dysfunction (Retnakaran et al., 2010). Previous studies have reported reduced adiponectin levels among those with GDM compared with their normoglycemic counterparts, even after adjusting for IR and traditional risk factors (Retnakaran et al., 2005).
The mechanistic processes that mediate adiponectin and other concomitant manifestations of diabetes, including IR and beta-cell dysfunction, remain to be fully clarified. Several studies have corroborated the clinical finding that low baseline adiponectin concentration is likely a precursor to the development of GDM, as it can predict future onset of IR and is an independent correlate of beta-cell dysfunction in late pregnancy (Weerakiet et al., 2006; Lain et al., 2008).

Limitations of this study include the use of self-reported pre-pregnancy height and weight, which is susceptible to reporting bias. Strengths of this study include the use of a standardized protocol to evaluate abdominal adiposity in a multi-ethnic population at the time of a routine prenatal ultrasound examination.

To our knowledge, this is the first report to describe the association between direct measures of abdominal adiposity and maternal adiponectin in pregnancy. We suggest that first-trimester ultrasound measurement of maternal abdominal adiposity may provide an accessible, safe and cost-effective approach to identify women with sub-clinical hypoadiponectinemia in mid-pregnancy, several weeks prior to the conventional screening for GDM. Moreover, first-trimester SAT and TAT may be superior to conventional measures of obesity, such as BMI, but this should be validated in a larger study.
Chapter 5  First-trimester maternal abdominal adiposity and dysglycemia and GDM in mid-gestation

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Segue

Elevated maternal abdominal adiposity on ultrasound in the first trimester of pregnancy predicts IR and low adiponectin later in the early gestational period pregnancy at 16-22 weeks. Whether this first-trimester ultrasound measure of maternal abdominal adiposity can also describe a continuum of dysmetabolic risk and IR later in pregnancy, during routine screening for GDM at 24-28 weeks and indeed predict GDM itself, remains unknown.

In the previous chapter we showed that our early pregnancy sonographic screening approach to measuring SAT, VAT and TAT can be used to detect early pregnancy IR well before the frank manifestation of GDM. We also showed that this method provided additional information about maternal risk for IR and hypoadiponectinemia beyond pre-pregnancy BMI alone.

In this chapter we explore whether this same first-trimester sonographic methodology for quantifying SAT, VAT and TAT can be used to detect downstream indicators of “pre-diabetes”, namely IFG, IGT and GDM in the late second trimester during routine screening for GDM.

If shown to be effective, this ultrasound screening method for elevated maternal abdominal adiposity, measured at a routine first-trimester ultrasound, could be used to describe a continuum of downstream dysmetabolic risk, making it eligible as a viable potential preventative approach to GDM management if further validated.
5.0 Abstract

Objective: Elevated abdominal adiposity in early pregnancy might predict GDM. We assessed the association between first-trimester abdominal adiposity with dysglycemia and IR in mid-pregnancy.

Research Design and Methods: In a prospective cohort study of 485 women, we measured SAT, VAT and TAT depth, using ultrasound at 11-14 weeks’ gestation. Logistic regression analysis assessed the relation between each quartile (Q) of SAT, VAT or TAT depth and the composite outcome of IFG, GIGT or GDM, based on a 75-g OGTT at 24-28 weeks. Multivariable linear regression analyzed the variance ($r^2$, 95% CI) explained by pre-pregnancy BMI, in addition to BMI, SAT, VAT and TAT depth at 11-14 weeks’ gestation, and individual measures of AUC-glucose, HOMA-IR and ISI_{OGTT} at 24-28 weeks.

Results: 52 women (10.7%) developed the composite outcome. Upon adjusting for maternal age, ethnicity, family history of diabetes and BMI, Q4 versus Q1 of VAT (aOR 3.1, 95% CI 1.1 to 9.5) and Q4 versus Q1 of TAT (aOR 2.7, 95% CI 1.1 to 7.8) were each significantly associated with the composite outcome of IFG, GIGT or GDM, but SAT was not (aOR 1.8, 95% CI 0.7 to 4.8). Adjusting for maternal BMI, VAT and TAT, each explained about 15% (95% CI 9 to 21) of the variance in AUC-glucose. TAT explained the greatest amount of the variance in HOMA-IR and ISI_{OGTT}.

Conclusions: Elevated first-trimester VAT and TAT depth independently predict the risk of developing dysglycemia in pregnancy beyond BMI alone, possibly because of greater IR.
5.1 Introduction

Maternal obesity is a primary risk factor for GDM and associated adverse pregnancy outcomes, and contributes to an elevated long-term risk of developing T2DM (Solomon et al., 1997; Kim et al. 2002). More than 40% of pregnant women are either overweight or obese in North America (Tjepkema, 2005; Gunderson; 2009).

Outside of pregnancy, excess visceral adiposity is considered a pathogenic determinant of dysglycemia and IR, beyond BMI alone (Tchernof & Després, 2013). During the second trimester of pregnancy—a time of maximal IR—some women are at higher risk of developing dysglycemia and GDM (Retnakaran et al., 2008).

Elevated central adiposity in early pregnancy may be one modifiable risk factor leading to onset of abnormal glucose homeostasis in the second trimester of pregnancy, as detailed within a few small-sample studies (Bartha et al., 2007; Martin et al., 2009; Cisneiros et al., 2013; De Souza et al., 2014; Gur et al., 2014).

Our group has previously shown, in a study of 62 women, that ultrasound-measured VAT depth above the upper quartile in early pregnancy was associated with a positive glucose challenge test in later pregnancy (adjusted OR 16.9, 95% CI 1.5 to 194.6), independent of BMI (Martin et al., 2009). Subsequently, we found that first-trimester VAT and TAT depth was associated with IR in early pregnancy (De Souza et al., 2014). Hence, maternal abdominal AT depth measured in the first trimester of pregnancy may characterize an upstream metabolic profile that precedes the onset of dysglycemia downstream in mid-pregnancy, when IR begins to peak.
Herein, we investigated the relation between early pregnancy central AT depth and both abnormal glucose homeostasis and IR at mid-pregnancy. We hypothesized that women with greater abdominal AT depth in early pregnancy would exhibit greater dysglycemia and IR in the second trimester, and that this would be so even after adjusting for BMI.

5.2 Research Design and Methods

We completed a prospective cohort study within the general Obstetrics Outpatient Clinic at St. Michael’s Hospital in Toronto, Ontario, Canada. The study was approved by the Research Ethics Board of St. Michael’s Hospital, and participants provided written informed consent.

Women aged 18 years and older were eligible for study entry if they had a viable singleton pregnancy at 11-14 weeks’ gestation. We excluded women with known pre-gestational DM or a prior pregnancy affected by GDM.

At 11-14 weeks’ gestation, abdominal AT depth was measured by trained ultrasound technicians, as previously described (Martin et al., 2009). SAT depth was measured from the outer border of the rectus abdominus muscle to the skin surface, at the intersection of the linea alba and the umbilicus. VAT depth was measured from the inner border of the rectus abdominus muscle to the anterior wall of the abdominal aorta. TAT depth was measured from the SAT layer surface to the anterior wall of the abdominal aorta, along the same plane as above. Depth and zoom settings were standardized, such that the aorta was at the bottom of the screen and the vertebral bodies were just visible. This technique has an inter-observer reliability of 0.79 (95% CI 0.69 to 0.88) for SAT and 0.87 (95% CI 0.82 to 0.93) for VAT (Martin et al., 2009). Measurements were taken using a Phillips IU22 ultrasound machine with either a 5-2 MHz or 9 MHz probe.
At 11-14 weeks, a questionnaire was also completed by each participant, detailing information about her ethnicity (Caucasian, Black, South Asian, East Asian or Other), self-reported pre-gestational height and weight, and family history of T2DM in a first-degree relative. Her current weight, at 11-14 weeks, was directly measured using a calibrated scale.

Subsequently, at 24-28 weeks’ gestation, each participant completed a 2-hour 75g OGTT following an overnight fast. Fasting, 1-hour and 2-hour serum glucose concentrations, measured within a single clinical laboratory, were used to calculate the area under the glucose curve (AUC-glucose), based on the trapezoidal rule (Purves, 1992). From the same sample, we calculated the HOMA-IR and OGTT-derived ISI\text{OGTT}, as previously described (Ozcimen et al., 2008; DeFronzo et al., 2010). The insulin concentration was measured in pmol/L, and then converted to µU/mL to calculate HOMA-IR. Glucose concentration was measured in mmol/L, and then converted to mg/dL to calculate ISI\text{OGTT}.

### 5.2.1 Data analysis

As HOMA-IR and ISI\text{OGTT} were positively skewed, data were log-transformed, and then back-transformed and reported as a percentage change. Pearson correlations were used to assess the association between anthropometric measures of maternal obesity and SAT, VAT and TAT.

We used two analytical approaches. First, logistic regression analysis was performed to assess the categorical relation between the respective quartiles of SAT, VAT or TAT depth and the development of the composite outcome of either IFG, IGT or GDM, based on the 75g OGTT at 24-28 weeks’ gestation.
IFG was defined as a fasting glucose ≥ 5.3 mmol/L, in isolation; GIGT was based on a glucose value at 1h ≥ 10.6 mmol/L or at 2h ≥ 8.9 mmol/L, in isolation; and GDM was defined as ≥ 2 abnormal serum glucose values (i.e. fasting ≥ 5.3 mmol/L, 1h ≥ 10.6 mmol/L and/or 2h ≥ 8.9 mmol/L) (CDA, 2008). In a post hoc sensitivity analysis, we examined the outcome of GDM alone using both the traditional (CDA, 2008) as well as the International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria for GDM (IADPSG, 2010). ORs were adjusted (aOR) for maternal age (continuous in years), ethnicity (Caucasian versus Non-Caucasian), family history of T2DM in a first-degree relative, change in BMI from 11-11 weeks’ gestation to 24-28 weeks’ gestation, and measured BMI at 11-14 weeks’ gestation (continuous in kg/m²). Quartile 1 served as the referent for each model.

In the second analytical approach, linear regression analysis was performed to compare the association between each continuous measure of maternal obesity, namely, self-reported pre-pregnancy BMI, as well as BMI, SAT, VAT and TAT depth measured at 11-14 weeks’ gestation, and the continuous outcomes of 1) glucose dysregulation (defined by AUC-glucose) at 24-28 weeks’ gestation; and 2) IR (defined by HOMA-IR and ISI_OGTT) at 24-28 weeks’ gestation. Multivariable linear regression models included maternal age (continuous in years), parity, ethnicity (Caucasian versus Non-Caucasian), family history of T2DM, and change in BMI from 11-14 weeks’ gestation to 24-28 weeks’ gestation (continuous in kg/m²) (Model #1). In the assessment of SAT, VAT and TAT, we further included pre-pregnancy BMI (Model #2) or BMI at 11-14 weeks (Model #3). When we assessed pre-pregnancy BMI or BMI at 11-14 weeks’ gestation, we did not include SAT, VAT or TAT in that model, given that the aim of the second analytical approach was to assess whether SAT, VAT or TAT more fully explained the variability (r² [95% CI]) in either AUC-glucose, HOMA-IR or ISI_OGTT than BMI, a commonly accepted and conventional measure of obesity.
All variables were included in the models *a priori*. Including gestational age and parity at the time of the OGTT in the linear or logistic regression models did not alter the effect sizes, so this covariate was excluded from all models. Variance inflation factors were under 2.5, indicating minimal multi-collinearity between measures of BMI and adiposity. Residual plots suggested adequate goodness-of-fit in the models.

Statistical analyses were performed using SAS (version 9.1.3; SAS Institute, Cary, NC).
Table 5.1: Physical, ultrasound and biochemical measures and associated references

<table>
<thead>
<tr>
<th>Measure (unit of analysis)</th>
<th>Detail of measurement</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Physical:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WC (cm)</td>
<td>After expiration at the midpoint between the lowest rib &amp; the iliac crest.</td>
<td>(Branchtein et al., 1997)</td>
</tr>
<tr>
<td>WHR</td>
<td>Hip circumference is obtained at the widest point between hip &amp; buttock.</td>
<td>(Branchtein et al., 1997)</td>
</tr>
<tr>
<td>Weight gain in pregnancy (kg)</td>
<td>Between 11-14 weeks &amp; 24-28 weeks gestation (or gestational age at delivery, if earlier)</td>
<td></td>
</tr>
<tr>
<td>Mean sitting blood pressure (mm Hg)</td>
<td>After a 5 minute rest, to be recorded 2 times over 10 minutes, measured with an automated, correct-fitting blood pressure cuff.</td>
<td>--</td>
</tr>
<tr>
<td><strong>Ultrasound-based measures:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdominal visceral cavity depth (cm)</td>
<td>Abdominal visceral cavity depth (cm).</td>
<td>(Martin et al., 2009)</td>
</tr>
<tr>
<td>Features of fatty liver (present or absent)</td>
<td>Based on presence of both: 1. Diffusely increased echogenic (“bright”) liver (liver echogenicity &gt; kidney); 2. Impaired visualization of the intrahepatic vessels.</td>
<td>(Sadeeh et al., 2002) (Charatcharoenwitthaya et al., 2007)</td>
</tr>
<tr>
<td><strong>Biochemical measures:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>75-g OGTT</td>
<td>75-g oral glucose tolerance test (OGTT), done after a 10-h overnight fast, using a standard oral solution containing 75 g of dextrose. Venous blood samples are drawn at 0, 1h and 2h.</td>
<td>(Reinblatt et al., 2006)</td>
</tr>
<tr>
<td>Total AUC-glucose</td>
<td>Calculated from the fasting, 1-h and 2-h blood glucose concentration at the time of the OGTT, using the trapezoid rule.</td>
<td>(Purves, 1992)</td>
</tr>
<tr>
<td>Plasma insulin</td>
<td>Specific insulin measured using Elecsys 1010 (Roche Diagnostics) immunoassay analyzer &amp; electrochemiluminescence immunoassay. Assay shows 0.05% cross-reactivity to intact human proinsulin &amp; the Des 31,32 circulating split form.</td>
<td>--</td>
</tr>
<tr>
<td>Calculated HOMA-IR</td>
<td>HOMA-IR = (fasting plasma insulin [μU/ml] X fasting glucose [mmol/L])/22.5.</td>
<td>(Ozcimen et al., 2008)</td>
</tr>
<tr>
<td>Calculated ISI</td>
<td>ISI = (10,000/sqrt [(fast glucose X fast insulin) X (mean glucose @ OGTT X mean insulin @ OGTT)])</td>
<td>(DeFronzo &amp; Matsuda, 2010)</td>
</tr>
<tr>
<td>Serum ALT</td>
<td>Analyzed using the Dimension RxL Max integrated chemistry system.</td>
<td>--</td>
</tr>
</tbody>
</table>
5.3 Results

A total of 485 out of 685 women completed all study measures (Figure 5.1).

