1 **Controlled Antenatal Thyroid Screening II: effect of treating maternal sub-**

2 **optimal thyroid function on child behaviour**

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24 **ABSTRACT**

25 *Context & Objectives* The Controlled Antenatal Thyroid Screening (CATS) study was

26 the first randomised controlled trial to investigate effects of treating suboptimal-

27 gestational-thyroid-function (SGTF) on child cognition. Since observational studies

28 indicated that SGTF may also increase symptoms of autism and attention deficit

29 hyperactivity disorder (ADHD), the CATS cohort was used to investigate whether

30 treatment of mothers affected their children's behavior.

31 *Design & Participants* Mothers (N=475) completed 3 questionnaires, strengths and

32 difficulties (SDQ), attention deficit hyperactivity disorder (ADHD) and social

33 communication (SCQ, used as a screen for autism spectrum disorder (ASD)), about

34 their children (aged 9.5 years). Group comparisons of total scores, numbers of children

35 above clinical thresholds and association between high maternal FT4 (>97.5th

36 percentile of the UK cohort, 'over-treated') and child neurodevelopment were reported.

37 *Results* There were no differences in total scores between normal-GTF (n=246),

38 treated (n=125) and untreated (n=104) SGTF groups. More children of treated mothers

39 scored above clinical thresholds, particularly the over-treated. Scores were above

40 thresholds in SDQ conduct 22% vs 7%, SCQ total scores 7% vs 1%, and ADHD

41 hyperactivity 17% vs 5% when comparing over-treated (n=40) and untreated (N=100)

42 respectively. We identified significantly higher mean scores for SDQ conduct (adjusted

43 mean difference (AMD) 0.74, 95% confidence interval (CI) 0.021 to 1.431, P=0.040,

44 effect size 0.018) and ADHD hyperactivity (AMD 1.60, CI 0.361 to 2.633, P=0.003,

45 effect size 0.028) comparing over-treated with normal-GTF children.

46 *Conclusions* There was no overall association between SGTF and offspring ADHD,

47 ASD or behavior questionnaire scores. However, children of 'over-treated' mothers

48 displayed significantly more ADHD symptoms and behavioral difficulties than normal-

49 GTF. Thyroxine supplementation during pregnancy requires monitoring to avoid over-

50 treatment.

51 **PRÉCIS**

52 We investigated effects of treating sub-optimal gestational thyroid function (SGTF)

53 on child behaviour. No associations found between SGTF & offspring behaviour;

54 children of 'over-treated' mothers had significantly more difficulties than untreated.

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INTRODUCTION

The cognitive impairment displayed in children with congenital hypothyroidism or born to mothers with iodine deficiency illustrates the essential role of thyroid hormones, triiodothyronine (T3) and thyroxine (T4), in brain development [1]. Although fetal T3 receptors in the human brain can be detected from about 10 weeks gestation and increase 50 fold by 16 weeks, the thyroid develops relatively late with full function being acquired at around 36 weeks gestation [1]; thus thyroid hormones must be supplied via placental transfer [2]. Sub-optimal gestational thyroid function (SGTF) (classified as low T4 and/or high thyroid stimulating hormone (TSH) values, or both) in pregnancy can occur in women with no previous thyroid dysfunction. Children born to mothers with SGTF have been reported to have lower IQ compared with children born to euthyroid mothers [3-11]. However, two large randomized controlled trials (RCTs) found no evidence of thyroid hormone treatment improving the IQ of children born to mothers with SGTF [12,13] tested at ages 3 and 5 respectively. These results were confirmed recently in a follow-up to the first study, in children aged 9 [14].

In addition to intelligence, SGTF may also affect other aspects of child neurodevelopment; specifically attention deficit hyperactivity disorder (ADHD, characterised by hyperactivity, inattention and impulsiveness) and autism spectrum disorder (ASD, characterised by social and communication problems, and repetitive behaviors). Multiple observational studies have reported higher ADHD scores in children born to mothers with SGTF, particularly hypothyroxinaemia, compared to controls [3,15-19]. Childhood ASD has been reported to be increased in those born to mothers with SGTF [20,21]. Maternal thyroid peroxidase autoantibodies, markers of thyroid autoimmunity, are also associated with increased ADHD [22] and ASD risk

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82 (23) in offspring. In contrast, others report no link between SGTF and ADHD or ASD

83 symptoms in the child (20,24,25).

