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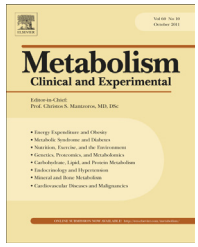

Translational

Effect of pre-existing maternal obesity, gestational diabetes and adipokines on the expression of genes involved in lipid metabolism in adipose tissue

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ABSTRACT

Objective. To determine the effect of maternal obesity, gestational diabetes mellitus (GDM) and adipokines on the expression of genes involved in fatty acid uptake, transport, synthesis and metabolism.

Materials/Methods. Human subcutaneous and omental adipose tissues were obtained from lean, overweight and obese normal glucose tolerant (NGT) women and women with GDM. Quantitative RT-PCR (qRT-PCR) was performed to determine the level of expression. Adipose tissue explants were performed to determine the effect of the adipokines TNFα, IL-1β and leptin on adipose tissue gene expression.

Results. Pre-existing maternal obesity and GDM are associated with decreased expression in genes involved in fatty acid uptake and intracellular transport (LPL, FATP2, FATP6, FABPpm and ASCL1), triacylglyceride (TAG) biosynthesis (MGAT1,7 MGAT2 and DGAT1), lipogenesis (FASN) and lipolysis (PNPLA2, HSL and MGLL). Decreased gene expression was also observed for the transcription factors involved in lipid metabolism (LXRα, PPARα, PPARδ, PPARγ, RXRα and SREBP1c). On the other hand, the gene expression of the adipokines TNFα, IL-1β and or leptin was increased in adipose tissue from obese and GDM women. Functional *in vitro* studies revealed that these adipokines decreased the gene expression of LPL, FATP2, FATP6, ASCL1, PNPLA2, PPARδ, PPARγ and RXRα.

Conclusions. Pregnancies complicated by pre-existing maternal obesity and GDM are associated with abnormal adipose tissue lipid metabolism, which may play a role in the pathogenesis of these diseases.

Abbreviations: GDM, gestational diabetes mellitus; NGT, normal glucose tolerant; qRT-PCR, quantitative RT-PCR; FFA, free fatty acid; FABP, fatty acid binding protein; FABPpm, plasma membrane fatty acid binding protein; FATP, fatty acid transport protein; ASCL1, acyl-CoA synthetase long-chain family member 1; LPL, lipoprotein lipase; FASN, fatty acid synthase; DGAT, diacylglycerol acyltransferases; MGAT, monoacyglycerol acyltransferases; PNPLA2, adipose triglyceride lipase; HSL, hormone-sensitive lipase; MGLL, monoglyceride lipase; LXR, liver X receptor; PPAR, proliferator activated receptor; RXR, retinoid X receptor; SREBP1c, sterol regulatory element binding protein; TAG, triacylglyceride; DAG, diacylglycerol; MAG, monoacylglycerol.

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1. Introduction

White adipose tissue plays a central role in regulating lipid and energy homeostasis, by storing triacylglycerides (TAGs) or releasing free fatty acids (FFAs) in response to changes in energy demands [1]. During human pregnancy, a number of metabolic changes occur in maternal adipose tissue that are essential for fetal growth and development. Early gestation is associated with increased maternal fat stores and insulin sensitivity. However, the second half of human gestation is characterised by increased insulin resistance, which results in increases in maternal circulating glucose, TAG and FFA concentrations, allowing for greater substrate availability for fetal growth [2,3]. In women with additional metabolic stress, such as those with gestational diabetes mellitus (GDM) and pre-existing obesity, there are alterations in adipose tissue glucose and lipid metabolism consistent with increased insulin resistance leading to increases in the circulating concentrations of fatty acids and lipids [4–7]. Of clinical significance, these infants have increased risk of later metabolic disease, including obesity, diabetes, cardiovascular disease, and certain cancers [8]. Thus, further understanding adipose tissue lipid metabolism is becoming increasingly important in light of the rising incidence of diabetes and obesity during pregnancy [9,10], and its associated disorders such as type 2 diabetes, dyslipidemia, and cardiovascular diseases later in life for both mother and offspring [11].