- Women aged 18+ years
- Singleton pregnancy
- No previous diabetes mellitus or chronic condition
- Underwent a first-trimester ultrasound for nuchal translucency

\((n = 685)\)

Excluded 180 women for the following reasons:

- Transferred to another hospital during study \((n = 30)\)
- Declined OGTT \((n = 150)\)

\(n = 505\)

Final cohort

\(n = 485\)

Figure 5.1: Flow chart of participant inclusion
The mean (SD) age was 32.9 (4.8) years (Table 5.2). BMI at 11-14 weeks’ gestation ranged from 17.2 to 49.9 kg/m², with a mean of 25.1 (5.1) kg/m². VAT depth ranged from 1.1 to 11.4 cm, with a mean of 4.1 (1.7) cm; SAT depth ranged from 0.56 to 5.1 cm, with a mean of 1.9 (0.80) cm; and TAT depth ranged from 2.0 to 14.2 cm, with a mean of 5.9 (2.1) cm.

### Table 5.2: Maternal characteristics, exposure and outcome variables for 485 study participants

<table>
<thead>
<tr>
<th>Variables</th>
<th>Measures</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td>Mean (SD) age at time of enrolment, years</td>
<td>32.9 (4.8)</td>
</tr>
<tr>
<td></td>
<td>No. (%) with a first degree relative with T2DM</td>
<td>110 (22.7)</td>
</tr>
<tr>
<td></td>
<td>No. (%) Caucasian</td>
<td>251 (51.8)</td>
</tr>
<tr>
<td></td>
<td>No. (%) with parity ≥ 1</td>
<td>212 (43.8)</td>
</tr>
<tr>
<td><strong>Exposures</strong></td>
<td>Mean (SD) self-reported pre-pregnancy BMI, kg/m²</td>
<td>23.8 (4.9)</td>
</tr>
<tr>
<td></td>
<td>Mean (SD) BMI at 11-14 weeks, kg/m²</td>
<td>25.1 (5.1)</td>
</tr>
<tr>
<td></td>
<td>Mean (SD) net change in BMI from 11-14 weeks to 24-28 weeks, kg/m²</td>
<td>2.6 (1.8)</td>
</tr>
<tr>
<td></td>
<td>Mean (SD) SAT depth at 11-14 weeks, cm</td>
<td>1.9 (0.8)</td>
</tr>
<tr>
<td></td>
<td>Mean (SD) VAT depth at 11-14 weeks, cm</td>
<td>4.1 (1.7)</td>
</tr>
<tr>
<td></td>
<td>Mean (SD) TAT depth at 11-14 weeks, cm</td>
<td>5.9 (2.1)</td>
</tr>
<tr>
<td><strong>Outcomes</strong></td>
<td>Mean (SD) fasting serum glucose concentration at 24-28 weeks, mmol/L</td>
<td>4.4 (0.44)</td>
</tr>
<tr>
<td></td>
<td>Mean (SD) 1-hour serum glucose concentration at 24-28 weeks, mmol/L</td>
<td>7.4 (1.9)</td>
</tr>
<tr>
<td></td>
<td>Mean (SD) 2-hour serum glucose concentration at 24-28 weeks, mmol/L</td>
<td>6.0 (1.5)</td>
</tr>
<tr>
<td></td>
<td>Geometric mean (SD) HOMA-IR at 24-28 weeks</td>
<td>1.8 (2.8)</td>
</tr>
<tr>
<td></td>
<td>Geometric mean (SD) ISI at 24-28 weeks</td>
<td>4.8 (6.8)</td>
</tr>
<tr>
<td></td>
<td>Mean (SD) AUC glucose at 24-28 weeks, mmol/L/hr</td>
<td>12.7 (2.6)</td>
</tr>
<tr>
<td></td>
<td>No. (%) meeting the criteria for GDM at 24-28 weeks*</td>
<td>45 (9.3)</td>
</tr>
<tr>
<td></td>
<td>No. (%) meeting the criteria for IFG, GIGT or GDM at 24-28 weeks†</td>
<td>52 (10.7)</td>
</tr>
</tbody>
</table>

*IFG was based on an abnormal fasting value ≥ 5.3 mmol/L, in isolation, and GIGT was based on an abnormal glucose value at 1h ≥ 10.6 mmol/L or 2h ≥ 8.9 mmol/L, in isolation.

†GDM was based on the presence of at least two abnormal serum glucose values: fasting ≥ 5.3 mmol/L; 1h ≥ 10.6 mmol/L and/or 2h ≥ 8.9 mmol/L.
When gestational age at OGTT or parity were included in the linear and logistic regression models, there was no change detected; therefore, models reported herein do not contain these covariates.

Fasting, 1-hour, 2-hour glucose measures, and AUC-glucose were each tested for normality (Figure 5.3a). Insulin, HOMA-IR and ISI_{OGTT} were skewed (Figure 5.3b) and therefore data were log-transformed (Figure 5.3c) and subsequently back transformed.
Figure 5.3a: Tests for normality for outcome variables fasting, 1-hour and 2-hour oral glucose tolerance test (OGTT) at 24-28 weeks’ gestation.

<table>
<thead>
<tr>
<th>Figure panel</th>
<th>Variable</th>
<th>Mean (SD)</th>
<th>Median</th>
<th>Skewness</th>
<th>Kurtosis</th>
<th>Kolmogorov-Smirnov (KS) Test</th>
<th>Shapiro-Wilk Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Fasting serum glucose concentration at 16-22 weeks’ gestation, mmol/L</td>
<td>4.43 (0.43)</td>
<td>4.40</td>
<td>0.99</td>
<td>2.39</td>
<td>0.11 p &lt; 0.01</td>
<td>0.95 p&lt;0.0001</td>
</tr>
<tr>
<td>B</td>
<td>1-hour serum glucose concentration at 16-22 weeks’ gestation, mmol/L</td>
<td>7.38 (1.85)</td>
<td>7.10</td>
<td>0.53</td>
<td>0.05</td>
<td>0.07 p &lt; 0.01</td>
<td>0.98 p&lt;0.0001</td>
</tr>
<tr>
<td>C</td>
<td>2-hour serum glucose concentration at 16-22 weeks’ gestation, mmol/L</td>
<td>5.98 (1.41)</td>
<td>5.80</td>
<td>0.64</td>
<td>0.64</td>
<td>0.76 p &lt; 0.01</td>
<td>0.98 p&lt;0.0001</td>
</tr>
</tbody>
</table>
Figure 5.3b: Tests for normality for outcome variables HOMA-IR and ISI at 24-28 weeks’ gestation.

<table>
<thead>
<tr>
<th>Figure panel</th>
<th>Variable</th>
<th>Tests for normality of outcome variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>HOMA-IR at 16-22 weeks’ gestation</td>
<td>Mean (SD) 1.82 (3.29)  Median 1.26  Skewness 16.50  Kurtosis 316.86  Kolmogorov-Smirnov (KS) Test 0.32  Shapiro-Wilk Test p &lt; 0.0001</td>
</tr>
<tr>
<td>B</td>
<td>ISI at 16-22 weeks’ gestation</td>
<td>Mean (SD) 20.90 (18.48)  Median 17.34  Skewness 4.26  Kurtosis 29.61  Kolmogorov-Smirnov (KS) Test 0.16  Shapiro-Wilk Test p &lt; 0.0001</td>
</tr>
</tbody>
</table>
Figure 5.3c: Tests for normality for outcome LOG-TRANSFORMED variables HOMA-IR, ISI and manually calculated AUC glucose at 24-28 weeks’ gestation

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean (SD)</th>
<th>Median</th>
<th>Skewness</th>
<th>Kurtosis</th>
<th>Kolmogorov-Smirnov (KS) Test</th>
<th>Shapiro-Wilk Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Log-transformed HOMA-IR at 16-22 weeks’ gestation</td>
<td>0.14 (0.27)</td>
<td>0.10</td>
<td>0.89</td>
<td>2.99</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>B</td>
<td>Log-transformed ISI at 16-22 weeks’ gestation**</td>
<td>1.20 (0.31)</td>
<td>1.2</td>
<td>-0.17</td>
<td>0.60</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>C</td>
<td>Calculated AUC glucose at 16-22 weeks’ gestation, mmol/L/hr</td>
<td>12.70 (2.60)</td>
<td>12.3</td>
<td>0.72</td>
<td>0.63</td>
<td>p &lt; 0.01</td>
</tr>
</tbody>
</table>

** Normal distribution assumed.
5.3.2 Correlations between measures of maternal adiposity and dysglycemia

On Pearson univariate correlation analysis, SAT, VAT and TAT were significantly correlated with other indices of maternal adiposity including pre-pregnancy BMI, BMI at 11-14 weeks, WC and WHR. Correlations between SAT, VAT and TAT and pre-pregnancy BMI were not significantly different from SAT, VAT and TAT and BMI at ultrasound (Table 5.4a). TAT was most significantly correlated with WC at 11-14 weeks’ gestation (0.72, 95% CI 0.68 to 0.74). All three measures of central adiposity (SAT, VAT, TAT) had the highest correlation with WC and the lowest significant correlation with WHR.

TAT depth emerged as the central adiposity measure that best correlated with metabolic parameters (AUC glucose, HOMA-IR, ISI\textsubscript{OGTT}) (Table 5.4b). Of these, the strongest univariate association was between TAT depth and HOMA-IR (r=0.54, 95% CI 0.47 to 0.60).
Table 5.4a: Pearson correlation coefficients, r and 95% CI between maternal central adiposity measures taken at 11-14 weeks’ gestation- SAT, VAT, TAT depth and other markers of maternal adiposity—pre-pregnancy BMI, BMI at 11 to 14 weeks’ gestation, WC and WHR at 11-14 weeks’ gestation for 485 study participants

<table>
<thead>
<tr>
<th>Adiposity Measure</th>
<th>SAT depth</th>
<th>VAT depth</th>
<th>TAT depth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-pregnancy BMI (kg/m²)</td>
<td>0.51 (0.44 to 0.58)</td>
<td>0.56 (0.50 to 0.62)</td>
<td>0.64 (0.58 to 0.69)</td>
</tr>
<tr>
<td>BMI at 11-14 weeks (kg/m²)</td>
<td>0.52 (0.45 to 0.56)</td>
<td>0.63 (0.57 to 0.68)</td>
<td>0.69 (0.64 to 0.75)</td>
</tr>
<tr>
<td>WC at 11-14 weeks (cm)</td>
<td>0.54 (0.47 to 0.60)</td>
<td>0.66 (0.60 to 0.70)</td>
<td>0.72 (0.68 to 0.74)</td>
</tr>
<tr>
<td>WHR at 11-14 weeks</td>
<td>0.22 (0.13 to 0.30)</td>
<td>0.34 (0.25 to 0.41)</td>
<td>0.35 (0.27 to 0.42)</td>
</tr>
<tr>
<td>Change in BMI from 11-14 to 24-28 weeks (kg/m²)</td>
<td>0.07 (-0.02 to 0.16)</td>
<td>-0.13 (-0.21 to -0.04)</td>
<td>-0.16 (-0.10 to 0.02)</td>
</tr>
</tbody>
</table>

Table 5.4b: Pearson correlation coefficients (r) and 95% CI for maternal glycemic parameters taken at 24 to 28 weeks’ gestation fasting glucose, AUC glucose, HOMA-IR and ISI<sub>OGTT</sub>, and maternal central adiposity measures taken at 11-14 weeks’ gestation- SAT, VAT and TAT depth, BMI and pre-pregnancy BMI for 485 study participants

<table>
<thead>
<tr>
<th>Adiposity Measure</th>
<th>Fasting glucose (mmol/L)</th>
<th>AUC glucose (mmol/L/hr)</th>
<th>Log HOMA-IR</th>
<th>Log ISI&lt;sub&gt;OGTT&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-pregnancy BMI (kg/m²)</td>
<td>0.28 (0.20 to 0.36)</td>
<td>0.21 (0.13 to 0.30)</td>
<td>0.47 (0.40 to 0.54)</td>
<td>-0.37 (-0.44 to -0.29)</td>
</tr>
<tr>
<td>BMI at 11-14 weeks (kg/m²)</td>
<td>0.31 (0.23 to 0.39)</td>
<td>0.22 (0.13 to 0.30)</td>
<td>0.49 (0.42 to 0.55)</td>
<td>-0.39 (-0.46 to -0.31)</td>
</tr>
<tr>
<td>SAT depth at 11-14 weeks (in cm)</td>
<td>0.20 (0.11 to 0.28)</td>
<td>0.22 (0.13 to 0.30)</td>
<td>0.41 (0.33 to 0.48)</td>
<td>-0.40 (-0.47 to -0.32)</td>
</tr>
<tr>
<td>VAT depth at 11-14 weeks (in cm)</td>
<td>0.32 (0.23 to 0.39)</td>
<td>0.31 (0.23 to 0.39)</td>
<td>0.48 (0.41 to 0.55)</td>
<td>-0.43 (-0.50 to -0.36)</td>
</tr>
<tr>
<td>TAT depth at 11-14 weeks (in cm)</td>
<td>0.33 (0.24 to 0.40)</td>
<td>0.33 (0.25 to 0.40)</td>
<td>0.54 (0.47 to 0.60)</td>
<td>-0.49 (-0.56 to -0.42)</td>
</tr>
</tbody>
</table>
5.3.3 Measures of maternal adiposity and abnormal glucose homeostasis—
AUC-glucose

In the unadjusted linear model, VAT and TAT each had a higher $r^2$ value than both pre-pregnancy and 11 to 14 weeks' gestation BMI measures, though not significantly. Upon adding other variables in the linear model, including either pre-pregnancy BMI (Model #2) or measured BMI at 11 to 14 weeks (Model #3), VAT and TAT each explained about 15% (95% CI 9 to 21) of the variance ($r^2$) in AUC-glucose (Model #3 in Figure 5.3, Table 5.5a).

