Maternal thyroid function in multifetal pregnancies before and after fetal reduction

O Ogueh¹, A P Hawkins¹, A Abbas², G D Carter³,
K H Nicolaidis² and M R Johnson¹

¹Section of Obstetrics and Gynaecology, Imperial College School of Medicine, Chelsea and Westminster Hospital, 369 Fulham Road, London SW10 9NH, UK

²The Harris Birthright Research Centre for Fetal Medicine, King's College Hospital Medical School, London SE5 8RX, UK

³Department of Chemical Pathology, Imperial College School of Medicine, Charing Cross Hospital, Fulham Palace Road, London W6 8RF, UK

(Requests for offprints should be addressed to M R Johnson)

Abstract

The aim of the study was to investigate maternal thyroid function in pregnancy by monitoring the circulating concentrations of thyroid stimulating hormone (TSH), free thyroxine (fT₄) and human chorionic gonadotrophin (hCG) in multifetal pregnancies before and after embryo reduction. We studied two groups of women: group 1 comprised singleton ($n=12$) and twin ($n=12$) pregnancies achieved after superovulation and *in vitro* fertilisation and embryo transfer (IVF-ET), and group 2 were multifetal pregnancies ($n=39$) undergoing selective fetal reduction to twin pregnancies. Blood samples were obtained initially at 10–12 weeks gestation (before fetal reduction) and then 4 and 8 weeks afterwards. Before fetal reduction, the circulating concentrations of fT₄ in multifetal pregnancies were significantly greater than those in singleton or twin pregnancies (singleton, mean 16.49 pmol/l (inter-quartile range 14.09–18.13 pmol/l); twins, 15.84 (15.36–

16.95 pmol/l); multifetal, 21.08 (16.64–26.29 pmol/l); $P<0.005$ for singleton and twins), and in a multiple regression analysis, fT₄ was significantly related to the number of fetuses ($F=23.739$, $P=0.0001$), but not to hCG. After fetal reduction to twins, the circulating concentrations of fT₄ in multifetal pregnancies decreased progressively towards those in control twin pregnancies, but remained significantly greater at both 4 ($P=0.003$) and 8 weeks ($P=0.050$). This pattern of change in the concentrations of fT₄ is similar to, but lags behind, that of hCG, which attains twin levels 4 weeks after fetal reduction. This may represent a delayed thyroid response to the decreasing concentrations of hCG, but the alternative is that the maternal thyroid function is controlled by a fetal factor in addition to hCG.

Journal of Endocrinology (2000) **164**, 7–11

Introduction

Thyroid activity is increased during the first trimester of normal pregnancy, reflected by a decrease in the circulating concentrations of thyroid stimulating hormone (TSH) and an increase in the concentrations of free thyroxine (fT₄) and tri-iodothyronine (fT₃). The origin of the increased maternal thyroid function is almost certainly stimulation of the TSH receptor by human chorionic gonadotrophin (hCG) (Guillame *et al.* 1985, Yoshikawa *et al.* 1989, Glinoe *et al.* 1990, Ballabio *et al.* 1991). As the fetal thyroid is active only after 14 weeks gestation, it is suggested that the increased maternal thyroid activity ensures that normal neuronal development occurs in the fetus (Shepard 1975). The circulating concentrations of TSH, fT₄ and fT₃ revert back to normal by the second trimester in most women, and remain within normal limits during the second and third trimesters (Harada *et al.* 1979).

The increase in thyroid activity in the first trimester is more profound in twin than in singleton pregnancy (Grun *et al.* 1997), probably because peak concentrations of hCG are greater and remain increased for longer in twin pregnancies (Grun *et al.* 1997). However, it has been suggested that factors other than hCG may be responsible for the pregnancy-associated increase in maternal thyroid function (Kennedy *et al.* 1992). The aim of the present study was to test the hypothesis that a factor other than hCG is responsible for the changes in maternal thyroid function. To achieve this, we measured the circulating concentrations of TSH, fT₄ and hCG before and after selective fetal reduction.

