Is a low level of free thyroxine in the maternal circulation associated with altered endothelial function in gestational diabetes?

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INTRODUCTION

Thyroid gland produces tetra-iodothyronine (T4 or thyroxine) and tri-iodothyronine (T3). In the human fetus, the synthesis of these hormones starts from 17 to 19th weeks of gestation (wg), therefore it is well accepted that before this period, the circulating T3/T4 in the fetus depends on the maternal levels of these hormones (Pérez-López, 2007). Thus, an altered function of the thyroid gland at the maternal side could prejudice physiological levels of T3/T4 at the fetal circulation, and impair fetal growth and development. Worldwide studies indicate that ∼10% of women may have hypothyroidism in their childbearing age (Mosso et al., 2012; Khalid et al., 2013; Ohashi et al., 2013). It has been also described that ∼35 or ∼3% of women with an apparent normal pregnancy have clinical hypothyroidism or exhibit maternal hypothyroxemia (low level of free T4), respectively (Mosso et al., 2012), both maternal conditions associated with several alterations in the fetus development (Parkes et al., 2012). Nevertheless, isolated maternal hypothyroxemia (IMH), a pathological condition manifested during pregnancy (Sahay and Nagesh, 2012), have been associated with occurrence of gestational diabetes (GD; Olivieri et al., 2000), pre-eclampsia (Sardana et al., 2009), or intrauterine growth restriction, IUGR (Chan et al., 2006). Moreover, pregnant women with IMH have higher risk (fourfold) to develop insulin resistance and GD (Karakaş et al., 2012; Tudela et al., 2012). In fact, reduced T4 level at the maternal circulation could result in an altered placental endothelial function during GD. In this review, we analyze available information regarding thyroid hormones and endothelial dysfunction in GD; and propose that low maternal levels of T4 observed in GD may be compensated by increased placental availability of T3/T4 via elevation in the activity of THT and/or reduction in deiodinases in the feto-placental circulation.

Keywords: thyroxine, gestational diabetes, endothelial dysfunction, pregnancy

Synthesis of thyroid hormones, thyroxine (T4) and tri-iodothyronine (T3), in the human fetus starts from 17 to 19th weeks of gestation. Despite the majority of normal pregnant women reaching adequate levels of circulating thyroid hormones, in some cases, women with normal pregnancies have low level of free T4 during first trimester of pregnancy, suggesting that T4 action may be compromised in those women and their fetuses. In addition, pathological low levels of thyroid hormones are detected in isolated maternal hypothyroxemia (IMH) and clinical hypothyroidism. Nevertheless, human placenta regulates T3/T4 concentration in the fetal circulation by modulating the expression and activity of both thyroid hormone transporters (THT) and deiodinases. Then, placenta can control the availability of T3/T4 in the feto-placental circulation, and therefore may generate an adaptive response in cases where the mother courses with low levels of T4. In addition, T3/T4 might control vascular response in the placenta, in particularly endothelial cells may induce the synthesis and release of vasodilators such as nitric oxide (NO) or vasoconstrictors such as endothelin-1 mediated by these hormones. On the other hand, low levels of T4 have been associated with increase in gestational diabetes (GD) markers. Since GD is associated with impaired placental vascular function characterized by increased NO synthesis in placental arteries and veins, as well as elevated placental angiogenesis, it is unknown whether reduced T4 level at the maternal circulation could result in an altered placental endothelial function during GD. In this review, we analyze available information regarding thyroid hormones and endothelial dysfunction in GD; and propose that low maternal levels of T4 observed in GD may be compensated by increased placental availability of T3/T4 via elevation in the activity of THT and/or reduction in deiodinases in the feto-placental circulation.
Gestational diabetes (GD) is associated with higher synthesis and release of vasodilators such as nitric oxide (NO) in the human fetal endothelium from GD (described as altered endothelial function) (De Vriese et al., 2000; Guzmán-Gutiérrez et al., 2011; Westermeier et al., 2011; Salomón et al., 2012). In addition, thyroid hormones are also involved in NO synthesis and release (Napoli et al., 2001; Fazio et al., 2004), but the potential contribution of reduced circulating T4 on deregulation of fetal endothelial function seen in GD pregnancies is unclear. We here analyze the available information regarding the potential relationship between maternal and fetal thyroid hormones with the occurrence of endothelial dysfunction in GD. We propose that the low maternal levels of T4 seen in GD may be compensated by higher placental availability of thyroid hormones via elevation in the activity of placental thyroid hormone transport and metabolism.

