T_3 secretion. Furthermore thyroid volume increased during pregnancy by about 20%, suggestive of a gland that was being stimulated to produce more thyroid hormone.

There are a number of other findings of interest in this study, but the most significant question is whether apparently normal pregnant women have difficulty in maintaining normal thyroid function during gestation. Belgium is an area of marginally low iodine intake (50–75 μg/day). Iodine is clearly important in the modulation of thyroid function beyond its role as a substrate for thyroid hormone synthesis. Whether the results are pertinent in the United States where there is a surfeit of iodine remains to be determined. Although longitudinal samples were obtained, the study was basically cross-sectional in design. One would like to know the results of thyroid function tests in these patients before the onset of pregnancy. An age-matched series of controls also would have been of interest.

Nevertheless, the question remains as to what to do with these pregnant women with relative hypothyroxinemia. Twenty years ago, Man and her co-workers (8) monitored thyroid function in pregnant women and subsequently obtained developmental data on the children born to these mothers. Hypothyroxinemia was identified in 41% of the pregnancies based on two low thyroid hormone values relative to normal pregnancy values or one low value in the presence of clinical hypothyroidism, previous reproductive failure or thyroidectomy. Thyroid hormone therapy was prescribed for 135 women with hypothyroxinemia. In a 7-yr follow-up, the progeny of hypothyroxinemic women who were inadequately treated had lower developmental scores. There was no compelling evidence that these women were actually hypothyroid. The possibility exists that socioeconomic situations might have played a role in the poor outcome. Further, these women did have at least one low thyroid hormone value.

The data in the present study suggest that there may be minor changes in thyroid hormone metabolism in pregnancy other than those that could be attributed directly to an increase in TBG. The authors comment that changes in maternal thyroid function are intricate and not well understood. Gertrude Stein is reputed to have said "A difference to be a difference should make a difference!" There is no evidence at present that these minor changes make a difference in thyroid function during normal pregnancy.

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Regulation of Maternal Thyroid during Pregnancy*

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ABSTRACT. A prospective study was undertaken in 606 healthy women during pregnancy to evaluate the changes occurring in maternal thyroid economy as a result of 1) the increased thyroid hormone-binding capacity of serum, 2) the effects of increased levels of hCG on TSH and on the thyroid, and 3) a marginally low iodine intake in the population (50–75 μg/day).

Four main features were observed. First, thyroidal activity adjusted to the marked increase in serum T_4-binding globulin: pregnancy was accompanied by an overall reduction in the T_4/T_4-binding globulin ratio, with lower free T_4 and T_3 levels, although in most cases free hormone levels remained within the normal range. The adjustment of thyroidal output of T_4 and T_3 did not occur similarly in all subjects. In approximately one third of the women, there was relative hypothyroxinemia, higher T_3/T_4 ratios (presumably indicating preferential T_3 secretion), and higher, although normal, serum TSH concentrations. Second, high hCG levels were associated with thyroid stimulation, both functionally (lower serum TSH) and anatomically (increased thyroid size). The data are consistent with a TSH-like effect of hCG on the thyroid. Hence, regulation of the maternal thyroid is complex, resulting from both elevated hCG (mainly in the first half of gestation) and increasing TSH (mainly in the second half of gestation). Third, a significant increase in serum thyroglobulin levels was observed throughout gestation, especially during the last trimester. Fourth, increased thyroid volume was common, and goiter formation not uncommon (goiter was found in 9% of women at delivery).

In conclusion, the alterations in maternal thyroid function during gestation are intricate and far from fully understood. In areas of marginally low iodine intake, gestation is associated in a significant number of women with relative hypothyroxinemia, increased thyroglobulin, and enlarged thyroid. (*J Clin Endocrinol Metab* **71:** 276–287, 1990)

THE UNDERSTANDING of thyroid physiology has greatly expanded during the past 2 decades, but the precise mechanisms regulating the maternal thyroid in pregnancy remain unclear (1–5). Alterations of biochemical parameters of thyroid function during gestation were recognized more than 30 yr ago (6, 7), and changes in thyroid volume were observed in pharaonic Egypt (8). It is well established that normal pregnancy is accompanied by a rise in the serum levels of T_4-binding globulin (TBG) and total T_4 and T_3. However, the changes in levels of free hormones (9–12) and TSH (13–15), and the potential role of thyroid stimulators of placental origin (3, 4, 14, 16, 17) remain debatable. Also, there have been only a few studies in small groups of women related to alterations in serum thyroglobulin levels (TG) or thyroid volume (TV) during pregnancy.

A prospective study was undertaken in a large cohort of pregnant women to obtain cross-sectional and longitudinal data on maternal thyroid function. The aims were to precisely evaluate the changes occurring in thyroid economy as a result of 1) the increased thyroid hormone-binding capacity of serum due to the increase in TBG levels, 2) the effects of increased hCG levels on serum TSH, and 3) a marginally low iodine intake in the population. The relationship between the functional alterations in thyroid parameters and the modifications in TV, estimated by ultrasonography, were also studied.

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Subjects and Methods

Subjects and design of study

Seven hundred and thirty-two consecutive pregnant women were enrolled between January and November 1988. At initial presentation at the out-patient gynecology clinic, the subjects gave informed consent. The protocol had first been accepted by the ethical committee of the Faculty of Medicine. During

the initial clinical evaluation a detailed history of past gynecological events was obtained, and gestational age was determined by fetal echography using standard procedures. The ages of the women ranged from 15–49 yr (mean ± SD, 28 ± 6 yr). Mean gestational age at booking was 17 ± 8 (±SD) weeks, with more than 70% of the pregnancies enrolled during the first 20 weeks of gestation. The cohort comprised 29% of primigravidae women, the 71% remaining having had 1–10 pregnancies (average, 2.7) and from 0–9 children (average, 2.1). Among the 520 women who had previously been pregnant, 37% had a positive obstetrical past history, including miscarriage (n = 102), premature delivery (n = 21), gravidic diabetes (n = 13), perinatal death (n = 11), preeclampsia (n = 7), molar pregnancy (n = 5), malformations (n = 5), trisomy (n = 3), and miscellaneous conditions (n = 14). A detailed history of thyroid-related past events was also recorded, and the thyroid gland was carefully palpated. The following parameters were determined at evaluation: total and free T_4 and T_3, TBG, TG, TSH, iodine concentration in a random urine sample, thyroid autoantibodies, hCG, and TV.

On the basis of the initial clinical and laboratory data, 100 pregnancies with positive thyroid features were excluded from the study: past history of thyroid disorder or therapy (n = 18), palpable goiter (n = 24), thyroid enlargement and/or nodularity detected only by ultrasonography (n = 41), hyper- or hypothyroidism (n = 3), and TG and/or microsomal autoantibodies (n = 15). Twenty-six patients were also excluded who did not complete gestation because of a miscarriage.