Patients and Methods

Two groups of women were examined. Group 1 comprised singleton ($n=12$) and twin ($n=12$) pregnancies

Table 1 Circulating concentrations of fT₄, TSH and hCG in sample 1, taken at 10–12 weeks gestation in all groups, and samples 2 and 3 taken at +4 and +8 weeks respectively. Data are median (interquartile range)

Hormone, and pregnancy type	Circulating hormone concentration		
	Sample 1 (pre-reduction)	Sample 2 (+4 weeks)	Sample 3 (+8 weeks)
fT ₄ (pmol/l)			
Singleton	16.49** (14.09–18.13)	14.12* (12.99–15.92)	13.48 (12.75–14.70)
Twin	15.84** (15.36–16.95)	13.04** (12.08–14.95)	12.86 (10.58–13.68)
Fetal reduction	21.08 (16.64–26.29)	17.60 (14.08–18.70)	13.97 (12.57–15.40)
TSH (mU/l)			
Singleton	1.077** (0.42–2.06)	1.45** (1.05–3.27)	1.07 (0.93–2.36)
Twin	0.70 (0.20–1.21)	1.275* (0.87–1.96)	0.813 (0.25–1.29)
Fetal reduction	0.113 (0.013–0.89)	0.382 (0.01–1.21)	1.145 (0.63–1.90)
hCG (IU/l)			
Singleton	90.6** (73.69–105.65)	41.69** (26.48–61.6)	16.6** (14.11–37.76)
Twin	138.6 (113.4–186.1)	83.3* (29.5–90.2)	57.6 (31.8–97.9)
Fetal reduction	201.9 (134.1–232.7)	123.3 (75.6–192.7)	53.9 (36.5–79.2)

P*<0.05, *P*<0.01 compared with multifetal values.

achieved after superovulation and *in vitro* fertilisation and embryo transfer (IVF-ET), recruited from the Assisted Conception Unit, King’s College Hospital, and group 2 were multifetal pregnancies (three to eight fetuses, *n*=39: three fetuses *n*=22, four fetuses *n*=6, five fetuses *n*=7, six fetuses *n*=3 and eight fetuses *n*=1) undergoing selective fetal reduction to twin pregnancies, recruited from The Harris Birthright Research Centre for Fetal Medicine, King’s College Hospital. The multifetal pregnancies occurred after a variety of assisted reproduction techniques that included ovulation induction, superovulation and intra-uterine insemination, and superovulation and IVF. They were referred from a variety of units. Fetal reduction to twins was carried out by ultrasound-guided trans-abdominal injection of potassium chloride into the fetal heart. Ultrasound demonstrated complete reabsorption of the fetoplacental units 8–12 weeks after reduction. In group 1, blood samples were obtained initially at 10–12 weeks gestation and then 4 and 8 weeks afterwards. In group 2, blood was obtained before reduction at 10–12 weeks gestation and then at 4 and 8 weeks after reduction. The Ethics Committee of King’s College Hospital approved the study and the women gave informed consent to participate.

TSH and fT₄ were measured using the Elecsys electrochemiluminescence immunoassay analyser (Roche

Diagnostic Ltd, Lewes, UK). Inter-assay precision (coefficient of variation) was as follows: fT₄, 4.7% (8.8 pmol/l) and 5.2% (34.5 pmol/l); TSH, 6.9% (0.32 mU/l), 5.3% (7.8 mU/l) and 5.1% (26.0 mU/l). hCG was measured by non-competitive fluoroimmunoassay (Pharmacia Wallac, Milton Keynes, Bucks, UK.). The variation (intra- and inter-assay) for these assays over the period of the study was less than 10%.

The data were not normally distributed, and non-parametric tests (Mann–Whitney *U*) were used to analyse the data. Simple and multiple regression analysis were used to investigate the associations between analytes and fetal number.

