國立臺灣大學醫學院臨床醫學研究所

碩士論文

Graduate Institute of Clinical Medicine

College of Medicine

National Taiwan University

Master Thesis

懷孕期間angiopoietin-like protein 4與葡萄糖和脂質代謝、胎盤功能及胎兒生長的關聯

The association between plasma angiopoietin-like protein 4, glucose and lipid metabolism during pregnancy, placental function, and fetal growth

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中華民國 112 年 6 月

June 2023

doi:10.6342/NTU202301373

國立臺灣大學碩士學位論文
口試委員會審定書
MASTER'S THESIS ACCEPTANCE CERTIFICATE
NATIONAL TAIWAN UNIVERSITY

懷孕期間 ANGPTL4 與葡萄糖和脂質代謝、胎盤功能及胎兒生長的關聯

The association between plasma ANGPTL4, glucose and lipid metabolism during pregnancy, placental function, and fetal growth

本論文係 嚴愛文 (姓名) P10421316 (學號) 在國立臺灣大學 臨床醫學研究所 完成之碩士學位論文，於民國 112 年 6 月 8 日承下列考試委員審查通過及口試及格，特此證明。

The undersigned, appointed by the Institute of Clinical Medicine on 8 June, 2023 have examined a Master's thesis entitled above presented by I-WENG YEN (P10421316) candidate and hereby certify that it is worthy of acceptance.

口試委員 Oral examination committee:

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中文摘要

背景：異常的胎兒生長，包括胎兒過大(large for gestational age)和胎兒過小(small for gestational age)，會增加周產期併發症的風險。然而，目前對於異常胎兒生長的致病機轉並不是完全了解，也沒有好用並且準確的預測模型。Angiopoietin-like protein 4 (ANGPTL4)是一種會被分泌至血漿中的蛋白質，已知會影響脂質和葡萄糖代謝，同時可能影響胎盤發育。在本研究中，我們將探討血漿中 ANGPTL4 在懷孕期間與胎兒生長是否有關，並探索其與葡萄糖代謝、脂質代謝和胎盤功能之間的關係。

方法：我們在國立臺灣大學醫院進行了一項前瞻性研究，收錄時間從 2013 年 11 月到 2018 年 4 月。我們在懷孕的第一和第二孕期紀錄了母親的臨床特徵和收集血液樣本，並在生產後測量胎兒出生體重和收集臍帶血樣本。以上資料被用來分析母親血漿中 ANGPTL4 濃度與 SGA、LGA、葡萄糖代謝、脂質代謝和胎盤功能之間的關係。

結果：本研究共招募了 852 名懷孕婦女。與正常胎兒大小組別相比，LGA 組和 SGA 組的母親血漿 ANGPTL4 濃度均較高。出生體重與懷孕第一孕期血漿 ANGPTL4 濃度呈曲線關係。在調整了其他相關因子後，母親血漿 ANGPTL4 與 LGA 和 SGA 均呈顯著相關。相較於其他傳統風險因子，母親血漿 ANGPTL4 可

doi:10.6342/NTU202301373

額外增加對 LGA 和 SGA 的預測力。此外，血漿 ANGPTL4 濃度與母親血中 growth hormone variant、糖化血色素、三酸甘油脂與游離脂肪酸濃度呈正相關，但與臍帶血內的各種生長相關因子無關連。

結論：母親血漿中 ANGPTL4 濃度與葡萄糖和脂質代謝、以及胎兒生長有關。懷孕早期母親的血漿 ANGPTL4 可以作為早期指標，用於預測 LGA 和 SGA 的風險。

doi:10.6342/NTU202301373

Abstract

Introduction: Abnormal fetal growth, including large-for-gestational-age (LGA) and small-for-gestational-age (SGA), is associated with adverse pregnancy outcomes. However, the underlying mechanisms and accurate predictive models for abnormal fetal growth remain limited. Angiopoietin-like protein 4 (ANGPTL4) is a secreted protein known to affect lipid and glucose metabolism and may have a role in placental development. In this study, we aimed to investigate the relationship between plasma ANGPTL4, glucose metabolism, lipid metabolism, placental function, and fetal growth during pregnancy.

Method: A prospective cohort study was conducted at National Taiwan University Hospital between November 2013 and April 2018. Clinical features and blood samples were collected during the first and second trimesters, while birth weight measurements and cord blood samples were obtained at delivery. We utilized the data to analyze the relationship between maternal plasma ANGPTL4 levels and SGA, LGA, glucose metabolism, lipid metabolism, and placental function.

Result: In this study, a total of 852 pregnant women were enrolled. Both the LGA and SGA groups had higher plasma ANGPTL4 concentrations compared to the appropriate-

doi:10.6342/NTU202301373

for-gestational-age group. A quadratic relationship was observed between birthweight and first trimester plasma ANGPTL4 concentration. Maternal plasma ANGPTL4 showed significant associations with LGA and SGA after adjusting for potential confounders. The predictive ability of maternal plasma ANGPTL4, measured by the area under the ROC curve, was superior to traditional risk factors in identifying LGA and SGA cases. Besides, plasma ANGPTL4 levels were positively correlated with plasma growth hormone variant, hemoglobin A1c, triglyceride, and free fatty acid levels but were not associated with cord blood growth factors.

Conclusion: Plasma ANGPTL4 is associated with glucose metabolism, lipid metabolism, and fetal growth during pregnancy. Plasma ANGPTL4 during the first trimester is an early biomarker to predict the risk of delivering LGA and SGA newborns.

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Introduction

1. Abnormal fetal growth increases the risk of adverse pregnancy outcomes.

The development of the fetus is subject to precise regulation, as both oversized and undersized fetuses can lead to unfavorable pregnancy outcomes[1, 2]. Abnormal fetal growth can lead to the delivery of newborns with large-for-gestational-age (LGA) and small-for-gestational-age (SGA). LGA refers to a newborn with a birth weight that exceeds the 90th percentile for their gestational age and sex, while SGA refers to a newborn with a birth weight below the 10th percentile for their gestational age and sex[3]. Both LGA and SGA are associated with increased risk of adverse pregnancy outcomes. LGA neonates have higher risk of birth trauma, mainly because of a mismatch between the size of the fetus and the maternal pelvis[4]. The large size of the fetus can lead to a higher incidence of shoulder dystocia, which in turn increases the risk of fetal asphyxia[5]. LGA is correlates with hyperinsulinemia[6] and can further lead to other morbidities such as polycythemia, hyperbilirubinemia, and hypoglycemia[7]. On the other hand, SGA neonates are linked to elevated risk of cerebral palsy, polycythemia, hyperbilirubinemia, and hypoglycemia[2]. Besides, certain condition can result in SGA and adverse pregnancy outcomes. For instance, congenital anomalies can lead to fetal growth restriction and increase the risk of perinatal death[8]. In the long-term outcomes, LGA represents an independent risk factor for developing metabolic

doi:10.6342/NTU202301373

syndrome in adulthood[9] and has a higher risk of overweight and obesity in childhood[10]. SGA has also been shown to be a risk factor for adult coronary heart disease and stroke[2].

The risk factors for LGA include a history of macrosomia, maternal overweight or obesity, excessive gestational weight gain, and the presence of gestational diabetes mellitus[11]; whereas the risk factors of SGA include lower maternal body mass index (BMI), pregnancy-induced hypertension (PIH), placental abnormalities, and maternal smoking habit[12]. Currently, fetal growth during pregnancy is monitored by ultrasonography. If restricted fetal growth is suspected, the fetal heart rate and flow velocity waveforms of the umbilical and cerebral arteries should be closely monitored to detect signs of fetal distress[13]. In the event of fetal distress, an emergent delivery should be performed. On the other hand, if ultrasound detects that the infant is large for gestational age, an earlier delivery theoretically can reduce the birth weight of the infant and may mitigate the primary risk factor for shoulder dystocia[14].

2. Regulation of fetal growth and the pathophysiology of LGA and SGA.

Several factors influence fetal growth and can lead to LGA or SGA. These factors include placental hormones, glucose and lipid metabolism, and placental function.

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2.1 Placental hormones

During pregnancy, placental is important for fetal growth[15]. Placenta can regulate maternal nutrients to be transported to the fetal circulation. Besides, placenta can also secrete a wide variety of substances. These substances can act locally in a paracrine- or autocrine-manner. In addition, they can enter the maternal circulation, the fetal circulation, or both, to have systemic effect remotely. Among these factors, placental growth hormone variant (GH-V), human placental lactogen (hPL), insulin-like growth factors (IGF) and IGF binding proteins (IGFBPs) are most important for both fetal development and placental function.

2.1.1 Placental growth hormone variant (GH-V) and human placental lactogen (hPL)

GH-V is expressed in the syncytiotrophoblast and extravillous cytotrophoblast layers of the human placenta. During pregnancy, the blood concentration of GH-V continues to rise and replaces the growth hormone secreted by the pituitary gland in the mid-pregnancy, becoming the major source of growth hormone in the body[16]. GH-V is considered a key factor in the signaling pathways of maternal metabolic adaptation to pregnancy. In experiments with transgenic mice that overexpressed GH-V[17], GH-V could induce insulin resistance by affecting insulin signaling in skeletal muscle and adipose tissue, resulting in increased lipolysis and elevated blood glucose levels. Thus,

doi:10.6342/NTU202301373

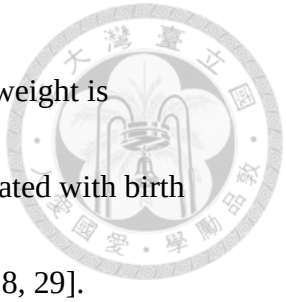
GH-V has been proposed to be an important pathogenesis factor which led to the development of gestational diabetes[18]. Besides, GH-V can also induce the expression and secretion of IGF-1. Overexpression of GH-V in mice increases body weight by 84% and plasma IGF-1 levels by 56%[17]. In pregnant women, maternal plasma concentrations of GH-V correlate significantly to plasma IGF-1 concentrations, and plasma GH-V concentrations are decreased in women who delivered SGA neonates[19].

hPL is also a peptide hormone secreted by placenta[20]. hPL plays a key role as a proliferative stimulator of maternal pancreatic beta cells during pregnancy, which helps to prevent the onset of glucose intolerance [21]. Besides, along with GH-V, hPL could induce systemic insulin resistance and subsequently elevating maternal blood glucose levels, facilitates the supply of energetic substrates to the fetus. The imbalance between hPL and GHV during pregnancy has been proven to be associated with the development of gestational diabetes in previous studies[22, 23].

