MATERNAL MACRONUTRIENT INTAKES,
GLUCOSE METABOLISM DURING PREGNANCY AND
METABOLIC HORMONES IN HUMAN MILK

by

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Substantial evidence supports a role of diet in glucose metabolism, but only a few reports have investigated the impact of diet during pregnancy on risk of gestational diabetes (GDM). Although metabolic hormones have been detected in milk, no studies have investigated the impact of maternal metabolic status assessed during pregnancy on insulin and adiponectin concentrations in human milk. The purpose of this thesis was to investigate the association of maternal macronutrient intakes with metabolic status during pregnancy and its subsequent impact on human milk hormones.

Participating women (n=216) underwent a 3-hour oral glucose tolerance test at 30 (95% confidence interval [CI] 25, 33) weeks gestation, recalled their second trimester dietary intake, and donated early (the first week) and mature (3 months postpartum) milk.

Higher vegetable and fruit fiber intake was associated with reduced insulin resistance (beta±SE -0.100±0.029, p=0.0008) and increased insulin sensitivity (0.029±0.012, p=0.01) among those with a family history of type 2 diabetes. Lower % carbohydrate and higher %
total fat were associated with increased GDM risk (odds ratio 0.60 [95% CI 0.40, 0.90] and 1.61 [1.06, 2.44], respectively). Prenatal metabolic abnormalities including higher pregravid body mass index (beta±SE 0.053±0.014, p=0.0003), in addition to gravid hyperglycemia (0.218±0.087, p=0.01), insulin resistance (0.255±0.047, p<0.0001), lower insulin sensitivity (-0.521±0.108, p<0.0001), and higher serum adiponectin (0.116±0.029, p<0.0001) were associated with higher insulin in mature milk. Obstetrical measures including nulliparity (0.171±0.058, p=0.004), longer duration of gestation (0.546±0.146, p=0.0002), and unscheduled caesarean section (0.387±0.162, p=0.02) were associated with higher adiponectin in early milk. Holder pasteurization, a process recommended by the Human Milk Bank Association of North America before distributing human donor milk, reduced milk adiponectin and insulin concentrations by 32.8% and 46.1%, respectively (both p<0.0001).

In conclusion, the distribution of macronutrient intakes during pregnancy was associated with risk for abnormal glucose metabolism later in pregnancy. In addition, maternal prenatal metabolic abnormalities were associated with high insulin concentrations in mature milk, while only obstetrical parameters were associated adiponectin concentrations in early milk. Our findings support the need for continued work to determine optimal prenatal nutritional strategies to prevent GDM and subsequently to improve infant nutrition.
ACKNOWLEDGEMENTS

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1. Ley, SH, Harris, SB, Connelly, PW, Mamakeesick, M, Gittelsohn, J, Wolever, TM, Hegele, RA, Zinman, B, Hanley, AJ. Utility of non-high density lipoprotein (HDL) cholesterol in assessing type 2 diabetes risk. *Diabetes Obes Metab* [Revision Submitted]


# LIST OF FREQUENTLY USED ABBREVIATIONS

<table>
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<th>Definition</th>
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<tr>
<td>A1C</td>
<td>glycosylated hemoglobin</td>
</tr>
<tr>
<td>AUCglu</td>
<td>total area under the glucose curve</td>
</tr>
<tr>
<td>BMI</td>
<td>body mass index</td>
</tr>
<tr>
<td>CDA</td>
<td>Canadian Diabetes Association</td>
</tr>
<tr>
<td>CI</td>
<td>confidence interval</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>FFQ</td>
<td>food frequency questionnaire</td>
</tr>
<tr>
<td>GCT</td>
<td>glucose challenge test</td>
</tr>
<tr>
<td>GDM</td>
<td>gestational diabetes</td>
</tr>
<tr>
<td>GI</td>
<td>glycemic index</td>
</tr>
<tr>
<td>HAPO</td>
<td>Hyperglycemia and Adverse Pregnancy Outcome</td>
</tr>
<tr>
<td>HDL</td>
<td>high density lipoprotein</td>
</tr>
<tr>
<td>HMW</td>
<td>high molecular weight</td>
</tr>
<tr>
<td>HOMA</td>
<td>homeostasis model assessment</td>
</tr>
<tr>
<td>IADPSG</td>
<td>International Association of Diabetes and Pregnancy Study Groups</td>
</tr>
<tr>
<td>IGT</td>
<td>impaired glucose tolerance</td>
</tr>
<tr>
<td>IR</td>
<td>insulin resistance</td>
</tr>
<tr>
<td>ISogtt</td>
<td>Matsuda insulin sensitivity index</td>
</tr>
<tr>
<td>ISSI-2</td>
<td>insulin secretion sensitivity index-2</td>
</tr>
<tr>
<td>LDL</td>
<td>low density lipoprotein</td>
</tr>
<tr>
<td>NDDG</td>
<td>National Diabetes Data Group</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>P : S ratio</td>
<td>polyunsaturated-to-saturated fat ratio</td>
</tr>
<tr>
<td>SE</td>
<td>standard error</td>
</tr>
<tr>
<td>SES</td>
<td>socioeconomic status</td>
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<tr>
<td>SD</td>
<td>standard deviation</td>
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Chapter 1

Introduction
Changes in metabolism occur during pregnancy to meet the demands of the growing fetus and to prepare maternal organs for delivery and lactation (1). Although insulin sensitivity deteriorates in normal pregnancy (2), most women are able to maintain glucose homeostasis by a compensatory increase in insulin secretion from pancreatic beta-cells (1, 3). However, women who have a compromised ability to compensate for increased insulin resistance of pregnancy develop gestational diabetes (GDM) (4-6). Women with GDM and their offspring are at increased risk not only for adverse pregnancy outcomes but also for long-term health consequences (5-8). Up to 50% of women with a history of GDM have been documented to develop type 2 diabetes within 5 years of the index pregnancy (9). Further, children with intrauterine exposure to maternal diabetes are more likely to be overweight (10) and to have impaired glucose tolerance in early adulthood (11). Since obesity and impaired glucose tolerance are risk factors for GDM (5, 6), these metabolic abnormalities in young adults likely contribute to the increasing rates of GDM (12-15). Therefore, it is critical to seek solutions to intervene and prevent this vicious cycle.

Evidence from observational studies and clinical trials indicates that dietary intake influences glucose metabolism (16). Randomized clinical trials that implemented dietary modifications as part of an overall lifestyle intervention successfully reduced risk of developing type 2 diabetes among high risk individuals (17-19). Although substantial evidence supports the role of diet in type 2 diabetes risk (20-22), only a few reports have investigated the impact of diet during pregnancy on risk of GDM (23-25). Substituting dietary fat intake for carbohydrate during the second trimester has been associated with increased GDM risk (23), but the first trimester dietary intake distributions of fat and carbohydrate were not associated with GDM in another study (24).
In addition to maternal prenatal nutrition, breastfeeding has been shown to have beneficial effects on short- and long-term maternal and infant health outcomes (26). For example, lactation has been shown to have beneficial effects on maternal glucose metabolism (27-29). Further, epidemiological evidence has shown a long-term protective effect of breastfeeding against obesity and type 2 diabetes in offspring (30-32). Human milk contains not only nutrients but also an array of bioactive substances including insulin (33, 34). More recently, adiponectin, an insulin-sensitizing hormone (35, 36), was detected in human milk (37-39). Previous studies have shown that oral administration of insulin early in life stimulates gut maturation (40) and that insulin and adiponectin receptors are present in the human intestine (41, 42). These hormones in milk, therefore, might be locally and/or systemically regulating tissue activities of infants while the endocrine system matures (43), and, therefore, contributing to the observed association of breastfeeding with reduced risk for metabolic disease later in life (26).

Since women with GDM and their offspring are at increased risk not only for adverse pregnancy outcomes but also for long-term health outcomes including future development of type 2 diabetes (5-8), it is critical to develop strategies for the prevention of GDM. Substantial evidence supports the role of diet in type 2 diabetes risk (20-22). However, only a few reports have investigated the impact of diet during pregnancy on risk of GDM (23-25), and no studies have evaluated this question using information from comprehensive glucose homeostasis profiles of participants including glycemia, insulin resistance / sensitivity, and beta-cell function. In addition to examining potential prenatal modifiable factors to improve maternal and offspring metabolic health, postnatal modifiable factors could be considered. Emerging evidence indicates that metabolic hormones are present in human milk (34, 37-39),
but no studies have investigated the impact of maternal metabolic status assessed during pregnancy on these hormone concentrations in human milk. In addition to the offspring exposed to maternal metabolic abnormalities, preterm very low birth weight infants, the primary recipients of donor human milk, are also at increased risk for insulin resistance and type 2 diabetes later in life (44-46). Although donor milk is pasteurized before distribution in North America to prevent disease transmission (47), the impact of the current pasteurization practice on insulin and adiponectin concentrations in milk has not been investigated. Therefore, it is import to determine the effect of pasteurization on these metabolic hormones, which may have a critical impact on infant metabolic trajectories (40, 48). The purpose of this thesis was to address these research gaps through a prospective investigation on the associations of maternal macronutrient intakes with metabolic status during pregnancy and its subsequent impact on human milk hormones and through an experimental study investigating the impact of pasteurization on metabolic hormones in milk.
Chapter 2

Literature review
2.1 Gestational glucose intolerance

Maternal metabolism changes during pregnancy to support the growing fetus and to prepare maternal organs for delivery and lactation (1). Although insulin sensitivity deteriorates in normal pregnancy (2), most women are able to compensate and maintain normoglycemia (1). Women who have a compromised ability to compensate for the increased insulin resistance of pregnancy, however, develop gestational diabetes (GDM) (4-6). Although GDM was previously regarded as a benign condition which causes unnecessary alarm in the screened population (49), emerging evidence linking GDM with perinatal morbidity underscores the seriousness of this condition (6).

2.1.1 Definition and diagnosis of gestational diabetes

GDM is characterized by glucose intolerance with onset or first recognition during pregnancy (5). Several screening and diagnostic tests for GDM have been proposed by national and international associations (5, 50, 51). Variants of these recommendations have been implemented in current practice of different jurisdictions.

The Canadian Diabetes Association (CDA) practice guidelines recommend universal screening for GDM in all pregnant women at 24-28 weeks gestation by a glucose challenge test (GCT) in which the plasma glucose concentration is measured 1 hour after ingestion of a 50g glucose load given at any time of day (52). If the GCT result is ≥10.3 mmol/L then the woman is diagnosed with GDM. If the GCT result is 7.8-10.2 mmol/L then the woman is referred to a 2-hour oral glucose tolerance test (OGTT) in which plasma glucose concentrations are measured while fasting and then hourly for 2 hours following ingestion of 75g glucose load (52). Women are diagnosed with GDM on the 2-hour OGTT if two or
more values equal to or exceeding the following thresholds (52): fasting blood glucose 5.3 mmol/L, 1-hour blood glucose 10.6 mmol/L, and 2-hour blood glucose 8.9 mmol/L. The CDA guidelines for screening and diagnosis of GDM are summarized in Figure 2.1.

In current Canadian obstetrical practice, a two-step approach to diagnosis involving a 3-hour OGTT is frequently used instead of the 2-hour OGTT recommended by the CDA (52). When the GCT result is greater than or equal to 7.8 mmol/L, pregnant women are referred to a 3-hour OGTT in which plasma glucose concentrations are measured while fasting and then hourly for 3 hours following ingestion of a 100g glucose load. Women are then diagnosed with GDM using the National Diabetes Data Group (NDDG) criteria (50), initially proposed by O’Sullivan and Mahan in 1964 (53), as two or more values equal to or exceeding the following: fasting glucose 5.8 mmol/L, 1-hour blood glucose 10.6 mmol/L, 2-hour blood glucose 9.2 mmol/L, or 3-hour blood glucose 8.1 mmol/L (Table 2.1).

Recently, the International Association of Diabetes and Pregnancy Study Groups (IADPSG) Consensus Panel reconsidered the pre-existing diagnostic thresholds after reviewing latest scientific evidence and formulated new criteria for diagnosing GDM (51). The IADPSG recommendations include performing a 2-hour 75g OGTT between 24 and 28 weeks gestation to diagnose GDM with the use of revised thresholds (51). To diagnose GDM by the IADPSG criteria (51), one or more values equal or exceed the following thresholds: fasting blood glucose 5.1 mmol/L, 1-hour blood glucose 10.0 mmol/L, and 2-hour blood glucose 8.5 mmol/L (Table 2.2). The application of glycosylated hemoglobin (A1C) during pregnancy was also considered by the IADPSG Consensus Panel (51). The panel favored an A1C value ≥6.5% (measured in a laboratory standardized with the Diabetes Control and Complications Trial/UK Prospective Diabetes Study assay) be used for detection
of overt diabetes in pregnancy (51), but the panel did not recommend the use of A1C for GDM diagnosis.

The new IADPSG criteria for GDM diagnosis are expected to increase the prevalence of GDM primarily because only one abnormal value, not two (50, 52), is used to diagnose GDM. Previous intervention trials that focused on women with mild hyperglycemia below the NDDG thresholds demonstrated benefits in maternal and infant health outcomes including a reduction in fetal overgrowth, shoulder dystocia, cesarean delivery, and hypertensive disorders (54) and improvement in maternal postpartum mood and quality of life (55). Therefore, the new IADPSG criteria have the potential to identify previously overlooked high risk individuals and reduce the prevalence of adverse pregnancy outcomes. However, additional well-designed clinical studies are warranted to determine the optimal intensity of monitoring and treatment of women with GDM diagnosed by the IADPSG new criteria (56).
Figure 2.1  Canadian Diabetes Association guidelines for the screening and diagnosis of gestational diabetes (GDM) (52)
Table 2.1  National Diabetes Data Group diagnostic criteria for gestational diabetes (50)

To diagnose gestational diabetes, two or more values equal or exceed the thresholds listed below using the 3-h 100g oral glucose tolerance test:

<table>
<thead>
<tr>
<th>Glucose concentration threshold</th>
<th>(mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting plasma glucose</td>
<td>5.8</td>
</tr>
<tr>
<td>1-h plasma glucose</td>
<td>10.6</td>
</tr>
<tr>
<td>2-h plasma glucose</td>
<td>9.2</td>
</tr>
<tr>
<td>3-h plasma glucose</td>
<td>8.1</td>
</tr>
</tbody>
</table>
To diagnose gestational diabetes, one or more values equal or exceed the thresholds listed below using the 2-h 75g oral glucose tolerance test performed between 24 and 28 weeks gestation:

<table>
<thead>
<tr>
<th>Glucose concentration threshold</th>
<th>Glucose concentration threshold (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting plasma glucose</td>
<td>5.1</td>
</tr>
<tr>
<td>1-h plasma glucose</td>
<td>10.0</td>
</tr>
<tr>
<td>2-h plasma glucose</td>
<td>8.5</td>
</tr>
</tbody>
</table>
2.1.2 Prevalence and incidence of gestational diabetes

An accurate estimation of the global incidence of GDM is not available because of the lack of uniform standards in screening and diagnosis for GDM worldwide (6). In a survey administered to diabetologists, obstetricians and others working on GDM in 173 countries (57), the national GDM prevalence estimates varied widely from <1% to 28%, likely attributed to variation in GDM screening approaches among countries (57). The prevalence rates of GDM previously reported in Canada were 3.7% in a non-Aboriginal population (58) and 6.4-11.5% in Aboriginal Canadian populations (58-60).