**OVERVIEW OF SYNTHESIS AND RELEASE OF THYROID HORMONES**

The thyroid hormones 3,5,3′,5′-tetraiodothyronine (T4 or thyroxine) and 3,5,3′-triiodothyronine (T3) are synthesized in the thyroid gland and is regulated by hypothalamus/pituitary/thyroid axis by a negative feedback. In this regulatory axis, hypothalamus releases thyrotropin releasing hormone (TRH), which interacts with TRH receptors in thyrotropin cells in the pituitary gland to release thyroid stimulating hormone (TSH). In turn, TSH is the main regulator of the release of thyroid hormones leading to TSH receptor (TSHr) activation and increased iodide (iodide) uptake in the thyroid gland (Szkudlinski et al., 2002).

Iodide intracellular uptake is mediated by cotransport with sodium (Na+ / I−) in the basal membrane of follicular cells in the thyroid gland. In these cells, iodides are oxidized by thyroid peroxidase (TPO) in the presence of hydrogen peroxide. Iodine (oxidized iodine) binds to thyroxine residues belong to the triiodothyronins (Tg), then thyroxine residues can be mono (MIT), di (DIT), or tetra-iodinated (T4) (Sugeno et al., 1984; Rouset, 1991). The release of thyroid hormones through the basolateral membrane in thyroid gland follicular cells requires endocytosis of iodinated Tg at the apical side of these cells. The Tg is then incorporated into phagolysosomes and digested by proteolytic proteins, with MIT and DIT being re-uptaked into Tg; however, T3 and T4 are released toward circulation (Rouset, 1991). T4 is the main thyroid hormone released by thyroid gland follicular cells (~40-fold compared with T3) and is almost all (99.97%) bound to thyroxine binding globulin (TBG), albumin and pre-albumin in the circulation. In addition, free T3 accounts for ~0.3% and the rest is bound to TBG and albumin.

Free T3 is the hormone with biological activity and is the active form of thyroid hormones. It is derived from 5′-deiodination of free T4 via iodothyronine deiodinases located in the target tissues (Sugeno et al., 1984; Rouset, 1991; Schussler, 2000; Bianco and Kim, 2006). Deiodinases are grouped in three subtypes: I, II, and III (or D1, D2, and D3, respectively), all of which are involved in the regulation of T3 activity (Bianco and Kim, 2006; Darras and Van Herck, 2012). For instance, D2 is specific to generate T3 from T4; however, D3 generates DIT from T3, and reverse T3 (rT3, inactive form of T3) from T4 (Kilby et al., 2005). D1 has been reported as an enzyme that is much less active compared with the other forms (Bianco and Kim, 2006; Dentice and Salvatore, 2011; Darras and Van Herck, 2012).

**VASCULAR EFFECTS OF THYROID HORMONES**

Thyroid hormones reduce peripheral vascular resistance by promoting relaxation in human and murine vascular smooth-muscle cells (Klemperer et al., 1995; Ojamaa et al., 1996; Park et al., 1997), and improve vascular reactivity by endothelium-dependent and -independent mechanisms (Napoli et al., 2001; Fazio et al., 2004). In rat, T4 released from the mesenteric arteries increases vasorelaxation when administrated at supraphysiological concentrations (Zwaveling et al., 1997). Moreover, T3 and T4 improve fibroblast growth factor-2 (FGF-2) expression, a recognized proangiogenic factor, in cultures of ECV304 cells (Davis et al., 2004). Interestingly, human umbilical vein endothelial cells (HUVEC) exposed to high T3 levels exhibit high expression of endothelin-1 (vasoconstrictor) and fibronectin (profibrotic molecule), suggesting that pathological conditions such as hyperthyroidism could be associated with vasocostriction (Baumgartner-Parzer et al., 1997; Diekman et al., 2001).