2.1.2 Insulin-like growth factors, and insulin-like growth factor binding proteins.

IGFs, including IGF-1 and IGF-2, are factors that could influence fetal growth. IGF-1 and IGF-2 are expressed in nearly all cell types of the placenta as early as 6 weeks' gestation[24]. Fetal organs started to express IGF-1 and IGF-2 as early as first trimester during gestation[25]. In human, cord blood IGF-1 concentration is correlated with birth

doi:10.6342/NTU202301373



weight[26], but the relationship between cord blood IGF-2 and birth weight is controversial. Some studies showed that cord blood IGF-2 was correlated with birth weight[19, 27], while in some others no such correlation was found[28, 29].

The effect of IGF is regulated by IGF-binding proteins (IGFBPs), which includes six distinct types in vertebrates, IGFBP1-IGFBP6[30]. Among the IGFBP family, IGFBP3 can prolong the half-life and action time of IGF by facilitating its binding with receptors. Within the serum, the majority of IGF circulates in a complex weighing about 150,000 Daltons, which consists of either IGF-2 or IGF-2, IGFBP-3, and a non-IGF binding component known as acid-labile subunit[31]. IGFBP1 is secreted by decidualized endometrium during the first trimester of pregnancy[32] and inhibits the binding of IGF-I to various types of cells[33]. Based on current in vitro data, it is suggested that IGFBP-4 functions also as an inhibitor of IGF activity and human study shows that increased IGFBP-4 in the maternal circulation in early pregnancy was associated with the development of fetal growth restriction[34], but little about IGFBP-4 was studied. The quantity of IGFBP-5 and 6 present in the serum of rats or humans is exceedingly small, and therefore, it is unlikely to hold any physiological significance[31]. Based on the aforementioned information, among all IGFBPs, IGFBP1 and IGFBP3 play more significant roles during pregnancy. Therefore, in our current study, we will focus on these two IGFBPs.

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2-2 Glucose and lipid metabolism

Previous research indicates that maternal caloric supply and metabolism are key factors influencing fetal growth[35, 36]. Glucose is quantitatively the most important substrate crossing the placenta[37]. In recent years, lipids have been proven to be a factor influencing fetal growth in addition to the well-established effect of glucose[38].

2.2.1 Glucose

The fetus primarily utilizes glucose as an energy substrate, and its transplacental transfer occurs through facilitated diffusion, making it highly dependent on maternal serum concentration[39]. In human, elevated maternal fasting glucose concentration[40] or a high post-prandial glucose[41] is associated with an increased risk of macrosomia. In contrast, lower maternal blood glucose level is correlated with increased incidence of delivering neonates with SGA[42].

2.2.2 Triglyceride and free fatty acids (FFA)

The developing embryo requires fatty acids for crucial energy and metabolic processes. Free fatty acids storage at adipocyte and adipocytes released newly synthesized and stored free fatty acids into the circulation to meet the fetal demand for fatty acids[43]. During normal pregnancy, placental hormones coordinate to bring

doi:10.6342/NTU202301373

marked increase in insulin resistance (IR)[44]. Aside from elevated plasma glucose levels, insulin resistance during pregnancy also results in higher plasma levels of free fatty acids (FFA) and triglycerides (TG). Since plasma TG cannot be transported across placenta directly, it must be hydrolyzed to FFAs by placental lipoprotein lipase (LPL). Diffusion is sufficient for the crossing of short-chain and saturated fatty acids (SFAs) through the placenta, whereas polyunsaturated fatty acids (PUFAs) require the presence of fatty acid transport proteins (FATPs), fatty acid translocases (FATs/CD36), and plasma membrane fatty acid-binding proteins (FABP) for crossing the placenta[43]. In addition to being an energy substrate, FFAs can induce IGF-1 secretion in human trophoblast cells to promote fetal growth. In human, maternal plasma FFA has been shown to be positively correlated with cord blood IGF-1 level[45], and maternal plasma triglyceride (TG) concentrations during pregnancy are associated with birth weight, as well as risk of LGA [46].

2.3 Placental function

As a bridge between a mother and her fetus, the placenta plays a crucial role in the development of the fetus. The connection between impaired blood flow in the utero-placental system and reduced fetal weight due to placental insufficiency was established[47]. Additionally, alterations in placental function can impact the transport

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of nutrients necessary for fetal growth, potentially resulting in the development of SGA or, conversely, LGA[48].

Studies conducted both in vivo and in vitro have demonstrated that IGFs exert endocrine as well as autocrine/paracrine effects on the regulation of placental function[49]. IGF-2 appears to play a particularly crucial role in establishing placental function, as evidenced by the fact that IGF-I deficient mice exhibit normal placental weight, whereas placental weight is reduced by 25% in mice deficient in IGF-2[50].

3 Angiopoietin-like protein 4 (ANGPTL4)

There is a group of proteins structurally similar to angiopoietins called ANGPTLs, and eight ANGPTLs (ANGPTL1-8) have been identified currently[51]. Among the ANGPTLs, previous studies have shown that ANGPTL4 is related to glucose and lipid metabolism. ANGPTL4 is mainly expressed in adipocytes, liver, and placenta in humans. Besides, it has a soluble form and can be detected in the circulation. ANGPTL4 can inhibit lipoprotein lipase (LPL) to prevent the breakdown of triglycerides[52]. Overexpression of ANGPTL4 increased plasma triglycerides by 24 folds in mice[53]. In addition, these mice also had an elevated plasma FFA levels[54]. In human, subjects with loss-of-function variants in ANGPTL4 have a significant lower plasma TG than noncarriers[55]. Besides, for glucose metabolism, overexpression of

doi:10.6342/NTU202301373

ANGPTL4 resulted in impaired glucose tolerance[54], whereas genetic inactivation of ANGPTL4 improves glucose tolerance in mice. In patients with type 2 diabetes, their plasma ANGPTL4 concentration are higher than subjects without diabetes[56]. Furthermore, ANGPTL4 is also involved in placental development. In cell models, treatment with human recombinant ANGPTL4 can induce trophoblast cell invasion [57] and enhance angiogenesis in human umbilical vein endothelial cells (HUVEC)[58].

4 The aim of the study

Since ANGPTL4 is involved in glucose and lipid metabolism, as well as placental development, it is likely that ANGPTL4 plays an important role in fetal growth during pregnancy. However, there is no report in the literature. Therefore, we investigated the relationship between plasma ANGPTL4 and fetal growth during pregnancy in this human cohort study. We analyzed the relationship between plasma ANGPTL4 and fetal growth, including the risk of LGA and SGA. Besides, we also studied the relationship between plasma ANGPTL4, glucose metabolism, lipid metabolism, growth factors and placental function.

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Material and Method

1. Study population

A prospective cohort study was conducted at National Taiwan University Hospital, from November 2013 to April 2018, which recruited pregnant women visiting the obstetric clinic for prenatal care before receiving a 75g oral glucose tolerance test at 24-28 gestational weeks. The inclusion criteria were pregnant women aged between 20 and 60 years old and had singleton pregnancies. Women with twin or multiple pregnancies, those with a history of cancer, and those whose pregnancies were complicated with preeclampsia and overt diabetes were excluded. Pregnant women who fulfilled the inclusion criteria, did not have any of the exclusion criteria, and agreed to participate were recruited consecutively. Medical history, physical examination findings, and laboratory test results were recorded in the first and second trimesters. All participants underwent a 75g OGTT at 24th to 28th gestational weeks to diagnose gestational diabetes. Plasma glucose, HbA1c, TC, HDL-C, LDL-C, plasma TG, and plasma insulin were measured after an 8-hour fast in the first and second trimester, using an automatic analyzer (Toshiba TBA 120 FR, Toshiba Medical Systems Co., Ltd., Tokyo, Japan). Plasma ANGPTL4 concentration was measured in duplicates with enzyme-linked immunosorbent assay (R&D system, USA, Catalog Number: DY3485). Plasma concentrations of GH-V and hPL were measured at first trimester using enzyme-linked

doi:10.6342/NTU202301373

immunosorbent assay (ELISA) kits (Aviva systems biology). Out of the group of study participants, we selected 20 women with the highest plasma triglyceride (TG) concentrations and 20 women with the lowest plasma TG concentrations. Blood samples collected during the second trimester were used to measure fasting plasma free fatty acids (FFAs). The measured plasma FFAs included palmitic acid (PA) (C16:0), stearic acid (SA) (C18:0), oleic acid (OA) (C18:1), and linoleic acid (LA) (C18:2) using liquid chromatography and mass spectrometry [45]. Cord blood IGF-1, IGF-2, IGFBP-1, and IGFBP-3 concentrations were measured by enzyme-linked immunosorbent (IGF-1, IGF-2, and IGFBP-3, R&D Systems, USA; IGFBP-1, Abcam, UK). Pregnancy complications, such as gestational diabetes (GDM) and pregnancy induced hypertension (PIH), as well as birthweight were recorded.