An increase in the prevalence and incidence of GDM has been recently documented in North America (12-15). According to the National Hospital Discharge Survey which comprised of annual maternal hospital discharge records of births across the United States (12), the prevalence of GDM increased from 1.9% in 1989-1990 to 4.2% in 2003-2004. The racial/ethnic disparity in the prevalence of GDM widened markedly in the 1997-2004 periods with a higher prevalence observed in black women compared to white women (12). In another report among women of various racial/ethnic backgrounds including white, Hispanic, African American, and Asian living in Colorado, the prevalence of GDM doubled between 1994 and 2002 (13). A gradual increasing trend in the prevalence of GDM was observed between 1999 and 2002 in a study from southern California (14), although the prevalence decreased slightly between 2002 and 2003 before increased again from 2003 to 2005. In a cohort study of 267 051 pregnancies screened for GDM from the Northern California Kaiser Permanente Medical Care Program (15), the age- and ethnicity-adjusted yearly cumulative incidence of GDM increased steadily from 5.1% in 1991 to 7.4% in 1997 before plateauing through 2000 at 6.9%. Considering these data from various geographical locations in North
America indicating that the rates of GDM are on the rise (12-15), it is important to improve our understanding about the disease.

2.1.3 Pathophysiology of gestational glucose intolerance

Changes in maternal metabolism occur during pregnancy to support the fetal growth and to prepare maternal organs for delivery and lactation (61). Early pregnancy is characterized as an anabolic state with deposition of maternal fat stores and increased insulin sensitivity in preparation for the high nutrient demands of late pregnancy and lactation (61). Conversely, late pregnancy is characterized as a catabolic state with increased insulin resistance to allow greater substrate availability for fetal growth (61). In a study which investigated longitudinal changes in insulin sensitivity using the hyperinsulinemic euglycemic clamp technique, a 56% reduction was reported in insulin sensitivity from pre-conception to 34-36 weeks gestation (62). Although insulin sensitivity deteriorates in late pregnancy (2, 62), most women are able to maintain glucose homeostasis by a compensatory increase in insulin secretion from pancreatic beta-cells (1, 3). The increased insulin secretion has been associated with hypertrophy and hyerplasia of the beta-cells likely triggered and maintained by placental hormones (3, 63).

Pregnant women who develop GDM, however, have a compromised ability to compensate for increased insulin resistance of pregnancy (1, 3, 64). Women with GDM have been estimated to have a 67% reduction in pancreatic beta-cell compensation for insulin resistance compared to pregnant women with normal glucose tolerance (65). The reason for this inadequate adaptation to insulin resistance of pregnancy is not entirely clear, although a number of potential mechanisms that may contribute to pathogenesis of GDM have been
proposed. Impairments in the insulin-signaling cascade have been reported in women with GDM including a reduction in insulin receptor tyrosine phosphorylation and decreased insulin-mediated glucose uptake in the skeletal muscle (66). Genetic variations in the glucokinase gene may also cause a secretory defect in pancreatic beta-cells, thereby contributing to the compromised ability to compensate for insulin resistance during pregnancy (67).

Robitaille and Grant (68) reviewed the emerging evidence indicating the role of genetics in the pathogenesis of GDM and suggested that pregnancy triggers a series of metabolic imbalances that lead to GDM in women who are genetically predisposed to develop diabetes. The authors reported that most genetic variants associated with GDM are also known to be involved in the development of type 2 diabetes (68). This notion supports the hypothesis that GDM serves as an early window to reveal a predisposition to the development of type 2 diabetes (6). With recent advances in genotyping techniques to detect genome-wide variant associations with diabetes (69-80), the field is increasingly improving its understanding on the genetic contribution to diabetes. The exposure to lifestyle and other environmental risk factors, in combination with genetic susceptibility, may contribute to the development of GDM, as illustrated in Figure 2.2.
Figure 2.2 Risk factors that might contribute to gestational diabetes (GDM) in genetically vulnerable individuals. The figure was modified from Reece (6).
2.1.4 Consequences of gestational glucose intolerance

Women with glucose intolerance during pregnancy and their offspring are at increased risk not only for short-term outcomes including macrosomia, cesarean delivery, and compromised lactation (5-7, 81) but also for long-term health consequences including future development of type 2 diabetes (5, 6, 8, 9).

The fetus in the environment of maternal GDM is likely exposed to higher concentrations of glucose because glucose travels freely across the placenta from the mother to the fetus (6). In response to increased glucose, the fetus increases its own insulin production which can cause the fetus to grow excessively resulting in a birth weight exceeding 4000-4500g, a condition which is referred to as macrosomia (6). Large-for-gestational-age fetuses, defined as those with an estimated weight above the 90th percentile or above two standard deviations for gestational age are at increased risk of injuries including shoulder dystocia and newborn asphyxia during vaginal delivery (82). Therefore, a cesarean section is often performed to deliver large-for-gestational-age infants (82). The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study, which enrolled 23 316 participants to assess maternal glucose concentrations at fasting, 1-hour, and 2-hour after a 75g glucose load at 24-32 weeks gestation, reported that elevated maternal glucose concentrations below diagnostic thresholds for GDM were associated with offspring birth weights above the 90th percentile (7). This result indicates that mild maternal glucose intolerance may also increase risk for adverse perinatal outcomes.

In addition to pregnancy and delivery complications, women with diabetes have been documented to experience a compromised initiation of lactation (81). The potential
consequences of maternal diabetes on lactation will be discussed in detail in the section 2.3.3, *Effect of maternal metabolic abnormalities on lactation.*

In addition to immediate consequences, women with a history of GDM are also at increased risk for subsequently developing type 2 diabetes later in life (5, 6, 9, 83). Based on Canadian registry data of hospital discharge information in Ontario between 1995 and 2002 (83), the probability of developing diabetes after GDM in the index pregnancy was 3.7% at 9 months postpartum and 18.9% at 9 years. According to a systematic review of articles published between January 1965 and August 2001 (9), about 20-50% of women with a history of GDM develop type 2 diabetes within 5 years of the index pregnancy. The incidence of type 2 diabetes plateaued after increasing steeply within the first 5 years of the index pregnancy (9). The authors, however, noted that the cumulative progression from GDM to type 2 diabetes varied widely among studies, a finding likely attributable to variations in the length of follow-up, retention rates, diagnostic criteria, and characteristics of the baseline population with GDM (9). The authors also reported that the rates of the progression to type 2 diabetes among women from mixed or non-white cohorts were similar to those of white cohorts (9). However, a more recent investigation conducted within the United States between 2001 and 2003 reported that significant racial/ethnic variation exists among women with a history of GDM pertaining to access to health care, presence of risk factors, and perceptions of health (84). Therefore, it is possible that we may observe an increase in long-term health disparity among ethnic groups with a history of GDM in the future. In a multicentre study from France (85), 1,009 women were recruited including 466 women diagnosed with GDM by a 3-hour 100g OGTT. After 6.75 years of follow-up, the investigators reported that having GDM in the index pregnancy increased risk of type 2 diabetes.
diabetes development, with an odds ratio (OR) of 3.91 (95% confidence interval [CI] 1.81, 8.42) with multiple adjustments including prepregnancy body mass index (BMI) and a family history of diabetes. In addition, women with a history of GDM are also at increased risk for cardiovascular disease later in life (86-88).

Offspring exposed to maternal diabetes are more likely to be overweight (10) and to have impaired glucose tolerance (IGT) and diabetes in early adulthood (11). The potential consequences of intrauterine exposure to maternal diabetes will be further discussed in the section 2.3.1, *Maternal diabetes, childhood obesity and type 2 diabetes*.

### 2.2 Nutrition and glucose metabolism during pregnancy

Women with GDM and their offspring are at increased risk not only for adverse pregnancy outcomes including macrosomia and cesarean sections but also for long-term health outcomes including the future development of type 2 diabetes (5-8). Therefore, it would be of considerable interest to prevent GDM. Dietary intake, a modifiable behavioral measure, is known to influence glucose metabolism (16).

#### 2.2.1 Dietary intake and glucose metabolism

Evidence from observational studies and clinical trials indicates that dietary intake influences glucose metabolism (16). Randomized clinical trials that implemented a low fat diet (17, 18) or a low fat and high carbohydrate diet (19) as part of an overall lifestyle intervention successfully reduced risk of developing type 2 diabetes among high risk individuals. In addition to the association of total fat intake with increased risk for glucose intolerance (16, 89), specific types of dietary fat including higher *trans* fat and lower polyunsaturated fat including omega-3 polyunsaturated fatty acids have been individually associated with risk of
type 2 diabetes (90, 91). In a randomized controlled dietary intervention study among 162 men and women aged 30-65 years (92), insulin sensitivity was significantly reduced among individuals who received a diet containing saturated fat intake of 17% of total daily energy and total fat of 37% for 3 months.

Dietary fiber intake is also known to reduce postprandial glucose responses (93). Higher intake of dietary fiber has been linked with improved insulin sensitivity (93-95) and reduced type 2 diabetes risk (20-22). A number of prospective cohort studies have reported reduced risk of developing type 2 diabetes among individuals who consumed higher intakes of total fiber (20-22) and cereal fiber (20, 21, 96-98). In addition, women who consumed lower total, cereal and fruit fiber were at increased risk for GDM in the Nurses’ Health Study II (99). The mechanism by which dietary fiber may impact glucose intolerance is not entirely clear. It has been proposed that dietary fiber may function to reduce the rate of carbohydrate absorption and subsequently to lower the postprandial glycemic and insulinemic responses (100, 101). Intervention studies using the euglycemic-hyperinsulinemic clamp technique have demonstrated that insulin sensitivity was higher among individuals who received a high fiber treatment for 4 weeks (95) and 6 weeks (94). Insulin resistance assessed using the homeostatic model assessment was also lower among those who consumed higher fiber intake (94). Therefore, improved insulin sensitivity might be an important mechanism whereby higher fiber intake might influence glucose metabolism and subsequently reduce the development of diabetes.

The glycemic index (GI), an indexing of the glycemic response of a fixed amount of available carbohydrate from a test food to the same amount of available carbohydrate from a standard food consumed by the same subject, has been viewed as an extension to beneficial
effects of dietary fiber intakes on glucose metabolism (102, 103). Large scale observational studies reported that diets high in GI (96, 97, 104) and glycemic load (20) were associated with increased risk of developing type 2 diabetes. Nurses’ Health Study II reported that women who consumed a diet of higher glycemic load prior to pregnancy were at increased risk for GDM although no association of GI with GDM was observed after covariate adjustment including a family history of diabetes (99). However, non-significant associations of GI with insulin sensitivity or resistance have been reported in large scale observational studies (105, 106). Liese et al (105) explained that free-living individuals in this population-based study might not have consumed a diet that was sufficiently low in GI values to reach preventive potential, thereby resulting in the non-significant association between GI and insulin sensitivity. In randomized clinical trials among patients with diabetes, choosing low GI foods has been shown to have beneficial effects on glucose metabolism and subsequently the management of diabetes (107, 108).

2.2.2 Nutrition practice guidelines for diabetes management

Based on evidence supporting the role of dietary intakes on glucose metabolism and risk for type 2 diabetes, the CDA Clinical Practice Guidelines include nutrition therapy for management of type 2 diabetes (5), although pharmacotherapy is recommended when glycemic targets are not achieved with lifestyle management alone (109). The CDA recommends nutrition therapy and meal planning to be individualized to accommodate the individual needs (110). The CDA also provides the acceptable macronutrient distribution range as the following: carbohydrate intake of 45-60% of total daily energy, maximum fat intake of 35%, and protein intake of 15-20% (110). The CDA encourages individuals to
consume high dietary fiber intake of 25-50g per day and to select low GI carbohydrate choices (110). Further, the CDA recommends limiting alcohol intake to moderation (1-2 standard drinks per day) and to consume alcohol with food to prevent hypoglycemia (110). Sucrose intake of up to 10% of total daily energy is recommended (110). Added fructose intake (e.g. fructose-sweetened beverages) is to be restricted to 60g (110), because it may increase circulating triglyceride concentrations (111). More recently, however, a pooled analysis of controlled experimental trials demonstrated no overall effect of isocaloric exchange of fructose for carbohydrate on triglyceride or other lipid profiles (112), although a modest triglyceride raising effect was observed in a subgroup with type 2 diabetes at doses >60g per day with follow-up of ≤4 weeks. Therefore, further well-designed investigations are needed to reassess and to provide clearer guidelines on fructose.

Routine vitamin and mineral supplementations are currently not recommended by the CDA for diabetes management (5), after considering previous clinical trials which have not demonstrated benefits of antioxidant supplements (vitamin E, vitamin C, and/or beta-carotene) on the prevention of cardiovascular disease (113-115). In view of increasing evidence supporting the pathogenesis of type 2 diabetes as an inflammatory disease (116-119), however, it has been suggested that antioxidants could be used for anti-inflammatory treatment among individuals with type 2 diabetes to prevent further development of complications by controlling free radical production and by increasing intracellular antioxidant defenses (120). In a pregnant population, low maternal plasma ascorbic acid and 25-hydroxyvitamin D concentrations in early pregnancy have been associated with risk of GDM (121, 122). Further, emerging observational evidence has reported the association of mineral intake with type 2 diabetes (123-125). A recent meta-analysis of prospective studies
provided evidence that magnesium intake was inversely associated with risk of type 2 diabetes in a dose-response manner (124). However, further clinical trials are warranted to confirm potential beneficial effects of these micronutrients in the prevention of diabetes and/or further progression of the disease.

2.2.3 Macronutrient intake during pregnancy and prevention of gestational diabetes

Despite the substantial evidence supporting the role of macronutrient intakes in type 2 diabetes risk (20-22), only a few studies have investigated the association of macronutrient intake distribution in pregnancy with risk of GDM (23-25). Previously, the Pregnancy, Infection, and Nutrition Study among black and white women reported that substituting fat for carbohydrate (per each 1% of total calories) during the second trimester resulted in increased risk for GDM of 1.1 (95% CI 1.02-1.10) (23). The authors further demonstrated that a macronutrient intake distribution of 30% fat and 50% carbohydrate resulted in an almost 50% reduction in the predicted probability of GDM compared to a diet of 40% fat and 40% carbohydrate (23). However, in Project Viva, another cohort study from the United States, the distribution of dietary fat and carbohydrate intake during first trimester of pregnancy was not associated with GDM (24).

Diets during pregnancy which are higher in saturated fat and lower in polyunsaturated fat and the ratio of polyunsaturated-to-saturated fat have been individually associated with glucose intolerance (25, 126, 127). Higher saturated fat intake was also associated with a higher fasting insulin concentration during pregnancy after adjustment for BMI in a study from Northern Italy (25). In a recent report from a case-control study nested within the Camden Study (126), women who developed GDM consumed diets higher in % saturated fat
and lower in % polyunsaturated fat during pregnancy compared to those who were free of GDM according to a single 24-hour recall obtained between 20 and 28 weeks gestation. However, the impact of other macronutrients on glucose metabolism was not provided in this report (126).

A recent pooled analysis of seven clinical trials demonstrated that a group that received any additional dietary counseling had reduce risk of GDM compared to those who received the standard of care (128). However, the interpretation of this meta-analysis is difficult because of the heterogeneity among study interventions (i.e. implemented interventions varied in the distribution of macronutrients, frequency of counseling, and duration of intervention) (129-135). Further, none of studies included in this meta-analysis were able to reach statistical significance individually (128-135). Therefore, dietary intervention may have preventative impact on GDM, but the effective intervention program for the prevention is unclear.

Although the CDA recommends consuming <35% of energy from fat and 45-60% from carbohydrate for diabetes management in general (5), others have suggest higher fat (≥40%) and lower carbohydrate (<40%) intakes for women with GDM (136). The American Diabetes Association and the American Congress of Obstetricians and Gynecologists recommend individualized nutritional therapy for metabolic management during pregnancy and provide no specific guidance on the optimal macronutrient distributions to manage GDM (137, 138). In addition to a lack of specific dietary guidelines for GDM management, evidence regarding optimal prenatal nutrition to prevent GDM has been limited (23, 24). Further, no studies have investigated the impact of diet during pregnancy on the metabolic status later in pregnancy using comprehensive glucose homeostasis profiles, including GDM
status, hyperglycemia, insulin resistance / sensitivity, and beta-cell function. Evaluation of these traits will provide novel information regarding the impact of macronutrient intakes on the continuum of metabolic disorders beyond dichotomous GDM status.