In addition, there is evidence that vasocostriction associated with high levels of thyroid hormones may result from a non-genomic action, which seems mediated by αvβ3 integrin as reported in HUVEC (Luidens et al., 2010). In this regard, αvβ3 integrin is a membrane protein that should activate the phospha-tidylinositol 3 kinase and protein kinase B/Akt (PI3K/Akt) pathway in this cell type (Hiroi et al., 2006; Luidens et al., 2010). Nevertheless, in another study HUVEC and bovine aortic endothelial cells (BAEC) seems to respond to T3 by increasing the phosphorylation of serine1177 (Ser1177; Ser1177-eNOS) at the endothelial nitric oxide synthase isotype (eNOS) in a time- and concentration-dependent manner (Hiroi et al., 2006). Similar results were seen in vascular smooth-muscle cells from rat thoracic aortae where T3 increases Ser1177-eNOS via PI3K/Akt pathway inducing eNOS, inducible (iNOS) and neuronal (nNOS) NOS expression. Thus, it is likely that T3 increases NOS expression via a genomic and a non-genomic (i.e., via αvβ3 integrin) action (Carrillo-Sepúlveda et al., 2010). Therefore, the thyroid hormone concentration is a determinant factor involved in the modulation of vascular function (Figure 1). However, there is not information addressing thyroid hormone effects on the human feto-placental vasculature.

**HUMAN THYROID HORMONES IN PREGNANCY**

Human fetal thyroid hormones are secreted from the 17 to 19 wg, indicating that the fetus requires thyroid hormones delivery from the mother during the first and beginning of the second trimester of pregnancy (Pérez-López, 2007). Pregnant women have TSH and free T4 levels that are normal and comparable to those in non-pregnant women; however, in the first trimester of pregnancy, there is an increase in the maternal free T4 level most likely in response to chorionic gonadotropin hormone (hCG; Fantz et al., 1999; Pérez-López,
FIGURE 1 | Thyroid hormones effects on endothelial cells. T4 (Tyrosine) is metabolized by deiodinase 2 (D2) to T3 (Triiodothyronine) of manner extra and intracellular. T3 and T4 (Tyrosine) could activate αvβ3 integrin receptor which initiate a signaling that involve PI3K, Akt and eNOS, generating nitric oxide (NO) as vasodilator. But, also T3 and T4 are uptake by thyroid hormones transporters (THT). T3 bind to DNA and activate transcriptional activity for endothelin-1 and fibronectin which are associated with vasoconstriction.

2007). It has been shown that increased hCG level leads to reduced hypothalamus/pituitary/thyroid axis activity, but improved thyroid hormones delivery to the fetus in the first trimester of pregnancy (Fantz et al., 1999; Pérez-López, 2007; Chan et al., 2009). In this period, the concentration of free T4 in the fetal circulation corresponds to a third of the level found in the maternal circulation (Chan et al., 2009). This phenomenon results from a reduced concentration of the TBG binding protein in the fetal circulation, which leads to a free T4 concentration enough to exert its biological effects in embryonic tissues (Kilby et al., 2005; Chan et al., 2009). In the second trimester of pregnancy, free T4 levels in the fetal circulation corresponds to about half of the concentration detected in the maternal circulation (Kilby et al., 2005; Chan et al., 2009). Therefore, it seems clear that thyroid hormone levels are regulated by the placenta tissue (Burrow et al., 1994). Thus, a role of this organ is crucial in the delivery of T4 to the fetus.

THYROID HORMONES METABOLISM IN THE HUMAN PLACENTA
In addition to the paracrine effect of hCG on the hypothalamus/pituitary/thyroid axis, the human placenta regulates directly the thyroid hormone concentration in the fetal circulation by modulation of thyroid hormone transporters (THT), and by thyroid hormones metabolism mediated by deiodinases (Burrow et al., 1994). THT are located at the apical and basolateral membranes of the cytotrophoblasts, syncytiotrophoblast and microvascular endothelial cells (James et al., 2007). There are several THT, including monocarboxylate transporters (MCT), where MCT8 and MCT10 are the main forms. Moreover, a role has been reported for L-amino acid transporters (LAT) and organic anion transporter polypeptides system (OATPs), which operates with less selectivity for T4 (James et al., 2007). Also in the human placenta MCT8 (Park and Chatterjee, 2005), MCT10, LAT1, LAT2 (Friesema et al., 2003), OATP1A2 and OATP4A1 (Hagenbuch and Meier, 2003; Hagenbuch, 2007) have been identified, but no studies addressing the role of these membrane transporters in any pathology of pregnancy have been documented. Moreover, D2 (located at the endoplasmatic reticulum) and D3 (located at the plasma membrane with a cytoplasmic active site; Koopdonk-Kool et al., 1996; Stulp et al., 1998; Chan et al., 2003) have been identified. D2 and D3 are referred as major factors controlling transplacental transport of T4 to the fetus (Mortimer et al., 1996). Interestingly, D2 and D3 expression is up regulated by T3 (Chan et al., 2003). In addition, since changes in the level of T3/T4 cause altered THT expression (Mortimer et al., 1996), modulation of D2/D3 and THT expression by T3/T4 could be a phenomenon serving as a defense mechanism for the fetus in pregnancies where the mother courses with hypothyroxemia.
ISOLATED MATERNAL HYPOTHYROIDISM AND CLINICAL HYPOTHYROIDISM