2. Definitions

LGA was defined as birth weight greater than the 90th percentile for gestational age and sex, and SGA was defined as birth weight less than the 10th percentile for gestational age and sex[3]. The distribution of birth weight was derived from the data of birth registration, Ministry of the Interior in Taiwan[59]. The diagnostic criteria for gestational diabetes mellitus (GDM) were based on the recommendations by the American Diabetes Association (ADA). GDM was diagnosed if any of the following

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criteria were met during a 75g OGTT at 24-28 gestational weeks: FPG levels ≥92 mg/dL, 1-hour plasma glucose levels during OGTT (1hPG) ≥180 mg/dL (10.0 mmol/L), or 2-hour plasma glucose levels during OGTT (2hPG) ≥153 mg/dL (8.5 mmol/L).[60]. Pregnancy-induced hypertension was defined if systolic blood pressure (SBP) ≥140 mmHg or diastolic blood pressure (DBP) ≥90 mmHg for the first time during pregnancy after 20th gestational week[61]. Hypertriglyceridemia was defined as plasma triglyceride ≥140 mg/dl in the first trimester or ≥ 220 mg/dl in the second trimester[62]

3. Statistical analysis

Plasma triglyceride, HOMA-IR, and cord blood IGF-1, IGF-2, IGFBP1, and IGFBP3 were subjected to log transformation to approximate a normal distribution for analysis. Continuous variables were presented as means and standard deviations for normally distributed variables, or medians and interquartile ranges for non-normally distributed variables. Categorical variables were presented as percentages. Differences in clinical characteristics among different birth weight groups were assessed using analysis of variance (ANOVA) and Chi-squared tests. The correlation between plasma ANGPTL4 and clinical characteristics was evaluated using Pearson's correlation test. The relationship between plasma ANGPTL4 and birth weight was analyzed using a quadratic model. Logistic regression analyses were conducted to explore the association

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between plasma ANGPTL4 and the occurrence of LGA or SGA. Potential confounders, identified either through significant associations in univariate analyses or reported as determinants of LGA or SGA in the literature, were adjusted for in the statistical models using forward selection. Additionally, receiver-operating characteristic analysis was performed to assess the predictive performance of plasma ANGPTL4 and other risk factors in predicting LGA or SGA.

Result

In the present study, there were 852 pregnant women recruited. Table 1 shows the clinical characteristics of the study subjects in the first and the second trimesters, classified into SGA, appropriate for gestational age (AGA), and LGA according to the fetal birth weight. When comparing the AGA group with the LGA group, it was observed that women in the LGA group were older and had higher BMI before pregnancy. Additionally, during the first trimester, the LGA group had higher BMI and plasma triglyceride levels, along with higher plasma ANGPTL4 concentration compared to the AGA group. In the second trimester, the LGA group had higher BMI, fasting glucose levels, post-loading glucose levels at 1 hour and 2 hours, HOMA2-IR and plasma triglyceride levels compared to the AGA group. On the other hand, when comparing the AGA group with the SGA group, it was found that the percentage of

doi:10.6342/NTU202301373

nulliparous women was higher in the SGA group. In the first trimester, SGA group had higher plasma ANGPTL4 concentration when comparing with AGA group. In the second trimester, the SGA group had lower BMI, plasma total cholesterol, and plasma LDL levels compared to the AGA group.

In Table 2, Pearson's correlation coefficients between plasma ANGPTL4 and clinical characteristic are shown. Plasma ANGPTL4 was significantly associated with BMI, HbA1c, total cholesterol, plasma triglyceride, and plasma HDL-C.

Figure 1 shows the relationship of birth weight to maternal plasma ANGPTL4 in the first and the second trimesters. There was a quadratic relationship between birth weight and plasma ANGPTL4 in the first trimester. Women who delivered neonates with a higher and a lower birth weight had higher plasma ANGPTL4 concentrations. However, there was no significant relationship between birth weight and plasma ANGPTL4 in the second trimester.

Table 3 presents the determinants of LGA in women who delivered newborn with LGA or AGA. In unadjusted analyses, first trimester plasma ANGPTL4, age, history of macrosomia, gestational diabetes, BMI before pregnancy, and post-loading glucose were significantly associated with LGA. However, plasma ANGPTL4 in the second trimester was not correlated with LGA. Model 1 was obtained through forward selection method. In this model, plasma ANGPTL4 was significantly associated with LGA,

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adjusted for history of macrosomia. Model 2 included all potential confounders, which shows that plasma AGNPTL4 was significantly correlated with LGA, adjusted for history of macrosomia, GDM, BMI and hypertriglyceridemia.

Table 4 presents the univariate crude odds ratios and two different multivariate models for risk factors associated with SGA. In unadjusted analyses, first trimester plasma ANGPTL4, parity and hypertriglyceridemia were significantly associated with SGA. Model 1 was obtained through forward selection method. In model 1, plasma ANGPTL4 was significantly associated with SGA, adjusted for parity. Model 2 included all potential confounders. In this model, plasma ANGPTL4 was significantly associated with SGA, adjusted for parity, GDM, BMI and hypertriglyceridemia.

The performance of plasma ANGPTL4 to predict LGA or SGA were shown in Table 5 (LGA) and Table 6 (SGA). In Table 5, the area under the ROC curve (AUC) for maternal plasma ANGPTL4 in the first trimester alone to predict LGA was 0.6743. In the full model including plasma ANGPTL4 in the first trimester, history of macrosomia, GDM, BMI before pregnancy and hypertriglyceridemia, the AUC to predict LGA was 0.7121. Deletion of plasma ANGPTL4 reduced the AUC by 0.0925, which was higher than that of history of macrosomia, GDM, BMI before pregnancy and hypertriglyceridemia. In Table 6, the area under the ROC curve (AUC) for maternal plasma ANGPT4 in the first trimester alone to predict SGA was 0.6449. In the full

doi:10.6342/NTU202301373

model including first trimester plasma ANGPTL4, parity, GDM, BMI before pregnancy and hypertriglyceridemia, the AUC to predict SGA was 0.6830. Deletion of plasma ANGPTL4 reduced the AUC by 0.0518, which was higher than parity, GDM, BMI before pregnancy and hypertriglyceridemia.

Figure 2 shows the relationship between first trimester maternal plasma ANGPTL4 and variables related to glucose metabolism. There was a positive correlation between plasma ANGPTL4 with GH-V and HbA1c in the first trimester. However, there was no significant relationship between maternal ANGPTL4 with hPL and fasting plasma glucose in the first trimester.

Figure 3 shows the relationship between maternal plasma ANGPTL4 concentrations in the first trimester with maternal plasma lipids. A positive association was found between plasma ANGPTL4 levels, plasma triglyceride, and plasma FFA levels, including palmitic acid, stearic acid, oleic acid, and linoleic acid (all $p<0.05$).

Figure 4 illustrates the correlation between maternal plasma ANGPTL4 concentration in the first trimester and cord blood growth factors. There was no significant correlation between plasma ANGPTL4 concentrations with cord blood IGF1, IGF2, IGFBP1, and IGFBP3.

doi:10.6342/NTU202301373

Discussion

In this study, we found a quadratic relationship between maternal plasma ANGPTL4 concentration and birth weight. Maternal plasma ANGPTL4 concentration in the first trimester was correlated with LGA and SGA, in both unadjusted and adjusted statistical models. Besides, plasma ANGPTL4 concentration improved the prediction of LGA on top of well-known risk factors such as history of macrosomia, GDM and hypertriglyceridemia. Similarly, plasma ANGPTL4 also improved the prediction of SGA in addition to traditional risk factors including parity, BMI, GDM and hypertriglyceridemia. To explain the relationship between plasma ANGPTL4 and risk of LGA and SGA, we found that plasma ANGPTL4 was significantly associated with variables of glucose and lipid metabolism, but not cord blood growth factors.

In Figure 5, we presented a summary of the potential mechanisms through which ANGPTL4 may influence fetal growth, as well as the findings from our study. First, ANGPTL4 may affect maternal nutrition conditions, including blood glucose and plasma lipids. Both human association study and transgenic mice studies[54, 56] had shown that ANGPTL4 is correlated with insulin resistance and hyperglycemia. In this report, we found that there was a correlation between plasma ANGPTL4 and HbA1c level. The lack of significant correlation between fasting glucose and plasma ANGPTL4 may be attributed to the low variability in fasting glucose levels among first trimester

doi:10.6342/NTU202301373

pregnant women. Besides, the relationship between plasma ANGPTL4, fasting plasma glucose, and HbA1c also suggest a potential relationship between plasma ANGPTL4 and post-prandial plasma glucose levels. In animal study, liver-specific silencing of ANGPTL4 decreased the post-prandial glucose level and had no influence on fasting glucose[63]. Findings from the present study and the literature suggest ANGPTL4 may be involved in glucose metabolism during pregnancy, and the elevated glucose in maternal circulation may provide more energy for fetal growth.

In addition to the role in glucose metabolism, ANGPTL4 also have a role in lipid metabolism. In animal studies, whole-body overexpression of ANGPTL4 raises plasma TG levels and reduces clearance of plasma TG through the inhibition of lipoprotein lipase [52, 53, 55]. Besides, fasting plasma FFAs and the expression of adipose triglyceride lipase (ATGL) were increased in mice overexpressing ANGPTL4 [54]. ATGL is expressed in adipocytes and catalyzes the breakdown of triglyceride into diglycerides and fatty acids. In a cell model, the concentrations of fatty acids and glycerol increased in the medium of adipose tissue explants derived from transgenic mice that overexpress Angptl4 [64]. Thus, the increased fasting plasma FFAs in mice overexpressing ANGPTL4 are likely a result of enhanced lipolysis in the adipocytes. In this report, we observed a correlation between plasma ANGPTL4, plasma TG and plasma FFAs, providing human evidence that ANGPTL4 may also regulate plasma TG

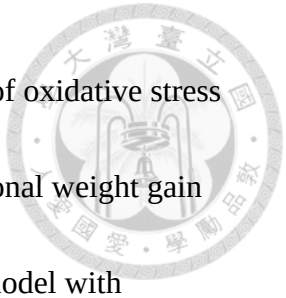
doi:10.6342/NTU202301373

and FFAs in human during pregnancy to provide lipid nutrients to the fetus.