2.3 Maternal diabetes, breastfeeding and childhood obesity

Children who experienced intrauterine exposure to maternal diabetes are more likely to be overweight (10) and to have IGT in early adulthood (11). Since obesity and IGT are risk factors for GDM (5, 6), these metabolic abnormalities in young adults likely contribute to the increasing rates of GDM (12-15). Therefore, it is important to seek solutions to intervene this vicious cycle. Breastfeeding has been shown to have beneficial effects on short- and long-term maternal and infant metabolic health outcomes (26). For example, lactation has been shown to have beneficial effects on maternal glucose metabolism (27-29). Further, epidemiological evidence has shown a long-term protective effect of breastfeeding against obesity and type 2 diabetes in offspring (30-32).

2.3.1 Maternal diabetes, childhood obesity and type 2 diabetes

Maternal diabetes in pregnancy has been associated with higher adiposity in offspring (10, 139-142). Further, in the multicenter multinational HAPO Study, maternal glucose intolerance between 24 and 32 weeks gestation at glucose concentrations less severe than traditional diabetes diagnostic thresholds was associated with neonatal adiposity (143). In addition, children exposed to maternal diabetes in pregnancy are more likely to have IGT later in life (11, 144). In the multi-ethnic SEARCH Case-Control Study, intrauterine exposure to maternal diabetes (OR 5.7 [95% CI 2.4-13.4]) and obesity (2.8 [1.5-5.2]) was
associated with type 2 diabetes in youth aged 10-22 years (8). The adolescent and young adult offspring of mothers with type 1 diabetes had an increased occurrence of IGT, a defective insulin secretory response (145), higher adiposity, and insulin resistance (146). Taken together, these studies indicate that the effect of the intrauterine and postnatal exposure to maternal diabetes and related disorders may be related to increased diabetes risk in offspring.

2.3.2 Breastfeeding, childhood obesity and type 2 diabetes

Breastfeeding has been shown to have beneficial effects on short- and long-term infant health outcomes (26). Several systemic reviews, including one by the World Health Organization, have demonstrated that breastfeeding has a protective effect on obesity over a wide range of ages (30, 31). However, a randomized breastfeeding promotion intervention in Belarus was not able to reduce childhood obesity assessed at age 6.5 years (147). The authors, therefore, have commented that previous findings on beneficial effects of breastfeeding on childhood obesity may have been caused by confounding and selection bias (147), including demographic, socioeconomic, educational, ethnic, cultural, and psychological factors in addition to maternal and infant physical and emotional health (148). However, differences in the maternal populations might also affect child care practices, access to medical care, and child health status (148), which could subsequently influence anthropometric and adiposity status of the child. Therefore, it is difficult to extrapolate the findings of the intervention study from Belarus to the setting of the childhood obesity epidemic in North America. In addition to childhood obesity, another systemic review reported that breastfeeding was associated with a reduced risk of type 2 diabetes later in life (28). Breastfeeding was
associated with lower preprandial blood glucose and insulin concentrations among infants (32), and breastfed children and adults without diabetes had lower fasting insulin concentrations than those who were formula fed (32).

In addition to sociodemographic factors (148), physiological factors including maternal metabolic status and components of human milk may contribute to the potential protective role of breastfeeding against childhood obesity (149, 150) and subsequently type 2 diabetes. However, previously published large scale observational studies have not assessed human milk components from women who have had the detailed characterization of metabolic status during pregnancy.

2.3.3 Effect of maternal metabolic abnormalities on lactation

Although growing evidence supports the benefits of breastfeeding for maternal and offspring metabolic health (26), women with obesity and/or diabetes often experience increased challenges in breastfeeding (81, 151-155). High prepregnancy BMI was associated with the delayed onset of lactogenesis II, defined as the time of onset of copious milk secretion ≥72 hours postpartum (156). Delayed lactogenesis II in obese women was subsequently associated with the early cessation of breastfeeding (154, 157, 158). The delayed onset of lactogenesis II among overweight/obese women compared to normal weight women was explained by disordered hormonal responses in early postpartum (159), specifically lower prolactin concentrations in response to suckling at 48 hours postpartum. Women with type 1 diabetes experienced delays in the onset of lactogenesis II (81) and an increased concentration of lactose, citrate, and total nitrogen in their milk early postpartum (160, 161). With the prevalence of obesity and related metabolic abnormalities among women of
reproductive age on the rise (12), it is critical to improve our understanding about the impact of maternal metabolic abnormalities on breastfeeding to assist women with increased lactation challenges.

2.3.4 Role of human milk on maternal and childhood metabolic abnormalities

A number of studies have investigated whether breastfeeding influences the association between maternal and offspring metabolic abnormalities (162-165). In Pima Indians, offspring who were not exposed to maternal diabetes and were exclusively breastfed for at least 2 months had about half the prevalence of diabetes by 10-39 years of age than those who were not breastfed, after adjustment for covariates including a family history of diabetes (162). Although the authors reported a similar trend among offspring who were exposed to maternal diabetes, the difference was not significant likely due to a small sample size (n=21) (162). In Aboriginal Canadians, the risk of type 2 diabetes was lower among adolescents who were breastfed for longer than 12 months compared to those who were not breastfed, after adjusting for maternal diabetes (163). In the Growing Up Today Study, breastfeeding was inversely associated with childhood obesity between ages of 9 and 14 years regardless of maternal diabetes or weight (164). In another study involving the offspring of mothers with GDM, exclusive breastfeeding for >3 months was associated with a lower risk of childhood overweight between ages of 2 and 8 years (165).

In a study from Germany, infants were supplemented with donor human milk from women with normal glucose tolerance when their own mothers with diabetes were not able to provide an adequate amount of milk. Children who consumed a higher amount of human milk from their own mothers with diabetes (type 1 diabetes, n=83; GDM, n=29) during the
first week of life were more likely to have a higher body weight at 2 years of age (166). The authors, therefore, suggested that the early neonatal ingestion of milk from mothers with diabetes might increase risk for childhood overweight. However, the specific components in human milk that might have contributed to this difference were not investigated in this study. In addition, findings from the studies of donor human milk supplementation are difficult to interpret and generalize to other practice settings since donor milk handling processes can alter concentrations of milk components (167, 168). In another report from this cohort, adjusting for the volume of human milk ingested by offspring of diabetic mothers during the first week of life attenuated the relation between breastfeeding duration and childhood overweight at 2 years of age (169). The authors, therefore, concluded that the first week of life is a critical window for the nutritional programming of offspring who ingested their diabetic mother’s milk which might have sub-optimal milk composition.

Taken together, these studies suggested that human milk that has potentially disordered composition of important components may influence the associations of maternal diabetes with childhood metabolic abnormalities. However, limited information is available on specific human milk components which may contribute to these associations.

2.4 Metabolic hormones and human milk

Based on evidence that human milk provides sufficient energy and the proper profile of nutrients to support normal growth of infants (170), the World Health Organization recommends exclusive breastfeeding for the first six months of life and the continuation of breastfeeding to two years of age or beyond (171). In addition to nutrients, however, human milk contains nonnutrient growth factors, immune factors, hormones, and other bioactive
components that can function as biological signals and confer protection against illness in infancy and later in life (33, 170, 172). More recently, metabolic hormones, known to be involved in pathophysiology of type 2 diabetes (173-175), were detected in human milk (40, 48). These hormones in milk might locally and/or systemically regulate tissue activities of infants while the endocrine system matures (43), and, therefore, may contribute to the observed association of breastfeeding with reduced risk for metabolic disease later in life (26).

### 2.4.1 Metabolic hormones and glucose metabolism

The hypothesis that obesity is an inflammatory condition leading to chronic activation of the innate immune system which subsequently contributes to progressive impairment of glucose tolerance has gained support in recent years (118). Further, bioactive proteins that are involved in the pathogenesis of type 2 diabetes have been identified (116, 118). In serum, these metabolic hormones regulate energy balance, glucose metabolism, and obesity-induced inflammatory signaling pathways which contribute to type 2 diabetes (117, 119, 176, 177). Adiponectin is one of these emerging metabolic hormones known to have insulin-sensitizing properties (36, 178). Insulin, a 51 amino acid 5.7 kDa protein comprised of two peptide chains linked by disulphide bonds (179), is synthesized and produced by pancreatic islet beta-cell in response to elevated plasma glucose concentrations (180). First isolated in 1921 and used as a treatment of type 1 diabetes in 1922, insulin is widely used to treat type 2 diabetes at various stages of disease progression (181).

Adiponectin is a 247 amino acid polypeptide hormone known to have anti-inflammatory, anti-atherogenic, and insulin-sensitizing properties (35, 36, 178, 182-184).
Serum adiponectin is inversely associated with obesity, insulin resistance, type 2 diabetes, and coronary artery disease (185-188). Low baseline serum adiponectin is associated with increased risk of developing diabetes in various populations (189-198). Adiponectin exists in different oligomeric forms in serum including a low molecular weight trimer, middle molecular weight hexamer, and high molecular weight (HMW) 12- to 18-mer (199, 200). In serum, HMW adiponectin is known to be the most biologically active form which has stronger associations with diabetes than total adiponectin (198, 201).

While the molecular mechanism of adiponectin is not completely understood, adiponectin has been associated with multiple signaling pathways (202-204). Adiponectin has been linked with p38 mitogen-activated protein kinase, AMP-activated protein kinase (AMPK), and peroxisome proliferator-activated receptor (PPAR)-α activations which are involved in glucose metabolism (205). Evidence has been provided using knockout mouse models that adiponectin receptor 1 (AdipoR1) mediated AMPK activity, while adiponectin receptor 2 (AdipoR2) mediated increased PPAR-α ligand activity in the liver (203). The disruption of both AdipoR1/R2 abolished adiponectin binding and action, increasing inflammation and oxidative stress, and therefore leading to insulin resistance and glucose intolerance (203). Adiponectin stimulated the Adaptor protein containing PH domain, PTB domain and Leucine zipper motif 1 (APPL1) binding to AdipoR1 which then modulated downstream insulin sensitizing and metabolic events (206). Adiponectin-stimulated APPL1 mediated glucose transporter-4 translocation, with involvement of Rab5 (GTPase), increasing glucose uptake (206). In another mouse model study, decreased expressions of AdipoR1 or AdipoR2 were associated with reduced insulin-sensitizing effects of adiponectin in hepatocytes and myocytes (204).
In addition, a feedback loop between insulin and adiponectin seems to be present whereby high insulin concentrations downregulate adiponectin, decreasing insulin sensitivity further and prompting an increase in insulin (207). Further, adiponectin modulated insulin secretion in beta-cells of insulin-resistant mice (202). This association of adiponectin with the beta-cell function has been also observed in human studies (208, 209).

2.4.2 Maternal metabolic status and metabolic hormones in milk

Metabolic hormones including insulin, adiponectin, leptin, resistin, obestatin, and ghrelin are present in human milk (34, 37, 210-213). However, limited information has been reported on whether these milk hormone concentrations are influenced by maternal metabolic status. One study reported that ghrelin concentrations in colostrum at 2 days postpartum were lower among women who had GDM compared to those without diabetes, but the difference was not observed in mature milk (214). This study suggests that the offspring of mothers who experience metabolic abnormalities during pregnancy might be exposed to compromised early nutrition. This study, however, was limited by a small sample size (GDM, n=12; no diabetes, n=14) which precluded adjustment for potential confounders. Since metabolic milk hormone concentrations during the first week postpartum vary considerably (34), variation in the time elapsed from delivery to milk collection might have influenced the differences reported.

2.4.3 Insulin in human milk

Insulin has been detected in human milk (34, 215, 216). Read et al (34) reported from 23 healthy mothers after delivery of full-term infants that human colostrum contained 40-fold
greater concentrations of insulin compared to human serum. Insulin concentrations in milk decline rapidly during the first few days of lactation, with insulin concentrations in mature milk of only 10% of those in colostrum, but remain constant thereafter in mature milk assessed up to 6 weeks postpartum (34). In another study, mature milk at 14 months postpartum was reported to have similar concentrations of insulin as those at 3 months postpartum (217). Among healthy women without diabetes who delivered at term (n=24), insulin concentrations in milk on day 3 postpartum were significantly higher than those on day 10 (216). It has been proposed that this high concentration of insulin in milk might be involved in regulating development of infants and subsequently in reducing susceptibility to future metabolic disease (40). The potential function of milk insulin will be further discussed in the section 2.4.5, Potential role of metabolic hormones in human milk.

2.4.4 Adiponectin in human milk

The presence of adiponectin in human milk was first reported in 2006 (37-39). Adiponectin in human milk is almost entirely composed of the HMW form (48). Martin et al (37) reported that adiponectin concentrations in milk decreased throughout the postpartum period from the first week to 7 months. In another study of 72 healthy mothers who had a non-complicated vaginal delivery of full term infants (218), milk adiponectin concentrations decreased from day 0 to 6 months postpartum, but concentrations were significantly higher at 12 months postpartum compared to 3 or 6 months postpartum (n=39 at 12 months). In a smaller sample size of 25 healthy non-obese women who delivered at full term (219), adiponectin concentrations in colostrum were not correlated with the birth weight of infants or with prepregnancy BMI of mothers. In another longitudinal study, high maternal
postpartum BMI was associated with higher adiponectin in milk (beta±SE 0.08±0.02, p<0.0001; n=14) (37). The interpretation of these study findings, however, is limited by a lack of detailed information on maternal metabolic status and small sample sizes.

Higher adiponectin in milk was associated with lower infant weight-for-age and weight-for-length Z-scores over the first 6 months (220). In the follow-up data from the same cohort, however, higher adiponectin in milk was associated with higher weight-for-age and weight-for-length Z-scores between age 1 and 2 years (221). The authors, therefore, concluded that milk adiponectin might be involved in catch up growth in the second year of life after slower weight gain during the first year of life (221). Similarly in another study, high adiponectin in mature milk was associated with risk of overweight in offspring at 2 years of age among those ever breastfed for ≥6 months, OR 2.1 (95% CI 1.1, 4.2), with adjustment including infant birth weight and maternal BMI (222). Taken together, a number of studies have confirmed the presence of adiponectin in human milk (37-39) and its potential involvement in infant growth (220-222), but no studies have assessed the impact of maternal metabolic status during pregnancy on milk adiponectin concentrations.

2.4.5 Potential role of metabolic hormones in human milk

While the molecular mechanisms through which metabolic hormones in milk may provide protection against the development of obesity and type 2 diabetes in the offspring later in life are not completely understood, the potential physiological roles of these hormones have been proposed in influencing infant metabolic trajectories (40, 48, 223). Insulin and adiponectin receptors are known to be present in the human intestine (41, 42). The immaturity of the neonatal gut barrier may also allow for absorption and survival of various hormones from
human milk (224-226). Further, the characteristics of the neonatal gastrointestinal tract, including delayed production of pancreatic proteases and gastric acid and higher permeability in the neonatal gut to macromolecules, in addition to the presence of antiproteases and inhibitors in human milk, would allow for survival of human milk components (224-226). Metabolic hormones in milk, therefore, might be locally and/or systemically regulating tissue activities of infants while the endocrine system matures (43).

It has been demonstrated that insulin is involved in the maintenance of normal rates of protein synthesis in small intestinal mucosa (227). In patients with type 1 diabetes, the synthesis rate of mucosal proteins was about 30% lower during insulin deprivation than during insulin treatment (227). In a study involving preterm infants (228), participants (n=8) who received insulin though a nasogastric feeding tube from 4 to 28 days of age had higher lactase activity, achieved full enteral feeding earlier, and had fewer gastric residuals compared to the control infants (228). The authors, therefore, concluded that insulin was efficacious in enhancing intestinal development and reducing feeding intolerance in preterm infants (228). However, this study had a number of limitations including a small treatment sample size, variation in infant feeding practice (human milk vs. premature infant formula), and retrospective collection of the control infant data. Therefore, additional investigations with a clearly defined study protocol are required to confirm the beneficial effect of enteral insulin in preterm infants.