Results

There was no significant difference in the circulating concentrations of fT₄ between singleton and twin pregnancies (*P*=0.95 at 10–12 weeks, *P*=0.40 at 14–16 weeks, and *P*=0.21 at 18–20 weeks). However, before fetal reduction, the fT₄ concentrations were greater in multifetal pregnancies when compared with singleton or twin pregnancies (*P*<0.005) (Table 1). After fetal reduction to twins, the circulating concentrations of fT₄ in multifetal pregnancies decreased progressively, but remained significantly different from those in twin

Figure 1 Relationship between the circulating concentrations of fT_4 and human chorionic gonadotrophin ($r = +0.52$, $P = 0.0006$).

Figure 2 Relationship between the circulating concentrations of fT_4 and fetal number ($r = +0.63$, $P < 0.0001$). Singleton ($n = 12$), twin ($n = 12$) and multifetal pregnancies ($n = 39$).

pregnancies at 8 weeks ($P = 0.003$ and $P = 0.0502$ at 4 and 8 weeks after reduction respectively) (Table 1).

There was no significant difference in the circulating concentrations of TSH between singleton and twin pregnancies ($P = 0.20$ at 10–12 weeks, $P = 0.41$ at 14–16 weeks, and $P = 0.14$ at 18–20 weeks). However, before fetal reduction, the TSH concentrations were significantly lower in multifetal pregnancies compared with singleton, but not twin, pregnancies ($P = 0.005$ and $P = 0.23$ respectively) (Table 1). After fetal reduction to twins, the circulating concentrations of TSH in multifetal pregnancies increased to twin pregnancy values by 8 weeks ($P = 0.025$ and $P = 0.19$ at 4 and 8 weeks after reduction) (Table 1).

Before fetal reduction, there was a positive association between the circulating concentrations of fT_4 and both hCG ($r = 0.52$, $P = 0.0006$; Fig. 1) and fetal number ($r = 0.63$ and $P < 0.0001$; Fig. 2); and a negative association with TSH concentrations ($r = -0.52$, $P = 0.0001$; Fig. 3). Thus, for a given hCG concentration, fT_4 concentration is proportionately greater when there are multiple fetuses. However, using multiple regression analysis, including all the above variables, fT_4 was significantly related to the number of fetuses (a positive relationship) and TSH (a negative relationship, Overall $F = 23.739$; for number of fetuses Partial $F = 18.199$ and $P = 0.0001$; for TSH Partial $F = 10.629$ and $P = 0.0019$), but not to hCG.

Discussion

It has been reported that hCG has a thyroid-stimulating effect *in vitro* (Davies & Platzer 1986). The thyrotrophic

activity of hCG is believed to be due to the homology between the TSH and hCG molecular structures, and receptors with only a 15% sequence difference between them (Nisula *et al.* 1974, Yoshikawa *et al.* 1989). However, thyroid stimulation by hCG is reported to be weak and to be effective only at high concentrations of hCG

Figure 3 Relationship between the circulating concentrations of fT_4 and log thyroid stimulating hormone concentration ($r = -0.52$, $P = 0.0001$).

(Guillaume *et al.* 1985). These data would explain why overt hyperthyroidism is seen only in some women and then only in the first trimester, when hCG concentrations are greatest, and the occurrence of clinical and biochemical hyperthyroidism in women with hCG-producing tumours such as hydatidiform mole and choriocarcinoma (Miyai *et al.* 1976). Indeed, the spectrum of increasing thyroid activity correlates with the severity of disease and the concentration of hCG, suggesting a causal relationship (Kenimer *et al.* 1975). At the cellular level, hCG is believed to increase intracellular release of cAMP in the thyroid, which leads to a degranulation response, and the secretion of thyroxine. *In vitro* data have not fully supported this view, as only a minimal increase in secretion occurs in response to hCG (Kraiem *et al.* 1994). However, the conflict may well be due to the isoform of hCG used, as its effect at the TSH receptor is determined by its glycosylation and can range from stimulation to inhibition (Hoermann *et al.* 1994, Poertl *et al.* 1995). Such data suggest that hCG is probably the only factor involved in the alteration of maternal thyroid function in early pregnancy, but the possibility remains that other factors are also involved.