For the remaining 606 healthy women without detectable thyroid abnormality, the study was designed prospectively to provide cross-sectional and longitudinal information. In women less than 29 weeks gestation at evaluation, second blood and urine samples were obtained at 30–33 weeks gestation, and the determinations described above were carried out (except for thyroid ultrasonography and hCG measurements). The third series of determinations was performed 1–4 days after delivery, including thyroid ultrasonography and biochemical parameters of thyroid function.

Methods

Total T_4, T_3, and TBG were measured by conventional RIA (18). Free T_4 was determined using the two-step Gamma-Coat [^{125}I]free T_4 (Clinical Assays, Baxter, Cambridge, MA), and free T_3 using the two-step chromatographic separation followed by RIA (Sclavo, Siena, Italy). Serum TG was measured using two methods: a conventional double antibody RIA (Techland, Liege, Belgium) and an immunoradiometric (IRMA) technique recently developed (Dynotest, Henning Gmbh, Berlin, West Germany). Serum TSH was determined using a sensitive IRMA (RIAbead II, Abbott, Chicago, IL), and hCG with a conventional double antibody RIA employing polyclonal rabbit anti-hCG antibody (19). TG and microsomal autoantibodies were measured by RIA and indirect immunofluorescence, respectively. The urinary iodine concentration was assessed using a fully automated Technicon Autoanalyzer, employing the Sandell-Kolthoff reaction (20). Conventional thyroid ultrasonography was carried out using a short focused, small parts 7.5-mHz transducer (SAL-77, Toshiba, Tokyo, Japan). The volume of each lobe was estimated using the geometric formula to calculate an ovoid: volume = $(\pi/6) \times W \times H \times T$, where W, H, and T represent width, height, and thickness. Total TV corresponded to the sum of both lobes (21, 22). Statistical analyses of the data were carried out using the SPSS program (Statistical Package for Social Sciences) (23), employing parametric and nonparametric tests, analyses of variance, and linear and mulitple regression tests as appropriate, on a PC-compatible Elite-AT computer (Compuline, Brussels, Belgium).

Results

The biochemical parameters of thyroid function, expressed as mean values per trimester of gestation, are given in Table 1.

Serum levels of thyroid hormones and TBG

The modifications in serum levels of TBG and total T_4 and T_3 as a function of gestational age are shown in Fig. 1. TBG gradually increased from 18.3 ± 5.5 (±SD) mg/L at 6 weeks to an average plateau value of 31.5 mg/L after 20 weeks. For the first 20 weeks of gestation, there was a significant correlation between TBG and

TABLE 1. Biochemical parameters of thyroid function during gestation

	Trimester		
	First	Second	Third
Total T_4 (50–150 nmol/L)	138 ± 3	148 ± 3[a]	148 ± 3[b]
Total T_3 (1.40–3.20 nmol/L)	3.15 ± 0.03	3.55 ± 0.05[a]	3.58 ± 0.03[b]
Molar T_3/T_4 ($10–23 \times 10^{-3}$)	23.1 ± 0.3	24.3 ± 0.3[c]	24.8 ± 0.3[b]
TBG (11–21 mg/L)	21.2 ± 0.3	28.5 ± 0.4[a]	31.5 ± 0.3[a]
TBG saturation (28–60%)	39.3 ± 0.6	30.9 ± 0.4[a]	27.9 ± 0.3[a]
Free T_4 (10–26 pmol/L)	17.9 ± 0.3	14.5 ± 0.1[a]	13.4 ± 0.1[a]
Free T_3 (3–11 pmol/L)	5.0 ± 0.1	4.2 ± 0.1[a]	3.8 ± 0.1[a]
TSH (0.2–4.0 mU/L)	0.75 ± 0.04	1.05 ± 0.04[a]	1.29 ± 0.04[a]
hCG (IU/L × 10^3)	38.5 ± 1.5	16.4 ± 0.9[a]	13.0 ± 1.5[b]
TG (≤30 μg/L)	31 ± 2	31 ± 2[b]	38 ± 2[a]
Urinary iodine (≤150 μg/L)	58 ± 3	58 ± 3[b]	53 ± 3[b]

Values are given as the mean ± SE. Reference ranges for nonpregnant subjects are indicated in parentheses; for hCG, it was less than 18 IU/L. TBG saturation corresponds to the fractional occupation of TBG binding sites by T_4, expressed as a percentage. It was calculated from individual T_4 and TBG determinations as the molar T_4/TBG ratio, using 57 kDa as the mol wt for TBG. *P* values were calculated from group *t* tests, comparing second to first trimester and third to second trimester. To calculate *P*, the separate or pooled variance estimates were employed, according to the two-tailed probability of F. The total numbers of determinations carried out were 230, 265, and 370, respectively, in the first, second, and third trimesters, for serum total T_4 and T_3, TBG, T_3/T_4 ratio, free T_4, TSH, and TG. For urinary iodine, determinations were performed at random in 50% of the cohort. For hCG, determinations were only performed at initial presentation (n = 498). For free T_3, determinations were performed in the first 350 serum samples available.

[a] $P < 0.001$.

[b] P = NS.

[c] $P < 0.005$.