The pathologies associated with low levels of free T4 correspond to IMH and clinical hypothyroidism. IMH (1–2% of normal pregnancies) is characterized by low free T4 (<10th percentile in normal range), but normal TSH level. Instead, clinical hypothyroidism characterizes by high levels of TSH, but low levels of free T4 (Casey et al., 2005). In another pathological condition referred as “low T3 syndrome,” an increase in the expression of the membrane transporters MCT8 is reported, which could be a compensatory response to low levels of thyroid hormones (Mebis et al., 2009). The latter seems paralleled by an increase in the D1 and D2 levels as reported in human skeletal muscle and liver (Peeters et al., 2003; Weetman et al., 2003). While, using a knock-out mice model for MCT8 (Mct8−/−) an increase in the plasma free T4 and T3 levels, and D1 and D2 expression and activity in the liver was shown (Dumitrescu et al., 2006). Therefore, these results support the fact that low levels of T4 lead to changes in the THT and deiodinase expression and activity in target organs. However, there is no information addressing this possibility in the human placenta from pregnancies coursing with maternal hypothyroxemia.

On the other hand, minor changes such as D2 gene polymorphism (Thr92Ala) are associated with human type 2 diabetes mellitus and insulin resistance (Mentuccia et al., 2002). In this regard, despite there is not information regarding D2 gene polymorphism in women coursing with pregnancies without a diagnosis of thyroid gland pathology, a negative correlation between free T4 level and metabolic markers of GD and insulin resistance (i.e., degree of glycosylated HbA1c, fasting insulin, and HOMA-IR) has been shown (Bassols et al., 2009). While, using a knock-out model for MCT8, an increase in the plasma free T4 and T3 levels, and D1 and D2 expression and activity in the liver was shown (Dumitrescu et al., 2006). Therefore, these results support the fact that low levels of T4 lead to changes in the THT and deiodinase expression and activity in target organs. However, there is no information addressing this possibility in the human placenta from pregnancies coursing with maternal hypothyroidism.

MATERNAL T4 LEVEL AND GD

GD is a disease coursing with glucose intolerance first recognized or manifested during pregnancy [Metzger et al., 2007; Reece et al., 2009; American Diabetes Association [ADA], 2012]. This pathology accounts for ~5% of pregnant women worldwide and it is associated with high risk of fetal perinatal alterations (e.g., macrosomia, insulin resistance) and higher incidence of diseases in the adulthood (e.g., GD, obesity, dyslipidemia, hypertension, metabolic syndrome) (Poston, 2010; Negrato et al., 2012). GD is associated with reduced maternal circulating T4 levels in the first trimester of pregnancy. To date, 5% of women couring with GD pregnancies have been shown to correlate with IMH (Krcma et al., 2010). In addition, free T4 levels are lower in women with GD pregnancy compared with women with normal pregnancies (Velkoska-Nakova et al., 2010) and a reduced free T4 level is shown in 70% of patients with GD pregnancies (Olivieri et al., 2000). Therefore, maternal hypothyroxemia could be associated with GD.

Other pathologies associated with low levels of free T4, such as clinical hypothyroidism (i.e., low free T4 and high TSH levels), also have been associated with GD. Indeed, 6–15% of GD pregnancies are associated with hypothyroidism (Velkoska-Nakova et al., 2010; Tudela et al., 2012; Stohl et al., 2013). Moreover, if pregnant women have hypothyroidism, they have 4.3-fold higher risk for developing GD (Karakosta et al., 2012). There are no publications addressing the T4 plasma levels at the fetal circulation in a pregnancy coursing with GD. However, since GD courses with endothelial dysfunction (De Vriese et al., 2000; Westermeier et al., 2011; Guzmán-Gutiérrez et al., 2011, 2014; Salomón et al., 2012) and thyroid hormones modulate endothelial function (Napoli et al., 2001; Fazio et al., 2004), it is likely that a low free T4 level at the maternal circulation eventually could result in altered endothelial function in GD pregnancies.