84 The two RCTs described above (12,13) included additional psychological

85 assessments of behavioral and social competency plus attention. Neither reported any

86 differences in these parameters in children of treated and untreated mothers; although

87 they were not powered specifically for these aspects. Furthermore, data from children

88 of mothers with normal-GTF were not collected. The current study is an extension of

89 the first RCT of treatment of SGTF during pregnancy (12) in which the offspring were

90 examined for behavior, social competency and attention after long-term follow-up. The

91 inclusion of children of normal-GTF mothers enabled us to assess whether there is a

92 deficit in neurodevelopment requiring treatment.

93 Furthermore, since a number of mothers demonstrated high FT4 concentrations

94 suggesting a degree of over-treatment, we were also interested in the effects of high-

95 FT4 on child neurodevelopment outcomes. The rationale for this derives from studies

96 by Korevaar and colleagues, who reported adverse effects on brain morphology and

97 cognition of both too little and too much thyroid hormone (26). In addition, a recent

98 study from Maraka et al, demonstrated worse obstetric outcomes such as pre-term

99 delivery and pre-eclampsia in treated women with SGTF (27).

100 **MATERIALS AND METHODS**

101 The Controlled Antenatal Thyroid Screening study

102 We report a follow-up to a treatment trial, the Controlled Antenatal Thyroid Screening

103 Study (CATS) (12), in which women were screened at a median of 12 weeks 3 days'

104 gestation between June 13, 2002 and May 31, 2006. Briefly, in CATS-I (12) midwives

105 and obstetric care-givers in the UK and Italy recruited ~22,000 women (excluding

106 history of thyroid disease, twin pregnancies, maternal age <18 years or gestational

107 age >15 weeks and 6 days), at their first antenatal hospital appointment. Participants

108 were randomized, by computer generated block design, either to screen or control

109 groups; both provided serum samples at recruitment with the screen group having

110 their thyroid function tested immediately and the controls after their child was born

111 (sera stored at -40°C). If the mother's FT4 was <2.5th percentile and/or TSH >97.5th

112 percentile, they were classified as having SGTF; percentiles being calculated from the

113 CATS cohort. Women in the screen group with SGTF were treated with levothyroxine

114 (starting dosage 150μg) for the remainder of their pregnancies. Dosages were

115 adjusted where necessary, to maintain a serum TSH of 0.1-1.0mIU/L, following

116 measurement of TSH and FT4 6 weeks after treatment was initiated and at 30 weeks

117 gestation. SGTF women diagnosed after delivery were advised to visit their general

118 practitioner for further management. The primary outcome was children's IQ at age 3

119 from 390 (303 in the UK) treated (screen) and 404 (306 in the UK) untreated (control)

120 mothers (assessors blinded to treatment group). A further 20,789 (15,593 in the UK)

121 women made up the normal-GTF group and recruitment ended when sufficient

122 numbers, determined by prior power calculation, had been reached. Figure 1 shows

123 the study flow chart of the UK participants.

124

125 Follow-up of children at ages 7-10 years; current data

126 Inclusion criteria were mothers living in the UK and from the CATS-I cohort whose

127 children were aged 7 to 10 years. Offspring follow-up data were collected between

128 August 8, 2011 and August 7, 2015. The target sample size of 480 participants was

129 pre-determined and informed by prior power calculations to assess cognition. Samples

130 of 120 from each of the treated and untreated SGTF groups provided 80% power with

131   a 5% two-sided significance level to detect a 1.97 increase in odds of IQ <85 in

132   untreated SGTF offspring assuming treated SGTF offspring have a mean IQ of 100

133   with a SD of 15. An additional 240 participants from the normal GTF group, randomly

134   selected from the 15,593 UK cohort, were used to assess interaction with maternal

135   thyroid status and levothyroxine treatment on offspring IQ.

136   For full details see Hales et al 2014 (28).

137   CATS mothers from the UK SGTF treated and untreated groups (n=609) were invited

138   to participate by letter. The Welsh Demographics Service and Patient Data Registrar

139   provided current addresses. Those without SGTF in the control and screen branches

140   of the RCT, were pooled (UK n=15,593), and named 'normal-GTF'; a random sample

141   of 4,000 from this group was also invited to participate. Data were collected at a

142   research centre, via a home visit, or by post; this approach was used to maximise the

143   follow-up rate. Informed consent was provided by all mothers who completed the

144   questionnaires.

145   Results of cognitive assessments in the children have been reported (14).

146   Cardiovascular, metabolic, bone data and DNA samples were also collected in those

147   participants attending the visit at the research centre but are not described here (ms

148   in preparation).