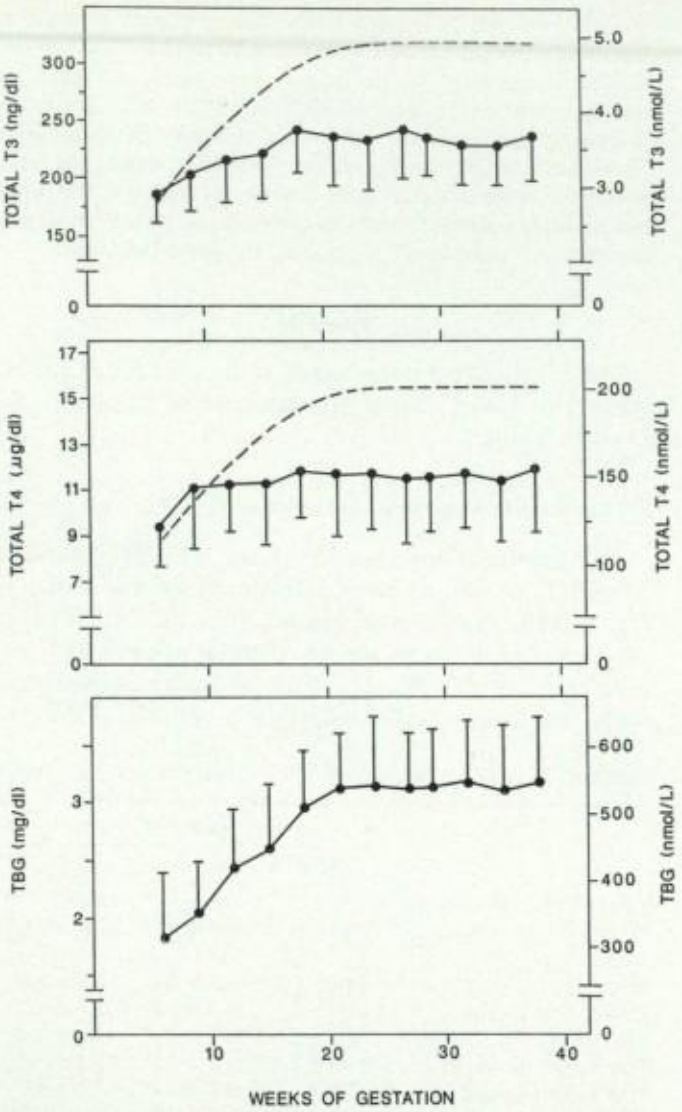

FIG. 1. Serum T_4, T_3, and TBG as a function of gestational age. Each *point* gives the mean value (±1 SD) of determinations performed at the initial presentation, pooled for 3 weeks, between 5–28 weeks (n = 510) and again for samples obtained between 28–39 weeks (n = 355). The latter samples include both late initial evaluations and the second series of determinations at 30–33 weeks. Each *point* represents an average of 72 individual determinations. The *dashed lines* illustrate the theoretical curves of T_3 and T_4 concentrations required to yield the average molar ratios of T_4/TBG and T_3/TBG that correspond to nonpregnant reference subjects (0.37 for T_4/TBG and 0.0089 for T_3/TBG, using a mol wt of 57 kDa for TBG).

gestational age (r = 0.60; $P < 0.001$). In the *two upper panels* of Fig. 1 are represented the actual changes in total T_4 and T_3. The *dashed lines* show the theoretical mean curves, calculated from the individual TBG levels, of the T_4 and T_3 concentrations required to yield a stable molar hormone/TBG ratio. Serum T_4 increased sharply between 6 and 9 weeks and thereafter only slowly, eventually reaching an average plateau value of 152 nmol/L at 18 weeks. In contrast to T_4, the rise in T_3 was more pronounced up to 18 weeks; thereafter, T_3 plateaued at an average value of 3.6 nmol/L. The correlation between T_3 and gestational age was higher than that between T_4 and gestational age (r = 0.38 for T_3 and r = 0.17 for T_4, respectively; $P < 0.001$), as was the correlation between T_3 and TBG compared to that between T_4 and TBG (r = 0.68 and r = 0.47, respectively; $P < 0.001$). Hence, the rise in serum TBG was not accompanied by comparable increases in hormone levels, and compared to the theoretical curves, the majority of women had lower serum T_4 and T_3 levels; only those women with hormone levels corresponding to the overall mean + 2 SD reached T_4 and T_3 levels similar to the theoretical values.

The modifications in serum free T_4 and T_3 concentrations and TBG saturation as a function of gestational age are shown in Fig. 2. There was a progressive decrease in TBG saturation, from 40% at 6–9 weeks to an average plateau level of 28% saturation after 20 weeks. Similarly, the mean free T_4 and T_3 concentrations decreased from 18 to 13 pmol/L and from 5.4 to 3.8 pmol/L, respectively. Compared to early gestation data, the decrements in

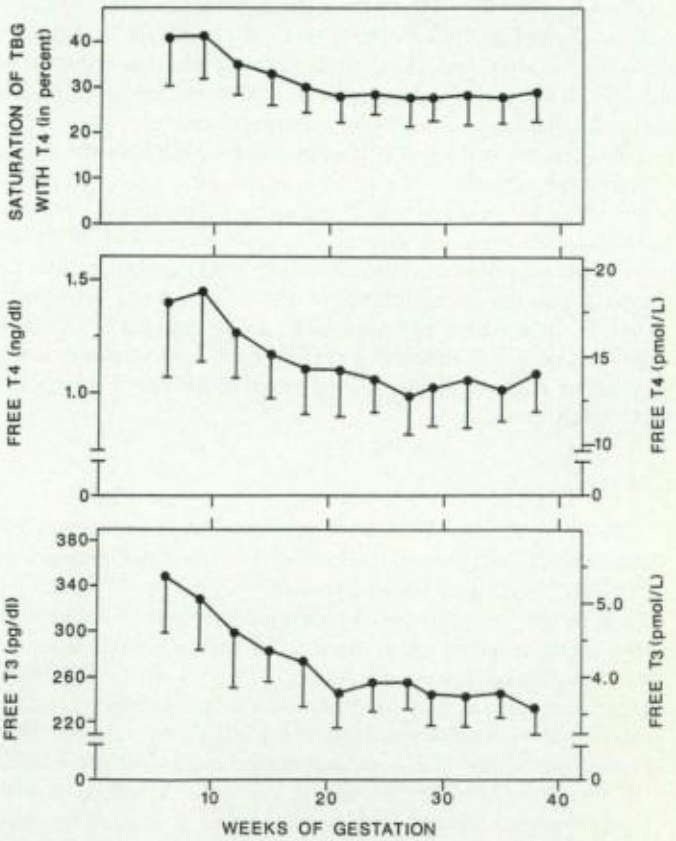

FIG. 2. Serum free T_4 and T_3 concentrations and TBG saturation as a function of gestational age. TBG saturation by T_4 corresponds to the molar T_4/TBG ratio expressed as a percentage, and was calculated from each individual set of data. Each *point* gives the mean value (±1 SD) of determinations performed at the initial presentation, pooled as indicated in Fig. 1. Each *point* represents an average of 72 individual determinations for TBG saturation, 64 for free T_4, and 24 for free T_3.

TBG saturation and free T_4 and T_3 concentrations during the second half of gestation were of the same magnitude (~30%). In addition, significant negative correlations were observed between the opposite changes in free hormones and TBG levels ($r = -0.34$ for free T_4 and $r = -0.35$ for free T_3; $P < 0.001$).

Iodine status

Figure 3 illustrates the distribution frequency of urinary iodine concentrations. The median concentrations were 50 and 45 μg/L during the first and second halves of gestation, respectively, corresponding to average daily urinary iodine excretion values of 50 and below 100 μg in over 90% of the cohort. Urinary iodine excretion in pregnancy was superimposable to that of nonpregnant women in Brussels.