A number of animal studies have demonstrated that the administration of oral insulin were able to regulate the gut maturation and function (229-232). Orally administrated insulin stimulated an increase in ileal mass and disaccharidase activity in 2-day-old miniature pigs (229). At the onset of the weaning period, the plasma insulin concentration and pancreatic
amylase activity increased in rats raised on milk formula supplemented with insulin and trypsin-inhibitor (231). In another experimental study, rats were provided with insulin supplemented drinking water for 1 week during the postweaning period (232). Insulin supplemented rats had a higher mucosal weight in duodenum and jejunum, duodenal circumference, and jejunal villous height compared to controls (232). Further, oral supplementation of insulin to apolipoprotein E-deficient 1-month-old mice resulted in decreased aortic atherosclerotic mean plaque area and reduced number of atherosclerotic lesions (233), supporting the systemic impact of oral insulin supplementation. It has been also proposed that disordered hormone concentrations in critical developmental periods may lead to permanent sub-optimal programming of fundamental regulatory systems in offspring (234). For example, neural projection pathways from the arcuate nucleus of the hypothalamus are permanently disrupted in leptin-deficient mice (235). Among these mice, leptin treatment in adulthood did not reverse the defects, whereas the treatment in neonates preserved the development of these pathways (235). Optimal milk hormone concentrations may be involved in the normal development of hypothalamic pathways during a critical window of development influencing the energy homeostasis throughout life. These milk metabolic hormones, therefore, may have a role through local or systemic mechanisms regulating the development of infants and subsequently in reducing the susceptibility to future diseases. However, additional human studies are needed to confirm a role of oral ingestion of these metabolic hormones in healthy term infants.

Since emerging evidence indicates that metabolic hormones are present in human milk (34, 37-39) and the offspring of women with GDM are at increased long-term risk for type 2 diabetes (5, 8), the impact of maternal metabolic status on these milk hormones would
be of interest to document. In addition to the scarce evidence available on the impact of maternal metabolic status during pregnancy on these hormones in milk, neither the World Health Organization (171) nor local organizations for obstetrics and pediatrics (236, 237) currently provide specific breastfeeding practice recommendations for those with metabolic abnormalities during pregnancy.

2.5 Human donor milk

To protect recipients against disease transmission, donor milk is pasteurized before distribution in North America (238). While various nutrient components remain intact after pasteurization, the pasteurization process is known to effect biological activity of a number of milk components (239). Since the primary recipients of donor milk, preterm very low birth weight infants, are at increased risk for insulin resistance and type 2 diabetes later in life (44-46), it might be beneficial to understand the effect of pasteurization on metabolic hormones in human milk.

2.5.1 Human milk donation and distribution

With improved knowledge about the benefits of breastfeeding, the demand for donor human milk has increased when mother’s own milk is not available (240). Although a resurgence of interest in human milk banks has recently emerged in North America (241, 242), human milk donation is a concept widely accepted and organized around the world including Australia (243-245), Brazil (246), Cape Verde (247), China (248), France (249, 250), Germany (251, 252), Italy (253), India (254, 255), Korea (256), Kuwait (257), Poland (258), the Scandinavian countries (259-261), and the United Kingdom (262, 263). Donor milk banks
have been organized to offer services of collecting, screening, processing, storing, and distributing donated human milk to meet the specific needs of recipients (241). The majority of donor milk recipients from member banks of the Human Milk Banking Association of North America is preterm and sick infants, but milk has been also requested for older infants and children with a variety of medical needs including metabolic disorders, food allergies, or feeding intolerance (240).

Donor milk banks have implemented safety screening and handling processes to prevent transmission of bacteria and pathogens (238, 251, 261, 262). These processes, however, vary among countries. Donor milk is pasteurized at 62.5°C for 30 minutes (Holder method) before distribution in North America and the United Kingdom (238, 262), while some countries have implemented more detailed screening processes of donors followed by a lower temperature pasteurization or no pasteurization (251, 261).

2.5.2 Pasteurization and human milk components

In North America, Holder pasteurization is used to eliminate viral contaminants including human immunodeficiency virus, human T-lymphotropic virus, cytomegalovirus, and tuberculosis (238, 251, 261, 262). Although a number of nutrients including various fatty acid components and lactose are unaffected by Holder pasteurization (168, 239, 264, 265), the pasteurization process deactivates or reduces the activity of several bioactive components in human milk including immunological proteins (i.e. secretory immunoglobulin [Ig], IgA, IgG, IgM, lactoferrin, and lysozyme) (266-268), antioxidants (269), lipases (265), interleukin-10 (270), and insulin-like growth factors (271). Variation in heat treatment options including a reduction in the length of pasteurization has been shown to preserve
insulin-like growth factors (271). In addition, reducing pasteurization temperature to 57°C improved immunological protein retention while effectively removing 99.9% of inoculated bacterial species (266).

Although the presence of adiponectin and insulin have been reported in human milk previously (34, 37-39), the effect of pasteurization on these metabolic hormones has not been investigated. Evidence indicates that oral administration of insulin stimulates gut maturation (40) and that adiponectin receptors are present in the fetal small intestine (48). These milk metabolic hormones, therefore, may have a direct role in the optimal metabolic development of infants and subsequently in reducing susceptibility to future metabolic disease. This may be especially important for the primary recipients of donor milk, preterm very low birth weight infants who are at increased risk for insulin resistance and type 2 diabetes later in life (44-46). With the increasing demands for donor human milk (240), it is important to understand the impact of pasteurization on milk metabolic hormones which may have a critical impact on infant metabolic trajectories (40, 48).

2.6 Summary of literature and study rationale

Evidence from observational studies and clinical trials indicates that dietary intake influences glucose metabolism (16). Randomized clinical trials that implemented dietary modification as part of an overall lifestyle intervention successfully reduced risk of developing type 2 diabetes among high risk individuals (17-19). Despite the substantial evidence supporting the role of macronutrient intake distribution in type 2 diabetes risk (20-22), only a few studies have investigated the impact of macronutrient intakes in pregnancy on the prevention of GDM (23-25).
In addition to maternal prenatal nutrition, breastfeeding has been shown to have beneficial effects on short- and long-term maternal and infant health outcomes (26). For example, lactation has been shown to have beneficial effects on maternal glucose metabolism (27-29). Further, epidemiological evidence has shown a long-term protective effect of breastfeeding against obesity and type 2 diabetes in offspring (30-32). Human milk contains an array of bioactive substances including insulin and adiponectin (33, 34, 37-39). Previous studies have shown that oral administration of insulin early in life stimulates gut maturation (40) and that adiponectin receptors are present in the human intestine (42). These hormones in milk might be locally and/or systemically regulating tissue activities of infants while the endocrine system matures (43), and, therefore, contributing to the observed association of breastfeeding with reduced risk for metabolic disease later in life (26). Despite a number of studies confirming the presence of insulin and adiponectin in human milk (34, 37-39), no studies have investigated the impact of maternal metabolic status assessed during pregnancy on these hormone concentrations in human milk.

In addition to offspring exposed to maternal metabolic abnormalities, preterm very low birth weight infants, the primary recipients of donor human milk, are also at increased risk for insulin resistance and type 2 diabetes later in life (44-46). The Holder pasteurization process used North America to prevent disease transmission is known to effect the biological activity of a number of milk components (239). However, the effect of pasteurization on insulin and adiponectin has not been investigated.

Since women with GDM and their offspring are at increased risk for adverse pregnancy outcomes and chronic disease development later in life (5-8), it is important to clarify the role of prenatal nutrition in the prevention of GDM. Nonetheless, only a few
reports have investigated the impact of diet during pregnancy on risk of GDM (23-25), and none have studied this question using comprehensive glucose homeostasis profiles including glycemia, insulin resistance / sensitivity, and beta-cell function. In addition to maternal prenatal nutrition, breastfeeding has been shown to have beneficial effects on short- and long-term maternal and infant health outcomes (26). Since emerging evidence indicates that metabolic hormones are present in human milk (34, 37-39) and the offspring of women with GDM are at increased long-term risk for type 2 diabetes (5, 8), the impact of maternal metabolic status on these milk hormones would be of interest to document. However, no studies have investigated the impact of maternal metabolic status assessed during pregnancy on these hormone concentrations in human milk. With recent evidence demonstrating that dietary intervention in infancy has long-term effects on beta-cell function (272, 273), it is important to understand the impact of the pasteurization process on metabolic hormone concentrations in donor human milk which may have a critical impact on infant metabolic trajectories (40, 48). The purpose of this thesis was to address these research gaps through a longitudinal study involving detailed metabolic and nutritional characterization of women during pregnancy and assessment of milk components during postpartum and through an experimental study assessing the impact of pasteurization practice on milk hormone concentrations.
Chapter 3

Objectives and hypotheses
3.1 Objectives

The overall objective of this thesis was to investigate the association of maternal macronutrient intakes with glucose metabolism during pregnancy and its subsequent impact on metabolic hormones in human milk. Specific objectives:

1 To investigate the association of macronutrient intake distribution in the second trimester of pregnancy with glucose metabolism (i.e. gestational diabetes [GDM] status, hyperglycemia, insulin resistance / sensitivity, and beta-cell function) later in pregnancy;

2 To assess the association of maternal metabolic status during pregnancy (i.e. overweight, insulin resistance / sensitivity, and glucose tolerance) with insulin and adiponectin concentrations in human milk at two different periods of lactation (during the first week and 3 months postpartum); and

3 To study whether pasteurization by the Holder method changes the concentrations of adiponectin and insulin in donor human milk.

3.2 Specific hypotheses

Specific hypotheses of the thesis are outlined below and are presented as a conceptual model in Figure 3.1. Specific hypotheses:

1 The sub-optimal distribution of macronutrient intakes during the second trimester of pregnancy will be associated with abnormal glucose metabolism (i.e. GDM, hyperglycemia, insulin resistance, lower insulin sensitivity, and beta-cell dysfunction) later in pregnancy;

2 Maternal metabolic status in pregnancy (i.e. overweight, insulin resistance, lower insulin sensitivity, and glucose intolerance) will be associated with variation in insulin and
adiponectin concentrations in early (first week of life) and mature milk (3 months postpartum); and

3 Pasteurization using the Holder method (62.5°C for 30 minutes), the current standard processing donor human milk for human consumption in North America, will reduce the concentrations of insulin and adiponectin in milk.

3.3 Organization of hypothesis testing and contribution of candidate

To test first two specific hypotheses, a prospective sub-study was conducted within ongoing cohort studies of women and their offspring. The overall protocol of the prospective sub-cohort study is presented in Chapter 4. The first specific hypothesis is tested in Chapter 5, and the second specific hypothesis is assessed in Chapter 6. To test the third specific hypothesis, an experimental study was conducted following the standardized donor human milk processing protocol used in North America (47). Chapter 7 addresses the third hypothesis.

The contribution of candidates on the prospective investigation in Chapters 4-6 includes conceptualizing and designing the study, leading study grant proposals, drafting documents for ethics approval, recruiting participants, collecting data, coordinating biochemical analysis and database development, managing study personnel and databases, performing data analysis and interpretation, and writing reports. In addition, the candidate developed and coordinated the assay protocol validation for milk adiponectin tested in Chapter 7.
Figure 3.1 Conceptual model of research hypotheses
Chapter 4

Impact of maternal metabolic abnormalities in pregnancy on human milk and subsequent infant metabolic development: methodology and design

This chapter is a prospective study protocol article previously published in BMC Public Health. The publisher of BMC Public Health is not responsible for any errors or omissions in this version of the manuscript or any version derived from it by third parties.


A link to the published manuscript can be found at

http://www.biomedcentral.com/1471-2458/10/590
4.1 Abstract

**Background and objective:** Childhood obesity is on the rise and is a major risk factor for type 2 diabetes later in life. Recent evidence indicates that abnormalities that increase risk for diabetes may be initiated early in infancy. Since the offspring of women with diabetes have an increased long-term risk for obesity and type 2 diabetes, the impact of maternal metabolic abnormalities on early nutrition and infant metabolic trajectories is of considerable interest. Human breast milk, the preferred food during infancy, contains not only nutrients but also an array of bioactive substances including metabolic hormones. Nonetheless, only a few studies have reported concentrations of metabolic hormones in human milk specifically from women with metabolic abnormalities. We aim to investigate the impact of maternal metabolic abnormalities in pregnancy on human milk hormones and subsequently on infant development over the first year of life. The objective of this report is to present the methodology and design of this study.

**Methods and design:** The current investigation is a prospective study conducted within ongoing cohort studies of women and their offspring. Pregnant women attending outpatient obstetrics clinics in Toronto, Canada were recruited. Between April 2009 and July 2010, a total of 216 pregnant women underwent a baseline oral glucose tolerance test and provided medical and lifestyle history. Follow-up visits and telephone interviews are conducted and expected to be completed in October 2011. Upon delivery, infant birth anthropometry measurements and human breast milk samples are collected. At 3 and 12 months postpartum, mothers and infants are invited for follow-up assessments. Interim telephone interviews are conducted during the first year of offspring life to characterize infant feeding and supplementation behaviors.
Discussion: An improved understanding of the link between maternal metabolic abnormalities in pregnancy and early infant nutrition may assist in the development of optimal prevention and intervention strategies and in the protection of nutritionally vulnerable offspring who are at risk for obesity and diabetes later in life.
4.2 Introduction

Childhood obesity has emerged as an epidemic which is reflected in the rapidly increasing rates of youth onset type 2 diabetes (274-276). Nutrition in infancy has been linked with lifetime effects on the pathogenesis of obesity, insulin resistance, and type 2 diabetes (30-32). It has been proposed that nutrition signals during the early postnatal period may influence metabolic developmental pathways and, thereby, induce permanent changes to metabolic disease susceptibility (234, 277). Human breast milk is the most preferred food during this early postnatal period (171, 237, 278). Several systemic reviews have reported that breastfeeding has a protective effect on obesity (30, 31) and type 2 diabetes later in life (32). The protective role of breastfeeding against childhood obesity and type 2 diabetes may be explained by a number of potential determinants including maternal metabolic abnormalities, infant feeding behaviors, and biological components of breast milk (149, 150). However, previously published large observational studies have not assessed whether maternal metabolic status in pregnancy and human milk components are responsible for the observed associations of breastfeeding with offspring obesity and type 2 diabetes.

Human milk contains not only nutrients but also an array of bioactive substances (33, 210). These human milk components function to transiently regulate tissue activities while the neonate’s endocrine system matures (43). Bioactive substances involved in metabolic regulation including adiponectin have been detected in human milk (37, 213, 279), and it has been hypothesized that these milk hormones may regulate growth and development in infancy and influence the programming of energy balance later in life (279). Interestingly, one of these milk hormones, ghrelin, was reduced in the early postpartum among women who had gestational diabetes mellitus (GDM) (214). In addition, mothers with diabetes often
experience delayed onset of lactogenesis II, defined as the time to onset of copious milk 
secretion \( \geq 72 \) hours postpartum (81). Taken together, these reports suggest that the offspring 
of women with metabolic abnormalities may be exposed to compromised early nutrition and, 
therefore, are vulnerable to altered metabolic development and increased susceptibility to 
metabolic disease later in life.

Since offspring of women with obesity and diabetes are at increased long-term risk 
for type 2 diabetes (5, 8), the impact of maternal metabolic abnormalities on early postnatal 
nutrition and infant metabolic trajectories is of considerable interest. Nonetheless, only a few 
studies have reported concentrations of metabolic hormones in human milk specifically from 
women with metabolic abnormalities in pregnancy (214). None have investigated the 
associations of metabolic milk hormones in colostrum (early human milk before onset of 
lactogenesis II), from women with metabolic abnormalities, with early metabolic 
development in infancy.