A number of genes are involved in adipocyte lipid metabolism [12–15]. Lipoprotein lipase (LPL) regulates the hydrolysis of circulating TAG into FFA. FFA entry into the adipocyte is the first step to lipid storage. Uptake of fatty acids into cells occurs via both passive diffusion and a protein-mediated mechanism. The most prominent and best characterised of these are three membrane-associated proteins, a 40 kDa plasma membrane fatty acid binding protein (FABPpm), FA translocase (FAT)/CD36, and 60 kDa fatty acid transport proteins (FATPs) also known as solute carrier proteins (SLC27A). Additionally, members of the fatty acid binding protein (FABP) family are intracellular carriers for fatty acids which likely transport them to their intracellular sites of metabolism. The synthesis and accumulation of TAGs in adipose tissue occur via a number of enzymes including fatty acid synthase (FASN), diacylglycerol acyltransferases (DGAT) and monoacyglycerol acyltransferases (MGATs). There are also a number of intracellular lipolytic enzymes that have an established function in the lipolytic breakdown of TAGs in adipose tissues, including adipose triglyceride lipase (ATGL or PNPLA2), hormone-sensitive lipase (HSL), and monoglyceride lipase (MGLL).

Peroxisome proliferator activated receptor (PPAR), liver X receptor (LXR) and their heterodimeric partner, retinoid X receptor (RXR), and sterol regulatory element binding proteins (SREBP1c and SREBP2) have emerged as metabolic sensors for lipid metabolism in adipocytes [16–21]. A change in the activity or abundance of these transcription factors leads to major changes in intracellular as well as whole body lipid levels.

Defective FFA uptake, transport, oxidation and lipolysis are features of obesity, insulin resistance and type 2 diabetes [16,22–24]. Despite the central physiological importance of these processes for for maintaining normal glucose, lipid and energy homeostasis during pregnancy, the expression of genes involved in FFA metabolism is insufficiently characterised in adipose tissue in association with diabetes and obesity during human pregnancy. Thus, the aim of this study was to determine the effect of pre-existing maternal obesity and GDM on the expression levels of genes involved in fatty acid uptake and transport, synthesis and metabolism. Both subcutaneous and visceral adipose tissue will be examined as there is marked heterogeneity with regard to lipolysis regulation and fatty acid flux from these sites [25]. In addition, as adipokines are among the numerous effectors that control the lipid metabolism in various tissues [26–29], the effect of TNFα, IL-β and leptin on adipose tissue gene expression will also be assessed. This is of relevance as low grade inflammation and increased levels of adipokines are key features of obese pregnancies and pregnancies complicated by diabetes [30–36].

2. Materials and methods

2.1. Tissue Collection and Preparation

Approval for this study was obtained from the Mercy Hospital for Women's Research and Ethics Committee and informed consent was obtained from all participating subjects. Human subcutaneous adipose tissue (from the anterior abdominal wall) and omental adipose tissue were obtained from a total of 46 pregnant women (28 NGT and 18 GDM). Tissues were obtained within ten minutes of delivery and dissected fragments were thoroughly washed in ice-cold PBS to remove any blood, snap frozen in liquid nitrogen and stored at −80 °C until required for RNA extraction. All tissues were obtained at the time of term Caesarean section before the onset of labour. Indications for Caesarean section included repeat Caesarean section or breech presentation. Women with any adverse underlying medical condition (i.e. including asthma, pre-eclampsia and pregestational diabetes) were excluded. Samples were collected from lean (BMI < 24.9 kg/m^2), overweight (BMI between 25 and 29.9 kg/m^2) and obese (BMI >30 kg/m^2) subjects. The women were classified as lean, overweight or obese based on their BMI, calculated at their first antenatal visit at approximately 12 weeks gestation. Women with GDM were diagnosed according to the criteria of the Australasian Diabetes in Pregnancy Society (ADIPS) by either a fasting venous plasma glucose level of ≥ 5.5 mmol/l glucose, and/or ≥ 8.0 mmol/l glucose 2 h after a 75 g oral glucose load at approximately 26–28 weeks gestation. All women with GDM were prescribed insulin in addition to dietary management. All pregnant women were screened for GDM, and women participating in the NGT group had a negative screen. The relevant clinical details of the subjects are detailed in Tables 1A and 1B.

2.2. Adipose tissue explants

For these studies, omental adipose tissue (n = 6 subjects) was obtained from lean NGT pregnant women, and tissue explants were performed as previously described [37–40]. Briefly, adipose tissues were finely diced and placed in DMEM at 37 °C in a humidified atmosphere of 21% O_2 and 5% CO_2 for