149   Mothers (mean age 31 (SD 5.12) years) were asked to complete three questionnaire

150   measures on their children (mean age 9.5 (SD 0.77) years): The Strengths and

151   Difficulties Questionnaire (SDQ) (29), The Child ADHD Questionnaire (ADHDq) (30),

152   and the Social Communication Questionnaire (SCQ) (31). The SDQ consisted of 25

153   items, scored by a Likert scale (0-2), grouped into five subscales; Hyperactivity (5

154   ADHD items), Emotional symptoms, Conduct problems, Peer problems, and Pro-

155 social behavior as well as a total score. The SDQ provides a measure of child mental

156 health difficulties, has been internationally validated and has good psychometric

157 properties (32-35). The ADHDq consists of 18 items addressing ICD-10 symptoms by

158 a Likert scale (0-3, Thapar et al.(30)); subscales include inattention, hyperactivity, and

159 impulsivity. The Social Communication Questionnaire has good external validity (36)

160 and is one of the most widely adopted screening tools for ASD (37). The SCQ

161 generates a total score from 'yes'/'no' answers; a score of ≥15, indicates possible

162 ASD (38). Data were collected, inputted, and cleaned by one researcher who was

163 blinded to participant study group.

164 Statistical analysis

165 All analyses (unless otherwise stated) were adjusted for child gender, maternal age at

166 time of consent into CATS, whether the mother breastfed for over one month, and

167 quintile of social deprivation (the lower the quintile the more deprived, quintiles

168 enabled comparisons between Welsh and English postcodes); these variables have

169 been reported previously to be associated with questionnaire scores (39-47). Child

170 age was not included as a covariate given the narrow age range of the children (3

171 years).

172 Descriptive statistics were calculated to identify any potential recruitment bias in the

173 three groups; continuous variables are presented as means (standard deviations) and

174 categorical data as percentages (unless otherwise stated). Thyroid function test

175 results at time of consent into CATS are presented per group, as well as the treated

176 SGTF group's additional test results. As a proportion of CATS mothers had high FT4

177 concentrations (26), the study was able to analyse an 'over-treatment' effect; a FT4

178 measurement >17.7pmol/L at either 20 weeks or 30 weeks' gestation as defined by

179 the top 2.5th percentile of FT4 in the entire CATS UK cohort at consent into the RCT.

180   The over-treatment value was calculated using a large sample-size (n=16,346) and

181   was higher than the upper limit of the regional reference range. Mean FT4 and median

182   TSH values of those who were over-treated were compared to the optimally treated

183   mothers (FT4 <17.6pmol/L). Effects of the treated SGTF group's thyroid function tests

184   throughout pregnancy were related to the questionnaire subdomains and total scores.

185   List-wise deletion was applied for questionnaires having >15% missing data

186   (previously reported 10-20% acceptable (48,49), with >15% requiring consideration

187   (50)), leading to exclusion of 21 participants. Of the remaining 454 participants

188   (normal-GTF=237, treated SGTF=117, untreated SGTF=100), 54 (11.9%) had

189   missing data; total values of missing data were n=85 (0.2%). The remaining

190   participants' data were analysed by Little's missing completely at random test (non-

191   significant), with missing values replaced by the 'series mean' in SPSS (51).

192   All Kolmogorov-Smirnov (and Shapiro-Wilk) normality tests were returned as p <0.001

193   for all questionnaires on every totalled domain. However, means and medians

194   appeared close and skewness and kurtosis ranges were largely just above ± 1 and

195   thus were returned as normal with caution.

196   In the primary analysis, we compared the mean total scores from the three

197   questionnaires administered to the normal-GTF, treated and untreated SGTF groups.

198   This was by a multivariate analysis of covariance (MANCOVA), which was followed by

199   univariate tests and pairwise comparisons that were Bonferroni corrected, where

200   relevant.

201   In the secondary analysis, the frequency of children scoring above the questionnaire

202   thresholds that indicate likely disorder was presented to enable comparisons between

203   the groups. For SDQ, the clinically significant cut-offs were adopted, those classified

204   as 'high' were in the 90-95th percentile range, and 'very high' were above the 95th

205   percentile (29). For the ADHDq, the authors calculated the SDs (30); scores >2SDs

206   were reported. For the SCQ, scores >15 were identified as the child potentially having

207   an ASD (31); a UK sample found a prevalence of 4-5% for scores >15 (52).