Maternal metabolic abnormalities and lactation

Women with obesity and/or diabetes often experience poor lactation performance (81, 151-
155). High prepregnancy body mass index (BMI) was associated with delayed onset of 
lactogenesis II (156). Delayed onset of lactogenesis II among overweight and obese women 
was explained by lower prolactin concentrations in response to suckling at 48 hours 
postpartum compared to normal-weight women (159). Delayed lactogenesis II among 
overweight women in addition to excessive gestational weight gain have been associated 
with the early cessation of breastfeeding (154, 157, 158). Studies which investigated milk 
composition of women with type 1 diabetes reported that the early milk samples had altered
lactose, citrate, and total nitrogen concentrations (160, 161). However, these studies were limited by small sample sizes which precluded adjustment for potential confounding variables including delivery complications, maternal behavior, and socioeconomic status (SES). In addition, these studies did not investigate concentrations of milk hormones that might be involved in metabolic regulation.

**Maternal metabolic abnormalities, breastfeeding and childhood obesity**

Although maternal metabolic abnormalities have been documented to affect offspring metabolic status (8), only a few studies have examined the impact of neonatal feeding practices by women with metabolic abnormalities on subsequent offspring obesity or diabetes (166, 169). In a study from Germany, infants were provided with human milk from their mothers with diabetes or donor human milk from women with normal glucose tolerance (166). Children who consumed a higher amount of human milk from mothers with diabetes during the first week of life were more likely to have a higher body weight at 2 years of age (166). Neither ingestion of human milk from women with diabetes during the first 2 to 4 weeks of life nor the duration of breastfeeding had an independent influence on risk of childhood overweight after adjusting for neonatal feeding practice during the first week of life (169). The authors, therefore, concluded that the first week of life was a critical window for nutritional programming of offspring who ingested milk from mothers with diabetes. Human milk composition during this first week of life, however, was not reported in this study. Therefore, it is unknown which components in human milk contributed to this difference.
**Metabolic hormones in human breast milk**

In addition to nutrients, human milk contains bioactive substances including metabolic hormones (33). A number of metabolic hormones, including leptin, adiponectin, and ghrelin (37, 39, 210, 213, 280-282), have been detected in human milk. In serum, these hormones regulate energy balance (176, 177) and obesity-induced inflammatory signaling pathways that contribute to type 2 diabetes (117).

Although several reports have been published on human milk leptin (210, 280, 283, 284), limited information is available on whether milk leptin concentrations vary from women across a spectrum of metabolic abnormalities in pregnancy, ranging from normal to obese/insulin resistant to the full range of glucose tolerance in pregnancy. Previous population-based longitudinal reports on the association of milk adiponectin with offspring anthropometric measures did not provide detailed metabolic characteristics of mothers nor did they study milk composition specifically in colostrum (220, 222). One study reported that ghrelin concentration in colostrum was reduced among women who had GDM, but the difference was normalized and not observed in mature milk (214). This study, however, was conducted in a small sample size and therefore the observation needs to be confirmed with a larger sample size. In addition, women who are obese and/or have diabetes are known to experience delayed onset of lactogenesis II (81, 154). Hence, offspring of mothers with metabolic abnormalities may be exposed to compromised early nutrition due to a lack of accessibility to human milk and/or to an altered composition of human milk (Figure 4.1).

With emerging evidence indicating that metabolic hormones are present in human milk, the impact of milk hormones on the early metabolic regulation and potential programming of energy balance requires an improved understanding. Since adiponectin and
leptin receptors are present in the human gastrointestinal tract (42, 285-287), it is conceivable that milk hormones may have a functional role locally via receptors in the stomach and intestine. The immaturity of the neonatal gut barrier may also allow for absorption and survival of various hormones from human milk, which would allow milk hormones to systemically influence metabolic development of the infant. Goldman (224, 225) and Koldovsky (226) have reviewed characteristics of the neonatal gastrointestinal tract that would allow for survival of human milk components in this setting, including delayed production of pancreatic proteases and gastric acid, the presence of antiproteases and inhibitors in human milk, and the possible existence of higher permeability in the neonatal gut to macromolecules. Disordered hormone concentrations in human milk during a critical period may lead to sub-optimal development of fundamental regulatory systems in offspring (234). For example, neural projection pathways from the arcuate nucleus of the hypothalamus are permanently disrupted in leptin-deficient mice (235). Among these mice, leptin treatment in adulthood did not reverse the defects, whereas the treatment in neonates preserved the development of these pathways (235). Optimal milk hormone levels may promote the normal development of hypothalamic pathways influencing energy homeostasis throughout life. Only a few studies have reported concentrations of metabolic hormones in human milk specifically from women with metabolic abnormalities in pregnancy (214). None have investigated the associations of metabolic milk hormones in colostrum, from women with metabolic abnormalities, with early metabolic development in infancy. We aim to address these knowledge gaps within the context of our ongoing mother and infant cohort studies. This study is conducted within a hospital setting which allows detailed characterization of clinical events during pregnancy and delivery in addition to lifestyle
information collected throughout late pregnancy and the first year postpartum through scheduled contacts with participants. The purpose of this report is to present the methodology and design of the study investigating the impact of maternal metabolic abnormalities in pregnancy on human milk hormones and subsequently on infant development over the first year of life.

**Study objectives**

Our overall aim is to investigate the impact of maternal metabolic status in pregnancy on breastfeeding, human milk composition, and infant development. The main specific objectives are as follows:

1. To investigate concentrations of metabolic hormones in colostrum of lactating women who had varying metabolic characteristics in pregnancy (i.e. a range of adiposity, subclinical inflammation, insulin resistance, beta-cell dysfunction, and glucose tolerance);
2. To compare metabolic hormone concentrations in colostrum with concentrations in mature breast milk at 3 months postpartum;
3. To study the associations of milk hormones in colostrum from mothers with varying metabolic characteristics with infant metabolic characteristics (i.e. adiposity and growth) over the first year of life; and
4. To investigate the associations of maternal metabolic abnormalities with delayed onset of lactogenesis II, defined as onset of copious milk secretion \(\geq 72\) hours postpartum.
4.3 Methods

4.3.1 Study setting and design

The current study is conducted within ongoing mother and infant prospective cohort studies investigating early events in the natural history of type 2 diabetes and vascular disease. These studies are being conducted at Mount Sinai Hospital and the Hospital for Sick Children in Toronto, Ontario, Canada (288-291). The current study protocol was approved by the Mount Sinai Hospital Research Ethics Board.

Standard obstetrical practice in Canada involves universal screening for GDM in all pregnant women at 24-28 weeks gestation by a glucose challenge test (GCT), wherein plasma glucose concentration is measured 1 hour after ingestion of a 50g glucose load (5). If the plasma glucose concentration is $\geq 7.8$ mmol/L, the patient is referred for a diagnostic oral glucose tolerance test (OGTT), in which plasma glucose values are measured while fasting and then hourly for 3 hours following ingestion of 100g of glucose. In clinical practice, the OGTT would typically only be ordered if the GCT were abnormal, whereas, in this study, the baseline pregnancy OGTT was completed in all participating women. In addition to the baseline OGTT visit, women were asked to participate in three follow-up visits and six telephone contacts from late pregnancy to one year postpartum (Figures 4.2).

4.3.2 Study participants and recruitment

Pregnant women were recruited in outpatient clinic waiting areas at Mount Sinai Hospital in Toronto, Canada, a large tertiary care centre where over 7000 pregnancies per year are followed. The inclusion criteria were singleton or twin pregnancy, aged 20 years or older at the time of recruitment, and intention to breastfeed. Women who reported to have pre-
existing diabetes were excluded. Written informed consents were collected from 271 pregnant women. A total of 55 participants were withdrawn from the study without completing the baseline visit. Forty seven of 55 were unable to visit the study testing unit during late pregnancy due to personal reasons including difficulty taking time off from work in addition to the time required for routine clinical obstetric visits. Eight of 55 women initiated the baseline visit but were unable to complete the 3-hour OGTT due to clinical reasons including nausea and vomiting. A total of 216 participants completed the baseline pregnancy characterization between April 2009 and July 2010.

4.3.3 Sample size justification

Sample size calculations were performed using PASS (Kaysville, UT). We estimated that baseline pregnancy characterization of between 210 and 220 women was required to address our four main specific objectives based on the following estimations.

To assess variation in metabolic hormones in colostrum from women with varying pregnancy metabolic characterization (objective 1), a sample size of 82 was required to provide 80% power to detect a slope of 0.10 at the significance level of 0.05; this sample size would allow detection of the correlation of 0.30. Based on previous literature, we expected that some women, especially overweight/obese women might experience delayed onset of lactogenesis II (153, 156, 157, 292), and therefore might have difficulty expressing colostrum samples. We considered that 50% of our participating women would be in overweight/obese pre-pregnancy BMI categories (i.e. BMI > 25 kg/m²) (288) and that 20% of normal weight women and 35% of overweight/obese women would experience delayed lactogenesis II (153,
156, 157, 292). After accounting for 15% loss to follow-up by the visit 2, a sample size of 135 was required to assess objective 1.

To test variation in metabolic hormone concentrations in colostrum and mature milk (objective 2), we considered that 25% of lactating women might discontinue providing human milk before 3 month postpartum based on previous local and national data (293, 294). After accounting for additional 10% loss to follow-up from visit 2 to 3, a sample size of 199 was required to assess objective 2.

To assess the impact of milk hormone concentrations on infant metabolic characteristics over the first year of life (objective 3), we accounted for additional 5% loss to follow-up from visit 3 to 4. This provided a sample size requirement of 210 women.

To study delayed onset of lactogenesis II from women with varying metabolic characteristics (objective 4), we aimed to complete pregnancy characterization for a sample that would provide 80% power at a 0.05 significance level to detect a difference in the outcome of delayed onset of lactogenesis II when the independent variable (e.g. BMI) was increased to one standard deviation above the mean. This change corresponded to an odds ratio of 1.8 in a logistic regression model with adjustment for other independent variables that themselves account for an R-Squared of 0.3 (or 30%). After considering a possible 20-30% overall loss to follow-up/missing data rate from baseline to the first week postpartum interview, we estimated that a sample size of 193-220 women was required to test objective 4.
4.3.4 Characterization of maternal metabolic status and data collection

Data collection for this prospective cohort study occurs during four study visits and six telephone contacts from late pregnancy to one year postpartum (Figure 4.2). Follow-up visits and interim telephone interviews are expected to be completed in October 2011.

Visit 1 (baseline): late second and early third trimester of pregnancy

Participating women underwent a 3-hour 100g OGTT in late pregnancy during which blood samples were drawn at fasting and at 30, 60, 120 and 180 minutes post-load. The OGTT on participating women in this study provides four categories of maternal glucose tolerance in pregnancy (288):

a. women with GDM defined as two or more of the following (50): fasting glucose ≥5.8 mmol/L, 1-hour blood glucose ≥10.6 mmol/L, 2-hour blood glucose ≥9.2 mmol/L, or 3-hour blood glucose ≥8.1 mmol/L;

b. women with an OGTT indicating gestational impaired glucose tolerance defined by exceeding only one of the above glycemic thresholds (288);

c. women with an abnormal GCT but a normal OGTT (i.e. none of the above criteria); and

d. women with a normal GCT and a normal OGTT.

Interviewers administered questionnaires to obtain information on demographic, medical history, smoking behavior, and physical activity using the Baecke instrument (288, 289). Dietary intake was collected using a modified Block food frequency questionnaire (FFQ) in which women were asked to recall usual intake during the second trimester of
pregnancy (295, 296). The Block FFQ has been validated in several populations (297, 298), including women in Ontario, Canada (299). The modified Block FFQ focuses on intake during the previous 3-month time period and has been validated within a pregnant population (23, 300). The questionnaire is designed to take approximately 30 minutes to complete and is self-administered. Women’s height and weight were measured following standardized anthropometric protocols (301).

**Phone contact: 36 weeks gestation**

Participating women are contacted at 36 weeks gestation via a telephone call. Participants are instructed to complete and return a modified Block FFQ recalling their usual dietary intake during the third trimester of pregnancy. Participants were provided with a FFQ in a postage-paid return-addressed envelop during the baseline visit for this purpose.

**Visit 2: the first 3 days postpartum**

Participating women and their neonates are visited in the hospital within the first 3 days postpartum. Infant anthropometry measurements are performed following standardized procedures (NHANES III Anthropometric Procedures) (302). For length, infants are placed supine on a measuring device, with a fixed headboard and a moveable footboard (Seca, Hamburg, Germany), and measured to the nearest 0.1 cm. Infants are weighed supine using an electronic infant scale (Medela BabyWeight; Medela Inc, Mississauga, Canada). For head and abdominal circumference, a non-stretchable, plasticized measuring tape is used to measure to the nearest 0.1 cm. The tape is wrapped around the head of the infant superior to the eyebrows and ears to assess the largest head circumference. For abdomen circumference,
the tape is wrapped around the abdomen above umbilicus with the infant in the supine position, and the expiration circumference is recorded. Three measurements are performed for each. Delivery and birth medical information of mothers and infants are obtained from a hospital clinical database.

Colostrum samples are collected by hand expression or an electric breastpump (Medela Electric Double Breastpump; Medela Inc, Mississauga, Canada), whichever is preferred by a participant, during their hospital stay. If the participating woman is unable to express milk during the hospital stay, a home visit is conducted by study personnel. Colostrum or an early human milk donation is accepted up until day 7 of infant life (first 24 hours is considered as day 0).

**Telephone interview: the first week of postpartum**

On day 3 postpartum, mothers are contacted via a telephone call (or visited in the hospital) to obtain information on the timing of onset of lactogenesis II and early postpartum infant feeding and supplementation behaviors. Mothers are asked to recall the nearest hour when they started to feel their “milk-coming-in”. Specifically, mothers are asked to recall the presence of a “tingling” feeling in the breast, fullness, and swelling. This technique for assessing onset of lactogenesis II by maternal perception has been validated with test weighing (303). If the mother has not experienced lactogenesis II by day 3 postpartum, the women is contacted again on day 7 postpartum (± 2 days). If the woman reports no “milk-coming-in” by day 7, the time to onset of lactogenesis II is recorded as greater than 7 days postpartum.
**Telephone interviews: 6 weeks postpartum**

Mothers are contacted at 6 weeks postpartum to obtain information on infant feeding, specifically, exclusivity and duration of breastfeeding and supplementation behaviors. Mothers are asked to recall dates of introduction to infant formula, cow’s milk, and other fluid and solids, if the event has taken place. If the mother reports to have discontinued breastfeeding, the last breastfeeding date and reasons for discontinuation are collected.

**Visit 3: 3 months postpartum**

Mothers and infants are invited to our research study unit for a follow-up assessment at 3 months postpartum (± 1 month). Mothers are asked to perform complete breast expression using a double electric breastpump. Mature human milk samples are collected for analysis. Mothers complete a 2-hour 75g OGTT. Interviewers administer questionnaires asking about parental SES status, paternal medical history, maternal postpartum physical activity/smoking, and infant feeding behaviors. A self-administered modified 3-month Block FFQ is completed asking mothers to recall their food intake during the first 3 months postpartum.

Infant anthropometric assessments are performed using standardized anthropometric procedures (NHANES III Anthropometric Procedures) (302), as described for visit 2. In addition, skinfold thickness measurements are performed using a skinfold caliper (Harpenden skinfold caliper; John Bull British Indicators Ltd, West Sussex, United Kingdom). Triceps, biceps, suprailiac, and subscapular skinfold thickness are measured on the right side of the body under standard conditions (304). Validation studies have shown that skinfold thickness measurements correlate with body fat assessed by direct measurements including dual-energy
x-ray absorptiometry in infants (305) and children (306). Three measurements are performed for each anthropometric variable.

**Telephone interviews: 5, 7 and 9 months postpartum**

Mothers are contacted at 5, 7 and 9 months postpartum via a telephone call and asked to recall infant feeding and supplementation behaviors as described for the 6 week phone interview.