Regulation of total and free T_4

In an attempt to delineate more precisely the parameters involved in the setting of total T_4, the cohort was arbitrarily subdivided into three groups according to individual total T_4 values: group L (low) corresponded to the lower third of the cohort (1st to 33rd percentile), group M (middle) to the 34th to 66th percentile, and group H (high) to the 67th to 100th percentile. Since T_4 levels were also modified with time during gestation, four time periods were considered: 6–12, 13–20, 21–29, and 30–40 weeks gestation. Analysis of variance was then carried out by comparing among subgroups the changes in TBG, TBG saturation, free T_4, molar T_3/T_4 ratio, TSH, hCG, and urinary iodine. Results are presented in Fig. 4, and statistical differences between subgroups L and H are indicated. This procedure, albeit somewhat intricate, allowed one to follow the evolution of each parameter considered and to integrate cross-sectional and longitudinal data during early and late gestation (see Fig. 4 for statistical validation). As expected, the differences in TBG among subgroups were highly significant throughout gestation ($P < 0.001$): TBG levels determined the setting of total T_4 levels. However, despite corresponding to the lowest TBG, women in subgroup L also exhibited lower TBG saturation ($P < 0.001$) as well as lower free T_4 ($P < 0.001$). Hence, compared to subgroup H, women in subgroup L presented a relative deficit in T_4 for their corresponding TBG level. Moreover, women in subgroup L also exhibited a significantly higher T_3/T_4 ratio. While in subgroup H, the mean ratio was 0.020 and was barely altered during gestation, it was greater than 0.025 in subgroup L and further increased with time, reflecting a higher degree of stimulation of the gland. Changes in TSH and hCG levels among subgroups were also analyzed. In subgroup L, the mean hCG level was significantly lower, and conversely, TSH was higher, particularly during early gestation. Multiple regression analysis (results not shown) confirmed that higher T_4 concentrations depended upon TBG and hCG being increased as well as TSH being decreased. Finally, no specific pattern of changes in urinary iodine was evident among subgroups L, M, and H.

A similar analysis of variance was carried out for free T_4 levels using the procedure described above for total T_4, with the cohort subdivided into the same subgroups L, M, and H according to the individual free T_4 concentrations. Results are presented in Fig. 5. Throughout gestation, the lowest free T_4 level was clearly associated with lower total T_4 ($P < 0.001$) as well as with lower TBG saturation ($P < 0.001$). In addition, low free T_4 was associated with higher TBG concentrations, at least during the phase of the rise in serum TBG, between 6–18 weeks. Women in subgroup L had a higher T_3/T_4 ratio than subgroup H ($P < 0.001$). Comparison of hCG levels between the subgroups indicated that the highest free T_4 level corresponded to significantly higher hCG, and, conversely, to lower TSH, during the first half of gestation (see below). There was no significant relationship be-

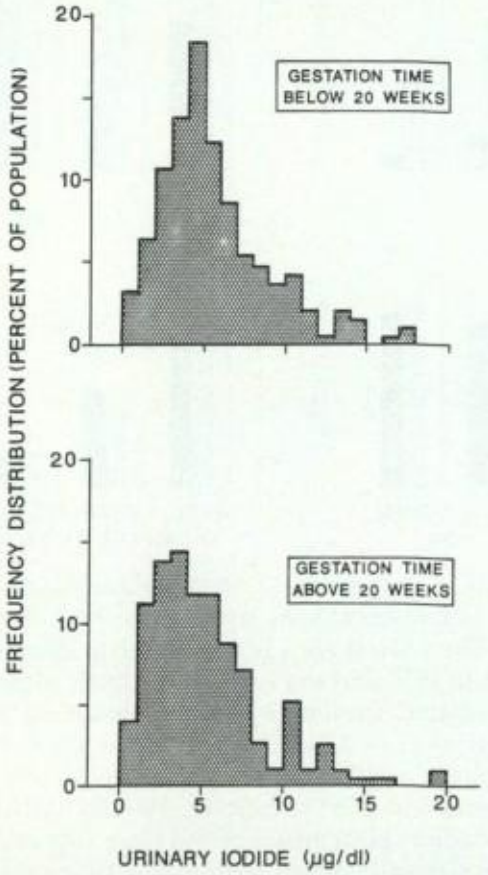

FIG. 3. Histogram of urinary iodine concentrations during the first and second halves of gestation (total of 334 urine samples assessed). Samples grossly contaminated with iodine (>250 μg/L) were not included.