208   The exploratory analysis investigated the effect of treatment. Mean scores from the

209   questionnaires (total and subdomains) were compared between the normal-GTF,

210   untreated SGTF, optimally treated SGTF (FT4 <17.6pmol/L), and over-treated SGTF

211   (FT4 >17.7pmol/L). Further exploratory analyses are in the Supplemental Material,

212   including effects of hypothyroxinemia or subclinical hypothyroidism and varying levels

213   of FT4 following treatment.

214   Standard regression analysis was performed to compare behavior of participating

215   children at age 3 (Child behavior check-list) and age 9 (strengths and difficulties

216   questionnaire).

217   All statistical analyses were performed with SPSS version 20 software. P<0.05 was

218   considered significant.

219   **RESULTS**

220   *Characteristics of the cohort*

221   A total of 485 women/child pairs participated but 10 were excluded (9 ineligible, one

222   whose results were deemed unreliable due to their approach to the assessment)

223   leaving 475 who completed the questionnaires (normal-GTF=246, treated SGTF=125,

224   and untreated SGTF=104); table 1 reports their demographic data. The SGTF groups

225   had younger children participating (by four months) than the normal-GTF group, and

226   the treated SGTF also had significantly younger mothers by ten months. The treated

227   SGTF had a higher median baseline TSH compared to the untreated SGTF group; this

228 suggested a potential recruitment bias and was reflected in SGTF group age 3 to age

229 9 comparisons; supplemental data table 1 (53).

230 Pregnancy thyroid function tests of the treated SGTF group at 20 weeks' gestation

231 identified a mean (SD) FT4 of 16.2pmol/L (2.89), reducing to 15.5pmol/L (2.47) at 30

232 weeks; median TSH values at 20 and 30 weeks gestation were 0.3mIU/L at both time

233 points. Of these women, 41 were over-treated, with FT4 concentration >17.7pmol/L at

234 20 or 30 weeks' gestation (see supplemental material for figures); with 13 of these

235 sustaining over-treatment throughout their pregnancies. The mean FT4

236 concentrations at 20 weeks and 30 weeks for the over-treated group were 19.2pmol/L

237 (2.53) and 17.8pmol/L (2.46), respectively, whilst median TSH values at these times

238 were below target at 0.08mIU/L and 0.05mIU/L, respectively. In the remaining

239 optimally treated SGTF group, the mean FT4 concentrations at 20 weeks' and 30

240 weeks' gestation were 14.8pmol/L (1.78) and 14.5pmol/L (1.73), respectively, with

241 median TSH values of 0.52mIU/L and 0.35mIU/L.

242 *Main analyses*

243 After removing participants with >15% missing data, 454 were analysed, revealing no

244 significant differences between the means of the 3 administered questionnaires (table

245 2) in the normal-GTF, treated SGTF and untreated SGTF groups (P=0.261). Total

246 questionnaire scores comparing the three participant groups were significantly

247 affected by child gender (/sex), mother age at time of consent and social deprivation

248 (P=0.001, P=0.030, and P=0.001, respectively). Higher questionnaire scores were

249 more likely for male children and children born to younger mothers during pregnancy

250 or to families with greater social deprivation.

251 *Secondary and Exploratory Analyses*

252 At the point of recruitment into CATS-I, there was a negative association between

253 maternal FT4 level and aspects of child behaviour assessed at age 9; i.e. children of

254 mothers with low FT4 before any treatment tended to have higher questionnaire

255 scores indicating more behaviour problems. However, the association between child

256 behaviour problems and FT4 in treated mothers was positive at 20 weeks' gestation

257 (figure 2). Thus we investigated the possible effects of over-treatment as defined by

258 the top 2.5th percentile of FT4 in the CATS UK cohort at recruitment (N=16,346,

259 FT4>17.7 pmol/L). We observed significantly higher mean scores for some domains:

260 SDQ conduct (adjusted mean difference (AMD) 0.74, 95% confidence interval (CI)

261 0.021 to 1.431, P=0.040, effect size 0.018) and ADHD hyperactivity (AMD 1.60, CI

262 0.361 to 2.633, P=0.003, effect size 0.028) in over-treated compared with normal-GTF.

263 Furthermore, individuals within each group had 'high scores' on domains reported in

264 the analyses (table 3); these were defined as clinically significant cut-offs (SDQ),

265 standard deviations calculated by Thapar et al.(30) (ADHDq), and a score >15

266 indicative of a child potentially having an ASD (31) (SCQ). The largest and significant

267 percentage differences were noted on SDQ Conduct, SDQ Hyperactivity, ADHDq

268 Hyperactivity, and SCQ Total; the treated SGTF had proportionately more than twice

269 as many individuals in the highest classifications compared to the normal-GTF and

270 untreated SGTF, which appeared to be driven by those children born to over-treated

271 mothers. When comparing the normal-GTF and untreated SGTF children, we did not

272 observe any difference between numbers of individuals in the highest classifications.

