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Hepatocyte growth factor is elevated in amniotic fluid from obese women and regulates placental glucose and fatty acid metabolism

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ABSTRACT

Introduction: To evaluate the impact of the pro-inflammatory cytokine hepatocyte growth factor (HGF) on the regulation of glucose and lipid placental metabolism.
Methods: HGF levels were quantified in amniotic fluid and placenta from control and obese women. 2-deoxy-glucose (2-DOG) uptake, glycolysis, fatty acid oxidation (FAO), fatty acid esterification, *de novo* fatty acid synthesis, triglyceride levels and carnitine palmitoyltransferase activities (CPT) were measured in placental explants upon addition of pathophysiological HGF levels.
Results: In obese women, total- and -activated-HGF levels in amniotic fluid were elevated ~24%, and placental HGF levels were ~3-fold higher than in control women. At a similar dose to that present in amniotic fluid of obese women, HGF (30 ng/mL) increased Glut-1 levels and 2-DOG uptake by ~25–30% in placental explants. HGF-mediated effect on 2-DOG uptake was dependent on the activation of phosphatidylinositol 3-kinase signaling pathway. In addition, HGF decreased ~20% FAO, whereas esterification and *de novo* fatty acid synthesis increased ~15% and ~25% respectively, leading to 2-fold triglyceride accumulation in placental explants. In parallel, HGF reduced CPT-I activity ~70%.
Discussion: HGF is a cytokine elevated in amniotic fluid and placental tissue of obese women, which through its ability to stimulate 2-DOG uptake and metabolism impairs FAO and enhances esterification and *de novo* fatty acid synthesis, leading to accumulation of placental triglycerides.

1. Introduction

Obesity is a growing health concern in women of reproductive age because is associated with a broad range of maternal and fetal complications, such as macrosomia, a condition characterized by excessive fetal fat accretion that predispose the newborn to suffer metabolic diseases later in life [1–4].

The mechanistic link between maternal obesity and fetal macrosomia is poorly understood. Maternal obesity is usually associated with hyperglycemia and hypertriglyceridemia, which may result in augmented transplacental nutrient transfer to the fetus. In the obesogenic–diabetogenic hypothesis proposed by Catalano et al. [2], changes in maternal availability of lipid surplus would facilitate non-esterified free fatty acids delivery to the adipocytes of the fetus, whereas maternal hyperglycemia and hyperinsulinemia would enhance lipogenesis leading to fetal adiposity. Hence, maternal hyperinsulinemia, and excessive circulating levels of glucose and lipids would play a direct role in the accumulation of fat in fetal adipose tissue. However, in pregnancies complicated with obesity, the expression of placental pro-inflammatory cytokines and certain immune cell populations are elevated leading to a chronic inflammatory milieu in which the fetus develops [5–7]. These observations have propelled the question whether excessive fetal adiposity can be explained solely as a result of higher circulating nutrients in maternal blood. Thus,

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it has been proposed that placental inflammation observed in obese women may modify the availability of nutrient supply at the maternal–fetal interface leading to augmented transplacental nutrient transfer to the fetus [2]. Unfortunately, this question remains to be addressed, and the contribution of intrauterine pro-inflammatory milieu on placental metabolism regulation has not been extensively investigated.

HGF is a pro-inflammatory cytokine elevated in serum of obese patients, which decline with weight loss and reduced body fat mass that occurs after gastroplasty [8–10]. The adipose tissue of these patients abnormally produces and secretes HGF, contributing to augmented serum HGF levels [9,11]. HGF is activated by serine proteases, such as the HGF activator (HGFA), to exert its biological functions [12]. Although HGF was initially identified as a circulating factor that stimulates hepatocyte proliferation after liver injury [13], HGF exhibits pleiotropic biological functions in a broad range of cell types. Among them, HGF is a potent regulator of glucose and lipid metabolism in pancreatic β-cells, intestine epithelial cells, adipocytes and skeletal muscle cells [14].

HGF is also expressed in placental mesenchymal cells, syncytiotrophoblast cells of the chorionic villi and in amniotic epithelium cells [15]. The biological effects of HGF are mediated by its receptor (c-met) a transmembrane protein encoded by the MET proto-oncogene [16]. The receptor for HGF is primary localized in placental cytotrophoblast cells and to a lesser degree in syncytiotrophoblast cells [17]. In the literature, it can be found few and contradictory reports about plasma HGF levels during normal pregnancy and in pregnancies complicated with obesity. In normal pregnancy, HGF levels increased with gestational age until term [18,19] or there was no change during pregnancy [20]. In pregnancies complicated with obesity, HGF levels were similar to normal women and remained unchanged with increasing BMI during the second trimester of pregnancy [21]. These results are in disagreement with the notion that in obese patients serum HGF associated with obesity [9,11] and had a linear relationship with BMI [9]. Finally, total-HGF and activated-HGF blood levels in neonates are regulated in a time-dependent manner along fetal development [22].

In this study, we aimed to further understand the mechanistic link between maternal obesity, through its associated inflammatory uteroplacental environment, and the regulation of placental metabolism. To this end, we tested the hypothesis that the pro-inflammatory cytokine HGF alters placental glucose and lipid metabolism leading to accumulation of placental triglycerides.