We hypothesized that ANGPTL4 is correlated with cord blood growth factors, particularly IGF-1, which is known to be important for fetal growth[26] and IGF-2, which is correlated with placental development[50]. However, maternal plasma ANGPTL4 was not associated with cord blood growth factors, including IGF-1, IGF-2, IGFBP1, and IGFBP3. This means that cord blood growth factors may have limited role in the relationship between plasma ANGPTL4 and fetal growth.

In the present study, we have demonstrated that maternal plasma ANGPTL4 in the first trimester is associated with the occurrence of LGA and SGA and can be considered as a biomarker for predicting LGA/SGA in early pregnancy. There have been several reports exploring the prediction model of LGA. Kristen S. Gibbons et al.[65] proposed a prediction model which included GDM, BMI, ethnicity, age, height, body weight, smoker, parity, family history of diabetes and blood pressure, and the AUC was 0.698. However, since GDM was detected after the second trimester, it cannot predict LGA in early pregnancy. Peng Ju Liu et al.[66] presented a model with the ratio of TG and HDL. The AUC of this model was only 0.646, which could possibly be attributed to the smaller number of parameters used. As for the prediction of SGA, due to poorer prognosis, there were many reports exploring for the prediction model of SGA. However, most of them was too complicate for clinical use. Otilia Perichart-Perera et

doi:10.6342/NTU202301373



al.[67] presented a prediction model in the first trimester, consisting of oxidative stress biomarkers (protein oxidation and total antioxidant capacity), gestational weight gain and vitamin D. Tracey J. Hanchard et al.[68] proposed a prediction model with trophoblast volume, maternal height and placental growth factor (PlGF). I Papastefanou et al.[69] constructed a competing risk model which was consisted of racial, maternal height, body weight, in-vitro fertilization, smoking, chronic hypertension, autoimmune disease, parity, gestational week at delivery of last pregnancy, birthweight of last pregnancy, interpregnancy interval, previous preeclampsia and previous intrauterine death. Our study found that ANGPTL4 could serve as a good biomarker for predicting SGA and LGA in early pregnancy. Importantly, it has the potential to be conveniently utilized by healthcare professionals. Once we can identify high-risk mothers for abnormal fetal growth, we can proactively manage the associated risk factors starting from early pregnancy. These risk factors may include blood glucose levels, plasma triglyceride levels, or maternal weight. Additionally, we can enhance prenatal care by closely monitoring the trajectory of fetal growth during the prenatal period. This allows for more comprehensive and attentive monitoring of the fetus's growth patterns.

The strength of this study is its relatively large sample size, with comprehensive clinical data during pregnancy and measures of glucose and lipid metabolism as well as growth factors. Besides, this is the first report that suggests a correlation between

doi:10.6342/NTU202301373

maternal plasma ANGPTL4 and birth weight. We also proposed potential mechanisms using the analysis of plasma ANGPTL4, glucose and lipid metabolism, and cord blood growth factors. The weakness of our study was we showed only association relationship, and the source of maternal plasma ANGPTL4 remains unclear. The pathophysiology between ANGPTL4 and abnormal fetal growth needs to be explored by future cell and animal studies.

Conclusion

In conclusion, this study has demonstrated that maternal plasma ANGPTL4 concentration in the first trimester is associated with plasma GH-V, HbA1c, plasma TG and FFAs, and the risk of LGA and SGA. Addition of plasma ANGPTL4 to traditional risk factors could improve the prediction of LGA and SGA. Our data suggest that plasma ANGPTL4 in the first trimester is an early biomarker during pregnancy for the prediction of LGA and SGA. Besides, these findings suggest that ANGPTL4 may affect fetal growth and birth weight through the regulation of glucose and lipid metabolism, which should be investigated in further studies in cell and animal models.

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Table 1. Clinical characteristics of the study subjects who delivered neonates with small-for-gestational-age (SGA), average-for-gestational-age (AGA), and large-for-gestational-age (LGA).

	SGA	AGA	LGA
Number	97	693	62
Age (years old)	33.4±4.1	33.8±4.0	**35.1±4.4***
Nulliparous (%)	**69.1***	53.7	41.9
BMI before pregnancy (kg/m^2)	21.1±3.0	21.6±3.1	**22.9±4.7***
First trimester			
BMI (kg/m^2)	21.3±3.1	21.9±3.0	**23.2±4.6***
FPG (mg/dl)	81.8±6.7	82.6±6.1	84.1±5.8
HbA1c (%)	5.24±0.27	5.30±0.25	5.25±0.33
HOMA2-IR[#]	0.71±0.29	0.76±0.32	0.87±0.37
Plasma total cholesterol (mg/dl)	171.5±29.5	178.4±34.3	175.6±35.4
Plasma triglyceride (mg/dl)†‡	105(76-118.5)	98(78-130)	**115(95-156)***

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Plasma LDL-C (mg/dl)	90.1±27.6	94.9±27.7	93.1±26.2
Plasma HDL-C (mg/dl)	70.0±14.7	71.4±15.2	66.9±14.9
SBP (mmHg)	113.7±10.4	113.3±11.9	115.5±13.7
DBP (mmHg)	68.3±8.6	67.1±8.7	67.9±9.3
Plasma ANGPTL4 (ng/ml)	**217.1±79.8***	177.3±117.0	**235.5±108.1***
Second trimester			
BMI (kg/m^2)	**23.3±2.9***	24.1±3.2	**25.6±4.6***
FPG (mg/dl)	77.9±6.2	78.9±5.8	**82.0±8.4***
OGTT 1hr (mg/dl)	134.8±34.8	133.7±28.5	**145.5±30.2***
OGTT 2hrs (mg/dl)	117.6±29.8	115.6±25.5	**125.8±27.9***
HbA1c (%)	4.86±0.42	4.88±0.38	4.95±0.40
HOMA2-IR‡	0.89±0.34	0.94±0.50	**1.16±0.59***
Plasma total cholesterol (mg/dl)	**227.3±38.7***	242.0±41.6	247.6±40.6
Plasma triglyceride (mg/dl)†‡	155(135-195)	170.5(135-211)	**188.5(154-232)***
Plasma LDL-C (mg/dl)	**128.6±37.3***	140.6±37.0	143.9±35.9

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Plasma HDL-C (mg/dl)	83.8±17.6	84.7±17.2	82.9±16.9
SBP (mmHg)	112.6±13.7	112.6±11.8	114.4±11.1
DBP (mmHg)	66.4±9.6	66.3±8.7	66.8±7.6
Plasma ANGPTL4 (ng/ml)	343.9±125.9	351.7±158.9	346.2±185.1
GDM (%)	11.5	11.9	19.4
PIH (%)	1	2.5	0

* $p<0.05$ vs. AGA

† Medians (interquartile ranges) were shown.

‡ Statistical analyses were done after logarithmic transformation.

BMI, body mass index; DBP, diastolic blood pressure; FPG, fasting plasma glucose; GDM, gestational diabetes; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; PIH: pregnancy-induced hypertension; SBP, systolic blood pressure.

doi:10.6342/NTU202301373

Table 2. Pearson's correlation coefficients and p values between plasma ANGPTL4 and metabolic factors in the first trimester.

Plasma ANGPTL4	Correlation coefficients	P value
Age	0.0768	0.1142
BMI	**0.1121**	**0.0217**
FPG	0.0351	0.4727
HbA1c	**0.1153**	**0.0182**
HOMA-IR*	0.0521	0.2884
Total cholesterol	**0.1209**	**0.0133**
Plasma triglyceride*	**0.2028**	**<0.001**
Plasma LDL-C	0.0810	0.0982
Plasma HDL-C	**0.0983**	**0.0445**

*Statistical analyses were done after logarithmic transformation.

BMI, body mass index; FPG, fasting plasma glucose; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol.

doi:10.6342/NTU202301373

Table 3. Odds ratios (p values) of plasma ANGPTL4 and clinical characteristics for the risk of LGA by simple and multiple logistic regression using LGA as the independent variable.

	Unadjusted	Model 1	Model 2
1st trimester plasma ANGPTL4*	**1.51 (0.010)**	**1.47 (0.024)**	**1.43 (0.039)**
2nd trimester plasma ANGPTL4*	0.97 (0.868)		
Age	**1.08 (0.016)**		
History of macrosomia	**8.76 (0.005)**	**17.55 (0.022)**	**18.25(0.024)**
Parity	1.38 (0.080)		
GDM	**1.98 (0.031)**		1.82 (0.203)
BMI before pregnancy	**1.10 (0.004)**		1.08 (0.145)
1st trimester FPG	1.04 (0.162)		
OGTT 1 hour	**1.01 (0.002)**		
OGTT 2 hors	**1.01 (0.003)**		
1st trimester HbA1c	0.46 (0.270)		
Hypertriglyceridemia	1.76 (0.092)		0.91 (0.826)

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BMI, body mass index; FPG, fasting plasma glucose; GDM, gestational diabetes;

OGTT: oral glucose tolerance test

*Odds ratios of every 1 SD increase in plasma ANGPTL4 was shown.

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Table 4. Odds ratios (p values) of plasma ANGPTL4 and clinical characteristics for the risk of SGA by simple and multiple logistic regression using SGA as the independent variable.

	Unadjusted	Model 1	Model 2
1st trimester plasma ANGPTL4*	**1.39 (0.019)**	**1.39 (0.028)**	**1.38 (0.033)**
2nd trimester plasma ANGPTL4*	0.95 (0.735)		
Age	0.98 (0.568)		
Parity	**0.65 (0.020)**	**0.46 (0.011)**	**0.500 (0.023)**
GDM	1.05 (0.885)		1.16(0.734)
BMI before pregnancy	0.95 (0.143)		0.97 (0.734)
1st trimester FPG	0.98 (0.384)		
OGTT 1hr	1.00 (0.730)		
OGTT 2hr	1.00 (0.474)		
1st trimester HbA1c	0.39 (0.077)		
Hypertriglyceridemia	**0.41 (0.006)**		0.64 (0.254)

BMI, body mass index; FPG, fasting plasma glucose; GDM, gestational diabetes;

doi:10.6342/NTU202301373

OGTT: oral glucose tolerance test

*Odds ratio of every 1 SD change was shown

doi:10.6342/NTU202301373

Table 5. The area under the receiver operating characteristic curve (AUC) with and without indicated variables in the model predicting LGA, including plasma ANGPTL4 in the first trimester, history of macrosomia, GDM, BMI before pregnancy and hypertriglyceridemia. Differences in AUC between the full model and the model without the indicated variable were shown in parentheses.