After fetal reduction, the concentrations of hCG decrease to those of control twin pregnancies by 4 weeks, suggesting that fetal reduction causes placental death and therefore production of hCG by the remaining pregnancies at a rate similar to that in control twins (Johnson *et al.* 1994). In contrast, after fetal reduction, the maternal concentrations of alpha fetoprotein (α -FP) increase, and remain increased for at least 8 weeks (Abbas *et al.* 1994). This pattern is probably due to the gradual breakdown of fetal tissue, the release of α -FP into amniotic fluid, and its subsequent absorption into the maternal circulation (Abbas *et al.* 1994). We found that multifetal pregnancy reduction was associated with a decrease in the circulating concentrations of fT_4 to those of the control group by 8 weeks, but at 4 weeks the concentrations of fT_4 were still significantly greater. The relative delay in the decrease in fT_4 compared with hCG may reflect a delay in the thyroid response to decreasing hCG concentrations. However, as fT_4 was related to the number of fetuses but not to hCG concentrations in the multiple regression analysis, it is possible that a fetal factor is also involved in the alteration of maternal thyroid function in pregnancy. If this were so, it would explain the similarity in the pattern of fT_4 decline to that of α -FP described above. A further possibility is that the form of hCG, which has thyroid stimulating-properties, may be cleared at a slower rate and so continues to stimulate thyroid activity for longer than anticipated by the total circulating concentrations of hCG. However, to account for the relationship between fT_4 concentrations and fetal number, concentrations of this form of hCG would have to be more closely related to fetal number than are total hCG concentrations.

We have shown that, in multiple pregnancy, thyroid activity increases with fetal number. This is consistent with the greater circulating concentrations of fT_4 and lesser circulating concentrations of TSH in twin, compared with singleton, pregnancies (the measurements between 8–15 weeks were pooled; Grun *et al.* 1997). However, the difference in TSH was not statistically significant and that of the fT_4 was significant at the $P=0.046$ level (Grun *et al.* 1997). With twin pregnancies, we have demonstrated a similar pattern for TSH, but not fT_4 . The lack of difference between singleton and twin pregnancies in our study may be because our samples were collected at 10–12 weeks gestation (rather than at 8 weeks onwards), when the altered concentrations of TSH and fT_4 may have reverted to normal (Harada *et al.* 1979). In contrast, the alteration in thyroid physiology is more profound in multifetal pregnancies and still apparent at 10–12 weeks.

As we do not have pre-pregnancy thyroid function tests, it is possible that the women with multifetal pregnancies had greater concentrations of fT_4 , that this promoted implantation of multiple fetuses, and that this is the origin of the observed differences in maternal thyroid function. However, we would not have expected to see a decline in maternal fT_4 concentrations after reduction, had this been the case. Similarly, it seems unlikely that maternal thyroid function is materially altered by the treatment used to achieve pregnancy, but this cannot be excluded.

In conclusion, there is profound alteration in thyroid physiology in multifetal pregnancies, which may be due to a combination of hCG and a fetal factor. Further studies are needed to elucidate whether such a fetal factor exists and, if so, what it is.