273 *Effect of subclinical hypothyroidism, hypothyroxinemia and overt hypothyroidism*

274 Similar non-significant differences were found between the normal GTF (n=237),

275 treated (n=67), and untreated (n=49) subclinical hypothyroid groups for total

276 questionnaire scores (P=0.909). When exploring the effect of treatment to the

277 17.7pmol/L threshold, there was also no difference in mean scores; supplemental data

278 table 2

279 Total questionnaire scores were not significantly different between the normal GTF

280 (n=237), treated (n=36) and untreated (n=44) hypothyroxinaemia groups;

281 supplemental data table 3.

282 There were 8.55% (n=10) of the treated SGTF group, and 9.00% (n=9) of the untreated

283 SGTF group classified as having overt hypothyroidism (OH). Comparing these groups

284 (combined) with the normal GTF for total questionnaire scores identified a significant

285 difference at the multivariate level (V=0.063, F(6,496)=2.669, P=0.015, η_p^2=0.031),

286 with a difference found for ADHD Total scores (F(2,249)=4.680, P=0.010, η_p^2=0.036).

287 The untreated OH group had significantly higher scores compared to the normal GTF

288 for ADHD Total (P=0.022 (0.945, 16.876)).

289 When exploring the effect of treatment to the 17.7pmol/L threshold for maternal FT4,

290 there was a difference at the multivariate level (V=0.203, F(27,726)=1.948, P=0.003

291 η_p^2=0.068). At the univariate level SDQ Conduct (F(3,248)=4.402, P=0.005, η_p^2=0.051),

292 ADHD Hyperactivity (F(3,248)=5.665, P=0.001, η_p^2=0.064), and ADHD Total

293 (F(3,248)=3.487, P=0.016, η_p^2=0.040) were significantly different. The over-treated OH

294 group had significantly higher scores for SDQ Conduct (P=0.038 (0.072, 4.273) and

295 ADHD Hyperactivity (P=0.020 (0.357, 6.597) compared to the normal GTF. Again, for

296 ADHD Total, the untreated OH had significantly higher scores than the normal GTF

297 (P=0.045 (0.117, 7.693).

298 Children born to mothers who were untreated for OH in pregnancy, had higher scores

299 for the Child ADHD Questionnaire, optimal treatment improved this with some means

13

300 moving closer to those of the normal GTF; supplemental data table 4. Over-treatment

301 for OH had a significant effect on the same domains identified with the entire SGTF

302 sample; SDQ Conduct and ADHD Hyperactivity. Caution is advised, however, as the

303 sample sizes are small for OH.

304 Finally, we applied standardised regression analysis to compare behavior assessed

305 at age 3, using the CBCL, and at age 9, using the SDQ. We observed that behavior

306 at ages 3 and 9 was highly correlated B (Std) = 0.54 (95%CI 0.48 – 0.66) p< 0.001,

307 with variation at age 3 predicting 30% of the variation at age 9. Adjusting for other key

308 factors such as sex, social class, maternal age and breastfeeding had no substantial

309 impact on relationship or effect estimates.

310 **DISCUSSION**

311 We have applied well-validated questionnaires to assess neurodevelopment and

312 behavior in a large cohort of children born to mothers recruited to CATS-I, the first

313 RCT to explore the effect of levothyroxine treatment on childhood outcomes. In

314 addition, our inclusion of children from normal-GTF mothers allowed us to investigate

315 whether SGTF has any effect on conduct and hyperactivity.

316 We found no difference in the mean total scores of questionnaires to assess ADHD

317 and ASD in children of mothers with normal-GTF compared with SGTF, whether

318 treated or not. Results were the same when considering treated and untreated

319 hypothyroxinemia and subclinical hypothyroidism separately, although numbers were

320 inevitably smaller in the sub-sets. Boys, children born to younger mothers or to socially

321 deprived families were more likely to have higher scores. FT4 was negatively

322 associated with measures of hyperactivity in children of treated mothers at recruitment

323 but positively associated after thyroxine supplementation. Reflecting this switch, there

324 were significantly more children of treated mothers with scores above clinical

325   thresholds for conduct and hyperactivity than normal-GTF mothers. In particular, there

326   were either significantly more children of over-treated mothers above defined cut-offs

327   for these parameters or with mean scores significantly higher than the untreated SGTF

328   or normal-GTF groups respectively.