5.3.4 Measures of maternal adiposity and markers of IR—HOMA-IR and ISI_OGGT

For HOMA-IR, each of the obesity measures had higher $r^2$ values than seen for AUC glucose. In the unadjusted model, TAT most explained HOMA-IR. Adjusting for BMI and other variables enhanced the $r^2$ values for all measures of adiposity depth, with TAT explaining the greatest amount of the variance in HOMA-IR (35%, 95% CI 28 to 42) (Model #3 in Figure 5.3, Table 5.5b).

VAT and TAT depth were each significantly associated with HOMA-IR—each 1 cm increase in either VAT or TAT depth was associated with a 9.7% increase in HOMA-IR (95% CI 7.2 to 14.8). SAT depth had a more pronounced association with HOMA-IR than both VAT and TAT depth—each 1 cm increase in SAT was associated with a 14.8% increase in HOMA-IR (95% CI 7.2 to 23.0).

All three domains of maternal abdominal adipose tissue were significantly more associated with HOMA-IR than early pregnancy BMI, for which each 1 cm increase was associated with a 7.2% increase in HOMA-IR (95% CI 4.7 to 7.2).
For $\text{ISI}_{\text{OGTT}}$, a similar pattern was seen as for HOMA-IR, with a slight diminution of the $r^2$ values (Model #3 in Figure 5.3, Table 5.5c).

In the model examining the association between maternal adiposity and insulin sensitivity, SAT and VAT depth described 28% (95% CI 0.21 to 0.35) of the variance in $\text{ISI}_{\text{OGTT}}$, while TAT depth explained 31% (95% CI 0.24 to 0.38) of the variance of $\text{ISI}_{\text{OGTT}}$ (Table 5.5c, adjusted model #3). The variance described by SAT, VAT and TAT depth were each slightly higher than the 15% variance (95% CI 0.09 to 0.21) described by early pregnancy BMI.

Each 1 cm increase in VAT resulted in a 10.9% decrease in $\text{ISI}_{\text{OGTT}}$ (95% CI 6.7 to 14.9) (Table 5.5c, Adjusted model #3). TAT followed a similar trend, where every 1 cm increase was also associated with a 10.9% decrease in $\text{ISI}_{\text{OGTT}}$ (95% CI 8.8 to 14.9). SAT depth had the highest association with $\text{ISI}_{\text{OGTT}}$; each 1 cm increase in SAT depth was associated with an 18.7% decrease in $\text{ISI}_{\text{OGTT}}$ (95% CI 10.9 to 25.9). SAT, VAT and TAT were all significantly more associated with $\text{ISI}_{\text{OGTT}}$ than early pregnancy BMI, for which each 1 cm increase was associated with a corresponding 4.5% increase in $\text{ISI}_{\text{OGTT}}$ (95% CI 4.5 to 6.7).

Overall, the variance explained by VAT depth for each of the adjusted models for markers of dysglycemia, AUC-glucose, HOMA-IR and $\text{ISI}_{\text{OGTT}}$, were slightly higher than the variance explained by the similarly adjusted models for pre-pregnancy or early pregnancy BMI. The $r^2$ in the adjusted models examining the relation between TAT depth with HOMA-IR or $\text{ISI}_{\text{OGTT}}$ were each slightly higher than the adjusted models examining the association between BMI, SAT and VAT depth with HOMA-IR and $\text{ISI}_{\text{OGTT}}$. 
Table 5.5a: Association between pre-pregnancy BMI, as well as BMI, abdominal SAT, VAT and TAT depth—each measured at 11-14 weeks’ gestation—and AUC glucose measured at 24-28 weeks’ gestation among 485 pregnant women

<table>
<thead>
<tr>
<th>Variable</th>
<th>AUC glucose (mmol/L/hr)</th>
<th>Unadjusted model</th>
<th>Model #1*</th>
<th>Model #2†</th>
<th>Model #3‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beta coefficient (95% CI) for AUC glucose</td>
<td>r² (95% CI)</td>
<td>Beta coefficient (95% CI) for AUC glucose</td>
<td>r² (95% CI)</td>
<td>Beta coefficient (95% CI) for AUC glucose</td>
</tr>
<tr>
<td>Pre-pregnancy BMI (kg/m²)</td>
<td><strong>0.11</strong> (0.07 to 0.15)</td>
<td><strong>0.05</strong> (0.01 to 0.09)</td>
<td><strong>0.11</strong> (0.05 to 0.15)</td>
<td><strong>0.11</strong> (0.06 to 0.16)</td>
<td><strong>--</strong></td>
</tr>
<tr>
<td>BMI at 11-14 weeks (kg/m²)</td>
<td><strong>0.11</strong> (0.07 to 0.16)</td>
<td><strong>0.05</strong> (0.01 to 0.09)</td>
<td><strong>0.11</strong> (0.06 to 0.15)</td>
<td><strong>0.12</strong> (0.07 to 0.17)</td>
<td><strong>--</strong></td>
</tr>
<tr>
<td>SAT depth at 11-14 weeks (cm)</td>
<td><strong>0.70</strong> (0.42 to 0.98)</td>
<td><strong>0.05</strong> (0.01 to 0.09)</td>
<td><strong>0.61</strong> (0.33 to 0.88)</td>
<td><strong>0.11</strong> (0.06 to 0.16)</td>
<td><strong>0.41</strong> (0.09 to 0.73)</td>
</tr>
<tr>
<td>VAT depth at 11-14 weeks (cm)</td>
<td><strong>0.48</strong> (0.35 to 0.61)</td>
<td><strong>0.10</strong> (0.05 to 0.15)</td>
<td><strong>0.43</strong> (0.29 to 0.57)</td>
<td><strong>0.14</strong> (0.08 to 0.20)</td>
<td><strong>0.38</strong> (0.22 to 0.55)</td>
</tr>
<tr>
<td>TAT depth at 11-14 weeks (cm)</td>
<td><strong>0.40</strong> (0.30 to 0.50)</td>
<td><strong>0.11</strong> (0.06 to 0.16)</td>
<td><strong>0.35</strong> (0.25 to 0.46)</td>
<td><strong>0.15</strong> (0.09 to 0.21)</td>
<td><strong>0.34</strong> (0.21 to 0.48)</td>
</tr>
</tbody>
</table>

* Linear regression analysis, maternal age, parity, ethnicity, family history of DM and change in BMI from 11-14 to 24-28 weeks.
† Linear regression analysis, maternal age, parity, ethnicity, family history of DM, change in BMI from 11-14 to 24-28 weeks and pre-pregnancy BMI.
‡ Linear regression analysis, maternal age, parity, ethnicity, family history of DM, change in BMI from 11-14 to 24-28 weeks and BMI at 11-14 weeks.
Table 5.5b: Association between BMI, abdominal SAT, VAT and TAT depth, all measured at 11-14 weeks’ gestation, and percent increase in HOMA-IR measured at 24-28 weeks’ gestation among 485 pregnant women

<table>
<thead>
<tr>
<th>Variable</th>
<th>Percent increase (95% CI) in HOMA-IR</th>
<th>r² (95% CI)</th>
<th>Percent increase (95% CI) in HOMA-IR</th>
<th>r² (95% CI)</th>
<th>Percent increase (95% CI) in HOMA-IR</th>
<th>r² (95% CI)</th>
<th>Percent increase (95% CI) in HOMA-IR</th>
<th>r² (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-pregnancy BMI (kg/m²)</td>
<td>7.2 (4.7 to 7.2)</td>
<td>0.23 (0.16 to 0.3)</td>
<td>7.2 (4.7 to 7.2)</td>
<td>0.27 (0.20 to 0.34)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>BMI at 11-14 weeks (kg/m²)</td>
<td>7.20 (4.7 to 7.2)</td>
<td>0.24 (0.17 to 0.31)</td>
<td>7.2 (4.7 to 7.2)</td>
<td>0.30 (0.23 to 0.37)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>SAT depth at 11-14 weeks (cm)</td>
<td>38.0 (28.8 to 44.5)</td>
<td>0.17 (0.11 to 0.23)</td>
<td>34.9 (25.9 to 44.5)</td>
<td>0.22 (0.16 to 0.29)</td>
<td>17.5 (9.7 to 25.9)</td>
<td>0.30 (0.23 to 0.37)</td>
<td>14.8 (7.2 to 23.0)</td>
<td>0.32 (0.25 to 0.39)</td>
</tr>
<tr>
<td>VAT depth at 11-14 weeks (cm)</td>
<td>20.2 (14.8 to 23.0)</td>
<td>0.23 (0.16 to 0.30)</td>
<td>20.2 (14.8 to 23.0)</td>
<td>0.27 (0.20 to 0.34)</td>
<td>12.2 (7.2 to 14.8)</td>
<td>0.33 (0.26 to 0.40)</td>
<td>9.7 (7.2 to 14.8)</td>
<td>0.34 (0.27 to 0.41)</td>
</tr>
<tr>
<td>TAT depth at 11-14 weeks (cm)</td>
<td>17.5 (14.8 to 20.2)</td>
<td>0.29 (0.22 to 0.36)</td>
<td>17.5 (14.8 to 20.2)</td>
<td>0.32 (0.25 to 0.39)</td>
<td>12.2 (7.2 to 14.8)</td>
<td>0.35 (0.28 to 0.42)</td>
<td>9.7 (7.2 to 14.8)</td>
<td>0.35 (0.28 to 0.42)</td>
</tr>
</tbody>
</table>

*Linear regression analysis, maternal age, parity, ethnicity, family history of DM and change in BMI from 11-14 to 24-28 weeks.
†Linear regression analysis, maternal age, parity, ethnicity, family history of DM, change in BMI from 11-14 to 24-28 weeks and pre-pregnancy BMI.
‡Linear regression analysis, maternal age, parity, ethnicity, family history of DM, change in BMI from 11-14 to 24-28 weeks and BMI at 11-14 weeks.
Table 5.5c: Association between BMI, abdominal SATVAT and TAT depth, all measured at 11-14 weeks’ gestation, and percent decrease in ISI\textsubscript{OGTT} measured at 24-28 weeks’ gestation among 485 pregnant women

<table>
<thead>
<tr>
<th>Variable</th>
<th>Percent decrease (95% CI) in ISI\textsubscript{OGTT}</th>
<th>r\textsuperscript{2} (95% CI)</th>
<th>Percent decrease (95% CI) ISI\textsubscript{OGTT}</th>
<th>r\textsuperscript{2} (95% CI)</th>
<th>Percent decrease (95% CI) ISI\textsubscript{OGTT}</th>
<th>r\textsuperscript{2} (95% CI)</th>
<th>Percent decrease (95% CI) ISI\textsubscript{OGTT}</th>
<th>r\textsuperscript{2} (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-pregnancy BMI (kg/m\textsuperscript{2})</td>
<td>4.5 (4.5 to 6.7)</td>
<td>0.14 (0.08 to 0.20)</td>
<td>4.5 (4.5 to 6.7)</td>
<td>0.23 (0.17 to 0.30)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>BMI at 11-14 weeks (kg/m\textsuperscript{2})</td>
<td>4.5 (4.5 to 6.7)</td>
<td>0.15 (0.09 to 0.21)</td>
<td>4.5 (4.5 to 6.7)</td>
<td>0.25 (0.18 to 0.32)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>SAT depth at 11-14 weeks (in cm)</td>
<td>29.2 (24.1 to 39.9)</td>
<td>0.16 (0.10 to 0.22)</td>
<td>27.6 (22.4 to 32.4)</td>
<td>0.24 (0.17 to 0.31)</td>
<td>20.6 (12.9 to 25.9)</td>
<td>0.28 (0.21 to 0.35)</td>
<td>18.7 (10.9 to 25.9)</td>
<td>0.28 (0.21 to 0.35)</td>
</tr>
<tr>
<td>VAT depth at 11-14 weeks (in cm)</td>
<td>16.8 (14.9 to 20.6)</td>
<td>0.19 (0.13 to 0.25)</td>
<td>14.9 (12.9 to 18.7)</td>
<td>0.25 (0.18 to 0.32)</td>
<td>10.9 (6.7 to 14.9)</td>
<td>0.28 (0.21 to 0.35)</td>
<td>10.9 (6.7 to 14.9)</td>
<td>0.28 (0.21 to 0.35)</td>
</tr>
<tr>
<td>TAT depth at 11-14 weeks (in cm)</td>
<td>16.8 (12.9 to 16.8)</td>
<td>0.24 (0.17 to 0.31)</td>
<td>14.9 (10.9 to 16.8)</td>
<td>0.30 (0.23 to 0.37)</td>
<td>10.9 (8.8 to 14.9)</td>
<td>0.31 (0.24 to 0.38)</td>
<td>10.9 (8.8 to 14.9)</td>
<td>0.31 (0.24 to 0.38)</td>
</tr>
</tbody>
</table>

\* Linear regression analysis, maternal age, parity, ethnicity, family history of DM and change in BMI from 11-14 to 24-28 weeks.