References

- Abbas A, Johnson M, Bersinger N & Nicolaides K 1994 Maternal alpha-fetoprotein levels in multiple pregnancies. *British Journal of Obstetrics and Gynaecology* **101** 156–158.
- Ballabio M, Poshychinda M & Ekins RP 1991 Pregnancy-induced changes in thyroid function: role of human chorionic gonadotropin as putative regulator of maternal thyroid. *Journal of Clinical Endocrinology and Metabolism* **73** 824–831.
- Davies TF & Platzer M 1986 HCG-induced TSH receptor activation and growth acceleration in FRTL-5 thyroid cells. *Endocrinology* **118** 2149–2151.
- Glinoe D, de Nayer P, Bourdoux P, Lemone M, Robyn C, van Steirteghem A, Kinthaert J & Lejeune B 1990 Regulation of maternal thyroid during pregnancy. *Journal of Clinical Endocrinology and Metabolism* **71** 276–287.
- Grun JP, Meuris S, De Nayer P & Glinoe D 1997 The thyrotrophic role of human chorionic gonadotropin (hCG) in the early stages of twin (versus single) pregnancies. *Clinical Endocrinology* **46** 719–725.
- Guillaume J, Schussler GC & Goldman J 1985 Components of the total serum thyroid hormone concentrations during pregnancy: high free thyroxine and blunted thyrotropin (TSH) response to TSH-releasing hormone in the first trimester. *Journal of Clinical Endocrinology and Metabolism* **60** 678–684.
- Harada A, Hershman JM, Reed AW, Braunstein GD, Dignam WJ, Derzko C, Friedman S, Jewelewicz R & Pekary AE 1979

- Comparison of thyroid stimulators and thyroid hormone concentrations in the sera of pregnant women. *Journal of Clinical Endocrinology and Metabolism* **48** 793–797.
- Hoermann R, Broecker M, Grossmann M, Mann K & Derwahl M 1994 Interaction of human chorionic gonadotropin (hCG) and asialo-hCG with recombinant human thyrotropin receptor. *Journal of Clinical Endocrinology and Metabolism* **78** 933–938.
- Johnson MR, Abbas A & Nicolaides KH 1994 Maternal plasma levels of human chorionic gonadotrophin, oestradiol and progesterone in multifetal pregnancies before and after fetal reduction. *Journal of Endocrinology* **143** 309–312.
- Kenimer JG, Hershman JM & Higgins HP 1975 The thyrotropin in hydatidiform moles is human chorionic gonadotropin. *Journal of Clinical Endocrinology and Metabolism* **40** 482–491.
- Kennedy RL, Darne J, Cohn M, Price A, Davies R, Blumsohn A & Griffiths H 1992 Human chorionic gonadotropin may not be responsible for thyroid-stimulating activity in normal pregnancy serum. *Journal of Clinical Endocrinology and Metabolism* **74** 260–265.
- Kraiem Z, Sadeh O, Blithe DL & Nisula BC 1994 Human chorionic gonadotropin stimulates thyroid hormone secretion, iodide uptake, organification, and adenosine 3',5'-monophosphate formation in cultured human thyrocytes. *Journal of Clinical Endocrinology and Metabolism* **79** 595–599.
- Miyai K, Tanizawa O, Yamamoto T, Azukizawa M & Kawai Y 1976 Pituitary–thyroid function in trophoblastic disease. *Journal of Clinical Endocrinology and Metabolism* **42** 254–259.
- Nisula BC, Morgan FJ & Canfield RE 1974 Evidence that chorionic gonadotropin has intrinsic thyrotropic activity. *Biochemical and Biophysical Research Communication* **59** 86–91.
- Poertl S, Liss L, Mann K & Hoermann R 1995 Crude urinary human chorionic gonadotropin contains variant forms of HCG with low sialic acid content that exhibit an increased thyrotropic activity in CHO cells expressing the human TSH receptor. *Experimental and Clinical Endocrinology and Diabetes* **103** 168–174.
- Shepard TH 1975 Development of the thyroid gland. In *Endocrine and Genetic Diseases of Childhood and Adolescence*, edn 2. Ed LI Gardner. Philadelphia: WB Saunders.
- Yoshikawa N, Nishikawa M, Horimoto M, Yoshimura M, Sawaragi S, Horikoshi Y, Sawaragi I & Inada M 1989 Thyroid-stimulating activity in sera of normal pregnant women. *Journal of Clinical Endocrinology and Metabolism* **69** 891–895.

Received 4 May 1999

Accepted 12 August 1999