	AUC
Plasma ANGPTL4	0.6743
Full Model	0.7121
Variable deleted from the model	
Plasma ANGPTL4	0.6196 (0.0925)
History of macrosomia	0.6948 (0.0173)
GDM	0.7116 (0.0005)
BMI before pregnancy	0.7015 (0.0106)
Hypertriglyceridemia	0.7067 (0.0054)

BMI: body mass index, GDM: gestational diabetes.

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Table 6. The area under the receiver operating characteristic curve (AUC) with and without indicated variables in models predicting SGA, including plasma ANGPTL4 in the first trimester, parity, GDM, BMI before pregnancy and hypertriglyceridemia. Differences in AUC between the full model and the model without the indicated variable were shown in parentheses.

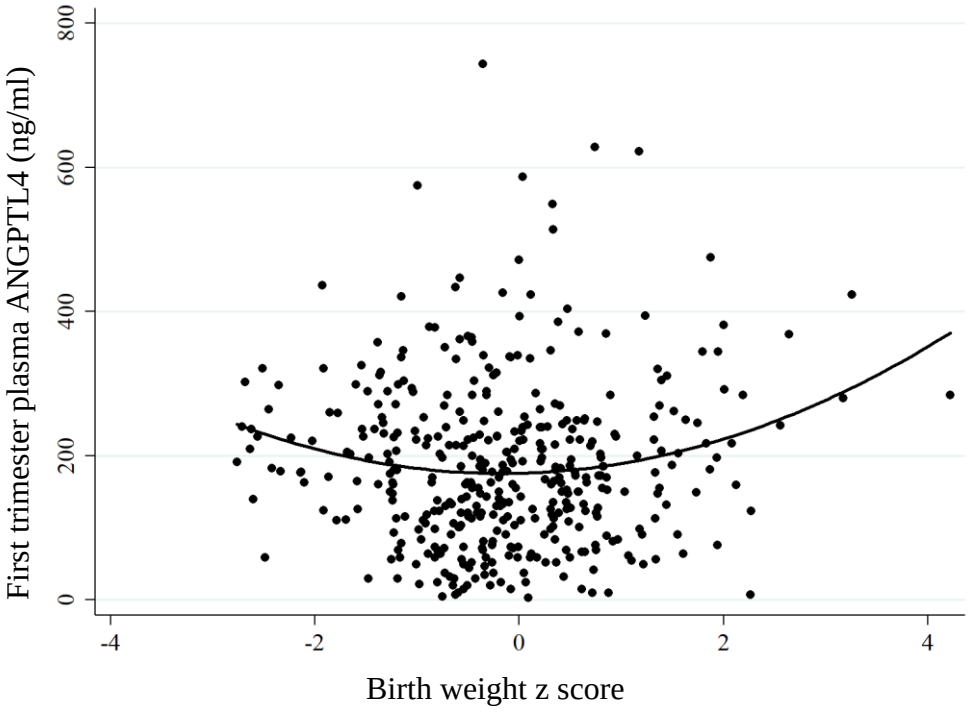
	AUC
Plasma ANGPTL4	0.6449
Full Model	0.6830
Variable deleted from the model	
Plasma ANGPTL4	0.6312 (0.0518)
Parity	0.6544 (0.0286)
GDM	0.6565 (0.0265)
BMI before pregnancy	0.6600 (0.0230)
Hypertriglyceridemia	0.6377 (0.0453)

BMI: body mass index; GDM: gestational diabetes

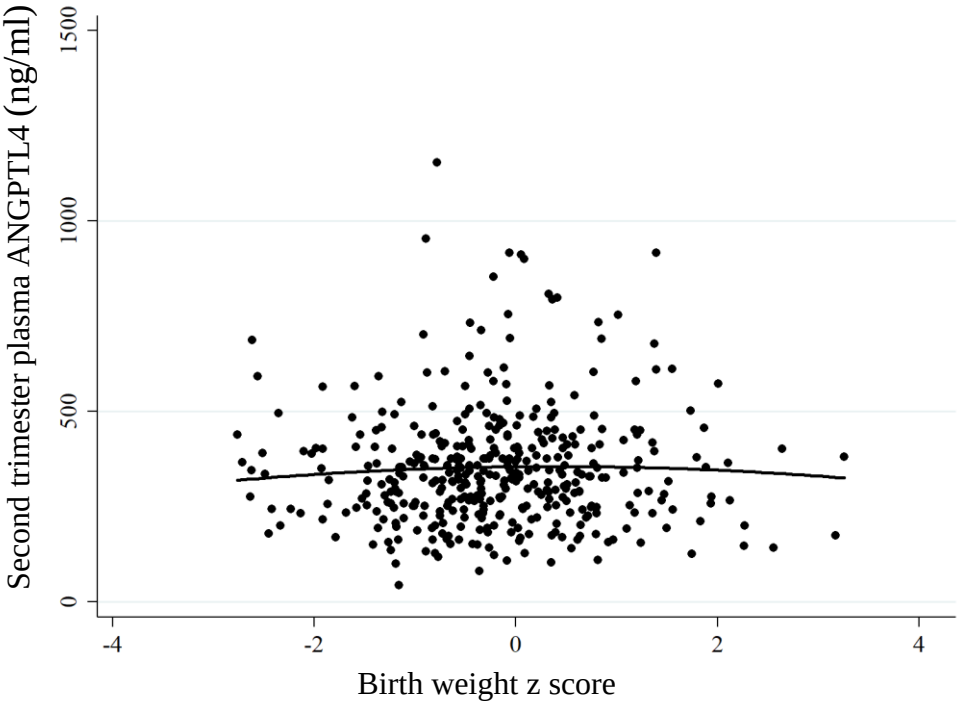
doi:10.6342/NTU202301373

Figure 1. The relationship between birth weight z score and plasma ANGPTL4 concentration in (A) the first trimester and (B) the second trimester.

(A)



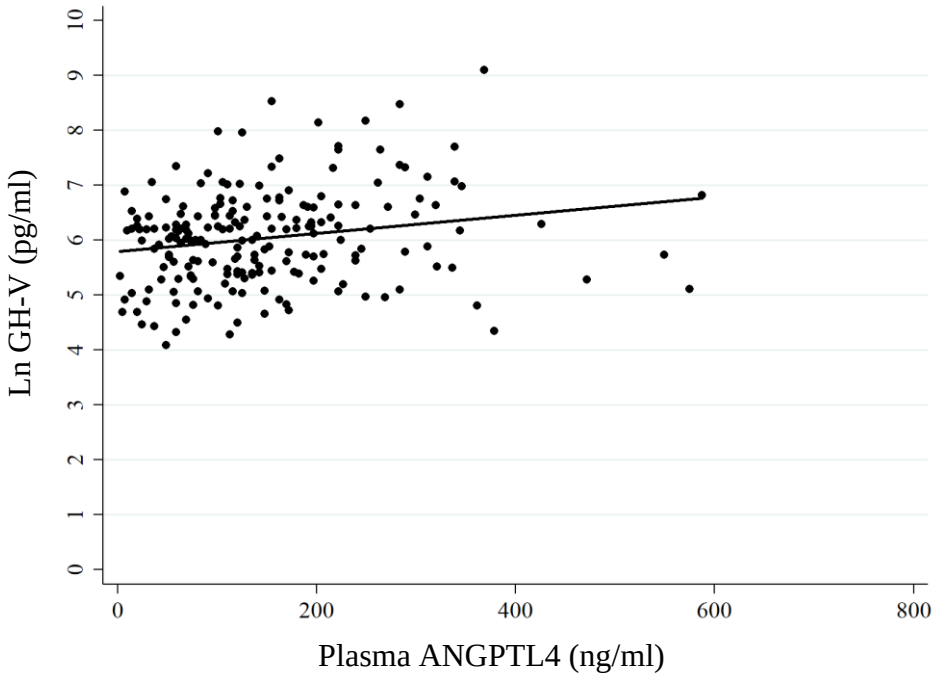
(B)



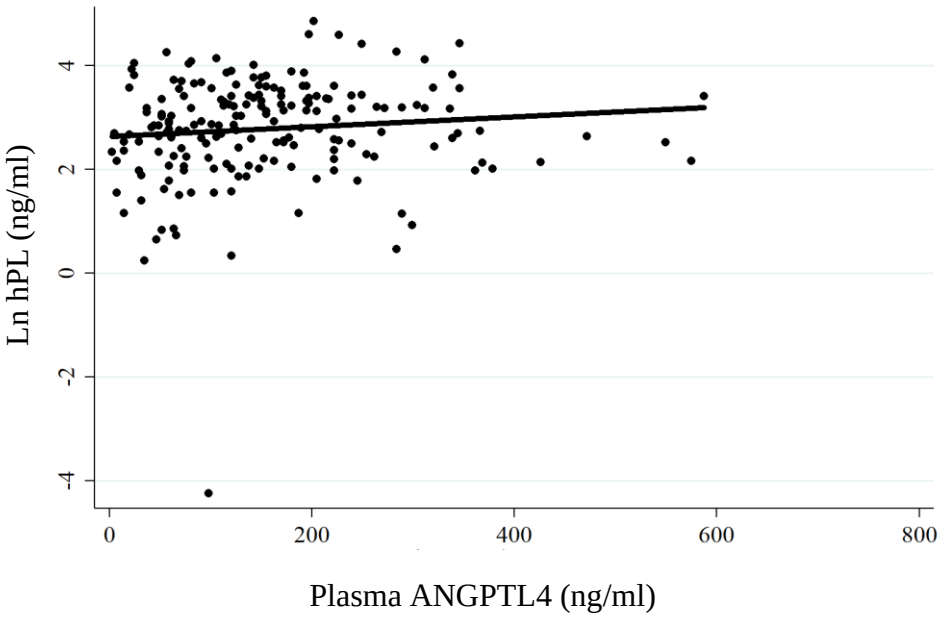
doi:10.6342/NTU202301373

Fig 2. The relationship between plasma ANGPTL4 and (A) plasma growth hormone variant (GH-V) (r=0.2005, p=0.0044), (B) plasma human placental lactogen (hPL) (r= 0.1033, p= 0.1571), (C) fasting plasma glucose (FPG) (r= 0.0351, p= 0.4727), and (D) plasma HbA1c (r= 0.1153, p= 0.0182) in the first trimester. Plasma GH-V and hPL were logarithmically transformed.