\† Linear regression analysis, maternal age, parity, ethnicity, family history of DM, change in BMI from 11-14 to 24-28 weeks and pre-pregnancy BMI.

\‡ Linear regression analysis, maternal age, parity, ethnicity, family history of DM, change in BMI from 11-14 to 24-28 weeks and BMI at 11-14 weeks.
5.3.5 Maternal abdominal adiposity and composite outcome of IFG or GIGT or GDM

A total of 52 out of 485 women developed the composite of IFG, GIGT or GDM, a rate of 10.7% (95% CI 8.1 to 13.8), of which 45 (9.3%, 95% CI 7.0 to 12.2) met the traditional criteria for GDM (CDA 2008). Relative to the bottom quartile, the highest quartile of SAT was significantly associated with a higher risk for the composite outcome (unadjusted OR 3.4, 95% CI 1.5 to 8.3), but not after adjusting for the covariates (aOR 1.8, 95% CI 0.70 to 4.8) (Table 5.6). The highest quartile of VAT depth (aOR 3.1, 95% CI 1.1 to 9.5) and TAT depth (aOR 2.7, 95% CI 1.1 to 7.8) were each associated with the composite outcome (Table 5.6).

In the post hoc sensitivity analysis, limiting the outcome to GDM using traditional criteria (CDA 2008; IADPSG, 2010), there was a slightly more pronounced effect seen for the highest quartiles of VAT (aOR 4.2, 95% CI 1.4 to 14.2) and TAT (aOR 3.0, 95% CI 1.1 to 8.9) (Table 5.7). Using the IADPSG criteria for GDM (IADPSG, 2010), the association remained significant, albeit less pronounced (Table 5.8).
Table 5.6: Association between abdominal SAT, VAT and TAT depth, all measured at 11-14 weeks’ gestation, and the risk of the composite outcome of either IFG, GIGT or GDM at 24-28 weeks’ gestation among 485 pregnant women

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Presence of composite outcome of IFG, GIGT or GDM at 24-28 week OGTT OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%) with the outcome</td>
</tr>
<tr>
<td>Subcutaneous adipose tissue depth</td>
<td></td>
</tr>
<tr>
<td>Quartile 1 (n = 124), ≥1.3 cm</td>
<td>8 (1.7)</td>
</tr>
<tr>
<td>Quartile 2 (n = 118), 1.4-1.7 cm</td>
<td>9 (1.9)</td>
</tr>
<tr>
<td>Quartile 3 (n = 121), 1.8-2.3 cm</td>
<td>12 (2.5)</td>
</tr>
<tr>
<td>Quartile 4 (n = 122) &gt;2.3 cm</td>
<td>23 (4.7)</td>
</tr>
<tr>
<td>Visceral adipose tissue depth</td>
<td></td>
</tr>
<tr>
<td>Quartile 1 (n = 120), ≥3.0 cm</td>
<td>6 (1.2)</td>
</tr>
<tr>
<td>Quartile 2 (n = 120), 3.1-3.8 cm</td>
<td>9 (1.9)</td>
</tr>
<tr>
<td>Quartile 3 (n = 120), 3.9-4.8 cm</td>
<td>7 (1.4)</td>
</tr>
<tr>
<td>Quartile 4 (n = 125) &gt;4.8 cm</td>
<td>30 (6.2)</td>
</tr>
<tr>
<td>Total adipose tissue depth</td>
<td></td>
</tr>
<tr>
<td>Quartile 1 (n = 121), ≥4.5 cm</td>
<td>8 (1.7)</td>
</tr>
<tr>
<td>Quartile 2 (n = 120), 4.6-5.5 cm</td>
<td>4 (0.82)</td>
</tr>
<tr>
<td>Quartile 3 (n = 120), 6.6-7.0 cm</td>
<td>10 (2.1)</td>
</tr>
<tr>
<td>Quartile 4 (n = 124) &gt;7.0 cm</td>
<td>30 (16.2)</td>
</tr>
</tbody>
</table>

*Adjusted for maternal age, ethnicity, family history of T2DM, change in BMI from 11-14 weeks’ to 24-28 weeks’ gestation, BMI at 11-14 weeks’ gestation
Table 5.7: *Post hoc* sensitivity analysis of the association between abdominal SAT, VAT or TAT depth, each measured at 11-14 weeks’ gestation, and the subsequent risk of GDM at 24-28 weeks’ gestation, using the traditional criteria for GDM (CDA, 2008).

<table>
<thead>
<tr>
<th>Exposure</th>
<th>GDM at 24-28 weeks gestation</th>
<th>OR (95% CI) for the outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%) with the outcome</td>
<td>Unadjusted</td>
</tr>
<tr>
<td>Subcutaneous adipose tissue depth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartile 1 (n = 124): ≤1.3 cm</td>
<td>7 (5.7)</td>
<td>1.00 (Referent)</td>
</tr>
<tr>
<td>Quartile 2 (n = 118): 1.4-1.7 cm</td>
<td>9 (7.6)</td>
<td>1.4 (0.50 to 4.0)</td>
</tr>
<tr>
<td>Quartile 3 (n = 121): 1.8-2.3 cm</td>
<td>12 (9.9)</td>
<td>1.8 (0.71 to 5.1)</td>
</tr>
<tr>
<td>Quartile 4 (n = 122): &gt; 2.3 cm</td>
<td>17 (14.0)</td>
<td>2.7 (1.1 to 7.2)</td>
</tr>
<tr>
<td>Visceral adipose tissue depth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartile 1 (n = 120): ≤3.0 cm</td>
<td>5 (4.2)</td>
<td>1.00 (Referent)</td>
</tr>
<tr>
<td>Quartile 2 (n = 122): 3.1-3.8 cm</td>
<td>8 (6.6)</td>
<td>1.6 (0.52 to 5.5)</td>
</tr>
<tr>
<td>Quartile 3 (n = 121): 3.9-4.8 cm</td>
<td>6 (5.0)</td>
<td>1.2 (0.35 to 4.3)</td>
</tr>
<tr>
<td>Quartile 4 (n = 122): &gt; 4.8 cm</td>
<td>26 (21.3)</td>
<td>6.2 (2.5 to 19.0)</td>
</tr>
<tr>
<td>Total adipose tissue depth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartile 1 (n = 121): ≤4.5 cm</td>
<td>7 (5.8)</td>
<td>1.00 (Referent)</td>
</tr>
<tr>
<td>Quartile 2 (n = 122): 4.6-5.5 cm</td>
<td>4 (3.3)</td>
<td>1.0 (0.14 to 1.9)</td>
</tr>
<tr>
<td>Quartile 3 (n = 120): 5.6-7.0 cm</td>
<td>9 (7.5)</td>
<td>1.3 (0.48 to 3.8)</td>
</tr>
<tr>
<td>Quartile 4 (n = 122): &gt; 7.0 cm</td>
<td>25 (20.5)</td>
<td>4.2 (1.8 to 10.9)</td>
</tr>
</tbody>
</table>

*Adjusted for maternal age, ethnicity, family history of T2DM, change in BMI from 11-14 weeks’ to 24-28 weeks’ gestation, and BMI at 11-14 weeks’ gestation*
Table 5.8: *Post hoc* sensitivity analysis of the association between abdominal SAT, VAT or TAT depth, each measured at 11-14 weeks’ gestation, and the subsequent risk of GDM at 24-28 weeks’ gestation, using the International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria for GDM (IADPSG, 2010).

<table>
<thead>
<tr>
<th>Exposure</th>
<th>GDM at 24-28 weeks’ gestation</th>
<th>OR (95% CI) for the outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%) with the outcome</td>
<td>Unadjusted</td>
</tr>
<tr>
<td>Subcutaneous adipose tissue depth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartile 1 (n = 124): ≤ 1.3 cm</td>
<td>14 (11.3)</td>
<td>1.00 (Referent)</td>
</tr>
<tr>
<td>Quartile 2 (n = 118): 1.4-1.7 cm</td>
<td>17 (14.4)</td>
<td>1.3 (0.62 to 2.9)</td>
</tr>
<tr>
<td>Quartile 3 (n = 121): 1.8-2.3 cm</td>
<td>22 (18.2)</td>
<td>1.8 (0.86 to 3.7)</td>
</tr>
<tr>
<td>Quartile 4 (n = 122): &gt; 2.3 cm</td>
<td>32 (26.2)</td>
<td><strong>2.8 (1.4 to 5.7)</strong></td>
</tr>
<tr>
<td>Visceral adipose tissue depth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartile 1 (n = 120): ≤ 3.0 cm</td>
<td>12 (10.0)</td>
<td>1.00 (Referent)</td>
</tr>
<tr>
<td>Quartile 2 (n = 122): 3.1-3.8 cm</td>
<td>18 (14.8)</td>
<td>1.6 (0.72 to 3.5)</td>
</tr>
<tr>
<td>Quartile 3 (n = 121): 3.9-4.8 cm</td>
<td>16 (13.2)</td>
<td>1.4 (0.62 to 3.1)</td>
</tr>
<tr>
<td>Quartile 4 (n = 122): &gt; 4.8 cm</td>
<td>39 (32.0)</td>
<td><strong>4.2 (2.1 to 8.9)</strong></td>
</tr>
<tr>
<td>Total adipose tissue depth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartile 1 (n = 121): ≤ 4.5 cm</td>
<td>15 (12.4)</td>
<td>1.00 (Referent)</td>
</tr>
<tr>
<td>Quartile 2 (n = 122): 4.6-5.5 cm</td>
<td>10 (8.2)</td>
<td>1.0 (0.30 to 1.5)</td>
</tr>
<tr>
<td>Quartile 3 (n = 120): 5.6-7.0 cm</td>
<td>19 (15.8)</td>
<td>1.3 (0.64 to 2.8)</td>
</tr>
<tr>
<td>Quartile 4 (n = 122): &gt; 7.0 cm</td>
<td>41 (33.6)</td>
<td><strong>3.6 (1.9 to 7.1)</strong></td>
</tr>
</tbody>
</table>

*Adjusted for maternal age, ethnicity, family history of T2DM, and BMI at 11-14 weeks’ gestation
Figure 5.3: Forest plot comparing variance (adjusted $r^2$, 95% CI) for different measures of maternal obesity in early pregnancy and their association with glucose intolerance and IR. Obesity measures included pre-pregnancy BMI, BMI at 11-14 weeks’ gestation, as well as SAT, VAT and TAT depth at 11-14 weeks’ gestation. Glucose tolerance was determined by AUC glucose and IR was determined at 24-28 weeks’ gestation using the HOMA-IR or the ISI after a 2-hour 75-gram oral glucose tolerance test. Measures of BMI were adjusted for maternal age, parity and ethnicity, change in BMI from 11-14 weeks to 24-28 weeks and family history of T2DM in a first degree relative. Measures of AT depth were adjusted for the same covariates with the addition of BMI at 11-14 weeks’ gestation.
5.4 Discussion

Elevated first-trimester measures of maternal abdominal adiposity—specifically the upper quartile of VAT and TAT—were associated with dysglycemia and GDM in mid-pregnancy. The associations were independent of maternal age, parity, ethnicity, family, history of T2DM, and change in BMI within pregnancy. The variation between abnormal glucose homeostasis and IR was slightly improved in models that included VAT or TAT depth rather than BMI alone.

A strength of the current study was its large sample size, derived from a multi-ethnic population, and use of a standardized ultrasound protocol that measures abdominal adiposity, coinciding with the time at which prenatal measurement of fetal nuchal translucency is performed. Ultrasound abdominal adipose tissue measurement is relatively simple to learn, cost-effective and could be applied in clinical practice, if further validated. Such validation can include the application of formal diagnostic test measurements, such as likelihood ratios and positive and negative predictive values. As a limitation, 16 out of 501 women did not have complete capture of all study variables, including the requisite 75 g OGTT.

Meta-analyses of non-pregnant individuals confirms that the metabolic syndrome increases the relative risk of T2DM by three to five times (Despres & Lemieux, 2006). Pregnancy has been described as a stress test, unmasking a latent tendency for the metabolic syndrome (Després et al., 2006; Alberti et al., 2009). In addition, 20-50% of women with GDM will go on to develop T2DM within 5 years (Kim et al., 2002), and many experience the metabolic syndrome postpartum (Retnakaran et al., 2010).
In non-pregnant populations, visceral adiposity has emerged as an independent predictor of IR, the metabolic syndrome, T2DM and CVD (Després, 1993; Pascot et al., 2001). Proposed mechanisms underlying the pathological association between VAT and IR include free fatty acid release into the hepatic portal circulation (Björntorp, 1991; Arner, 1999), adipokine imbalance, and pro-inflammatory cytokine release from the VAT depot (Armellini et al., 1990; Retnakaran et al., 2005).