**Visit 4: 12 months postpartum**

Mothers and infants are invited to the research study unit for a follow-up assessment at 12 months postpartum. Mothers complete a 2-hour 75g OGTT. Infant anthropometric assessments are performed using standardized anthropometric protocols as described for the 3 month postpartum assessments. Infants are weighed using an electronic infant scale appropriate for infants at 1-year (Seca, Hamburg, Germany). Infant feeding questionnaires are administered asking mothers to recall infant feeding and supplementation behaviors.

**4.3.5 Biochemical Procedures and Analyses**

Maternal serum samples are processed and analyzed for glucose and insulin concentrations according to established protocols at the Banting and Best Diabetes Centre laboratory, Mount Sinai Hospital, Toronto, Ontario, Canada. Aliquots of serum/plasma are prepared and frozen immediately at -80°C for additional assays (e.g. inflammatory markers, adipokines, and lipids). Insulin sensitivity and beta-cell function are assessed by validated indices derived from insulin and glucose measurements during the OGTT (307, 308).
For human milk samples, whole milk samples are divided into aliquots and stored at -80°C until analysis. Before performing assays to measure metabolic hormone concentrations, milk samples are sonicated to disrupt the membrane vesicles and to allow immunodetection of milk hormones because adipokines may be present in milk fat globules (309). Skim milk is prepared by centrifugation to separate milk fat from the liquid phase.

### 4.3.6 Statistical Analyses

Distributions of continuous variables will be assessed for normality, and transformations of skewed variables will be used in statistical analyses as appropriate. Descriptive statistics for continuous variables will be summarized as mean ± standard deviation or median (25th-75th percentile) for variables with a skewed distribution. Categorical variables will be summarized using proportions. Welch’s modified t test or Chi-Square test will be performed for continuous and categorical pairwise group comparisons as appropriate. Bivariate associations of continuous variables will be assessed using Spearman correlation analysis.

The following statistical analyses will be performed to address the main objectives. Multiple linear regression will be performed to assess study objectives 1, 2, and 3 with adjustment for potential covariates including maternal lifestyle, SES, and medical history. The main outcome and exposure variables and covariates for these models, by objective, are presented in detail in Table 4.1. For objective 1, the outcome variable will be metabolic hormone concentrations in colostrum, while the exposure variables will be maternal pregnancy metabolic characteristics. For objective 2, the outcome variable will be concentrations of metabolic hormones in mature breast milk at 3 months postpartum, while the exposure variables will be concentrations of metabolic hormones in colostrum adjusting
for covariates including maternal metabolic characteristics at 3 months postpartum. For objective 3, the outcome variable will be metabolic characteristics of offspring during the first year of life, while the main exposure variables will be concentrations of metabolic hormones in colostrum adjusting for potential covariates including maternal gestational metabolic characteristics. This covariate adjustment will enable us to assess the associations between milk hormones and offspring metabolic characteristics independent of maternal gestational metabolic variables. Other potential confounders to be evaluated in these models are listed in Table 4.1. For study objective 4, multiple logistic regression will be performed with potential adjustment for covariates including maternal lifestyle, SES, and medical history. In these models, the outcome variable will be defined as the presence of delayed lactogenesis II; the main exposures will be maternal metabolic abnormalities (i.e. obesity, hyperglycemia, insulin resistance) (Table 4.1).

4.4 Discussion

We anticipate that this study will allow us to investigate the impact of maternal metabolic characteristics in pregnancy on breastfeeding and infant development. An improved understanding of maternal metabolic abnormalities and infant nutrition may assist in developing prevention and intervention strategies which will protect vulnerable offspring exposed to suboptimal nutrition.

The main challenge of this study is that we have a narrow biological window of time to collect colostrum samples. We, therefore, have developed a computerized notification protocol to alert the study personnel when participants are admitted to the hospital maternity unit and delivered their infants. However, it is expected that some women may experience
difficulty expressing and donating milk samples during the hospital stay. We have considered this aspect into our sample size power calculation. In addition, we have also developed an additional step within our protocol to visit mothers at home to increase the opportunity to collect early human milk samples within the first week postpartum. The strength of the study is that it will yield novel insights into the impact of maternal metabolic abnormalities on human milk hormones and subsequently on the offspring’s early metabolic trajectory. This study may assist in developing optimal strategies to support the development of nutritionally vulnerable offspring exposed to maternal metabolic abnormalities and, therefore, reducing risk for obesity, type 2 diabetes, and vascular disease later in life.
Figure 4.1 Conceptual model
Figure 4.2 Schedule of four assessment visits and interim telephone calls from late pregnancy to 1-year postpartum
Table 4.1 Summary of statistical analysis plans

<table>
<thead>
<tr>
<th>Objective</th>
<th>Statistical technique</th>
<th>Outcome variable</th>
<th>Main exposures</th>
<th>Covariates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Linear regression</td>
<td>concentrations of metabolic hormones in colostrum (e.g. adiponectin)</td>
<td>maternal metabolic characteristics (e.g., glucose intolerance, insulin resistance, obesity)</td>
<td>maternal lifestyle, SES, medical history</td>
</tr>
<tr>
<td>2</td>
<td>Linear regression</td>
<td>concentrations of metabolic hormones in mature breast milk at 3 months postpartum</td>
<td>concentrations of metabolic hormones in colostrum</td>
<td>maternal lifestyle, SES, medical history, maternal metabolic characteristics</td>
</tr>
<tr>
<td>3</td>
<td>Linear regression</td>
<td>metabolic characteristics in offspring (e.g. infant growth, adiposity) during the first year of life</td>
<td>concentrations of metabolic hormones in colostrum</td>
<td>maternal lifestyle, SES, medical history, maternal metabolic characteristics, infant feeding / supplementation behaviors</td>
</tr>
<tr>
<td>4</td>
<td>Logistic regression</td>
<td>delayed onset of lactogenesis II</td>
<td>maternal metabolic characteristics (e.g., glucose intolerance, insulin resistance, obesity)</td>
<td>maternal lifestyle, SES, medical history</td>
</tr>
</tbody>
</table>

Abbreviations: SES, socioeconomic status
Chapter 5

Effect of macronutrient intake during the second trimester on glucose metabolism later in pregnancy

Contents of this chapter have been published in The American Journal of Clinical Nutrition.


A link to the published manuscript can be found at

[http://www.ajcn.org/content/94/5/1232.long](http://www.ajcn.org/content/94/5/1232.long)
5.1 Abstract

**Background**: Dietary intake is known to influence glucose metabolism, but there is little consensus on the optimal distribution of macronutrient intake during pregnancy for gestational diabetes (GDM) prevention.

**Objective**: We aimed to investigate whether the macronutrient intake distribution during the second trimester of pregnancy was associated with glucose metabolism later in pregnancy.

**Design**: Women with singleton pregnancies and without pre-existing type 1 or type 2 diabetes were included. Participants underwent a 3-hour oral glucose tolerance test at 30 (95% CI 25, 33) weeks gestation and were asked to recall their second trimester dietary intake using a validated food frequency questionnaire.

**Results**: Of 205 participants, 47 (22.9%) were diagnosed with GDM. Higher % calorie intake of saturated fat (beta±SE 0.059±0.021, p=0.005), % trans fat (0.381±0.145, p=0.009), and added sugar (0.017±0.007, p=0.02) and lower vegetable and fruit fiber (-0.026±0.012, p=0.03) were individually associated with increased fasting glucose after multiple adjustment. Among those with a family history of type 2 diabetes, higher vegetable and fruit fiber intake was associated with reduced insulin resistance (-0.100±0.029, p=0.0008) and increased insulin sensitivity (0.029±0.012, p=0.01) with similar adjustment. Lower % carbohydrate and higher % total fat were associated with increased GDM risk (odds ratio per 1-SD change 0.60 [95% CI 0.40, 0.90] and 1.61 [1.06, 2.44], respectively) with similar adjustment.

**Conclusions**: Macronutrient intake during the second trimester of pregnancy was associated with risk for abnormal glucose metabolism later in pregnancy. This finding supports the need for continued work to determine optimal prenatal nutritional strategies to prevent GDM.
Please go to the journal website to read the contents of Chapter 5.

http://www.ajcn.org/content/94/5/1232.long
Chapter 6

Associations of prenatal metabolic abnormalities with insulin and adiponectin in human milk

Contents of this chapter have been published in The American Journal of Clinical Nutrition.


A link to the published manuscript can be found at

http://www.ajcn.org/content/early/2012/02/28/ajcn.111.028431.long
6.1 Abstract

**Background:** Emerging evidence indicates that metabolic hormones are present in human milk, but no studies have investigated the impact of maternal metabolic status assessed during pregnancy on insulin and adiponectin concentrations in milk.

**Objectives:** We aimed to investigate the associations of prenatal metabolic abnormalities with insulin and adiponectin in human milk and to compare the concentrations of these hormones in early and mature milk.

**Design:** Pregnant women aged ≥20 years, with intention to breastfeed and without pre-existing type 1 or type 2 diabetes were recruited. Participants (n=170) underwent a 3-hour oral glucose tolerance test at 30 (95% CI 25, 33) weeks gestation and donated early (the first week) and mature (3 months postpartum) milk.

**Results:** Adiponectin and insulin concentrations in early milk were higher compared to those in mature milk (both p<0.0001). Prenatal metabolic abnormalities including higher pregravid BMI (beta±SEE 0.053±0.014, p=0.0003), in addition to gravid hyperglycemia (0.218±0.087, p=0.01), insulin resistance (0.255±0.047, p<0.0001), lower insulin sensitivity (-0.521±0.108, p<0.0001), and higher serum adiponectin (0.116±0.029, p<0.0001) were associated with higher insulin in mature milk with covariate adjustment. Prenatal metabolic measures were not associated with milk adiponectin, but obstetrical measures including nulliparity (0.171±0.058, p=0.004), longer duration of gestation (0.546±0.146, p=0.0002), and unscheduled caesarean section (0.387±0.162, p=0.02) were associated with higher adiponectin in early milk with covariate adjustment including the time elapsed from delivery to milk collection.
**Conclusions:** Maternal prenatal metabolic abnormalities are associated with high insulin concentrations in mature milk, while only obstetrical parameters are associated with adiponectin concentrations in early milk.

Please go to the journal website to read the contents of Chapter 6.

[http://www.ajcn.org/content/early/2012/02/28/ajcn.111.028431.long](http://www.ajcn.org/content/early/2012/02/28/ajcn.111.028431.long)
Chapter 7

Effects of pasteurization on adiponectin and insulin concentrations in donor human milk

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A link to the published manuscript can be found at
http://www.nature.com/pr/journal/v70/n3/full/pr2011172a.html
7.1 Abstract

Objective: Although pasteurization is recommended before distributing donor human milk in North America, limited data are available on its impact on metabolic hormones in milk. We aimed to investigate the effects of pasteurization on adiponectin and insulin concentrations in donor human milk.

Study design: The study investigates concentrations of components in donor human milk before and after Holder pasteurization. Following the guidelines of the Human Milk Bank Association of North America, human milk samples were pooled to produce 17 distinct batches (4 individuals per batch) and pasteurized at 62.5°C for 30 minutes. Adiponectin, insulin, energy, fat, total protein and glucose concentrations were measured pre- and post-pasteurization.

Results: Pasteurization reduced milk adiponectin and insulin by 32.8% and 46.1%, respectively (both p<0.0001). Adiponectin and insulin were significantly correlated with energy and fat milk composition (r=0.36-0.47; all p<0.05). Pasteurization effects on milk hormone concentrations remained significant after adjusting for fat and energy (beta±SE -4.11±1.27, p=0.003 for adiponectin; -70.0±15.0, p<0.0001 for insulin).

Conclusions: Holder pasteurization reduced adiponectin and insulin concentrations in donor human milk. In view of emerging knowledge on the importance of milk components, continued work to find the optimal pasteurization process which mitigates risks but promotes retention of bioactive components is needed.
7.2 Introduction

The epidemic in childhood obesity is a major public health challenge which is reflected in the rapidly increasing rates of youth onset type 2 diabetes (274, 275). Epidemiological evidence has shown a protective effect of breastfeeding on obesity (30, 31) and type 2 diabetes later in life (32). Human milk contains not only macro and micronutrients but also an array of bioactive substances including insulin (33, 34). More recently, adiponectin, an insulin-sensitizing hormone in serum (198), has been detected in human milk (37-39). Therefore, these metabolic hormones may explain the observed association of breastfeeding with reduced risk for metabolic disease in offspring later in life (223).

With improved knowledge about the benefits of breastfeeding, there has been increasing demand for donor milk when mother’s own milk is not available (240). To protect recipients against disease transmission, donor milk undergoes safety screening and handling processes (238, 251, 261, 262). These processes, however, vary among countries: Donor milk is pasteurized at 62.5°C for 30 minutes (Holder method) before distribution in North America and the United Kingdom (238, 262), whereas donor milk is pasteurized at a lower temperature or unpasteurized in other countries (251, 261). While various nutrient components remain intact after pasteurization, the pasteurization process is known to effect biological activity of a number of milk components (239). Although the concentrations of adiponectin and insulin have been reported in human milk previously (37, 38), the effect of pasteurization on these metabolic hormones has not been investigated. With recent evidence from a randomized controlled study demonstrating that dietary intervention in infancy has long-term effects on beta-cell autoimmunity (272), it is important to understand the impact of pasteurization on milk metabolic hormones which may have a critical impact on infant...
metabolic trajectories (40, 48). We therefore aimed to investigate the effects of Holder pasteurization on adiponectin and insulin concentrations in donor human milk.

7.3 Methods

The study was approved by the Hospital for Sick Children Ethics Review Committee, and informed consent was obtained from all milk donors. The inclusion criteria of donor milk samples were that milk needed to be expressed at more than one month postpartum and less than one year. This was to avoid inclusion of colostrum and transitional milk samples and milk from an involuting mammary gland. Donor milk from thirty-four women, frozen immediately and stored at -20°C for less than 6 months, were used for the current analysis. After thawing individual milk samples in a water bath at 37.5°C, milk was pooled and processed following the guidelines of the Human Milk Bank Association of North America (238). Distinct batches of 17 pooled samples were produced with each batch containing milk from four women. Samples were divided into two sets of 17 batches to assess pre- and post-pasteurization effects. Samples for post-pasteurization analysis were processed in a Breast Milk Pasteurizer (T30/USA, Sterifeed, Medicare Colgate Ltd, UK) which involved submerging bottles into a preheated water bath (63.2°C) followed by a cool water bath (<9°C). A temperature probe was positioned in a centrally placed non-sample bottle to ensure milk samples were maintained at 62.5°C for 30 minutes. Pre- and post-pasteurization samples were aliquoted and stored at -80°C until biochemical analysis.
**Biochemical analysis and validation of adiponectin assay**

Adiponectin concentration was measured using a radioimmunoassay (Millipore, Linco Research, MO, USA). This assay has an interassay coefficient of variation of 9.3% at 7.5µg/l. To validate assay methods for adiponectin, whole milk samples were spiked with 5, 10, 20, or 40ng/mL human adiponectin standards (Millipore, Linco Research, MO, USA) to determine the recovery of the added volume. To account for sample dilution effects due to the added spiking volume, an equivalent volume of physiological saline containing 0ng/mL adiponectin was added to control non-spiked samples. Therefore, each adiponectin spiked sample had its own control designated as baseline. To assess whether lipids interfered with the adiponectin assay, 12 non-spiked study samples (6 pre- and 6 post-pasteurization pooled batches) were assayed using both whole and skim milk samples. Skim milk samples were centrifuged (3000 revolutions per minute for 15 minutes), and the fat layer was removed.