(A)

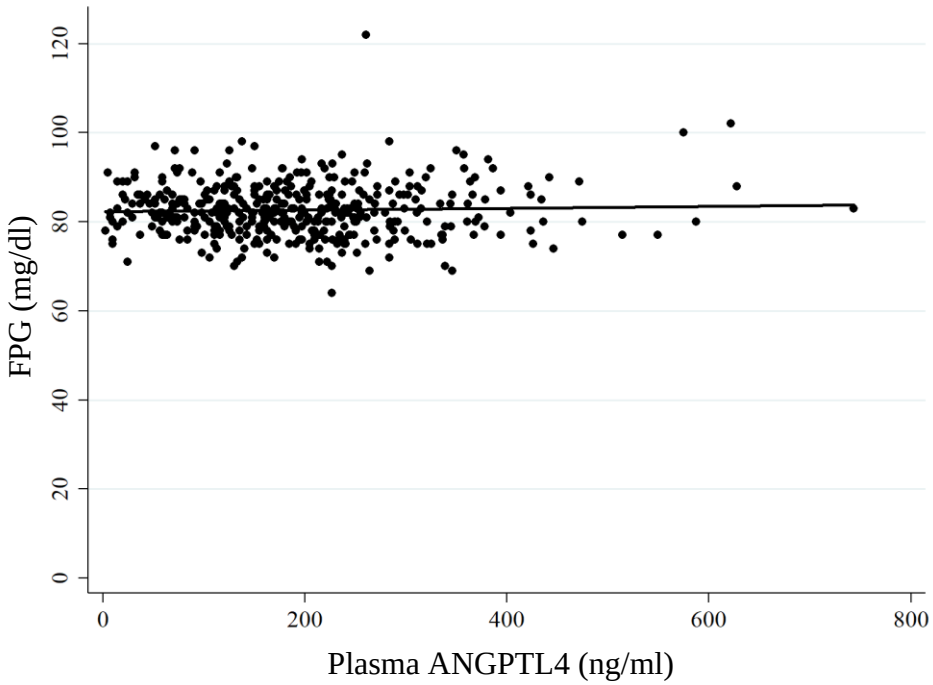


(B)

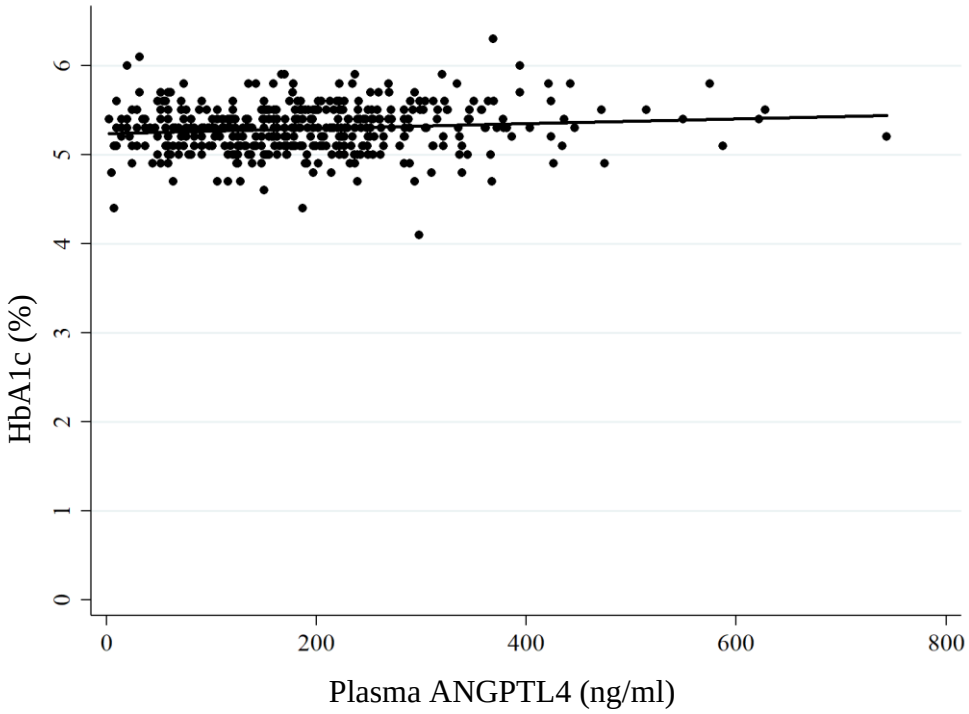


doi:10.6342/NTU202301373

(C)



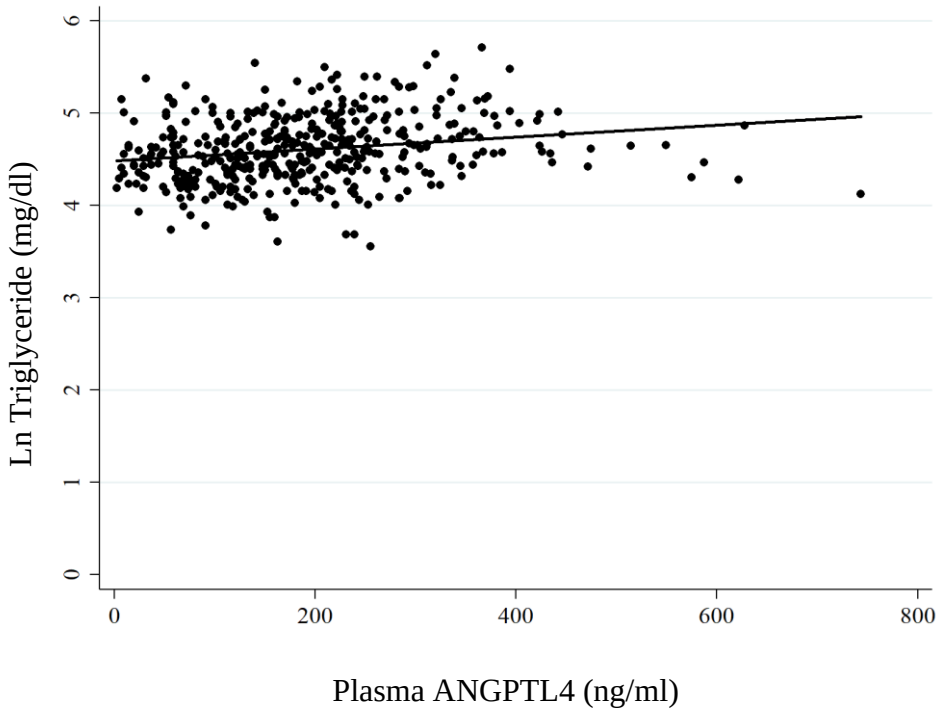
(D)



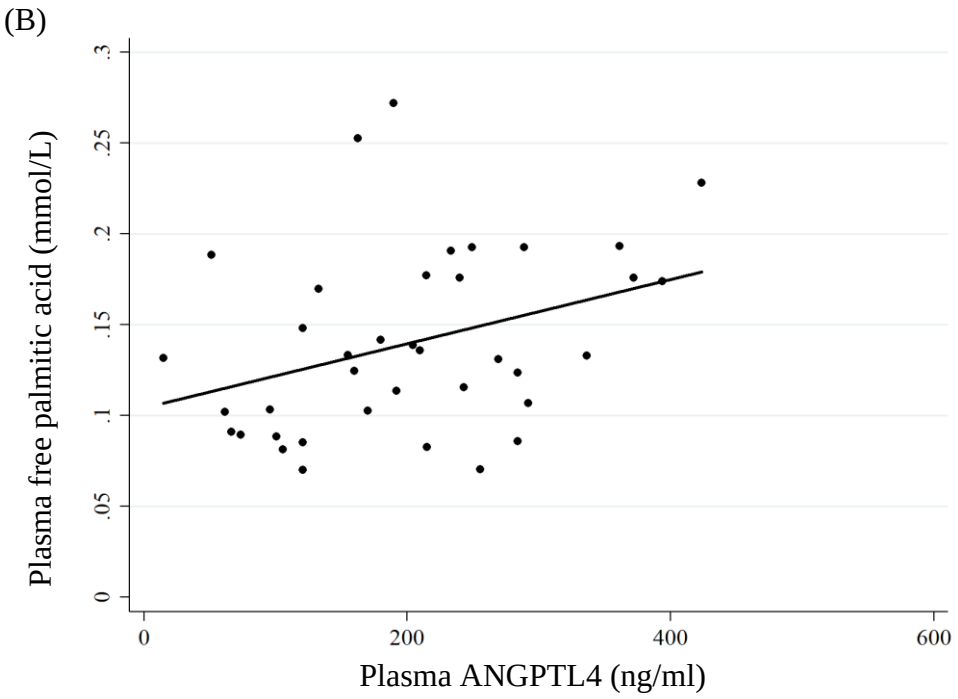
doi:10.6342/NTU202301373

Fig 3. The relationship between plasma ANGPTL4 in the first trimester and (A) plasma triglyceride in the first trimester (r= 0.2028, p<0.001), (B) plasma free palmitic acid (r= 0.3527, p= 0.0299), (C) plasma free stearic acid (r= 0.5063, p= 0.0012), (D) plasma free oleic acid (r= 0.4240, p= 0.0080), and (E) plasma free linoleic acid (r= 0.3360, p= 0.0392) in the second trimester. Plasma triglyceride was analyzed after log transformation.