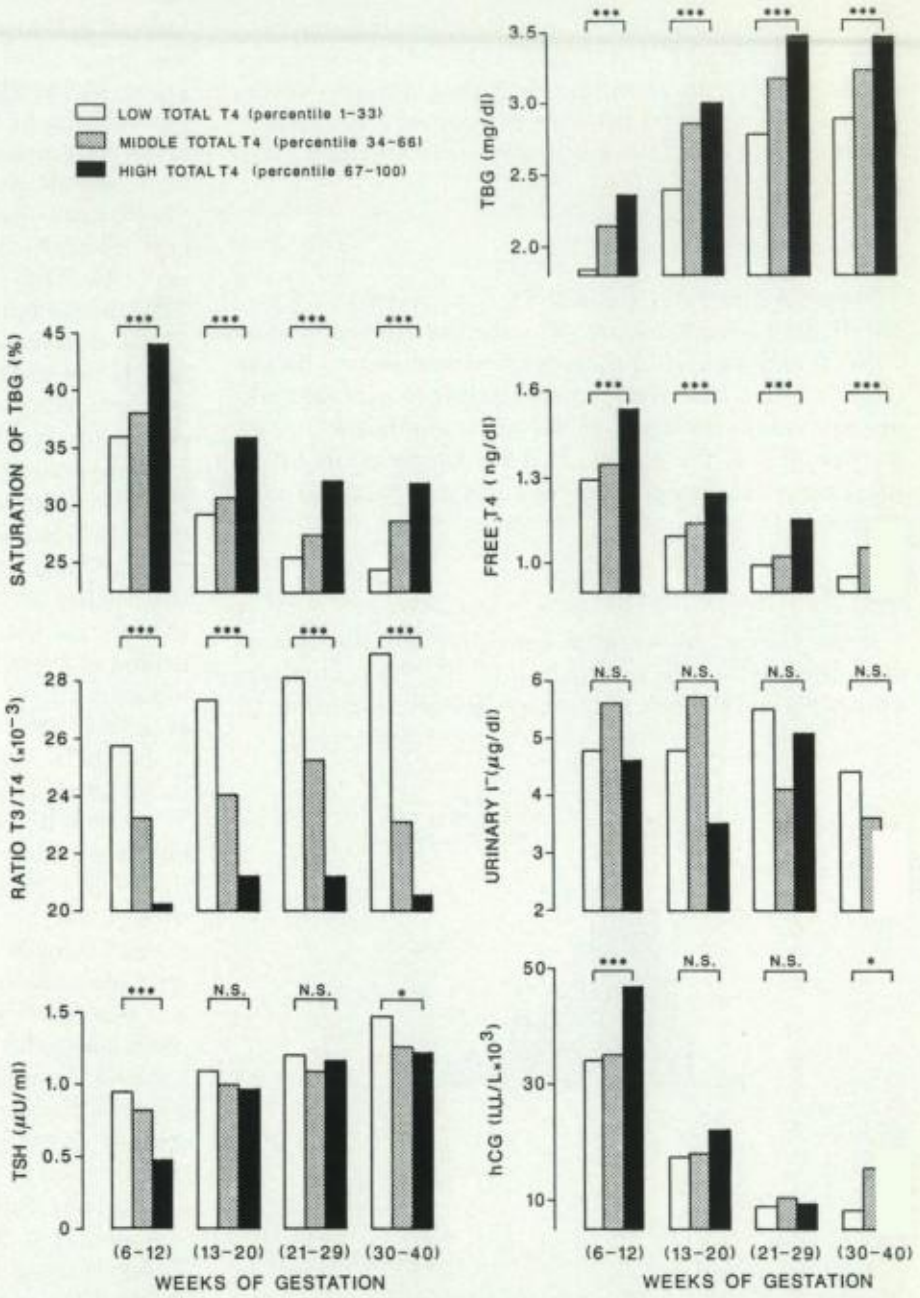

FIG. 4. The cohort was arbitrarily subdivided into three groups of similar size, corresponding to the three sets of columns, on the basis of individual total T_4 levels at different times during gestation. Since it was necessary to avoid any preconceived hypothesis concerning the actual levels of T_4 to be used in defining the subgroups, the subdivision technique employed consisted in categorizing T_4 (a quantitative continuous parameter) according to percentiles. The less arbitrary method was to use tertiles, allowing for the statistical comparison between the upper and lower tertiles around a central subgroup. For example, between 6–12 weeks gestation, subgroup L was defined by total T_4 less than 123 nmol/L and subgroup H by T_4 more than 147 nmol/L. This figure shows the analysis of variance for the following parameters: serum TBG, TBG saturation, free T_4, molar T_3/T_4 ratio, TSH, urinary iodine, and hCG in relation to total T_4. Each column gives the mean value for each parameter examined. To clarify the presentation, only the statistical comparisons between sugroups L and H are indicated (***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$).

tween free T_4 and iodine concentrations. The longitudinal evolution of serum free T_4 in a given woman was evaluated by comparing late to early gestational data; after 30 weeks gestation, 47% of the women in subgroup H already belonged to subgroup H at initial evaluation; similarly, 39% of the women in subgroup L already belonged to subgroup L at initial evaluation.

Serum TSH and hCG

The modifications in serum TSH and hCG as a function of gestational age are illustrated in Fig. 6. The classical pattern of hCG in pregnancy was observed, with peak values between 8–14 weeks gestation, and a wide scatter in individual hCG levels (between 5,000–115,000 IU/L). For TSH, there was a clear mirror image between the rise in hCG and the lowering in TSH, with a significant negative correlation between individual TSH and hCG values ($r = 0.34$; $P < 0.001$; $n = 228$). After the initial phase, a gradual rise in TSH levels occurred during the second and third trimesters. Median TSH levels in the immediate postpartum period were 1.89 mU/L ($n = 323$), significantly higher than at any time point during gestation. It is of interest to note that TSH was undetectable in 13%, 4.1%, and 1.2% of women, respectively, during the first, second, and third trimesters of gestation.

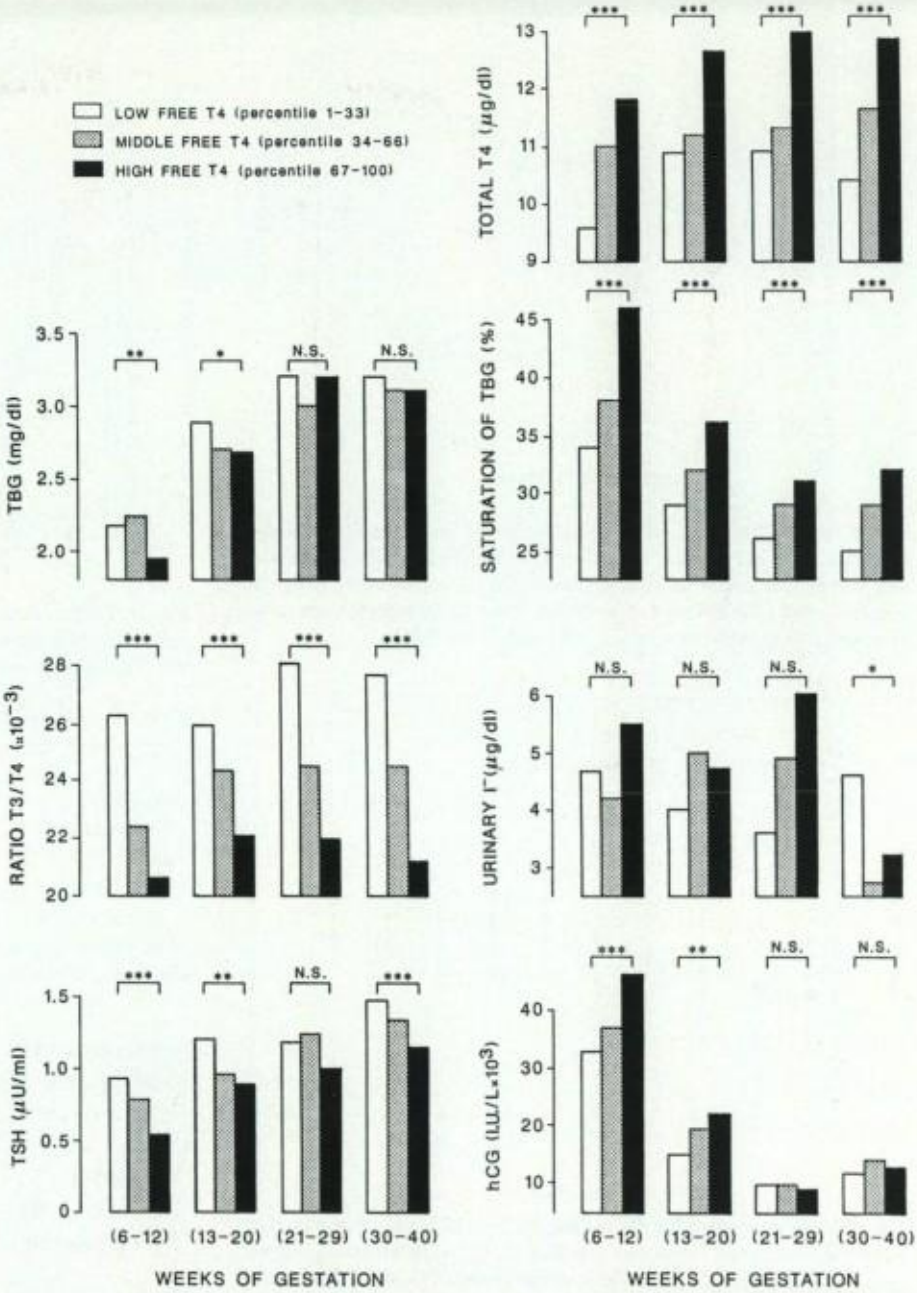

FIG. 5. Analysis of variance for total T_4, TBG, TBG saturation, T_3/T_4 ratio, TSH, urinary iodine, and hCG in relation to free T_4 levels. The three sets of columns correspond to the cohort subdivided on the basis of free T_4 concentrations at different time intervals during gestation, using a procedure similar to that for total T_4 (see Fig. 4). For example, between 6–12 weeks gestation, subgroup L was defined by free T_4 less than 15 pmol/L and subgroup H by free T_4 more than 19 pmol/L.