Insulin concentration in skim milk was measured using the electrochemiluminescence immunoassay (Roche Modular Analytics E170, NJ, USA). This assay shows 0.05% cross-reactivity to intact human proinsulin and the primary circulating split form (des 31, 32). Total energy was determined by bomb calorimetry using the 1241 Automatic Adiabatic Calorimeter (Parr Instrument Company, IL, USA) according to the method described by Garza et al (326). Total fat was determined using the Creamatocrit methodology described by Lucas et al (324). Total protein was measured using the Bicinchoninic Acid protein assay kit (Sigma, MO, USA) (323). Glucose concentration in skim milk was determined using the hexokinase enzymatic method (Roche Modular Analytics E170, NJ, USA). All study sample assays including nutrient compositions were performed on two sets (pre- and post-pasteurization) of 17 pooled batches.
Statistical analysis

Data analyses were performed using SAS software, version 9.2 (SAS Institute, NC, USA) and with the consideration of two-sided p<0.05 as statistically significant for all analyses. Distributions of continuous variables were assessed for normality and were determined to follow a normal distribution.

Descriptive statistics for continuous variables were summarized as mean ± SD. Concentrations of milk components pre- and post-pasteurization were compared using paired t tests. To assess correlations of adiponectin and insulin with potential covariate milk components, Spearman rank correlation analysis was performed.

To evaluate effects of pasteurization as a main exposure on the outcomes of adiponectin and insulin with adjustment for potential covariates, we used mixed model analysis which is similar to analysis of covariance except the analysis accounts for correlation within the batches related to the repeated measure design. Three models were tested for each outcome variable: 1) an unadjusted model; 2) a model adjusted for total fat; and 3) a model adjusted for total fat and energy.

7.4 Results

Average recovery rates of spiked adiponectin from whole and skim milk samples were 105.5 ± 18.2% (mean ± SD) and 118.4 ± 36.0%, respectively. Non-spiked adiponectin concentrations of whole and skim milk samples were highly correlated (r_{spearman}=0.90 and r_{pearson}=0.97, both p<0.0001) (Figure 7.1), and mean adiponectin concentrations of whole (12.3 ± 6.1 ng/mL) and skim (12.1 ± 5.4) milk were not significantly different. Based on
these similar findings, adiponectin concentrations in study milk samples were measured using whole milk.

Pasteurization reduced the concentration of milk adiponectin by 32.8% (pre and post mean ± SD: 13.9 ± 4.8 v. 9.3 ± 3.0 ng/mL; p<0.0001) and insulin by 46.1% (162.8 ± 64.2 v. 87.8 ± 26.3 pmol/L; p<0.0001), while changes in energy, total fat, total protein and glucose concentrations were modest, ranging 0-8.9% (Table 7.1).

As assessed by Spearman correlation, adiponectin was significantly correlated with energy and fat composition in milk (r=0.47 and 0.41, respectively; both p<0.05), but not with protein and glucose (r=0.04 and -0.08, respectively). Similarly, insulin was significantly correlated with energy and fat composition in milk (r=0.41 and 0.36, respectively; both p<0.05), but not with protein and glucose (r=-0.21 and 0.09, respectively).

The effects of pasteurization as a main exposure on the outcome of adiponectin or insulin were assessed with adjustment for significantly correlated potential covariates. Pasteurization effects on milk hormone concentrations remained significant after adjusting for fat and energy and accounting for correlations within the same batches (beta ± SE: -4.11 ± 1.27, p=0.003 for adiponectin; -70.0 ± 15.0, p<0.0001 for insulin) (table 2).

7.5 Discussion

We report that Holder pasteurization recommended in North America reduced adiponectin and insulin concentrations in donor human milk. Pasteurization effects on milk hormone concentrations remained significant with adjustment for potential covariates including milk fat.
To prevent transmission of bacteria and pathogens including human immunodeficiency virus and human T-lymphotropic virus, donor milk banks have implemented safety screening and handling processes (238, 251, 261, 262). While donor milk is Holder pasteurized before distribution in North America and the United Kingdom (238, 262), some countries have implemented more detailed screening processes followed by a lower temperature pasteurization or no pasteurization (251, 261). Although a number of nutrients are unaffected by pasteurization (239), the pasteurization process deactivates or reduces the activity of several bioactive components in human milk including immunological proteins (239, 266). Our results, which demonstrate that pasteurization reduced adiponectin and insulin concentrations in donor milk, raise concerns considering these hormones may have an important role in the metabolic development of infants (40, 48). While the molecular mechanism by which these metabolic hormones in milk may provide protection against developing metabolic disease later in life is not completely understood, potential mechanisms and physiological roles of these hormones influencing infant metabolic trajectories have been reviewed (40, 48, 223). Evidence indicates that oral administration of insulin stimulates gut maturation (40) and that adiponectin receptors are present in the fetal small intestine (48). These milk metabolic hormones, therefore, may have a direct role in the optimal metabolic development of infants and subsequently in reducing susceptibility to future metabolic disease. This may be especially important for the primary recipients of donor milk, preterm very low birth weight infants who are at increased risk for insulin resistance and type 2 diabetes later in life (44-46).

In human serum, high-molecular weight (HMW) adiponectin is known to be the most biologically active form which has stronger associations with diabetes risk than total
adiponectin (198). It must be noted that we used the total adiponectin assay which is not specific to the HMW form of adiponectin. Adiponectin in human milk, however, has been reported to be almost entirely composed of the HMW form (48). We also note that the heat treatment at 70°C for 10 minutes denatures adiponectin from trimeric to monomeric forms (200). Therefore, it is possible that Holder pasteurization might have reconfigured the adiponectin molecule such that it was unrecognizable to the assay. This unrecognizable molecule, however, is likely to be also non-functional. If this non-functional molecule in pasteurized milk was detected by the assay, pasteurized milk would have even greater reduction in its bioactivity. However, the functional analysis was not performed in the current study which is another limitation of the study. In addition, we report the pasteurization effects on donor milk components, but not other donor milk handling processes including freeze-thaw cycles. Previously, Bronsky et al (38) reported a strong correlation between adiponectin concentrations before and after two freeze-thaw cycles (r=0.894, p<0.0001). Although Holder pasteurization is likely a major attributor that alters adiponectin and insulin concentrations, we cannot conclude that the impact of the overall donor milk processing protocol on milk composition is not greater than the pasteurization effects reported here. We also cannot separate effects of each pasteurization step, including heat treatment and container changes, on concentrations of milk components based on our results. Although the magnitude of changes in pre- and post-pasteurization fat and glucose concentrations were small, they were significant. We speculate that the fat content might have been reduced during the donor milk handling process as a result of multiple container transfers. As for glucose concentrations, the pasteurization process might have caused molecular reconfigurations exposing more glucose molecules to be recognized by the assay.
These hypotheses, however, were not tested in the current study. The strength of our report is that we investigated the practical impact of Holder pasteurization on donor milk following the standardized processing protocol (238).

In conclusion, Holder pasteurization, currently recommended by the Human Milk Banking Association of North America, reduced adiponectin and insulin concentrations in donor human milk. Since insulin and adiponectin are known to be involved in the pathophysiology of diabetes (198), the impact of the reduction in these milk hormones on susceptibility for developing type 2 diabetes later in life warrants further investigation. Variation in heat treatment options including a reduction in the length of pasteurization has been shown to preserve insulin-like growth factors (271). In addition, reducing pasteurization temperature to 57°C improved immunological protein retention while effectively removing 99.9% of inoculated bacterial species (266). With recent evidence from a randomized controlled study demonstrating long-term benefit of dietary intervention in infancy (272), it is important to understand the effect of pasteurization on milk components which may have a critical impact on infant metabolic trajectories (40, 48). In view of emerging knowledge on the importance of milk components on human health outcomes, comprehensive risk and benefit assessments to find the optimal pasteurization process which mitigates risks but promotes retention of bioactive components is needed.
Table 7.1  Concentrations of human milk components pre- and post-pasteurization\(^1\)

<table>
<thead>
<tr>
<th>Component</th>
<th>Mean ± SD</th>
<th>Mean of differences ± SE</th>
<th>P</th>
<th>% Observed change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>pre</td>
<td>post</td>
<td></td>
</tr>
<tr>
<td>Adiponectin, ng/mL</td>
<td>13.91 ± 4.84</td>
<td>9.34 ± 2.96</td>
<td>-4.57 ± 0.61</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Insulin, pmol/L</td>
<td>162.8 ± 64.2</td>
<td>87.8 ± 26.3</td>
<td>-74.9 ± 11.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Energy, Kcal/dL</td>
<td>71.5 ± 9.9</td>
<td>69.4 ± 8.8</td>
<td>-2.2 ± 1.5</td>
<td>0.17</td>
</tr>
<tr>
<td>Fat, g/L</td>
<td>4.29 ± 0.95</td>
<td>3.91 ± 0.81</td>
<td>-0.38 ± 0.15</td>
<td>0.02</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>0.97 ± 0.25</td>
<td>1.11 ± 0.22</td>
<td>0.13 ± 0.03</td>
<td>0.0007</td>
</tr>
<tr>
<td>Protein, g/L</td>
<td>14.8 ± 1.5</td>
<td>14.8 ± 1.1</td>
<td>-0.02 ± 0.37</td>
<td>0.97</td>
</tr>
</tbody>
</table>

\(^1\)Abbreviations: SD, standard error; SE, standard error of the mean. Paired t test was performed.
Table 7.2  Milk adiponectin and insulin concentrations pre- and post-pasteurization

<table>
<thead>
<tr>
<th></th>
<th>Adiponectin (95% CI), ng/mL</th>
<th>Insulin (95% CI), pmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>beta±SE</td>
<td>pre</td>
</tr>
<tr>
<td>1</td>
<td>-4.57±1.36</td>
<td>14.8 (11.9-17.6)</td>
</tr>
<tr>
<td>2</td>
<td>-4.03±1.30</td>
<td>15.0 (12.2-17.9)</td>
</tr>
<tr>
<td>3</td>
<td>-4.11±1.27</td>
<td>14.5 (11.8-17.1)</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; SE, standard error of the mean. Mixed models were used accounting for correlations within the same batches. Models are 1) unadjusted, 2) adjusted for total fat, and 3) adjusted for total fat and energy.
Chapter 8

Discussion and conclusions
8.1 Summary of findings

The overall objective of this thesis was to investigate the association of maternal macronutrient intakes with glucose metabolism during pregnancy and its subsequent impact on metabolic hormones in human milk. We report here that macronutrient intake during pregnancy was associated with risk for abnormal glucose metabolism later in pregnancy. In addition, maternal prenatal metabolic abnormalities were associated with high insulin concentrations in mature milk. We tested three specific hypotheses in this thesis:

**Hypothesis 1:** The sub-optimal distribution of macronutrient intakes during the second trimester of pregnancy will be associated with abnormal glucose metabolism (i.e. gestational diabetes [GDM], hyperglycemia, insulin resistance, lower insulin sensitivity, and beta-cell dysfunction) later in pregnancy (Figure 8.1).

The first hypothesis was tested in Chapter 5. Higher vegetable and fruit fiber intake in the second trimester of pregnancy was associated with reduced insulin resistance and increased insulin sensitivity later in pregnancy among those with a family history of type 2 diabetes after adjustment for potential confounders. In addition, lower % carbohydrate and higher % total fat were associated with increased GDM risk with covariate adjustment. Therefore, we concluded that the distribution of macronutrient intakes during the second trimester of pregnancy was associated with risk for abnormal glucose metabolism later in pregnancy.

**Hypothesis 2:** Maternal metabolic status in pregnancy (i.e. overweight, insulin resistance, lower insulin sensitivity, and glucose intolerance) will be associated with variation in insulin
and adiponectin concentrations in early (first week of life) and mature milk (3 months postpartum) (Figure 8.1).

The second hypothesis was tested in Chapter 6. Prenatal metabolic abnormalities including higher pregravid body mass index, in addition to gravid hyperglycemia, insulin resistance, lower insulin sensitivity, and higher serum adiponectin assessed in late pregnancy were associated with higher insulin in mature milk after adjustment for covariates. Prenatal metabolic measures were not associated with milk adiponectin, but obstetrical measures including nulliparity, longer duration of gestation, and unscheduled cesarean section were associated with higher adiponectin in early milk with covariate adjustment. Therefore, we concluded that maternal prenatal metabolic abnormalities were associated with high insulin concentrations in mature milk, while only obstetrical parameters were associated adiponectin concentrations in early milk.

**Hypothesis 3**: Pasteurization using the Holder method (62.5°C for 30 minutes), the current standard processing donor human milk for human consumption in North America, will reduce the concentrations of insulin and adiponectin in milk (Figure 8.1).

The third hypothesis was tested in Chapter 7. Holder pasteurization reduced adiponectin and insulin concentrations in donor human milk by 32.8% and 46.1%, respectively. Pasteurization effects on milk hormone concentrations remained significant after adjusting for fat and energy and accounting for correlated observations within the same batches.
Therefore, we concluded that adiponectin and insulin concentrations in milk were reduced by Holder pasteurization, which is used in North America before distributing donor human milk.
Figure 8.1  Conceptual model of tested research hypotheses
8.2  General discussion

8.2.1  Macronutrient intake and glucose metabolism during pregnancy

Since evidence from observational studies and clinical trials indicates that macronutrient intake influences glucose metabolism (16), it would be of interest to intervene on and prevent GDM through diet. However, the American Diabetes Association and the American Congress of Obstetricians and Gynecologists currently provide no specific guidance on the optimal macronutrient intake distribution ranges to manage GDM (137, 138). The Canadian Diabetes Association recommends consuming ≤35% of energy from fat and 45-60% from carbohydrate for diabetes management in general (5), while others suggest higher fat (≥40%) and lower carbohydrate (<40%) intakes for women with GDM (136).

In addition to a lack of clarity in dietary guidelines for the management of GDM, evidence regarding optimal prenatal nutrition to prevent GDM has been limited (23, 24). In Chapter 5, lower % carbohydrate and higher % total fat were associated with increased GDM risk with covariate adjustment. Similar to our results, the Pregnancy, Infection, and Nutrition Study reported that substituting fat for carbohydrate (per each 1% of total calories) during the second trimester resulted in increased relative risk of GDM of 1.1 (95% CI 1.02, 1.10) (23). The authors further demonstrated that a macronutrient intake distribution of 30% fat and 50% carbohydrate resulted in an almost 50% reduction in the predicted probability of GDM compared to a 40% fat and 40% carbohydrate diet (23). However, in Project Viva, another cohort study from United States, distributions of dietary fat and carbohydrate intakes in early pregnancy were not associated with GDM (24). In this study, the authors assessed dietary intake from the time of conception (last menstrual period) to a mean of 12 weeks of gestation using a Food Frequency Questionnaire (24, 319). The dietary intake reported in Project
Viva, therefore, reflected the diet during the first trimester of pregnancy, while the Pregnancy, Infection, and Nutrition study and our investigation in Chapter 5 captured second trimester intake. It is possible that the inconsistent findings yielded among these studies might be explained by differences in the impact of diet on GDM risk according to trimester. It must also be noted that the metabolic effects of exchanging fat for carbohydrate are complex because different types of fat and carbohydrate may have different effects on glucose metabolism (16).

In addition to the association of total dietary fat with increased risk for glucose intolerance (16), dietary fat compositions including higher trans fat and lower polyunsaturated fat have been individually associated with risk of type 2 diabetes (90). In Chapter 5, high % saturated fat and % trans fat intakes during the second trimester of pregnancy were associated with hyperglycemia later in pregnancy. Similar to our results, diets higher in saturated fat and lower in polyunsaturated fat and the ratio of polyunsaturated-to-saturated fat have been individually associated with glucose intolerance in other pregnant populations (25, 126, 127). In addition, higher saturated fat intake was associated with a higher fasting insulin concentration during pregnancy after adjustment for body mass index in a study from Northern Italy (25). In a randomized controlled dietary intervention study among 162 men and women aged 30-65 years (92), insulin sensitivity was significantly reduced among individuals who received a diet 17% saturated and 37% total fat for 3 months. Our results, however, indicated that slightly higher % fat intakes might be beneficial to improved insulin sensitivity among those without a family history of type 2 diabetes, while this association was not present among those with a family history. Our results further indicated that higher postprandial glucose response assessed using the total area under the
glucose curve was significantly associated with lower % carbohydrate, higher % total fat, and higher % saturated fat intakes among those with a family history of diabetes, while this was not present among those without a family history. Therefore, the distribution of macronutrient intake during pregnancy may have different metabolic effects on healthy women free of a family history of diabetes compared to those who are predisposed based on a positive family history. Genetic variation might influence the observed nutrient and metabolic abnormality associations (6, 68), but we did not have relevant genetic information nor an appropriate sample size to further investigate. We recommend that future studies on maternal nutrition and glucose metabolism during pregnancy include genotyping of participants to understand the impact of genetic predisposition on these associations of nutrients with metabolic abnormalities.