A few small studies have examined the association between ultrasound-measured VAT and glucose homeostasis in pregnancy (Bartha et al., 2007; Martin et al., 2009; Cisneiros et al., 2013; De Souza et al., 2014; Gur et al., 2014). We previously completed a small pilot study showing that higher VAT depth, but not SAT depth, in early pregnancy was associated with a positive glucose challenge test later in pregnancy (Martin et al., 2009). We then pursued a second study and found that first-trimester VAT and TAT depth each explained 42% and 46% of the variance in IR at 16 weeks’ gestation along with covariates (De Souza et al., 2014). What was lacking was a large study sample, use of a standardized OGTT and measures of IR at 24 to 28 weeks’ gestation, at the conventional time when one screens for GDM.

Herein, we demonstrated that TAT, and especially VAT, are important pathogenic markers of abnormal glucose homeostasis and IR in pregnancy. SAT alone was less important. The SAT compartment is comprised of a metabolically and morphologically heterogeneous histology, with two distinct layers, deep and superficial (Kelley et al., 1996). The metabolic action of the deep SAT layer is thought to behave synonymously with VAT (Carey et al., 2000; Misra et al., 2004).
Studies have highlighted considerable variability in the extent to which SAT and VAT each contributes to IR (Garg, 1997). The metabolic activity within the deep SAT layer may explain the slightly more pronounced association between TAT depth and measures of IR—something worthy of examination in a future study that has the capacity to distinguish between deep and superficial SAT layers.

As a surrogate measure of IR, HOMA-IR reflects hepatic glucose output and IR, while ISI\textsubscript{OGTT} describes whole body insulin sensitivity in both hepatic and peripheral tissue (Fulghesu et al., 2006; Radaelli et al., 2010). In a previous study, we found that ISI\textsubscript{OGTT}, but not HOMA-IR, was associated with VAT and TAT depth in the first trimester. Herein, we observed an association between first-trimester VAT and TAT and both ISI\textsubscript{OGTT} and HOMA-IR measured in the second trimester. The discrepancy between studies may be explained by the observation that women often experience some degree of improved insulin sensitivity in early pregnancy and, accordingly, a relatively low HOMA-IR in that period (Fulghesu et al., 2006). Endogenous IR rises into the late second and early third trimester of pregnancy (Phelps et al., 1981). Given that the state of IR in GDM and T2DM includes loss of both hepatic and peripheral insulin sensitivity, HOMA-IR and ISI\textsubscript{OGTT} collectively provide a reasonable estimate of both hepatic and total body insulin sensitivity (Fulghesu et al., 2006; Radaelli et al., 2010). Using a direct sonographic estimate of maternal abdominal AT compartments, it seems that VAT offers a reflection of IR in both early (De Souza et al., 2014) and mid-pregnancy, further manifesting as GDM.
Ultrasound measurement of maternal abdominal adiposity affords the opportunity to overcome some of the limitations posed by using either BMI or other conventional metabolic syndrome characteristics, which may be obscured by pregnancy. Our proposed screening approach exploits a practical and validated ultrasound-based screening tool to quantify SAT, VAT and TAT depth at the time of routine fetal nuchal translucency assessment (Martin et al., 2009; De Souza et al., 2014). Such a screening tool, used in early pregnancy, might mitigate the onset of GDM by allowing the identification and management of upstream risk factors for GDM. This approach should be evaluated in comparison to the current “downstream” approach of screening and treating GDM in the second trimester of pregnancy.
Chapter 6  First-trimester maternal NAFLD and dysglycemia and GDM in mid-gestation

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Segue

In the previous chapters we demonstrated that early first-trimester sonographic screening for maternal abdominal adiposity can be used to detect the development of impaired dysglycemia, IR and GDM in the late second trimester. We also supported our previous work that this method done at the time of routine ultrasound screening for nuchal translucency is a viable approach to quantifying early pregnancy presence of maternal abdominal AT.

NAFLD is the liver’s manifestation of IR and the metabolic syndrome. NAFLD is associated with obesity and is chronic in nature, remaining asymptomatic in most populations. Over 60% of women in North America are overweight and many have features of the metabolic syndrome prior to conception. NAFLD is associated with previous GDM and T2DM.

Evidence shows that NAFLD can be safely and reliably measured using ultrasound. The following chapter is the first study to examine NAFLD in early pregnancy in a large prospective cohort. We examined whether maternal fatty liver, measured by ultrasound in the first trimester of pregnancy, during routine screening for fetal nuchal translucency, is associated with a “pre-diabetes” state as indicated by IFG and GIGT as well as the frank manifestation of GDM in the second trimester.

In this chapter we explore whether our first-trimester, upstream ultrasound methodological approach for measuring maternal abdominal adiposity at the time of routine first-trimester prenatal ultrasound, can also be used to identify sonographic evidence of NAFLD and whether presence of first-trimester maternal fatty liver can describe an upstream dysmetabolic risk as a possible prevention approach to GDM management.
6.0 Abstract

Objective: NAFLD is the most common liver disease worldwide. As a hallmark of IR, the subclinical manifestation of NAFLD in early gestation may contribute to the development of GDM later in pregnancy. First-trimester sonographic evidence of maternal fatty liver and its association with dysglycemia and GDM in mid-pregnancy were assessed.

Research Design and Methods: In a prospective cohort study of 476 pregnant women, the qualitative presence of hepatic fat was assessed by ultrasonography at 11-14 weeks’ gestation, during routine sonographic screening for fetal nuchal translucency. Ultrasounds were then scored by independent sonographers according to the presence or absence (score=0) of hepatorenal contrast and / or impaired visualization of the intrahepatic vessels (score = 1 or 2). We used logistic regression analysis to assess the relation between the semi-quantitative presence of first-trimester hepatic fat and the composite outcome of IFG, GIGT or GDM, based on the 75-g OGTT at 24-28 weeks’ gestation. Fasting serum ALT was measured during the OGTT.

Results: After adjusting for maternal age, ethnicity, family history of T2DM, early pregnancy BMI and change in BMI between 11-14 weeks’ and 24-28 weeks’ gestation, women with a score of 1 (adjusted OR 2.0, 95% CI 1.0 to 4.1) or 2 (aOR 2.9, 95% CI 1.0 to 18.4) features of NAFLD had a higher odds of developing IFG, GIGT or GDM in mid-pregnancy compared with those who had no evidence of fatty liver in early pregnancy. When analyzed for one or more features of NAFLD, the odds remained significantly higher (aOR 2.2, 95% CI 1.1 to 4.3) than for those women without any evidence of fatty liver. ALT was not associated with the composite outcome.
Conclusions: First-trimester sonographic evidence of maternal hepatic fat accumulation may reveal sub-clinical NAFLD and an increased upstream risk for the development of impaired glucose homeostasis and GDM later in pregnancy.
6.1 Introduction

Maternal obesity has pathogenic consequences, including IR, GDM and T2DM (Kim et al., 2002; Solomon et al., 1997). IR and the metabolic syndrome are antecedents in the natural history of DM, yet they are not routinely measured in obstetric clinical settings (Retnakaran et al., 2008; CDA, 2013). Novel markers of metabolic dysfunction and possible estimates of IR are emerging as maternal risk factors for GDM and subsequent T2DM. Both visceral adiposity and NAFLD have a shared pathogenesis and are established determinants of DM and CVD (Kantartzis et al., 2010).

Our research group has demonstrated that sonographic measurement of first-trimester maternal abdominal adiposity contributes to early and mid-pregnancy IR, and dysglycemia (Martin et al., 2009; De Souza et al., 2014; De Souza et al., 2015).

NAFLD is the most common liver disease worldwide (Targher et al., 2007) and is the principal liver manifestation of the metabolic syndrome, since it requires the presence of IR (Kalhan et al., 2008). The histological spectrum of NAFLD ranges from simple steatosis (fatty infiltration) to inflammatory steatohepatitis (NASH) and long-term liver injury (Targher et al., 2007; Wahren et al., 2007; Kalhan et al., 2008). Physiological transfer of free fatty acids and pro-inflammatory cytokines from the abdominal visceral adipose compartment to the liver via portal venous circulation, as well as from systemic circulation via the digestive tract, are considered predominant sources of ectopic fat accumulation in the liver, and consequent NAFLD (Wahren et al., 2007; Kalhan et al., 2008).
Various epidemiological studies of non-pregnant populations have corroborated evidence of NAFLD in the pathogenesis of metabolic disease. Among obese adolescents, IR and glucose dysregulation increases in parallel to hepatic fat content (Cali et al., 2009). In our study of adults with newly diagnosed diabetes we observed a significant relation between T2DM and the risk of serious liver injury, the latter likely explained by NAFLD; moreover, the degree of liver injury was worse in those with T2DM combined with other features of the metabolic syndrome (Porepa et al., 2010). Among young non-pregnant women with previous GDM who underwent MRI of the liver, those with high hepatic fat had elevated fasting serum triglyceride, and insulin concentrations, and lower whole-body insulin sensitivity than those with low hepatic fat (Tiikkainen et al., 2002).

Ultrasonography of the liver is universally used as a qualitative screening assessment for the presence of hepatic fat in asymptomatic patients and remains the reference standard when liver biopsy or radiation-based imaging are not feasible, as in pregnancy (Harrison et al., 2008; Ix et al., 2008; Stefan et al., 2008).

We investigated, for the first time, the relation between first-trimester sonographic semi-quantitative presence of maternal hepatic fat and its association with IR and GDM in mid-gestation. We hypothesized that women with sonographic evidence of hepatic fat accumulation in early pregnancy would exhibit dysglycemia and GDM in the second trimester, and that this association would persist even after adjusting for BMI.
6.2 Research Design and Methods

We conducted a prospective cohort study at a general obstetrics outpatient clinic at St. Michael’s Hospital in Toronto, Ontario. The study was approved by the Research Ethics Board of St. Michael’s Hospital, and participants provided written informed consent.

Healthy women aged 18 years and older were eligible for study entry if they had a viable singleton pregnancy at 11-14 weeks’ gestation. We excluded those who had known pre-pregnancy T2DM, a prior pregnancy affected by GDM or any chronic or pregnancy-specific disorder that might affect liver function.

Maternal hepatic fat was assessed in two stages. In the first stage, at 11-14 weeks' gestation, hepatic adiposity was distinguished and measured by four trained ultrasound technicians during routine ultrasound assessment for fetal nuchal translucency. Qualitative identification of fatty liver was based on the presence of i) diffusely increased echogenic (“bright”) liver, in which hepatic echogenicity is greater than the right kidney (“hepatorenal echo contrast”), and ii) impaired visualization of the portal and hepatic veins, in which intrahepatic vessels appear blurred (Chang et al., 2007). This sonographic approach has within-observer reliability of 0.95 and between-observer reliability of 0.95 (Chang et al., 2007). Measurements were obtained using a Phillips IU22 ultrasound machine with either a 5-2 MHz or 9 MHz probe (Martin et al., 2009) (Table 1, Figure 1a, 1b).
In the second stage of assessment, two independent sonographers reviewed the hard copies of each ultrasound image for all participants and used a semi-quantitative scoring method to assign a score of 0 for no evidence of hepatic fat and 1 or 2 for either one or both qualitative indicators of hepatic fat—hepatorenal echo contrast and impaired visualization of the intrahepatic vessels. These sonographic scoring criteria have a sensitivity for detecting NAFLD ranging from 60-95% and a specificity ranging from 84-100%; typically the specificity is higher than 90% (Hamaguchi et al., 2007) (Tables 1 and 2).

A brief questionnaire was completed by each participant immediately following the 11-14-week ultrasound. Therein, we collected information about maternal age, ethnicity (Caucasian, Black, South Asian, East Asian or Other), self-reported pre-gestational height and weight, and family history of T2DM in a first-degree relative. Current weight was measured in person using a calibrated scale during the routine ultrasound appointment.

At 24-28 weeks’ gestation, at the time of routine blood sampling for integrated prenatal screening, all study participants completed a 2-hour 75g OGTT following an overnight fast as per study protocol. In addition to serum glucose, we measured the fasting, 1-hour and 2-hour serum insulin concentration, and fasting serum ALT to test liver function.
6.2.1 Data analysis

A multiple logistic regression model was used to assess the association between a fatty liver score of 0, 1 or 2 NAFLD features and the development of the composite outcome of either IFG, GIGT or GDM, based on the same 75-g OGTT at 24-28 weeks’ gestation. IFG was defined as a fasting glucose ≥ 5.3 mmol/L, in isolation; GIGT was based on a glucose value at 1h ≥ 10.6 mmol/L or at 2h ≥ 8.9 mmol/L, in isolation; and GDM was defined as ≥ 2 abnormal serum glucose values (i.e. fasting ≥ 5.3 mmol/L, 1h ≥ 10.6 mmol/L and/or 2h ≥ 8.9 mmol/L) (CDA, 2008).

ORs were adjusted (aOR) for maternal age (continuous in years), ethnicity (Caucasian versus Non-Caucasian), family history of T2DM, change in BMI from 11-14 weeks’ to 24-28 weeks’ gestation, and measured BMI at 11-14 weeks’ gestation (continuous in kg/m²).

A similar adjusted multiple logistic regression model assessed the association between quartiles, quantiles and sextiles of ALT and the composite outcome of IFG, GIGT and GDM. All variables were included a priori.