GD AND ENDOTHELIAL DYSFUNCTION

One of the main alterations detected in GD pregnancies is the associated endothelial dysfunction of the fetoplacental circulation (De Vriese et al., 2000; Guzmán-Gutiérrez et al., 2011, 2014; Westermeier et al., 2011; Salomón et al., 2012). Since the vasculature in the human placenta lacks innervation (Marzioni et al., 2004), several local metabolic mechanisms, such as synthesis and release of vasoactive molecules (e.g., NO, adenosine) (Vásquez et al., 2004; Guzmán-Gutiérrez et al., 2011, 2014; Sobrevia et al., 2011) or release of nanovesicles (e.g., exosomes), most likely mediating autocrine and/or paracrine modulation of vasculature (Salomon et al., 2013), could lead to acute and rapid modulation of vascular tone in this vascular bed (Guzmán-Gutiérrez et al., 2011, 2014; Burnstock and Novak, 2013).

Arteries and veins in the human placenta from pregnancies with GD exhibit increased NO synthesis (Figueroa et al., 2000). Furthermore, similar results were early reported in primary cultures of HUVEC from pregnant women diagnosed with GD (Sobrevia et al., 1995). Therefore, it has been proposed that vascular dysfunction in GD could result from a functional dissociation between NO synthesis and its bioavailability in the human placental circulation (Guzmán-Gutiérrez et al., 2011, 2014; Sobrevia et al., 2011). Even when endothelial dysfunction, referred to as an alteration of NO synthesis and the uptake of cationic amino acid l-arginine (i.e., l-arginine/NO pathway), is associated with GD, a clear mechanism behind these effects of GD is still unavailable (Guzmán-Gutiérrez et al., 2014).

HUMAN PLACENTA ENDOTHELIAL FUNCTION AND T4 IN GD: A HYPOTHESIS TO BE TESTED

In normal pregnancies maternal free T4 is taken up by THT by the syncytiotrophoblast, where it is metabolized by D2 and D3 to be converted into T3 or rT3, respectively. T4 is then released via THT at the basolateral membrane of the syncytiotrophoblast into the intervillous space from where it is taken up by the microvascular endothelial cells via THT. In these cells a fraction of T4 is metabolized again to produce T3 and rT3 via D2 and D3, respectively (Figure 2). As a result of this process, T4 and T3 are released into the fetal blood. However, currently there is no information regarding transport of thyroid hormones across the human placenta in GD pregnancies. We propose that low levels of T4 lead to an increase in the number and activity of...
THT membrane transporters available at the plasma membrane of the human placental endothelial cells, and reduced deiodinase expression and activity, in order to supply T₄ necessities associated with fetal development in GD. These changes could constitute a mechanism by which the endothelium from the human placenta intends to maintain normal intracellular and circulating levels of T₄ in the fetus. The latter would be potentially reached by a greater delivery of maternal T₄ to the fetal blood.

**FINAL COMMENTS AND CONCLUSION**

Based on what was described in this review, our central research questions are: (1) is a low level of free T₄ in the maternal circulation associated with GD? (2) is GD a disease associated with increased placental THT, but reduced deiodinase expression and activity? and (3) would the potential changes caused by reduced free T₄ level in the maternal circulation and altered THT and deiodinases in the placenta in GD lead to placental endothelial dysfunction? Furthermore, nothing is known regarding the feto-placental vascular function/dysfunction in pregnancies where the mother courses with hypothyroxemia. Despite many benefits for using human placental tissue after birth, we acknowledge that information behind cellular mechanisms and adaptative response occurred at the beginning of pregnancy is difficult to extrapolate; however, it offers a good approximation for studying consequences of human pathologies. Potential more complex models, might include analysis of placentas collected from animal deficient in leptin receptor (db/db), since they develop GD during pregnancy (Bobadilla et al., 2010), offering a model that may to understand molecular mechanisms of THT and deiodinases in first trimester of pregnancy. Moreover, a therapeutical approach of pregnant women coursing with hypothyroxemia targeted to improve free T₄ circulating levels will likely reduce the risk of developing GD and the deleterious consequences of this disease in the feto-placental endothelial function. We also speculate that a normalization of free T₄ levels in the first trimester of pregnancy could reduce the risk to face GD-associated complication.

**AUTHOR CONTRIBUTIONS**

Enrique Guzmán-Gutiérrez and Luis Sobrevia generated the text and figures, Carlos Vees, Andrea Leiva, and Carlos Escudero contributed for design of text.

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