(A)

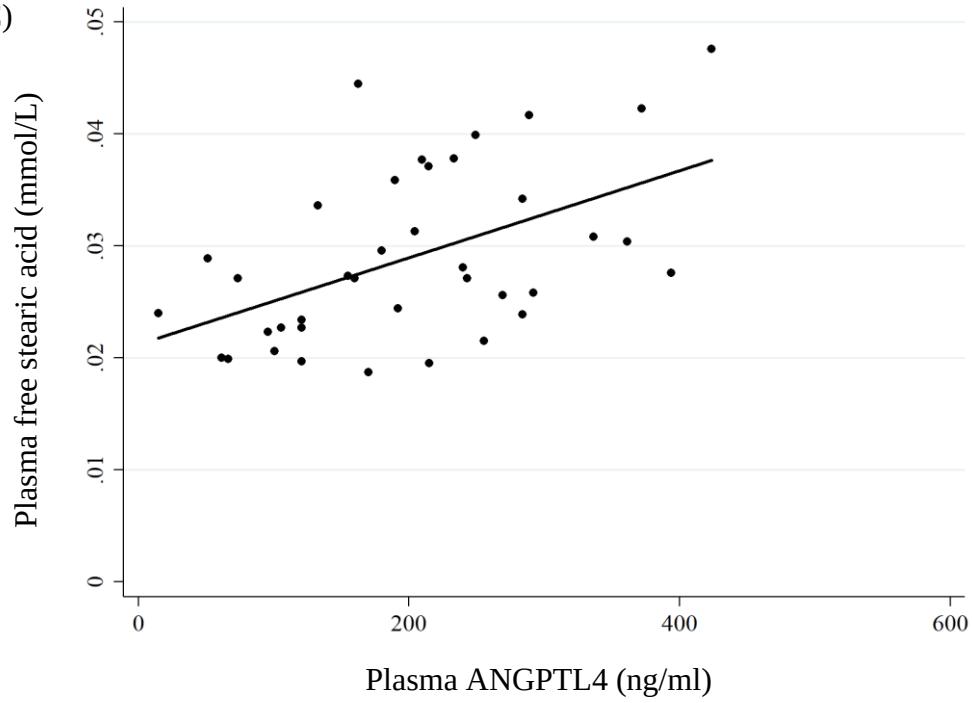


(B)

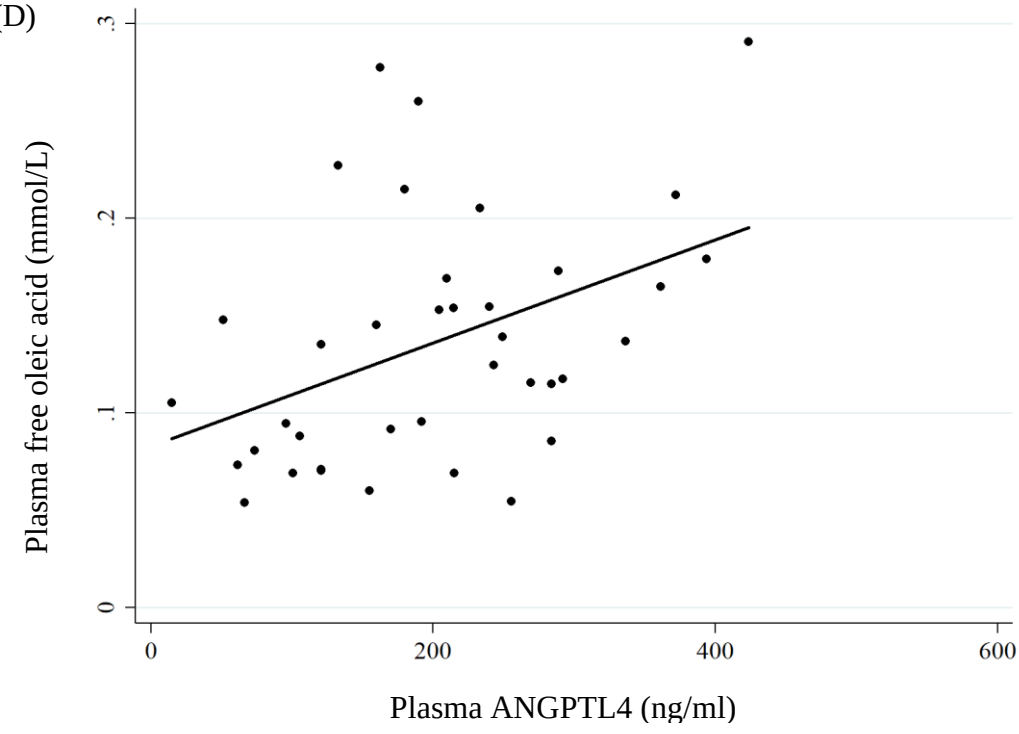


doi:10.6342/NTU202301373

(C)

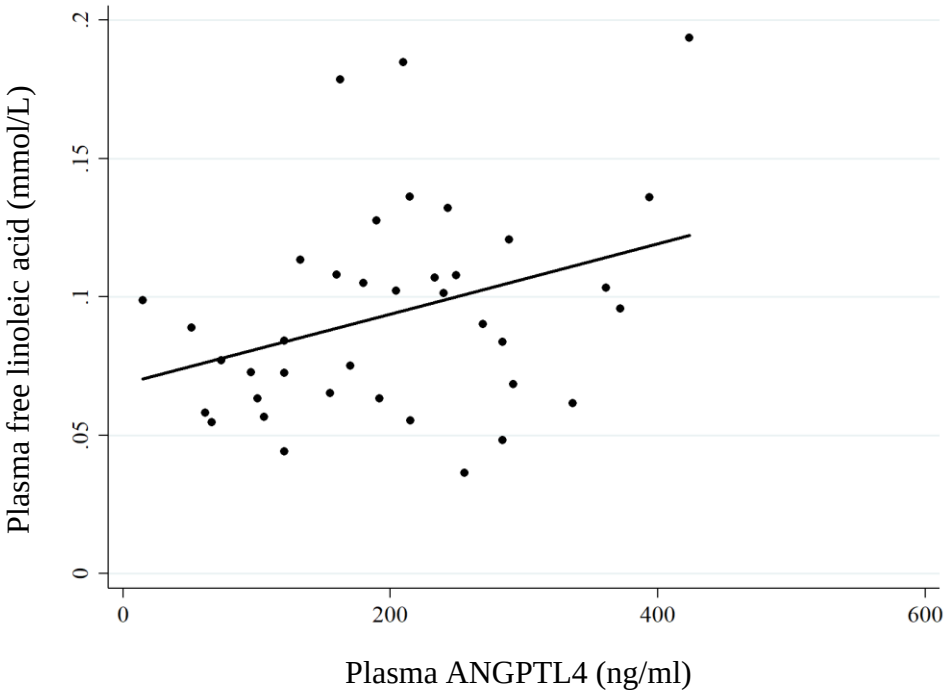


(D)



doi:10.6342/NTU202301373

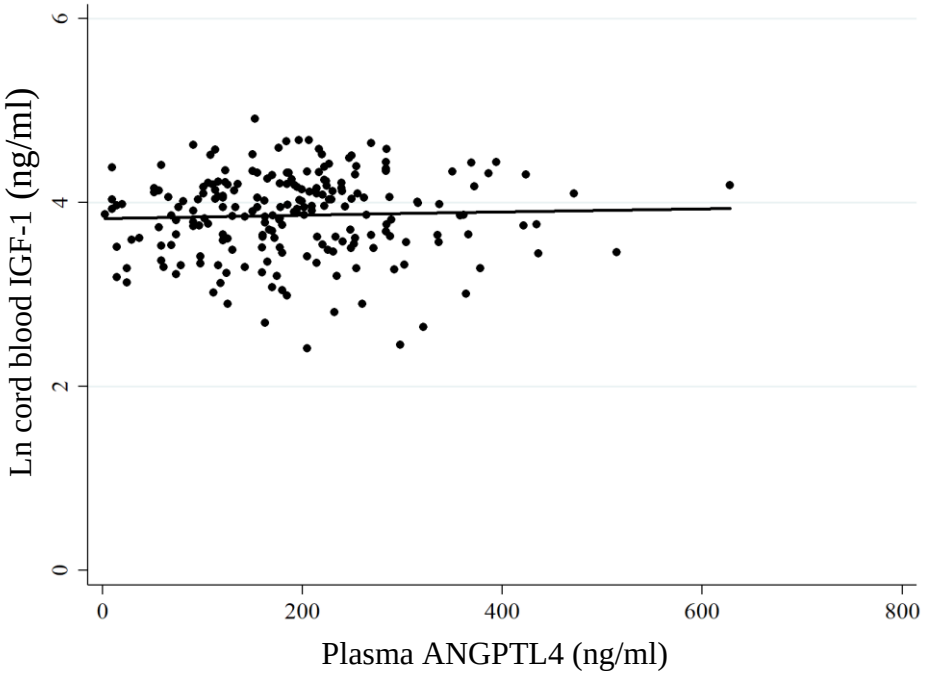
(E)