The increase in TSH was not restricted to a small fraction of the cohort, but represented a general phenomenon; the sequential data (from initial evaluation to late gestation and delivery) indicated that TSH increased in more than 82% of the women, even though levels remained in the normal range (by nonparametric Friedman rank test, $P < 0.0001$).

The effect of hCG to stimulate the thyroid and thereby increase free T_4 levels is illustrated in Fig. 7. Mean free T_4 concentrations increased from 15.0 to 18.5 pmol/L when women with hCG peak levels below 20,000 or above 60,000 IU/L, respectively, were compared. In addition, there was a positive correlation between individual free T_4 and hCG levels ($r = 0.35$; $P < 0.001$). The data were consistent with a TSH-like activity of hCG, but indicated that the hCG effect was of relatively small amplitude; at the time of peak hCG levels, only 3% of women had free T_4 values above the upper limit of the reference values.

TG concentrations

The distribution of serum TG levels during gestation is illustrated in Fig. 8. At booking there was a wide scatter in serum TG, from 4–182 μg/L, but the median TG concentration was already high (25 μg/L) compared to the reference range in nonpregnant females (1–30 μg/

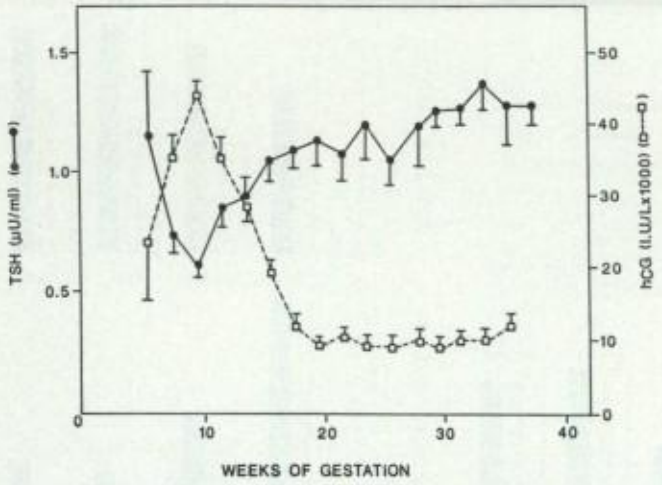

FIG. 6. Serum TSH and hCG as a function of gestational age. Serum hCG was determined at initial evaluation, and TSH at initial evaluation and during late gestation. The symbols give the mean value (±SE) for samples pooled for 2 weeks of gestation. Each *point* corresponds to the average of 33 determinations for hCG and 49 for TSH.

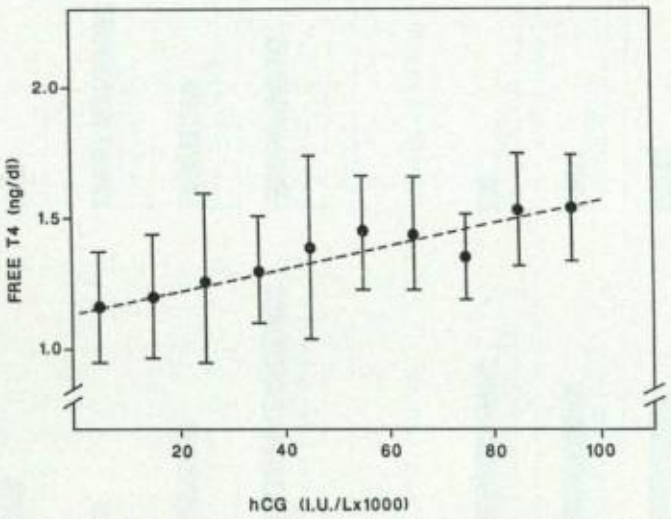

FIG. 7. Scattergram of free T_4 in relation to hCG concentrations. Each *point* represents the mean (±1 SD) free T_4 value, determined between 6-20 weeks gestation, for 10,000 IU/L increments in hCG. The *dashed line* indicates the linear regression curve.

L). Indeed, TG levels at initial evaluation were above 30 μg/L in 39% of the cohort and above 100 μg/L in 3%. TG values tended to increase, mainly during the last weeks of gestation. Between 30–33 weeks, TG levels were above 30 μg/L in 49% and above 100 μg/L in 5% of the women. Immediately after delivery, only 39% of the women had TG values within the reference range, and as many as 9% had values above 100 μg/L. The longitudinal analysis of data indicated that two thirds of women exhibited a significant increase in serum TG between initial evaluation and delivery ($P < 0.001$, by nonparametric Wilcoxon test). There was no direct correlation

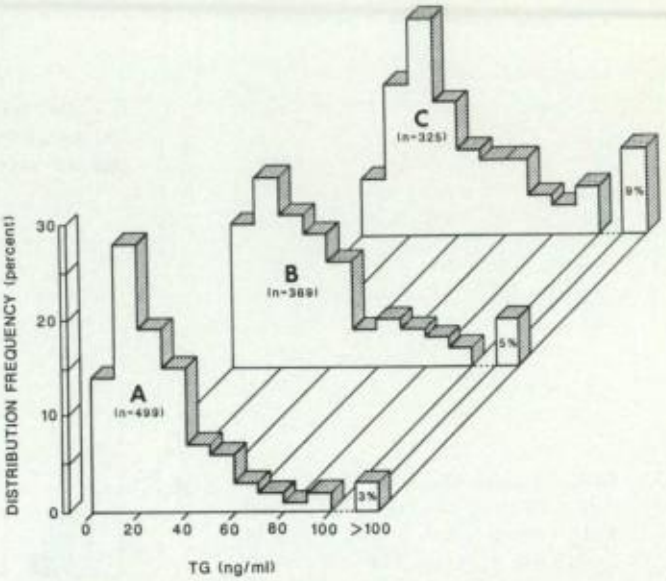

FIG. 8. Distribution frequency of serum TG determined at initial evaluation (A), during late gestation (B), and immediately after delivery (C). The number of determinations is in *parentheses*.

TABLE 2. TV (milliliters)

	n	Left lobe	Right lobe	Total vol	P^a
First trimester (a)	168	5.7 ± 2.3	6.3 ± 2.6	12.1 ± 4.5	
Second trimester	172	5.9 ± 2.4	6.9 ± 2.4	12.8 ± 4.5	NS
Third trimester	33	6.8 ± 2.8	7.2 ± 2.5	13.9 ± 4.8	<0.03
Delivery	179	7.0 ± 3.1	8.0 ± 4.1	15.0 ± 6.8	<0.001

Ultrasonographies from first to third trimesters are cross-sectional data; ultrasonographies at delivery are longitudinal data. Values are the mean volume ± SD.