329

330   We are aware that our study sample may be biased but group means were within

331   ±1SD of the ADHDq mean (24) or similar to the means reported for the SDQ and SCQ

332   from other large UK cohorts (52) suggesting that participants are representative.

333   However, numbers in the SGTF groups, especially untreated, were below our target

334   and recruitment was a challenge from the outset, mainly because participants had re-

335   located and did not respond to invitation.

336

337   Incomplete questionnaires were minimised by spot-checks and thus less than 1% of

338   participants were excluded. However, questionnaires were completed only by the

339   mothers and the study would have benefited from the child's mental health and

340   neurodevelopment being assessed by another independent source, such as their

341   teacher. Furthermore, significant mean effects are mainly seen on individual domains

342   of the questionnaires, although these sub-domains mostly relate to hyperactivity.

343

344   In the two RCTs (12, 13) of the effects of SGTF treatment on child outcomes, neither

345   reported any differences (mean scores) in behavior, social competency or attention in

346   children of treated and untreated mothers at age 3 and 5 respectively. Our results in

347   children aged 9 confirm both studies and demonstrate that behavior scores at age 3

348   predict 30% of the variability in behavior scores at age 9.

349   In contrast to other studies, we found no differences in ADHD (3,15-18) and ASD

350   (20,21) questionnaire domains between the SGTF and normal-GTF groups. However,

351   we recognise that our findings are based on a smaller sample size, particularly of

352   normal-GTF, than the large observational cohorts.

353   A small number of mothers in our study had overt hypothyroidism (n=19) and their

354   children, whether in the untreated or over-treated groups, had more conduct, total

355   ADHDq and hyperactivity symptoms then those in the normal-GTF group

356   (supplemental material). In studies of congenital hypothyroidism, similar findings have

357   also been reported: increased hyperactivity with higher levothyroxine dosages (49),

358   and if the child had a high T4, they were significantly more distractible (50). Haddow

359   et al.(3) identified more children with attention difficulties born to mothers who were

360   treated in pregnancy, compared to untreated; however this was not an RCT and there

361   was limited information on thyroid hormone levels of those who were treated. Recent

362   research confirms that both high and low maternal FT4 may be detrimental to offspring

363   in respect of intelligence and brain morphology (26). Our results also hint at a biphasic

364   effect of maternal FT4 on neurodevelopment as illustrated by the switch from negative

365   to positive association between FT4 and measures of hyperactivity before and after

366   initiation of treatment. Mothers received a starting Levothyroxine dose of 150 µg daily

367   after which the dose was adjusted to maintain TSH in the lower end of the reference

368   range according to recommended treatment targets at the time of the study (54). This

369   resulted in one third having an FT4 concentration above 17.7pmol/L, a threshold

370   derived from the top 2.5th percentile of the UK CATS-I cohort (n=16 346), a cohort

371   larger than those used to set reference ranges, and which excluded mothers with a

372   history of thyroid disease. Thus subclinical hyperthyroidism may have inadvertently

373   been induced in the over-treated group (based on current guidelines of a TSH value

374   <0.1mIL/l (55). A nationwide study in Europe (n=857, 014) reported that if the mother

375   was hyperthyroid throughout pregnancy, the child was at an increased risk (OR 1.23,

376   1.05 to 1.44) of being diagnosed with ADHD (20). Yau et al.(25) reported that lower

377   maternal TSH values were associated with more ASD symptoms in the offspring;

378   whilst individuals with resistance to thyroid hormone (n=75) displayed a significant

379   positive correlation between T4 levels and both ADHD hyperactivity and impulsivity

380   symptoms (56). The same study also found a significant positive correlation between

381   T3 and both ADHD hyperactivity and impulsivity in individuals without resistance to

382   thyroid hormone (case family members, n=77). Thus, our results confirm these earlier

383   studies indicating that higher levels of thyroid hormones can affect ADHD symptoms.

384   Our results are relevant to clinicians managing pregnant women with hypothyroidism.

385   If a woman is already prescribed levothyroxine before pregnancy, current guidelines

386   (55) advise an increase in the levothyroxine dose of 20-30% during pregnancy.