In Chapter 5, higher vegetable and fruit fiber intake during the second trimester of pregnancy was associated with reduced insulin resistance and increased insulin sensitivity later in pregnancy among those with a family history of type 2 diabetes after adjustment for potential confounders. Similar to our findings, women who consumed lower total, cereal and fruit fiber prior to pregnancy were at increased risk for GDM in the Nurses’ Health Study II (99). A number of prospective cohort studies have reported reduced risk of developing type 2 diabetes among individuals who consumed higher intakes of total fiber (20-22) and cereal fiber (20, 21, 97). The mechanism by which dietary fiber may impact glucose intolerance is not entirely clear. It has been proposed that dietary fiber may act to reduce the rate of carbohydrate absorption and subsequently to lower the postprandial glycemic and insulinemic responses (100, 101). Intervention studies using the euglycemic-hyperinsulinemic clamp technique have demonstrated that insulin sensitivity was higher
among individuals who received a high fiber treatment for 4-6 weeks (94, 95). Insulin resistance assessed using the homeostasis model assessment of insulin resistance was also lower among higher fiber consumers (94). Therefore, improved insulin sensitivity might be an important mechanism whereby higher fiber intake might influence glucose metabolism and subsequently reduce the development of diabetes.

### 8.2.2 Metabolic hormones in human milk

The World Health Organization recommends breastfeeding as the ideal food source for the growth and development of infants and an integral part of the reproductive process (171). Breastfeeding has been shown to have beneficial effects on short- and long-term maternal and infant health outcomes (26). Although the recommendations for optimal breastfeeding practices continue to be revisited (236), evidence linking maternal metabolic abnormalities with human milk components is limited. In addition to nutrients, a number of metabolic hormones including insulin, adiponectin, leptin, and ghrelin are present in human milk (33, 34, 37, 210, 213). In serum, these hormones regulate energy balance (176, 177) and obesity-induced inflammatory signaling pathways which contribute to type 2 diabetes (117).

It has been previously reported that ghrelin concentrations in colostrum at 2 days postpartum were lower among women who had GDM compared to those without diabetes, but the difference was not observed in mature milk (214). This study suggests that the offspring of mothers who experience metabolic abnormalities during pregnancy might be exposed to compromised early nutrition. This study, however, was limited by a small sample size (GDM, n=12; no diabetes, n=14) which precluded adjustment for potential confounders. Since metabolic milk hormone concentrations during the first week postpartum vary
considerably (34), variation in the time elapsed from delivery to milk collection might have influenced the differences reported. After an investigation of a larger number of colostrum samples in Chapter 6, we report that metabolic status did not influence insulin and adiponectin concentrations in early milk after adjustment for multiple confounders including the time elapsed from delivery to milk collection. Adiponectin in early milk, however, was associated with obstetrical parameters in our investigation. Since women with diabetes have been shown to experience higher rates of delivery complications including cesarean section (5), it is possible that women with GDM in the previous investigation might have experienced obstetrical complications, thereby indirectly influencing metabolic hormone concentrations in milk. However, obstetrical parameters were not reported in that study (214).

In another study, infants were supplemented with donor human milk from women with normal glucose tolerance when their own mothers with diabetes were not able to provide an adequate amount of milk. Children of mothers with diabetes who consumed a higher amount of their own mothers milk (type 1 diabetes, n=83; GDM, n=29) during the first week of life were more likely to have a higher body weight at 2 years of age (166). The authors suggested that human milk ingested during the first week of life might have a critical impact on the nutritional programming in offspring. However, specific components in human milk were not investigated in this study (166). In our investigation in Chapter 6, maternal prenatal metabolic abnormalities were associated with high insulin concentrations in mature milk at 3 months postpartum. It is, therefore, possible that the disordered milk composition in mature milk might have an impact on the offspring development beyond the first week of life. However, the effect of milk insulin concentrations on offspring was not
investigated in Chapter 6, and, therefore, further research is warranted to determine its impact on offspring. However, it must be also noted that findings from the study of donor human milk supplementation by Plagemann et al (166) are difficult to interpret and generalize to other practice settings since donor milk handling processes can alter concentrations of milk components (167, 168).

In addition to the offspring exposed to maternal metabolic abnormalities, preterm very low birth weight infants, the primary recipients of donor human milk, are also at increased risk for insulin resistance and type 2 diabetes later in life (44-46). To prevent transmission of bacteria and pathogens including human immunodeficiency virus and human T-lymphotropic virus, donor milk banks have implemented safety screening and handling processes (47, 251, 261, 262). In North America, donor milk is Holder pasteurized before distribution (47). Although a number of nutrients are unaffected by pasteurization (239), the pasteurization process deactivates or reduces the activity of several bioactive components in human milk including immunological proteins (239, 266). Our results in Chapter 7, which demonstrate that pasteurization by the Holder method reduced adiponectin and insulin concentrations in donor milk, raise concerns considering these hormones may have an important role in the metabolic development of infants (40, 48).

While the molecular mechanisms through which these metabolic hormones in milk may provide protection against development of obesity and type 2 diabetes in offspring later in life are not completely understood, the potential physiological roles of these hormones in influencing infant metabolic trajectories have been reviewed (40, 48, 223). Evidence indicates that oral administration of insulin early in life stimulates gut maturation (40) and that adiponectin receptors are present in the human intestine (42). These milk metabolic
hormones, therefore, may have a role through local or systemic mechanisms regulating development of infants and subsequently in reducing susceptibility to future metabolic disease. In addition, the immaturity of the neonatal gut barrier may also allow for absorption and survival of various hormones from human milk, which would allow milk hormones to systemically influence metabolic development of the infant. Goldman (224, 225) and Koldovsky (226) have reviewed characteristics of the neonatal gastrointestinal tract that would allow for survival of human milk components in neonates, including delayed production of pancreatic proteases and gastric acid, the presence of antiproteases and inhibitors in human milk, and higher permeability in the neonatal gut to macromolecules.

Therefore, consuming compromised concentrations of metabolic hormones in human milk could have short- and long-term implications in offspring. These milk metabolic hormones may have a direct role in the optimal metabolic development of infants thus subsequently reducing susceptibility to future metabolic disease (40, 48). This may be critical for the primary recipients of donor milk, preterm very low birth weight infants, who are at increased risk for insulin resistance and type 2 diabetes later in life (44-46), in addition to infants who are exposed to maternal metabolic abnormalities.

8.3 Strengths and limitations

8.3.1 Strengths

Since women with GDM and their offspring are at increased risk for adverse pregnancy outcomes and chronic disease development later in life (5-8), it is important to clarify the role of prenatal nutrition in the prevention of GDM. However, only a few reports have investigated the impact of diet during pregnancy on risk of GDM (23-25), and none have
studied this question using comprehensive glucose homeostasis profiles. In Chapter 5, we were able to extend this literature by assessing the detailed macronutrient intake distributions during a clearly defined trimester of pregnancy and study their associations with insulin sensitivity / resistance, beta-cell function, and glucose profiles.

In addition, insulin and adiponectin have been detected in human milk (34, 37-39), but no studies have investigated the impact of maternal metabolic status assessed during pregnancy on these hormone concentrations in human milk. We reported in Chapter 6 that insulin in mature milk is influenced by maternal prenatal metabolic status using detailed assessments of gravid metabolic and obstetrical parameters. We were also able to obtain milk samples at two clearly defined periods of lactation (during the first week and at 3 months postpartum) from women to investigate the impact of prenatal metabolic and obstetrical parameters on metabolic hormones in human milk.

With the growing demand for donor human milk in North America (240), it is also important to understand the impact of the pasteurization process. In Chapter 7, we investigated the practical impact of Holder pasteurization on insulin and adiponectin concentrations in donor milk following the standardized processing protocol used in North America (47). While previous research had shown that Holder pasteurization deactivates or reduces the activity of several bioactive components in human milk including immunological proteins (266-268), antioxidants (269), lipases (265), interleukin-10 (270), and insulin-like growth factors (271), no previous studies had examined the impact of Holder pasteurization on concentrations of insulin and adiponectin in milk.
8.3.2 Limitations

The present investigations, however, have limitations that must be noted. We cannot exclude the possibility that our observed associations of vegetable and fruit fiber intake with insulin sensitivity reported in Chapter 5 might have been confounded by other components in vegetables and/or fruits. For example, antioxidants in vegetables and fruits might have influenced this association through effecting anti-inflammatory pathways (120). However, it is difficult to isolate this potential confounder in a pregnant population with a frequent use of supplementations. Our lack of association between grain fiber and metabolic status during pregnancy could also be attributed to a lack of variation in grain fiber intakes among our study participants. Similarly, a lack of significant associations of macronutrients with postprandial glucose response or insulin resistance among those without a family history of diabetes might be attributed to a narrower degree of variation in metabolic parameters among women in this subgroup. It is possible that logistic regression, which assessed the dichotomous outcome of GDM status, might have been under powered to assess small effect changes in % saturated and trans fat, vegetable and fruit fiber, and added sugar intakes that were detectable with the continuous outcome variable (i.e. fasting glucose). In addition, we were unable to further investigate potential ethnic specific eating habits in relation to metabolic responses because of our small sample size and lack of detailed ethnic origin information. Another methodological limitation of our study is potential under-reporting of dietary intake, as it has been documented that overweight and obese individuals are more likely to under-report dietary intake (320, 321). We have employed energy adjusted intake values to assess diet and metabolic status associations to minimize the potential effect of under-reporting. However, under-reporting might have reduced our reported effect sizes by
narrowing the difference in exposure between those with normal and abnormal glucose metabolism. Another methodological limitation of our study was that pregravid body mass index and gestational weight gain variables were calculated using measured height but self-reported weight. It is possible that overweight and obese women might have under-reported weight (322), thereby attenuating effect sizes by narrowing the degree of variation in reported weight.

According to standard practice at our study hospital, dietary advice is not provided to women with a positive glucose challenge test (GCT), or with a higher risk for GDM (5), but we cannot rule out the possibility that women with an abnormal GCT may have independently altered their dietary intakes between GCT and OGTT. If women took the initiative to change their dietary intake, it would have had a minimal impact on the assessed dietary intakes between 13 and 26 weeks of gestation because an average wait period between the GCT and OGTT is only 2 weeks and the majority of our study participants would likely have completed the GCT at \( \geq 26 \) weeks of gestation (as 87.3% completed the OGTT at \( \geq 28 \) weeks of gestation).

Although both women with and without normal GCT were eligible to participate in our study, we had a greater proportion of women with an abnormal GCT among those who participated in the 3-h OGTT visit during pregnancy. This is likely because normal GCT women were not clinically indicated for the diagnostic OGTT but asked to complete an additional 3-h OGTT as a part of the study protocol. This might have contributed to a high rate of GDM (22.9%) reported in Chapter 5.

For the collection of milk samples, we had variation in the number of hours elapsed between delivery and early milk collection because a number of participants experienced
difficulty expressing their colostrum shortly after delivery. To account for this variation, we included adjustment for the time elapsed from delivery to milk collection in multiple regression analysis in Chapter 6. In addition, a sub-analysis was performed among those who donated early milk within first 3 days postpartum. Further, an assay was used which detects total adiponectin and which is not specific to the high-molecular weight (HMW) form of adiponectin. In human serum, HMW adiponectin is known to be the most biologically active form and to have stronger associations with diabetes risk compared to total adiponectin (198). Adiponectin in human milk, however, has been reported to be almost entirely composed of the HMW form (48). Therefore, the total adiponectin in milk reported here would likely be predominantly the HMW form.

In the pasteurization study in Chapter 7, we reported the pasteurization effects on donor milk components, but not other donor milk handling processes including freeze-thaw cycles. Previously, Bronsky et al (38) reported a strong correlation between adiponectin concentrations before and after two freeze-thaw cycles ($r=0.894$, $p<0.0001$). Although Holder pasteurization is likely a major attributor that alters adiponectin and insulin concentrations, we cannot exclude the possibility that the impact of the overall donor milk processing protocol on milk composition might be greater than the pasteurization effects reported here. We also cannot separate effects of each pasteurization step, including heat treatment and container changes, on concentrations of milk components based on our results.

### 8.4 Conclusions and implications for future directions

In addition to limited evidence regarding optimal prenatal nutrition to prevent GDM (23, 24), no previous studies have used a comprehensive set of glucose homeostasis indicators. Using
comprehensive assessments of diet during a clearly defined trimester of pregnancy and
detailed assessments of the glucose metabolic profile in Chapter 5, including GDM status,
hyperglycemia, insulin resistance / sensitivity, and beta-cell function, we report that
macronutrient intake during the second trimester of pregnancy was associated with risk for
abnormal glucose metabolism later in pregnancy. Our results add to growing knowledge on
the importance of nutrition during pregnancy on the metabolic health of women and
emphasize the need for continued work to determine optimal prenatal nutritional strategies to
prevent GDM, with a subsequent goal of preventing adverse pregnancy outcomes and long-
term health consequences including the development of type 2 diabetes (5-8).

Although metabolic hormones were previously detected in human milk (34, 37-39),
we report for the first time in Chapter 6, to our knowledge, that these milk hormones are
influenced by maternal prenatal metabolic status or obstetrical events using samples assessed
at two different periods of lactation. Maternal prenatal metabolic abnormalities were
associated with high insulin concentrations in mature milk, while only obstetrical parameters
were associated adiponectin concentrations in colostrum. These findings indicate that a
metabolically complicated pregnancy does not affect the metabolic hormone concentration in
early milk and hence supports current clinical practice recommendations for all women,
including those with prenatal metabolic abnormalities, to be encouraged to breastfeed (171,
236, 237). Our findings linking maternal prenatal metabolic abnormalities with elevated
insulin in mature milk, in addition to recent evidence from a randomized controlled study
indicating long-term benefits of dietary intervention in infancy (272), demonstrate the need
for future investigation assessing the impact of compromised metabolic hormones in milk on
the short-term metabolic development in infants and long-term susceptibility to obesity and type 2 diabetes later in life.

Holder pasteurization, currently recommended by the Human Milk Banking Association of North America (238), reduced adiponectin and insulin concentrations in donor human milk in Chapter 7. Since insulin and adiponectin are known to be involved in the pathophysiology of diabetes (198), the impact of the reduction in these milk hormones on susceptibility for developing type 2 diabetes later in life warrants further investigation. Variation in heat treatment options including a reduction in the length of pasteurization has been shown to preserve insulin-like growth factors (271). In addition, reducing pasteurization temperature to 57°C improved immunological protein retention while effectively removing 99.9% of inoculated bacterial species (266). With recent evidence from a randomized controlled study demonstrating long-term benefit of dietary intervention in infancy (272), it is important to understand the effect of pasteurization on milk components which may have a critical impact on infant metabolic trajectories (40, 48). In view of emerging knowledge on the importance of milk components on human health outcomes, comprehensive risk and benefit assessments to find the optimal pasteurization process which mitigates risks but promotes retention of bioactive components is needed.

In summary, we reported in this thesis that the distribution of macronutrient intakes during pregnancy was associated with risk for abnormal glucose metabolism later in pregnancy. In addition, maternal prenatal metabolic abnormalities were associated with high insulin concentrations in mature milk, while only obstetrical parameters were associated adiponectin concentrations in early milk. Our findings support the need for continued work
to determine optimal prenatal nutritional strategies to prevent GDM and subsequently to improve infant nutrition.
Chapter 9

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