Odds ratios were reported with associated 95% confidence intervals. Statistical analyses were performed using SAS (version 9.1.3; SAS Institute, Cary, NC).
<table>
<thead>
<tr>
<th>Name of measure</th>
<th>Position of the Patient</th>
<th>Description of the measure</th>
<th>Result</th>
<th>Score for the result</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hepato-renal contrast</strong></td>
<td>Left lateral to oblique (turned leftward, to access the liver—use wedges if needed)</td>
<td>Ultrasonographic contrast right renal cortex and the adjacent hepatic parenchyma as visualized in the right intercostal space in the mid-axillary line.</td>
<td>Absent</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Position to enable the scan to penetrate the posterior aspect of the upper pole in the mid-axillary sagittal plane of the kidney</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Present</td>
<td>1</td>
</tr>
<tr>
<td><strong>Impaired visualization of the portal &amp; hepatic veins</strong></td>
<td>Position to enable the scan to penetrate the posterior aspect of the upper pole in the mid-axillary sagittal plane of the kidney</td>
<td>Blurring of the borders of the portal &amp; hepatic veins and narrowing of their lumen</td>
<td>No vessel blurring</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Borders of the portal &amp; hepatic veins are unclear and the lumen of those vessels is narrowed</td>
<td>1</td>
</tr>
</tbody>
</table>
Table 6.2: Ultrasound method to capturing maternal fatty liver during ultrasound

<table>
<thead>
<tr>
<th>Name of measure</th>
<th>Position of the Patient</th>
<th>Description of the measure**</th>
<th>Result</th>
<th>Result Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepato-renal contrast</td>
<td>Left Lateral Oblique (LPO)*</td>
<td>Compare ultrasonographic right renal cortex with the adjacent hepatic parenchyma</td>
<td>Liver to kidney: Equal echogenicity</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Liver to kidney: Liver greater echogenicity</td>
<td>1</td>
</tr>
<tr>
<td>Impaired visualization of the portal &amp; hepatic veins</td>
<td>Left Lateral Oblique (LPO)*</td>
<td>Blurring of the borders of the portal &amp; hepatic veins and narrowing of their lumen</td>
<td>No vessel blurring</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Borders of the portal &amp; hepatic veins are unclear and the lumen of those vessels is narrowed</td>
<td>1</td>
</tr>
</tbody>
</table>

*Position of the patient is LPO: The left posterior aspect of the patient in contact with the table. Use the Green Transvaginal Wedge (30° wedge) and place it with the lower (pointy) margin of the wedge at the level of the patient’s left ASIS. Have the left knee flexed and right leg extended. The patient is rolled posteriorly onto wedge.

**Capturing the image: The posterior aspect of the image should show the posterior aspect of the superior pole of the right kidney, in the sagittal plane, taken in the mid-axillary line.
Figure 6.1: Abdominal ultrasound images of qualitative measures of hepatic fat measured at 11-14 weeks’ gestation. Panel a shows no liver to kidney contrast while panel b shows the appearance of liver to kidney contrast.

Standard Photographs 1-2.

a) No liver to kidney contrast  b) Presence of liver to kidney contrast

Figure 6.1: Abdominal ultrasound images of qualitative measures of hepatic fat measured at 11-14 weeks’ gestation. Panel a shows no liver to kidney contrast while panel b shows the appearance of liver to kidney contrast.
Figure 6.2: Abdominal ultrasound images of qualitative measures of hepatic fat measured at 11-14 weeks’ gestation showing visibility of the anatomy as well as visualization of the intrahepatic vessels (panel b) and impaired visualization of the intrahepatic vessels (panel a).
6.3 Results

There were 476 women who completed the study (Figure 6.3).

- Women aged 18+ years
- Singleton pregnancy
- No previous diabetes mellitus or chronic condition
- Underwent a first-trimester ultrasound for nuchal translucency chronic condition  
  \( (n = 685) \)

Excluded 180 women for the following reasons:
- Transferred to another hospital during study  
  \( (n = 30) \)
- Declined OGTT  \( (n = 150) \)

n = 505

- Incomplete OGTT  \( (n = 20) \)
- Unusable fatty liver ultrasounds  \( (n = 9) \)

Final cohort

n=476

Figure 6.3: Flow chart of participant inclusion

The mean (SD) age was 32.9 (4.8) years (Table 6.3). BMI at 11-14 weeks’ gestation ranged from 17.2 to 49.9 kg/m\(^2\), with a mean of 25.1 (5.1) kg/m\(^2\).
Table 6.3: Maternal characteristics and both exposure and outcome variables for all 476 study participants.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Measures</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td>Mean (SD) age at time of enrolment, years</td>
<td>32.9 (4.8)</td>
</tr>
<tr>
<td></td>
<td>No. (%) with a first degree relative with T2DM</td>
<td>110 (22.7)</td>
</tr>
<tr>
<td></td>
<td>No. (%) Caucasian</td>
<td>251 (51.8)</td>
</tr>
<tr>
<td></td>
<td>No. (%) with parity ≥ 1</td>
<td>212 (43.8)</td>
</tr>
<tr>
<td><strong>Exposures</strong></td>
<td>Mean (SD) self-reported pre-pregnancy BMI, kg/m$^2$</td>
<td>23.8 (4.9)</td>
</tr>
<tr>
<td></td>
<td>Mean (SD) BMI at 11-14 weeks, kg/m$^2$</td>
<td>25.1 (5.1)</td>
</tr>
<tr>
<td></td>
<td>Mean (SD) net change in BMI from 11-14 weeks to 24-28 weeks, kg/m$^2$</td>
<td>2.6 (1.8)</td>
</tr>
<tr>
<td><strong>Outcomes</strong></td>
<td>Mean (SD) fasting serum glucose concentration at 24-28 weeks, mmol/L</td>
<td>4.4 (0.44)</td>
</tr>
<tr>
<td></td>
<td>Mean (SD) 1-hour serum glucose concentration at 24-28 weeks, mmol/L</td>
<td>7.4 (1.9)</td>
</tr>
<tr>
<td></td>
<td>Mean (SD) 2-hour serum glucose concentration at 24-28 weeks, mmol/L</td>
<td>6.0 (1.5)</td>
</tr>
<tr>
<td></td>
<td>Mean (SD) fasting serum alanine transaminase (ALT), U/L</td>
<td>14.7 (8.6)</td>
</tr>
<tr>
<td></td>
<td>No. (%) hepatorenal contrast</td>
<td>71 (15.0)</td>
</tr>
<tr>
<td></td>
<td>No. (%) visualization of the intrahepatic vessels</td>
<td>6 (1.3)</td>
</tr>
<tr>
<td></td>
<td>No. (%) meeting the criteria for GDM at 24-28 weeks*</td>
<td>43 (9.0)</td>
</tr>
<tr>
<td></td>
<td>No. (%) meeting the criteria for IFG, GIGT or GDM at 24-28 weeks†</td>
<td>50 (10.5)</td>
</tr>
</tbody>
</table>

*GDM was based on the presence of at least two abnormal serum glucose values: fasting ≥ 5.3 mmol/L; 1h ≥ 10.6 mmol/L and/or 2h ≥ 8.9 mmol/L.

†IFG was based on an abnormal fasting value ≥ 5.3 mmol/L, in isolation, and GIGT was based on an abnormal glucose value at 1h ≥ 10.6 mmol/L or 2h ≥ 8.9 mmol/L, in isolation.
Of the 476 women analyzed (Table 6.4), 399 (84%) had no evidence of fatty liver; 71 (15%) women had evidence of diffuse echogenesisis of the liver as indicated by hepatorenal contrast (fatty liver score=1) or blurring of the intrahepatic vessels as indicated by impaired visualization of the intrahepatic vessels, and 6 (1%) women had a score of 2, demonstrating both diffuse echogenesisis of the liver and blurring of the intrahepatic vessels on ultrasound (Table 6.4a).

A total of 50 out of 476 women (10.5%, 95% CI 7.8 to 13.6) developed the composite of IFG, IGT or GDM. Moreover, 43 out of 476 women (9.0%, 95% CI 6.6 to 12.0) had GDM, according to the CDA 2008 diagnostic criteria.

In the unadjusted logistic regression model, having 1 or 2 features of NAFLD conferred a significantly higher odds of developing the composite outcome of IFG, IGT or GDM (unadjusted model, Table 6.4a). After adjusting for maternal age, ethnicity, family history of T2DM, early pregnancy BMI and change in BMI from 11-14 weeks’ to 24-28 weeks’ gestation, women with an ultrasound fatty liver score of 1 had a greater odds (adjusted OR 2.0, 95% CI 1.0 to 4.1) of developing the composite outcome compared with their counterparts with an ultrasound liver score of 0 (adjusted model, Table 6.4a). A similar effect was observed for women with a fatty liver score of 2 (aOR 2.9, 95% CI 1.0 to 18.4) (adjusted model, Table 6.4a).

When the data were collapsed to assess a fatty liver score of 1 or more features of NAFLD, the unadjusted model revealed that women had a higher odds (OR 2.5, 95% CI 1.3 to 4.8) of developing the composite outcome compared with those who had an ultrasound fatty liver score of 0 (unadjusted model, Table 6.4b) and this effect remained true in the adjusted model (aOR 2.2, 95% CI 1.1 to 4.3) (adjusted model, Table 6.4b).
Table 6.4a: Development of the composite outcome of impaired fasting glucose (IFG), impaired glucose tolerance (IGT) or GDM in relation to the presence of 0, 1 or 2 sonographic features of fatty liver at 11-14 weeks gestation, among 476 pregnant women.

<table>
<thead>
<tr>
<th>Exposure Variable</th>
<th>Presence of composite outcome of IFG, GIGT or GDM at 24-28 week OGTT OR (95% CI)</th>
<th>Crude</th>
<th>Adjusted*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 features (n=399)</td>
<td>35 (8.8)</td>
<td>1.00 (referent)</td>
<td>1.00 (referent)</td>
</tr>
<tr>
<td>1 feature (n=71)</td>
<td>12 (16.9)</td>
<td>2.1 (1.0 to 4.2)</td>
<td>2.0 (1.0 to 4.1)</td>
</tr>
<tr>
<td>2 features (n=6)</td>
<td>3 (0.50)</td>
<td>10.4 (1.9 to 58.1)</td>
<td>2.9 (1.0 to 18.4)</td>
</tr>
</tbody>
</table>

*Adjusted for maternal age, ethnicity, family history of T2DM, BMI at 11-14 weeks’ gestation and change BMI from 11-14 weeks’ to 24-28 weeks’ gestation

Table 6.4b: Development of the composite outcome of impaired fasting glucose (IFG), gestational impaired glucose tolerance (IGT) or gestational diabetes mellitus (GDM) in relation to the presence of 0 or ≥ 1 sonographic features of fatty liver at 11-14 weeks gestation, among 476 pregnant women.

<table>
<thead>
<tr>
<th>Exposure Variable</th>
<th>Presence of composite outcome of IFG, GIGT or GDM at 24-28 week OGTT OR (95% CI)</th>
<th>Crude</th>
<th>Adjusted*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 features (n=399)</td>
<td>35 (8.8)</td>
<td>1.00 (referent)</td>
<td>1.00 (referent)</td>
</tr>
<tr>
<td>≥1 feature(s) (n=77)</td>
<td>15 (19.5)</td>
<td>2.5 (1.3 to 4.8)</td>
<td>2.2 (1.1 to 4.3)</td>
</tr>
</tbody>
</table>

*Adjusted for maternal age, ethnicity, family history of T2DM, BMI at 11-14 weeks’ gestation and change in BMI from 11-14 weeks’ to 24-28 weeks’ gestation
Table 6.5: Development of gestational diabetes mellitus (GDM) using the CDA 2008 criteria in relation to the presence of 0, 1 or 2 sonographic features of fatty liver at 11-14 weeks' gestation, among 476 pregnant women.

<table>
<thead>
<tr>
<th>No. sonographic features of fatty liver at 11-14 weeks gestation</th>
<th>Presence of GDM at 24-28 weeks gestation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%)</td>
</tr>
<tr>
<td>0 features (n = 399)</td>
<td>33 (8.3)</td>
</tr>
<tr>
<td>1 feature (n = 71)</td>
<td>8 (11.3)</td>
</tr>
<tr>
<td>2 features (n = 6)</td>
<td>2 (33.3)</td>
</tr>
</tbody>
</table>

*Adjusted for maternal age, ethnicity, family history of T2DM, BMI at 11-14 weeks gestation and change in BMI from 11-14 weeks' to 24-28 weeks' gestation
In the sensitivity analysis, while there was no significant association between the sonographic score for hepatic fat and GDM according to the CDA 2008 criteria (Table 6.6), yet there was a relation when GDM was defined by the IADPSG criteria with an adjusted OR of 2.0 (95% CI 1.1 to 3.6) (Table 6.8).
Table 6.6 Development of gestational diabetes mellitus (GDM) using the CDA 2008 criteria in relation to the presence of 0 or ≥ 1 sonographic features of fatty liver at 11-14 weeks gestation, among 476 pregnant women.

<table>
<thead>
<tr>
<th>No. sonographic features of fatty liver at 11-14 weeks gestation</th>
<th>Presence of GDM at 24-28 weeks gestation</th>
<th>Unadjusted OR (95% CI)</th>
<th>Adjusted OR (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 features (n = 399)</td>
<td>33 (8.3)</td>
<td>1.0 (referent)</td>
<td>1.0 (referent)</td>
</tr>
<tr>
<td>≥ 1 feature(s) (n = 77)</td>
<td>10 (13.0)</td>
<td>1.7 (0.74 to 3.4)</td>
<td>1.4 (0.61 to 3.0)</td>
</tr>
</tbody>
</table>

*Adjusted for maternal age, ethnicity, family history of T2DM, BMI at 11-14 weeks’ gestation and change in BMI from 11-14 weeks’ to 24-28 weeks’ gestation

Table 6.7: Development of gestational diabetes mellitus (GDM) using the IADPSG criteria in relation to the presence of 0, 1 or 2 sonographic features of fatty liver at 11-14 weeks gestation, among 476 pregnant women.