387   Recommended starting doses for women diagnosed during pregnancy range from 100

388   µg for OH but 25-50 µg daily for subclinical hypothyroidism and depend on 'normal

389   reference ranges' for T4 and TSH according to geographic region and ethnic origin

390   (57,58). In all cases treatment focuses on reducing TSH levels, and FT4 levels are

391   seldom monitored, hence over-treatment in pregnancy may be more commonplace

392   than anticipated (57,59,60). Of note, 17% of the women found to be over-treated in

393   the present study had TSH values within the normal reference range. We hypothesise

394   that there could be a beneficial mid-range of maternal FT4 during pregnancy for the

395   offspring; too little or too much may affect the child. To investigate a possible 'tipping

396   point', when differences in mental health and neurodevelopment between groups were

397   no longer significant, FT4 thresholds of 17, 16, 15 and 14pmol/L at 20 weeks' gestation

398   were applied (supplemental data table 5). It was identified that most differences

399   diminished below the 17.7pmol/L category, with lowering FT4 also increasing

400   questionnaire scores; this also supports the biphasic effect of maternal FT4.

401   While screening and treatment of thyroid dysfunction in pregnancy will clearly benefit

402   patients with overt hypothyroidism, the benefits for SGTF remain to be proven. In the

403   light of our findings screening programmes should be carefully considered in order to

404   avoid the unintended consequences of over-treatment. To fully explore the impact of

405   SGTF and its treatment on behavioral problems requires large-scale studies with early

406   intervention. Before embarking on such studies, consensus needs to be reached on

407   trimester-specific reference ranges for TSH, FT4 and FT3, all derived from large

408   groups of healthy women, free of thyroid autoantibodies, in an iodine sufficient area

409   and with no history of thyroid disease.

410   We conclude that SGTF and optimal management of levothyroxine treatment during

411   pregnancy have no effect on ADHD and ASD-symptoms of offspring at age 9. The

412   children of women with FT4>97.5th percentile had more conduct, ADHDq, and ASD-

413   symptoms and suggests that levothyroxine dose in pregnancy needs to be carefully

414   monitored.

415

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DATA AVAILABILITY

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

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620

LEGENDS FOR FIGURES AND TABLES

622   Figure 1: Flow diagram of the CATS studies showing UK participants only.

623   Figure 2: Correlations between elements of child behaviour and FT4 levels at

624   recruitment and following several weeks of thyroxine treatment.

625   Table 1: Characteristics of the cohort.

626   Table 2: Group total scores for the questionnaires

627   Table 3: High questionnaire scores from the normal gestational thyroid function

628   (GTF), treated, and untreated suboptimal GTF groups

629

SUPPLEMENTARY MATERIALS

Characteristics of the follow-on cohort

The SGTF groups were compared between first participation of offspring, and at follow-up (current). The sample appeared mostly similar between the two time points (table 1). Those in the treated SGTF group who continued participation into the follow-up of CATS had significantly raised TSH values. A potential reason for this could have been that those who felt most benefit from treatment, continued to aid the study. The mothers in the untreated SGTF group who participated in the follow-up were significantly older at time of pregnancy consent compared to the original CATS sample, this was by an age increase of 1.1 years.

Characteristics at time of study entry	Treated SGTF		P for difference	Untreated SGTF		P for difference
	CATS N=303	Follow-up N=125		CATS N=306	Follow-up N=104	
Male children (%)	54.1	52.0	0.921*	51.3	48.1	0.569
Social deprivation**	3 IQR=2-5 (mean=3.3)	4 IQR=3-5 (mean=3.8)	0.358*	3 IQR=2-5 (mean=3.3)	4 IQR=2-5 (mean=3.4)	0.981
Mother age	29.8 (5.6)	30.2 (5.1)	0.447	29.8 (5.4)	30.9 (4.8)	0.050 (95% CI -0.002, 2.364)
Maternal TSH	3.6 IQR=1.5-4.7	4.1 IQR=1.9-5.1	0.026†	3.4 IQR=1.2-4.1	3.6 IQR=1.1-4.5	0.372†
Maternal FT4	11.8 (1.8)	12.0 (1.9)	0.262	12.0 (1.9)	11.7 (1.9)	0.310

Table 1 | Comparison of study population to original cohort. Figures are mean values (standard deviation). P for difference by t-test unless stated otherwise.

*Significance tested by chi square.
**The social deprivation scores used were calculated from postcodes at time of pregnancy, rather than the participants' current deprivation scores as used in the main analysis.
†Significance for TSH at consent test by Mann Whitney.
SGTF=suboptimal gestational thyroid function, CATS=controlled antenatal thyroid screening, TSH=thyroid stimulating hormone, FT4= free thyroxine

Thyroid function tests during pregnancy of the treated suboptimal gestational thyroid function group

Thyroid function tests in pregnancy in the treated SGTF group were analysed by repeated measures ANOVAs (FT4) and Friedman's test (TSH).