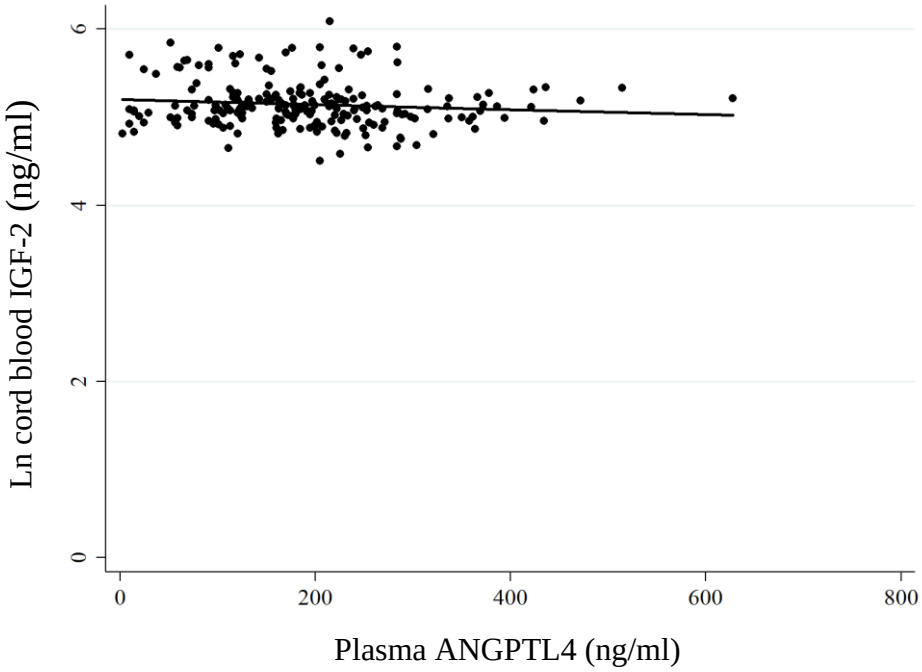
doi:10.6342/NTU202301373

Fig.4 The relationship between plasma ANGPTL4 in the first trimester and (A) cord blood IGF-1 (r= 0.0398, p= 0.5678), (B) cord blood IGF-2 (r= -0.1077, p= 0.1206), (C) cord blood IGFBP1 (r=0.0777, p= 0.2645), and (D) cord blood IGFBP3 (r= 0.0081, p= 0.9073). Cord blood IGF-1, IGF-2, IGFBP1 and IGFBP3 were analyzed after log transformation.

(A)

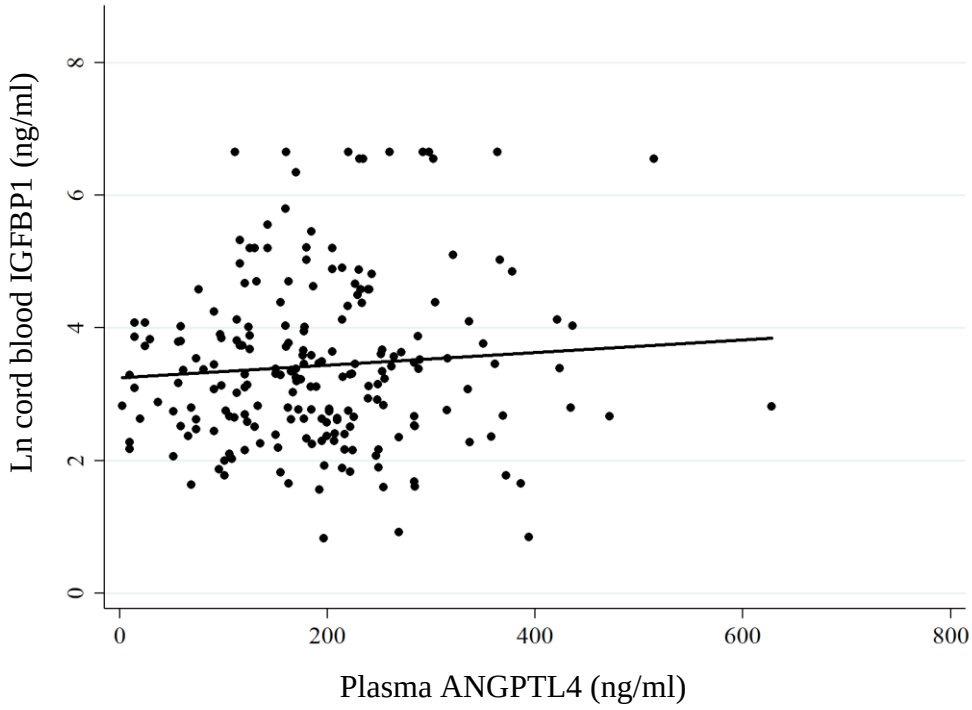


(B)

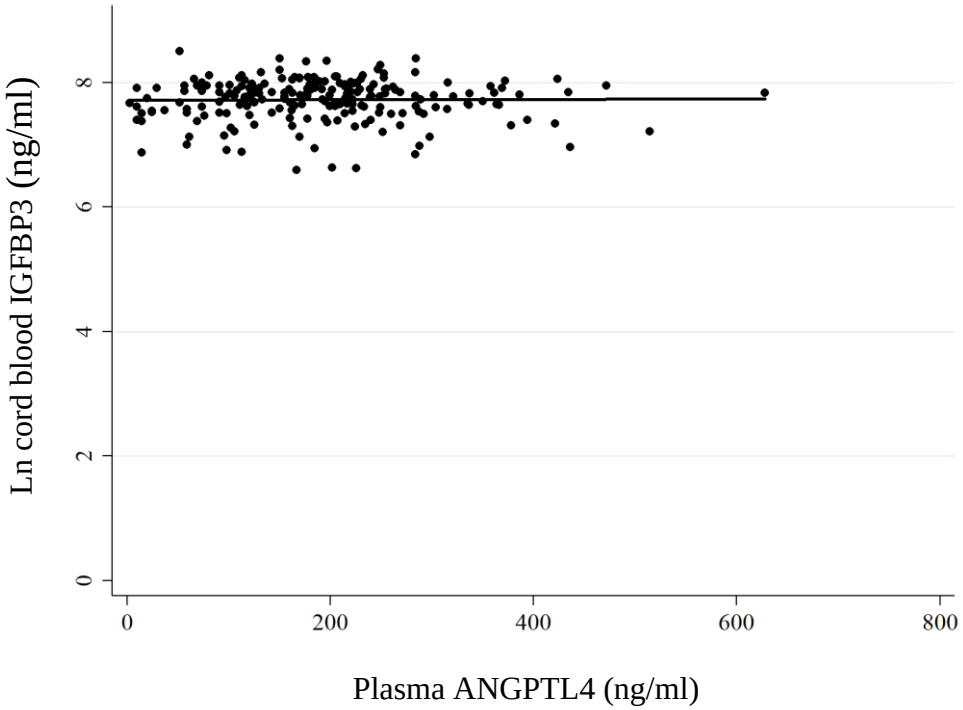


doi:10.6342/NTU202301373

(C)

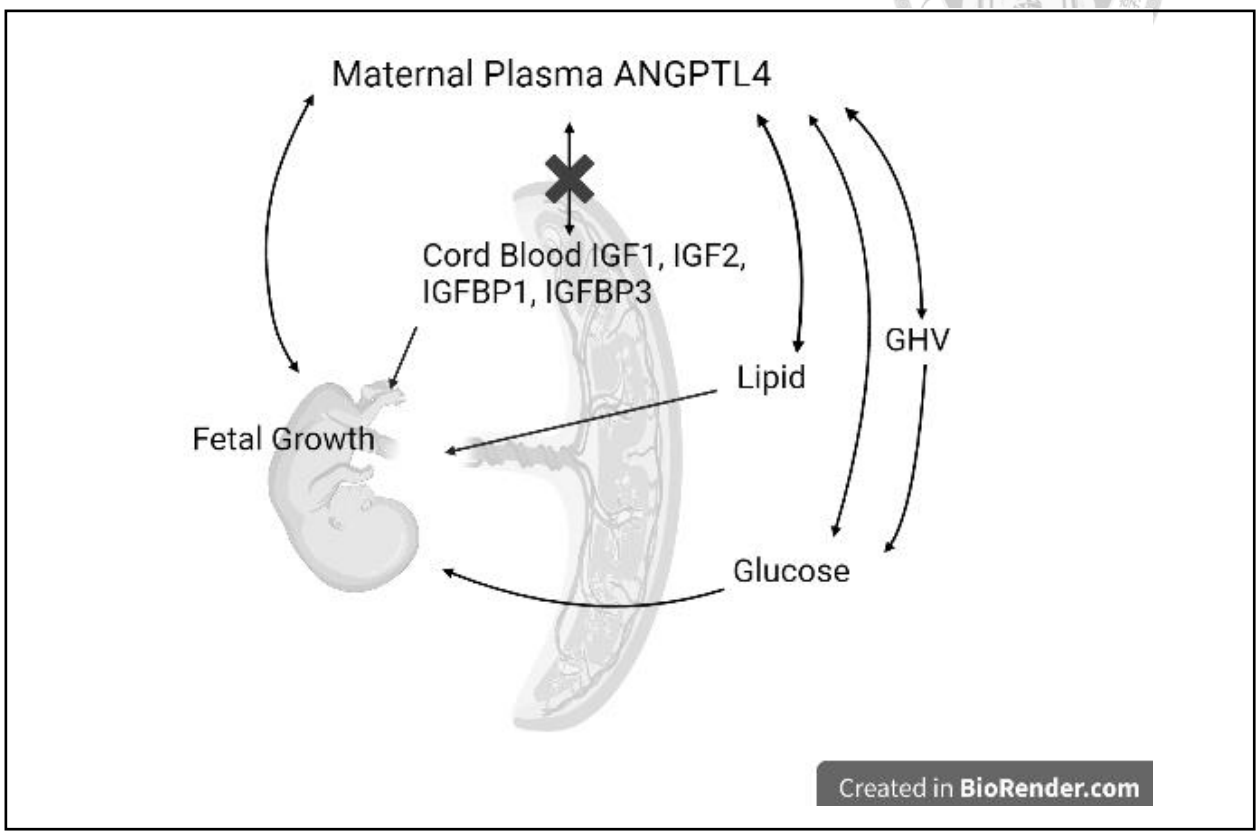


(D)



doi:10.6342/NTU202301373

Fig 5. Summary of findings in the present study.



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