[a] By *t* test, by groups, compared to volumes of first trimester.

between serum TG, at any time during gestation, and total or free thyroid hormones, iodine status, or hCG. Only a modest correlation was observed at delivery between TG and TSH ($r = 0.15$; $P < 0.05$; $n = 310$) and TG and T_3/T_4 ratio ($r = 0.16$; $P < 0.01$; $n = 325$). The increase in TG was, however, correlated to changes in TV (see below).

TV

Table 2 shows the changes in TV, assessed from the analysis of 552 ultrasonographies. There was a progressive increase in the size of each lobe, and TV increased, on the average, by 18% of the initial size determined at initial evaluation. The increase was significant and occurred in a majority of women (73%). Conmparisons between TV at initial evaluation and delivery indicated that the increment varied up to 130%, exceeded 25% in one quarter of the cohort, and exceeded 50% in as much as 14% of the cohort. Furthermore, the increase in TV was significantly correlated to the initial volume; for TV at initial evaluation up to 10 mL, the increment was 2.7-

fold greater than for TV between 10–20 mL (+32% *vs.* +12%; $P < 0.01$). Four features characterized TV changes and their relationship with the biochemical parameters of thyroid function. There was no direct correlation between urinary iodine and TV. Second, TV was negatively correlated to TSH throughout gestation, so that at delivery women with TSH levels below 0.9 mU/L had a mean TV of 18.1 mL, compared to 12.7 mL ($P < 0.001$) in women with TSH levels above 1.6 mU/L. Third, high TG levels were more frequently associated with larger increases in TV; while TG levels above 50 μg/L at delivery were found in 15% of the women with an increment in size greater than 25%, only 5% had TG values above 50 μg/L when the increment was small (χls2, 11.2; $P < 0.001$). Finally, there was an association between a high T_3/T_4 ratio at delivery and the increase in TV (Table 3). This was particularly evident for women with the highest T_3/T_4 ratios, in whom the increase in TV was 2- to 3-fold greater than for the remainder of the cohort.

Discussion

The present studies represent a large prospective evaluation of the regulation of maternal thyroid function in pregnant women without detectable thyroid abnormalities. Pregnancy constitutes a unique experimental model in humans, wherein a normal thyroid is faced with a triple challenge. First, important modifications occur in thyroidal economy due to the marked increase in circulating levels of the major T_4 transport protein (TBG) in response to high estrogen levels (6, 7, 24). Second, several thyroidal stimulatory factors of placental origin (mainly hCG) are produced in excess. Although it is well recognized that hyperthyroidism may occur in patients with elevated hCG due to trophoblastic disease, the role of hCG in normal pregnancy is still debated (14, 16, 25–28). Third, pregnancy is accompanied by a decrease in the availability of iodide for the maternal thyroid, due to increased renal clearance (29) and losses to the feto-placental complex during late gestation (4), resulting in a relative iodine deficiency state (30, 31). The moderately low iodine supply in Brussels (50–70 μg/day), as in most Western European cities, constituted an additional motivation for performing such a study in our country (32), with the hypothesis that a marginally low iodine supply might become relatively insufficient in a prolonged physiological condition in which the maternal thyroid requirements as well as iodide losses are increased.

TABLE 3. TV and T_3/T_4 ratio

Population studied (percentiles)	At delivery		Vol increment (%)
	Molar T_3/T_4 ratio ($\times 10^{-3}$)	Thyroid vol (ml; mean ± SE)	
1–25[a]	11.0–20.5	13.6 ± 0.81	+15
26–50	20.6–23.3	14.2 ± 0.87	+17
51–75	23.4–26.7	14.1 ± 0.75	+11
76–100	26.8–38.0	18.3 ± 1.43[b]	+33[c]

[a] The cohort was subdivided in quartiles with increasing T_3/T_4 ratio.
[b] Analysis of variance; F = 4.74; $P < 0.005$.
[c] Analysis of variance; F = 3.04, $P < 0.04$.

The dramatic increase in serum TBG during pregnancy results from both an increased TBG production rate by hepatocytes (33) and a reduced peripheral TBG degradation rate (due to oligosaccharide modification) (34) under the influence of high estrogen conditions. As a consequence, the TBG content of the extracellular distribution space increases from 2700 to 7400 nmol (7). To maintain a stable T_4/TBG ratio of 37–40%, the increase in the binding capacity of the system leads to a necessary adjustment of the extrathyroidal T_4 pool, from 1000 to 2700 nmol (7, 35). In pregnancy, changes in TBG levels take place over a period of 3 months, and it can be calculated that the thyroidal adjustment represents an enhanced T_4 output above baseline values of 1–3% day, during the first 3 months. Furthermore, because of the 100-fold greater binding affinity of TBG for T_4, compared to T_3, it can be expected that changes in T_4 would more closely follow the changes in TBG and that the T_3/T_4 ratio would remain essentially unaltered or even decrease slightly during pregnancy (36–39). In the present study, the expected rise in TBG during the first half of gestation was confirmed. However, an important finding was that after 10 weeks gestation, serum T_4 (and, to a lesser extent, T_3) did not catch up, and total hormone levels trailed behind TBG changes. During the second half of gestation, a plateau was reached, with total T_4 and T_3 remaining below the expected theoretical values in most women. Furthermore, the present work provided clear information that variable pattterns of thyroidal adjustment take place in normal pregnancy. By analyzing in greater detail three arbitrarily defined subgroups among the cohort, the data indicated that although the level of TBG constituted the main determinant of total T_4 levels, it was not the sole factor involved. At least one third of the subjects were characterized as having lower TBG saturation, lower free T_4 concentrations, increased TSH levels, albeit within the normal range, and higher T_3/T_4 ratios during the first 3 months of gestation. These women could be referred to as presenting together relative hypothyroxinemia, preferential T_3 secretion, and a higher setting of the pituitary thyrostat, *i.e.* a pattern of increased glandular stimulation.

Modifications of free hormone levels during pregnancy have been the subject of controversies over the past years, partially biased by methodological flaws in the techniques used for determining free hormones, which were affected by the changes in TBG, albumin, and FFA associated with pregnancy (40–43). Several reports in-

dicated that free hormones remained unchanged (44–46), decreased (11, 12, 15, 47, 48), or even increased (16, 49, 50). Although controversies will not be resolved by the present study, it may help to clarify the debate. The present data confirmed earlier reports indicating that pregnancy was associated with a decrease in free T_4 and T_3 levels by about 30% in late pregnancy compared to values in early pregnancy and to those in nonpregnant women (15, 47, 51, 52). The decrease in free hormones was a logical consequence of TBG desaturation, although free T_4 concentrations remained within the reference range of nonpregnant subjects in most women. In addition, this overall view ought to be reexamined more closely: women with initially higher total T_4 tended to maintain higher free hormone levels and had stable T_3/T_4 ratios throughout pregnancy. In contrast, the fraction of the cohort with low free T_4 levels also had total T_4 below 150 nmol/L, despite a rise in TBG comparable to that in the other subgroups. Additionally, these women displayed a significantly higher T_3/T_4 ratio as well as higher TSH, and both further increased with time. The present results, therefore, suggest that variable patterns of thyroidal adjustment occur in normal pregnancy. A reliable and simple index to predict subjects with a higher degree of glandular stimulation could be provided by the T_3/T_4 ratio, especially when it exceeds 0.025 in the first trimester and increases further during gestation.