<table>
<thead>
<tr>
<th>Exposure Variable</th>
<th>Presence of GDM at 24-28 week OGGT OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes (%)</td>
</tr>
<tr>
<td>0 features (n=399)</td>
<td>59 (14.8)</td>
</tr>
<tr>
<td>1 feature (n=71)</td>
<td>17 (23.9)</td>
</tr>
<tr>
<td>2 features (n=6)</td>
<td>4 (66.6)</td>
</tr>
</tbody>
</table>

*Adjusted for maternal age, ethnicity, family history of T2DM, BMI at 11-14 weeks’ gestation and change in BMI from 11-14 weeks’ to 24-28 weeks’ gestation.
Table 6.8: Development of gestational diabetes mellitus (GDM) using the IADPSG criteria in relation to the presence of 0 or ≥ 1 sonographic features of fatty liver at 11-14 weeks gestation, among 476 pregnant women.

<table>
<thead>
<tr>
<th>Exposure Variable</th>
<th>Presence of GDM at 24-28 week OGTT OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes (%)</td>
</tr>
<tr>
<td></td>
<td>Crude</td>
</tr>
<tr>
<td></td>
<td>Adjusted*</td>
</tr>
<tr>
<td>0 features (n = 399)</td>
<td>59 (14.8)</td>
</tr>
<tr>
<td></td>
<td>1.00 (referent)</td>
</tr>
<tr>
<td></td>
<td>1.00 (referent)</td>
</tr>
<tr>
<td>≥ 1 feature(s) (n = 77)</td>
<td>21 (27.3)</td>
</tr>
<tr>
<td></td>
<td>2.2 (1.2 to 3.8)</td>
</tr>
<tr>
<td></td>
<td>2.0 (1.1 to 3.6)</td>
</tr>
</tbody>
</table>

*Adjusted for maternal age, ethnicity, family history of T2DM, BMI at 11-14 weeks’ gestation and change in BMI from 11-14 weeks’ to 24-28 weeks’ gestation.
There was no association between ALT quartiles and the composite outcome of IFG, GIGT or GDM (Table 6.9), nor was there an association between ALT quintiles, sextiles or deciles, data not shown.

Table 6.9. Association between ALT measured at 11-14 weeks’ gestation, and the risk of the composite outcome of IFG, GIGT or GDM at 24-28 weeks’ gestation among 454 pregnant women.

<table>
<thead>
<tr>
<th>Alanine transaminase (ALT), u/L</th>
<th>Presence of composite outcome of IFG, GIGT or GDM at 24-28 week OGTT OR (95% CI)</th>
<th>No. (%)</th>
<th>Crude</th>
<th>Adjusted*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quartile 1 (n = 77), &lt; 9 u/L</td>
<td></td>
<td>6 (7.8)</td>
<td>1.00 (Referent)</td>
<td>1.00 (Referent)</td>
</tr>
<tr>
<td>Quartile 2 (n = 115), 10-12 u/L</td>
<td></td>
<td>13 (11.3)</td>
<td>1.5 (0.57 to 4.5)</td>
<td>1.5 (0.53 to 5.2)</td>
</tr>
<tr>
<td>Quartile 3 (n = 147), 13-17 u/L</td>
<td></td>
<td>13 (8.8)</td>
<td>1.2 (0.43 to 3.4)</td>
<td>1.4 (0.49 to 4.9)</td>
</tr>
<tr>
<td>Quartile 4 (n = 115) &gt;17 u/L</td>
<td></td>
<td>12 (10.4)</td>
<td>1.4 (0.51 to 4.1)</td>
<td>1.6 (0.52 to 5.5)</td>
</tr>
</tbody>
</table>

*Adjusted for gestational age at oral OGTT, maternal age, ethnicity, family history of T2DM, BMI at 11-14 weeks’ gestation and change in BMI from 11-14 weeks’ to 24-28 weeks’ gestation
6.4 Discussion

This is the first study to examine the association between early-pregnancy sonographic evidence of NAFLD and the development of dysglycemia and GDM in mid-gestation in a large prospective cohort. Sonographic evidence of hepatic fat in early pregnancy, especially the presence of hepato-renal contrast, was found to predict dysglycemia and GDM in mid-pregnancy. This remained so, despite adjusting for maternal age, ethnicity, family history of T2DM, change in BMI, and maternal BMI.

A strength of this study was our large sample size, derived from a multi-ethnic population, and prospectively followed from the first trimester of pregnancy. We used a simple and standardized assessment of hepatic fat that conveniently coincided with the time at which a woman underwent measurement of fetal nuchal translucency. The presence of hepatic fat on ultrasound should not have influenced our assessment of dysglycemia in mid-pregnancy, since the results of the former were not shared with the woman or her practitioner.

As a limitation, sonographic assessment of fatty liver is a semi-quantitative measure, and cannot detect small amounts of hepatic steatosis or distinguish various stages of NAFLD (Sadeeh et al., 2002; Charatcharoenwitthaya et al., 2007). However, ultrasound typically yields positive results when adipose tissue in the liver exceeds 30% (IDF, 2010).

As another limitation, 22 of 476 women did not undergo measurement of serum ALT as they declined additional biochemistry testing.
Current diagnosis of GDM occurs at 24-28 weeks’ gestation, which may be too late to favorably impact maternal and fetal outcomes. Pregnancy is considered a stress test for future onset of GDM and may reveal a latent, preconception dysmetabolism (Retnakaran et al., 2008). While the classical definition of the metabolic syndrome has not been validated in pregnancy, emerging metabolic risk factors such as NAFLD, the liver’s manifestation of the metabolic syndrome, may characterize early gestation IR and ectopic fat accumulation and thereby predict downstream development of dysglycemia and GDM.

Moreover, apparent features of NAFLD in early pregnancy implicate a subclinical condition that should be monitored through pregnancy and postpartum (Castracane et al., 2012).

Only two small studies have examined NAFLD in pregnancy. In one study, hepatic fat was detected in a sample of 5 women with elevated BMI (Page & Girling, 2011). In another study of 57 women who underwent ultrasound of the liver in the first or third trimester of pregnancy, there was no difference in semi-quantitative measure of NAFLD between the obese and normal BMI groups (Castracane et al., 2012); those with NAFLD also had normal serum ALT levels (Castracane et al., 2012). Therefore NAFLD can exist in the absence of overt obesity and may not always be detected by ALT.

In the present study, we were unable to detect a relation between ALT and the composite study outcome. This is consistent with the latter study described above (Castracane et al., 2012) and another case-control study (Sridhar et al., 2014), in which there was no association between ALT and GDM, but where there was a possible association between gamma-glutamyl transferase and GDM (Sridhar et al., 2014).
It is possible that the lack of association between ALT and the composite outcome of GDM in our study is due to the relatively younger age of our study participants, since older age may increase risk and susceptibility to certain liver diseases (23). Another reason may be attributed to mechanistic differences in ALT during pregnancy (Sridhar et al., 2014).

The observed association between ALT and incident T2DM in other published reports may be explained by the inclusion of high-risk populations that are not representative of the general population (Schindhelm et al., 2005) or in young, reproductive-aged females, as studied herein.

Very few studies have examined the association between previous GDM and NAFLD (Forbes et al., 2011). Forbes et al. (2011) found that NAFLD was greater in women with previous GDM (38%; 95% CI 28 to 47%) compared with those without previous GDM (17%; 95% CI 10 to 24%).

Recently, Hagstrom et al. (2015) conducted a large retrospective study identifying 110 pregnancies in women with NAFLD wherein previous GDM was associated with an adjusted relative risk of 2.28 (95% CI 1.25 to 6.15).

Increased VAT leads to the pathogenesis of NAFLD, and both are established determinants of DM and CVD. In recent decades, VAT has emerged as an independent predictor of T2DM and the metabolic syndrome (Després, 1993; Hamaguchi et al., 2007). The pathology of VAT and IR remains unclear, although it appears to involve the release of free fatty acids into the hepatic portal circulation, alongside other metabolic mediating factors that include the release of pro-inflammatory cytokines from portal and systemic circulation to the liver (Després, 1993; Hamaguchi et al., 2007).
Incidentally, these same obesity-mediated mechanistic pathways also initiate the pathogenesis of inflammation and fibrosis in NAFLD (Hamaguchi et al., 2007). NAFLD treatment efforts aim to minimize inflammation and fibrosis through control of features of the metabolic syndrome, including obesity, IR, hyperglycemia, hyperinsulinemia, and T2DM (Mishra & Younossi, 2007).

Research on ultrasound-measured maternal central obesity and dysglycemia is also scarce (Bartha et al., 2007; Martin et al., 2009; De Souza et al., 2014). In a pilot study, we showed that first-trimester sonographic measures of VAT depth above the upper quartile in early pregnancy were associated with a positive GCT in later pregnancy (Martin et al., 2009), independent of BMI. We subsequently investigated whether this first-trimester measurement of VAT along with SAT and the combination of these, TAT, could contribute to early pregnancy IR and found that –along with covariates - VAT and TAT each contributed to 42% and 46% of the variance in IR during the first trimester (De Souza et al., 2014).

While CT scanning or MRI are largely used in research to quantify hepatic fat, a practical advantage of using ultrasound in early pregnancy is that it can be done safely along with the sonographic measurement of abdominal AT depth, and we have demonstrated that both measures can be done during routine first-trimester ultrasound measurement of nuchal translucency (Martin et al., 2009; De Souza et al., 2014; De Souza et al., 2015).

NAFLD may not be completely understood (Mach, 2000), but excess VAT is thought to generate a surplus free fatty acid and pro-inflammatory cytokine release into the hepatic portal circulation, with the potential for hepatic inflammation and fibrosis (Després, 1993; McCullough, 2004; Bartha et al., 2007). The control of
NAFLD is focused on reducing the number and degree of features of the metabolic syndrome (Targher et al., 2007).

Whether this is true in pregnancy is not known. The opportunity to measure hepatic adiposity in early pregnancy is important, since this population of young, otherwise healthy women represents a population in which we would not expect to find any presence of fatty liver.

Clinical practice guidelines recommend screening for GDM at 24-28 weeks' gestation (IDF, 2010; CDA, 2013), but this may be too late to favorably impact on maternal and fetal outcomes. Evidence suggests that subtle abnormalities of glucose homeostasis may be predictive of a “pre-diabetic” state in pregnancy, such as IFG or IGT, before the overt onset of GDM (Ray et al., 2010). The presence of fatty liver in early pregnancy suggests the presence of IR, a subclinical condition that can influence a woman’s health in pregnancy and postpartum (Page & Girling, 2011). Measuring hepatic fat in early pregnancy may distinguish women with established IR, and who are more likely to develop glucose mishandling thereafter.

Certainly, assessment of related downstream adverse outcomes is warranted, including fetal large-for-gestational-age measurement, maternal or newborn birth trauma, and newborn hypoglycemia. Validation measures should include metrics such as likelihood ratios and positive and negative predictive values.

The earliest origins of NAFLD may occur in utero as transmission of metabolic phenotypes from mother to offspring leads to general transfer of risk for metabolic outcomes including NAFLD (Brumbaugh & Friedman, 2014). Studies using MRI have established that maternal obesity and GDM predict neonatal hepatic fat storage, with significant associations among those with elevated pre-pregnancy BMI (Modi et al., 2011; Brumbaugh et al., 2013). More than 60% of women in
North America are overweight at the time of conception (Hinkle et al., 2012). Like the deleterious effects of IR, chronic inflammation in the liver occurs long before its diagnosis (Brumbaugh & Friedman, 2014). With escalating rates of obesity, NAFLD is expected to be the most common etiology for liver transplantation in the 21st century (Agopian et al., 2012).

Early pregnancy monitoring of dysmetabolism may help manage a pre-existing sequelae of IR that includes NAFLD which, if left unattended, could potentiate postnatal metabolic disease for mothers and their offspring.
Chapter 7  Implications and Future Directions


7.1 Implications

Research outside of pregnancy has greatly expanded our understanding of abdominal obesity in the pathogenesis of obesity-mediated metabolic diseases and its relevance to the metabolic syndrome. Indeed, including WC in the metabolic syndrome definition attempts to approximate abdominal adipose tissue.

While only a few small studies have investigated NAFLD and pregnancy, they have shown that GDM is associated with NAFLD (Tiikkainen et al., 2002). Given that NAFLD is the liver’s manifestation of IR, it may be a viable screening tool in early pregnancy for the future onset of GDM and may additionally indicate future risk of NAFLD, in a manner analogous to the risk conferred by GDM for the subsequent development of T2DM (Page & Girling, 2011).

The main objective of this thesis was to identify an early pregnancy risk identification tool for the development of impaired glucose tolerance and GDM. Another objective of this thesis was to expand the scientific literature related to abdominal adiposity and NAFLD in non-pregnant populations to the context of pregnancy. We examined the association between first-trimester abdominal adiposity and NAFLD with impaired glucose homeostasis, IR and the development of GDM. Explanatory analyses were used to describe these associations and to establish the potential use of both as early screening tools for GDM and thus warrants validation in future studies.

The most constant theme throughout this body of work is the role of maternal obesity as an early indicator of a dysmetabolic risk profile and the development of GDM. This is reviewed in the introductory and background material of Chapter 2, and is most directly addressed experimentally in Chapters 3, 4, 5 and 6. The conclusion suggested by the data in Chapters 3 and 5 that first-trimester VAT and TAT depth is associated with the development of impaired glucose homeostasis,
IR and GDM later in pregnancy, provides some added perspective on how maternal abdominal adiposity might influence the pathogenesis of GDM.

Outside of pregnancy for instance, theories that ascribe a functional role of abdominal adiposity to the development of T2DM and CVD (Tchernof & Després, 2013), predict that expansion of the VAT compartment leads to a cascade of dysmetabolism that is manifested in the liver and is indicative of a high dysmetabolic risk profile well before diagnosis of T2DM and CVD. In a parallel manner, the findings reported herein extend the literature outside of pregnancy to suggest that elevated early pregnancy AT may lead to dysglycemia and GDM which is routinely screened for and diagnosed late in the etiological trajectory of GDM.