2. Methods

2.1. Study subjects

The study was performed on placentas and amniotic fluid from pregnant women recruited at the Department of Obstetrics and Gynecology, University Hospital "Puerta del Mar" (HUPM). Patient samples were obtained after written informed consent in accordance with the HUPM Ethics Committee requirements and the Declaration of Helsinki. Specific exclusion criteria included women under the age of 18, smokers, a history of long-chain 3-hydroxyacyl-CoA deficiency, hemolysis elevated liver function syndrome or acute fatty liver of pregnancy, preeclampsia, chronic hypertension, pregestational diabetes, GDM, other co-morbid disease, abnormal karyotype, fetal malformations and multiple pregnancy.

In the studies conducted using placental explants, pregnant women who planned to deliver by an elective Caesarean section due to clinical reasons such as breech presentation or prior Caesarean section were recruited. All Caesarean sections were performed at term. Placental samples and fasting maternal blood samples were obtained at the time of the elective Caesarean section. Neonatal anthropometric measurements were performed immediately at delivery as usual. In total, 26 women with no pregnancy complications (BMI 20–24.9) participated in the study for placental explants experiments. In addition, placentas from 10 obese women (pre-pregnancy BMI >30) were used in experiments showed in Fig. 1. Randomly chosen subsets of placentas were used for the experiments as indicated in the figure legends. In Table 1 are listed demographics and baseline data, as well as perinatal variables.

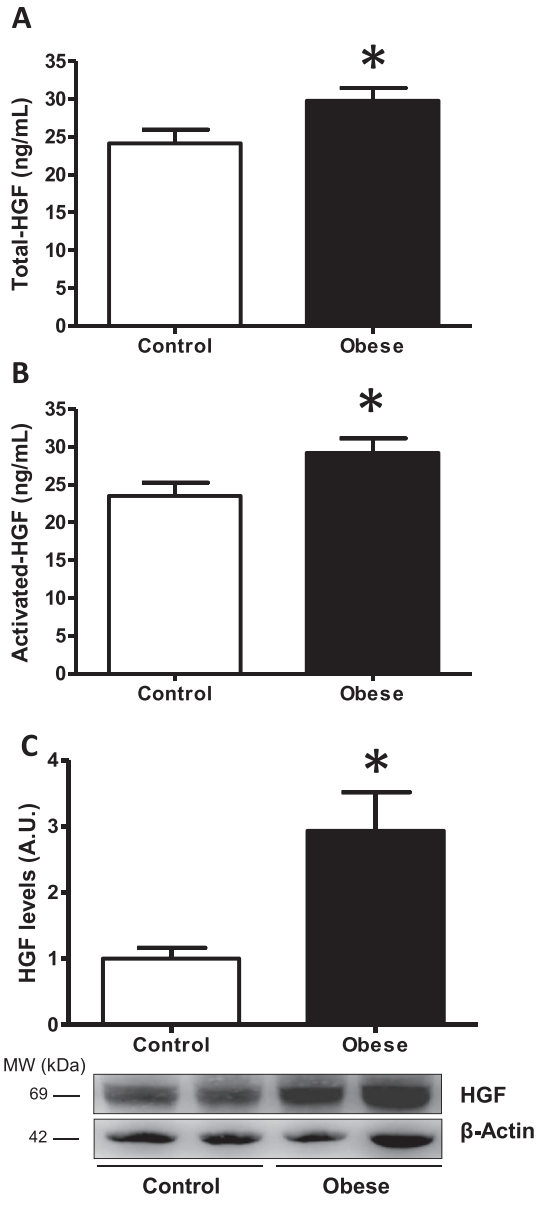

Fig. 1. HGF levels in amniotic fluid and placentas from obese pregnant women. HGF levels in amniotic fluid. t-HGF (A) and a-HGF (B) were measured in amniotic fluid from control (n = 29) and obese (n = 12) women by ELISA (see details in Table 2). Values are means ± SEM. *p < 0.05 relative to control group by unpaired t-test. (C) Western blot analysis of HGF in placental tissue from control and obese women. Frozen placental tissues (~100 mg) from control (n = 8) and obese (n = 10) groups (see details in Table 1) were used to quantify placental HGF content. β-actin expression was determined to ensure similar protein loading. Top: the y-axis represents the ratio of HGF vs. β-actin in arbitrary units (A.U). Bottom: a representative picture of the western blot is shown. Data are means ± SEM.*p < 0.05 relative to control group by unpaired t-test.

In the studies conducted using amniotic fluid, patients were eligible among pregnant women attending our antenatal clinic undergoing elective amniocentesis at 15–20 weeks of gestation for karyotype analysis, most of them due to advanced maternal age or combined screening showing high risk for trisomy 21. All fetuses were chromosomally and anatomically normal at delivery. Women were asked to give an extra amount of 3 mL of amniotic fluid for the study. Women were divided into two groups according to their pre-pregnancy BMI: the control group composed of 29 normal weight women (BMI 20–24.9); and the obese group, composed of 12 obese pregnant women (BMI >30). Demographics and baseline data, as well as perinatal variables, are shown in Table 2. Specific exclusion criteria included women under the age of 18, smokers, a history of long-chain 3-hydroxyacyl-CoA deficiency, hemolysis elevated liver function syndrome or acute fatty liver of pregnancy, preeclampsia, chronic hypertension, pregestational diabetes, GDM, other co-morbid disease, abnormal karyotype, fetal malformations and multiple pregnancy.