Relative iodine deficiency in several European countries has recently been documented (53, 54). In the present studies, the daily urinary iodide excretion was less than 100 μg in 90% of subjects. In a study of pregnant women in an iodine-deficient area in Chile (55), the researchers suggested that the inability of T_4 concentrations to increase during gestation resulted from iodine deficiency, thereby enhancing TSH secretion and, in turn, T_3 production. The Belgian population is certainly much less iodine deficient, since serum T_4 did increase during early gestation, and TSH remained normal. Because there was no direct correlation between urinary iodine and the alterations in thyroid function, it is assumed that the marginally low iodine intake in Brussels had an overall permissive role in allowing for the regulatory changes in thyroid economy to be enhanced.

On the basis of structural similarities between hCG and TSH, it has been suggested that hCG possesses intrinsic TSH-like activity (27, 28). In trophoblastic disease, extremely high hCG levels are often accompanied by abnormal thyroidal stimulation and hyperthyroidism (25, 26, 28), and a direct effect of hCG to stimulate thyroid cells in culture was recently demonstrated (56, 57). In normal pregnancy, the putative role of hCG on maternal thyroid, originally proposed by Braunstein and Hershman (27), was recently confirmed (58, 59). The present data are consistent with an intrinsic TSH-like activity of hCG and suggest that hCG acts on the thyroid in normal pregnancy. There was a decrease in serum TSH corresponding to peak hCG levels, with a clear mirror image between TSH and hCG and a significant negative correlation in individual samples, from 8–14 weeks gestation. The results also indicated a linear relationship between hCG and free T_4 concentrations, although the stimulatory action of hCG was relatively weak; a 10,000 IU/L hCG increment corresponded to a mean free T_4 increment of 0.6 pmol/L and, in turn, to a lowering of TSH of 0.1 mU/L. These estimates are comparable to earlier calculations of the TSH-like activity of hCG (14, 27, 59). Finally, free T_4 levels in the thyrotoxic range were exceedingly rare, and the effect of hCG was probably confined to the first half of gestation.

Data on changes in TSH during pregnancy are conflicting, with some investigators reporting an increase (60–62) and others no increase in TSH (14, 15, 63). We observed a significant and progressive rise in TSH, within the limits of the reference range, in more than 80% of the women. Thyrotropic regulation of the thyroid in normal pregnancy remains unclear and complex, exhibiting a possible dual role for both hCG during the first half of gestation and TSH later on.

Early studies have suggested that TG levels are higher in the serum of pregnant women and rise further near term (64–67). In two recent small studies, the researchers have attempted, without success, to correlate TG changes with the level of iodine excretion and TSH changes (68, 69). Our study confirmed that TG levels were increased during the first, but mostly during the last, trimester of pregnancy. At delivery, two thirds of the cohort had TG levels above the upper limit of the reference range. Sequential data demonstrated that changes in TG were common, occurring in more than two thirds of pregnancies. No direct correlation was found between changes in TG and levels of thyroid hormones, urinary iodine, or hCG. There was a weak but significant correlation at delivery between TG and biochemical indices of glandular stimulation, specifically increased TSH and T_3/T_4 ratio. However, the most significant correlation was between TG and thyroid volume; the greater the changes in TV, the more frequently TG was elevated. The results, therefore, suggested that TG elevation in pregnancy reflects anatomical changes and could be considered a marker of an increase in TV.

Data on alterations in TV during pregnancy are scanty. Goiter is rarely observed in the U.S., and its prevalence among pregnant teenagers is comparable to that in the rest of the population (70). In Europe, many clinicians give the impression that goiter is perhaps more frequent among pregnant women, but systematic studies are lacking (71). Rasmussen *et al.* (69) reported a 30% increase in TV in a group of 20 pregnant women, but the research-

ers were unable to correlate changes in TV with parameters of thyroid function. The present studies comprised 552 ultrasonographies of the thyroid, including a longitudinal evaluation of TV changes. The data clearly indicated that the size of each lobe, and, hence, total volume, did increase significantly by an average of 20%. The TV increase was observed in the majority of women, but the amplitude of the increment varied widely. True goiter, defined as TV greater than 23 mL, was found in 9% of the cohort at delivery, with volumes up to 46 mL. Changes in size were negatively correlated to initial size, so that for TV initially under 10 mL, the average increase exceeded 30%. These changes were also associated with hCG stimulation and biochemical features of thyroid stimulation, specifically high T_3/T_4 ratio and TG levels. An apparent paradox was that the largest volumes at delivery were negatively correlated to TSH, presumably as a result of high hCG levels. By measuring TV with echography and in the absence of histological data, it was impossible to distinguish glandular hyperplasia from hypertrophy or appreciate the role of changes in blood flow occurring during pregnancy. However, the association of biochemical features of thyroidal stimulation with volumetric changes in the gland in conditions of marginally low iodine intake strongly suggests that pregnancy truly induces goitrogenesis rather than vascular swelling alone.

In conclusion, the present studies were undertaken in a large cohort of healthy pregnant women to evaluate the regulation of maternal thyroid function, with the hypothesis that increased thyroid requirements would be enhanced by the marginally low iodine intake. Three main features were observed. First, thyroidal activity had to adjust to a marked increase in serum TBG. The adjustment of thyroidal output of T_4 and T_3 did not occur similarly in all subjects; in approximately one third of the women, there was relative hypothyroxinemia, higher T_3/T_4 ratios, presumably indicating preferential T_3 secretion, and higher, albeit normal, serum TSH levels. Pregnancy was accompanied by an overall TBG desaturation, with lower free T_4 and T_3 levels, although in most cases they remained within normal limits. Second, high hCG levels were associated with thyroidal stimulation, both functionally (with lowering of TSH) and anatomically (with increased glandular size). Hence, the thyrotropic regulation of the maternal thyroid is complex and unclear, resulting from both elevated hCG (mainly in the first half of gestation) and increasing TSH (mainly in the second half of gestation). Third, changes in thyroid volume were common, and goiter formation was not uncommon, related in part to the thyrotropic effect of hCG. In conditions of marginally low iodine intake, pregnancy constituted a goitrogenic stimulus. This study, therefore, provides additional arguments to suggest that iodine supply during pregnancy be increased. Finally, the question of the reversibility, during the postpartum period, of the alterations demonstrated during gestation remains open for further investigation. If the changes occurring during gestation were not entirely reversible and were to be repeated during later pregnancies, the present study may have provided a clue to understanding the higher prevalence of thyroid disorders in women compared to men, at least in predisposed subjects.

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