The central abdominal adipose compartment has a heterogeneous anatomy comprised of SAT and VAT layers, both of which are believed to contribute to IR and ectopic fat accumulation in organs outside of the abdominal cavity. However, whether these compartments are functionally equivalent or distinct in their contribution to IR, glucose dysregulation or GDM was an important unanswered question that this thesis aimed to address. Should differences between abdominal compartments exist, they would be evident through their respective associations with surrogate markers of IR and insulin sensitivity as well as the outcome of GDM. These associations should also be detectable at different stages of gestation to reflect their contribution to an increasingly more insulin-resistant state that is beyond what is adaptive and normal in pregnancy and one that is in addition to measures of BMI alone. The following summarizes the findings herein that help answer these hypotheses.
7.1.1 First-trimester maternal abdominal AT is associated with early pregnancy IR

In chapter 3, we quantified SAT, VAT and TAT using ultrasound at 11-14 weeks’ gestation during a routine ultrasound assessment of fetal nuchal translucency test for trisomy 21. These measures were evaluated for their association with early pregnancy OGTT-derived surrogate markers of IR and insulin sensitivity, using the HOMA-IR and Matsuda ISI respectively, at 16-22 weeks’ gestation. We aimed to determine whether first-trimester maternal adiposity could describe early- to mid-gestation IR and insulin sensitivity, well before the conventional screening for GDM at 24-28 weeks’ gestation. In accordance with the literature in non-pregnant populations, we expected that VAT would be most strongly associated with IR in early pregnancy than that observed with pre-pregnancy BMI alone. We confirmed our hypothesis, but with the added finding that TAT was also similarly associated with IR. One possible explanation for this is that the deep SAT compartment exhibits a metabolic activity akin to that of VAT with similar associations to IR (Kelley et al., 2000). Measures of TAT would inherently quantify both deep and superficial SAT in addition to VAT. Importantly, we demonstrated that the TAT and SAT measures should not be overlooked in future research.

Other important emerging obesity markers may mediate the pathology between AT and dysmetabolism. Therefore, we investigated the notion that early pregnancy maternal abdominal adiposity would also predict biochemical biomarkers of dysmetabolism in early pregnancy well before the onset of GDM.
7.1.2 First-trimester maternal abdominal AT is associated with early pregnancy hypoadiponectinemia

In chapter 4, we examined the association between the ultrasound-measured first-trimester maternal abdominal adiposity and serum adiponectin a few weeks later. SAT and TAT were most strongly associated with low adiponectin levels at 16-22 weeks’ gestation. This finding confirmed our hypothesis that TAT would be associated with adiponectin based on our previous study, which showed that the association between VAT and TAT with early pregnancy IR was additive to pre-pregnancy BMI. However, it was unexpected that SAT, but not VAT, would be associated with low adiponectin in early pregnancy. This reinforced the interpretation that SAT morphology and metabolism is a noteworthy mediators in the pathogenesis of abdominal obesity.

Obesity is a complex phenotype that is incompletely characterized by BMI. BMI does not discern between pathogenic and non-pathogenic AT accumulation. Visceral adiposity manifests long before the diagnosis of T2DM and CVD in non-pregnant populations. In chapter 3, we demonstrated that first-trimester VAT and TAT reflected in early pregnancy IR, beyond that described by pre-pregnancy BMI alone; what remained unclear was whether these first-trimester measures of maternal abdominal adiposity compartments could also describe dysglycemia and IR late into the second and early third trimesters, at the time of routine screening for GDM.
7.1.3 First-trimester maternal abdominal AT is associated with the development of dysglycemia in mid-gestation and to GDM

In a subsequent larger, prospective cohort study (chapter 5) we examined whether sonographically measured abdominal adiposity in the first trimester is associated with the downstream development of impaired glucose homeostasis, insulin resistance and insulin sensitivity. We confirmed our hypothesis that both VAT and TAT were important mediators in the development of dysglycemia and GDM, with the highest quartiles of VAT conferring the greatest risk of GDM followed by the highest quartiles of TAT, but not SAT.

7.1.4 First-trimester maternal NAFLD is associated with the development of dysglycemia in mid-gestation and GDM

Outside of pregnancy, fatty liver is associated with T2DM and CVD and is considered the liver's principal manifestation of IR. In chapter 6, we measured hepatic fat at 11-14 weeks' gestation to determine its association with the later development of dysglycemia and GDM at 24-28 weeks' gestation. The identification of maternal fatty liver would be unexpected in this young, otherwise healthy population; therefore, its presence in early pregnancy might be indicative of dysfunctional AT and liver dysmetabolism. We confirmed our expected finding that the presence of first-trimester maternal NALFD is associated with dysglycemia and GDM later in pregnancy.
Using validated ultrasound techniques for capturing upstream, first-trimester maternal abdominal adipose tissue compartments, SAT, VAT and TAT, as well as maternal fatty liver, we obtained several lines of evidence to suggest that pathologically elevated SAT and especially VAT and TAT, and the presence of fatty liver, each contributes to dysglycemia and IR during pregnancy. These prospective cohort studies jointly suggest that first-trimester maternal abdominal adiposity and fatty liver, conveniently measured by ultrasound during a routine prenatal care visit, might help to identify a high-risk phenotype for dysmetabolism in early pregnancy.

Ectopic adipose tissue accretion is a potential mechanism underlying NAFLD and is considered to be, at least in part, attributed to increased abdominal AT, particularly VAT expansion (Edmison & McCullough, 2007). By using an ultrasound technique to capture and quantify maternal SAT, VAT and TAT compartments, as well as the sonographic presence of fatty liver, our findings suggest that the subclinical manifestations of IR and the frank outcome of GDM may be mediated by abdominal AT expansion and fatty liver.

Using a standardized clinical protocol during routine prenatal care that is cost-effective, readily accomplished with simple training and taking just a few minutes to complete, has the potential to concentrate on early pregnancy prevention of GDM, especially in combination with traditional risk criteria such as elevated maternal age, non-Caucasian ethnicity, family history of T2DM and elevated maternal BMI. This is especially intriguing as routine management of GDM involves screening and diagnosis in the late second trimester without any early pregnancy prevention.
7.2 Future Directions

While the association between maternal abdominal adiposity and hepatic liver fat accretion does not necessarily indicate a causative role of abdominal obesity and liver fat in the development of dysglycemia and GDM, the modest association between VAT, TAT and fatty liver in the first trimester with IR and GDM, described in chapters 3, 4, 5 and 6, shed insights into the pathogenesis of GDM and potentiates a clinical prevention model—a concept that deserves further study and clarification. Translating and interpreting findings reported herein to tangible diagnostic clinical tools is outside of the scope of this thesis; however, maternal abdominal AT, particularly VAT and TAT, and the presence of maternal fatty liver, can be examined in a future study testing a clinical prediction model, examined alongside conventional risk factors for IR in early pregnancy.

One possible perspective that cannot be critically evaluated by this work is the role of other novel biochemical markers, some of which arise from adipose tissue dysfunction, such as adiponectin, in the development of GDM in addition to or beyond abdominal adiposity itself. For example, we showed in chapter 4 that there is an association between first-trimester maternal abdominal adiposity and serum adiponectin in mid-pregnancy. Whether other biochemistry markers can predict GDM is a growing area of ongoing research.

Studies of the abdominal AT compartment outside of pregnancy have focused on MRI and CT imaging, which allows for a robust, comprehensive
characterization of specific AT distribution in the entire truncal region (reviewed in Tchernof & Després, 2013).

In contrast, our clinical protocol involves the use of ultrasound, which nonetheless, has been validated as a diagnostic tool for capturing abdominal adiposity and hepatic fat, and which overcomes radiation and is more practical in pregnancy. Ultrasound imaging techniques have been supported in many therapeutic areas, and are increasingly utilized in large epidemiological studies as a validated, useful and accessible tool. An important caveat to these exciting developments is that the complex phenotype of obesity harbors many mechanistic pathways that may be affected by the hormones of pregnancy and, therefore, research in pregnancy should attempt to explain mechanisms of preexisting IR manifesting as abdominal and hepatic fat well before the onset of IR in pregnancy and GDM.

Questions remain about how abdominal adiposity and liver fat might influence, or be influenced by, the natural hormonal changes of pregnancy and other pathologic pathways that together exacerbate an otherwise adaptive IR state. For instance, while reduced insulin sensitivity (ISI) was detected in women with higher amounts of VAT and TAT, there was a more modest association between VAT and early pregnancy HOMA-IR, the latter of which is a strong indicator of IR in the liver (Chapter 3). In contrast, we observed stronger associations between VAT and TAT with HOMA-IR measured at a later time point in pregnancy in the larger cohort study (chapter 5).

Evaluating several hormonal and metabolic mechanisms at once is certainly one of the next challenges for research in this area.

Several experiments are ongoing or planned for the near future and reflect our general aim to further evaluate the importance and potential preventive utility of ultrasound measures of maternal obesogenic characteristics that lead to
maladaptive IR and GDM. In keeping with this general aim, numerous avenues for further study could be explored moving forward, including those described below.

7.2.1 Predictive analyses and targeted population assessments

Recruiting obese, overweight and normal weight pregnant women in a future study, based on BMI, might shed light on whether they exhibit proportionate differences in not only abdominal AT and hepatic fat, but also the relative risk of developing GDM.

Our research group is currently investigating the combined effect of elevated AT and the co-presence of NAFLD on the development of IFG, IGT and GDM at 24-28 weeks’ gestation.

7.2.2 Biochemistry of AT

VAT is considered an endocrine organ because of its apparent metabolic activity, secreting adipokines and vasoactive constituents. In addition to adiponectin, other metabolic markers, such as sex-hormone binding globulin, Fetu-in-A, leptin, c-reactive protein, omentin-1 and retinol binding protein 4 (RBP4), offer promising opportunities for identifying early in pregnancy IR and related risk of GDM (Simas & Covera, 2014).
7.2.3 Ethnicity and acculturation in the study of maternal obesity and GDM

A multi-center study collecting abdominal adiposity and hepatic fat measures in early pregnancy would enhance the external validity of our current findings. This approach could also facilitate the evaluation of geographic and ethnic-specific variation in observed associations with GDM risk. Particular ethnic groups, such as those women with South Asian and East Asian ancestry, are highly susceptible to GDM.

Unfortunately, we were unable to examine the respective associations between maternal obesity and dysglycemia and GDM across individual ethnic groups, due to sample size limitations. In a future multi-centre study, one could also evaluate the “healthy immigrant effect” and acculturation, to see if ethnic-specific effects change with duration of residence among immigrant populations.

7.2.4 Post-partum weight retention and dysglycemia

Abdominal VAT and an elevated ratio of VAT to SAT are each significantly associated with increased cardiometabolic risk, independent of BMI (Wajchenberg, 2000; Taksali et al., 2008; Liu et al., 2010; Kaess et al., 2012). Postpartum weight retention (PPWR) is an important determinant of lifetime obesity risk (Gunderson et al., 2000; Gore et al., 2003). Gestational weight gain and post-partum BMI are predictors of PPWR (Williamson et al., 1994; Schmitt et al., 2007).

Several studies have shown an increase in obesity using postpartum CT scanning and bioelectric impedance after pregnancy. Other studies have investigated the change in WC and BMI before pregnancy through postpartum (Gunderson et al., 2008; Cho et al., 2011). To date, no study has directly
evaluated the change in VAT between early pregnancy and that measured postpartum, or compared how that change is correlated to change in WC and BMI. This line of inquiry could provide further evidence of the long-term consequences of elevated first-trimester maternal abdominal adiposity, including the presence of IFG, IGT and T2DM.

7.2.5 Heritability

Genetic studies present another opportunity for future study in this research area. Heritability quantifies the proportion of trait variability resulting from the additive effects of genes—that is, the contribution of both genes and the common environment (Fox et al., 2007). Genetic imprinting for pregnancy occurs during fetal programming and many young women follow the gestational patterns of their mothers. In their study of SAT and VAT, Fox and colleagues found that both SAT and VAT are highly heritable (Fox et al., 2007). They also demonstrated that SAT is an important cardiometabolic risk factor, and as such, should not be overlooked in studies of abdominal adiposity and metabolic dysfunction.

Obese and overweight states continue to surpass global projected estimates (Fox et al., 2007). The gradual development of obesity-mediated disease over many years highlights the reality that the full extent of the obesity epidemic is not yet realized (Fox et al., 2007). There is a clear need and opportunity for advances in preventive efforts against GDM. Obesity affects all populations, and therefore, research toward prevention, starting at the root of life – pregnancy – may, conceivably, change the global landscape and trajectory of this global pandemic.
References


Brand, JS.; van der Tweel, I., Grobbee, DE., Emmelot-Vonk, MH. & van der Schouw, YT. (2011). Testosterone, sex-hormone-binding globulin and the metabolic syndrome: A systematic review and meta-analysis of observational


Kaess, BM.; Pedley, A., Massaro, JM., Murabito, J., Hoffmann, U. & Fox, CS. (2012). The ratio of visceral to subcutaneous fat, a metric of body fat distribution, is a unique correlate of cardiometabolic risk. Diabetologia, Vol. 55, No. 10, pp. 2622-2630.


Ridker, PM.; Buring, JE., Cook, NR. & Rifai, N. (2003). C-Reactive Protein, the metabolic syndrome, and risk of incident cardiovascular events. Circulation, Vol. 107, No. 3, pp. 391-397, doi:10.1161/01.CIR.0000055014.62083.05