Mean (SD) FT4 levels at time of consent, at 20 weeks' gestation, and 30 weeks' gestation were 12.0pmol/L (1..91), 16.2pmol/L (2.89), and 15.5pmol/L (2.47), respectively). Maternal FT4 was different at the multivariate level throughout pregnancy for the treated SGTF group (Pillai's Trace=0.687, $F(2,116)=127.93$, P=0.001, $\eta_p^2=0.687$). Pairwise comparisons that were Bonferroni corrected, identified that at time of consent FT4 was significantly lower than at 20 weeks gestation (P=0.001, -5.038 to -3..627), and 30 weeks gestation (P=0.001, -4.151 to -2.929). FT4 at 20 weeks gestation was significantly higher than at 30 weeks gestation (P=0.003, 0.231 to 1.353).
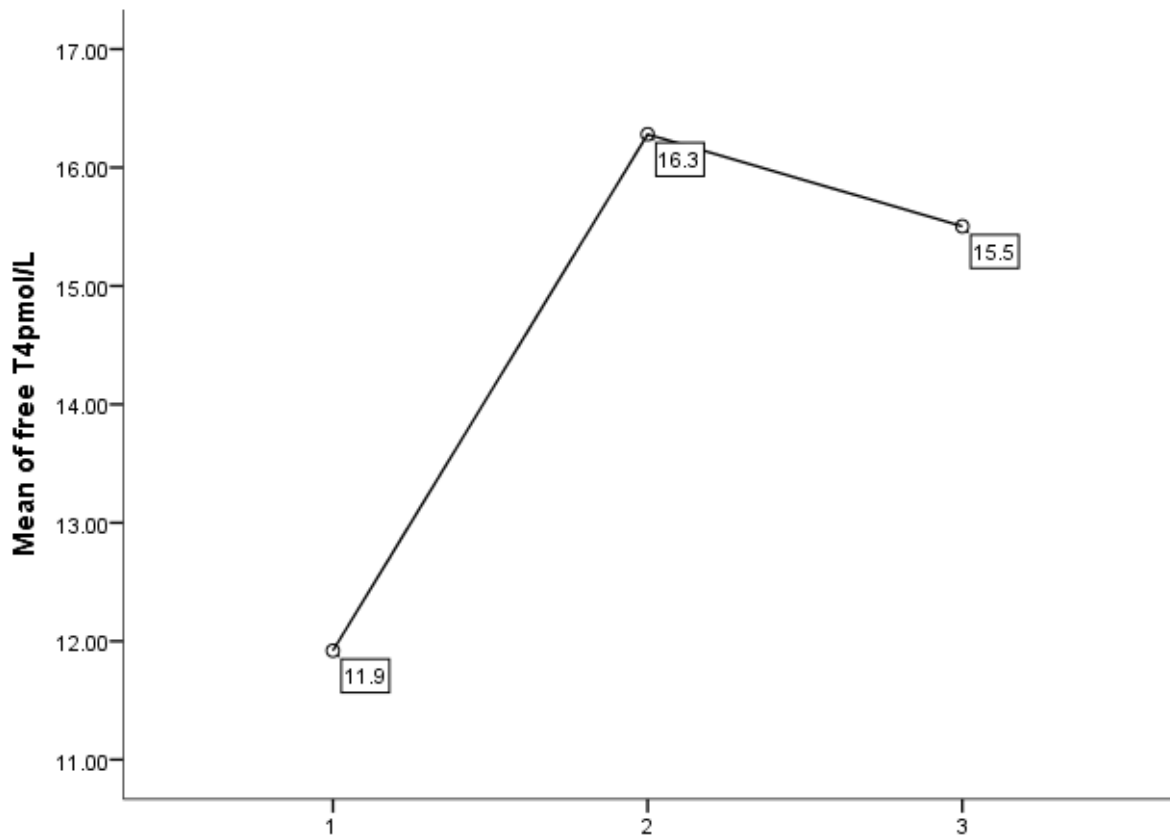
Fig 1|Mean free thyroxine (T4) values of the treated suboptimal gestational thyroid function group taken at: (1) consent into the Controlled Antenatal Thyroid Screening study (median 12 weeks 3 days gestation), (2) 20 weeks (0 days) gestation, and (3) 30 weeks (1 day) gestation. T4 was measured in pmol/L.

There was a statistically significant difference in TSH levels throughout the pregnancies of those from the treated SGTF group, $\chi^2(2)=173.207$, $P=0.001$. Post hoc analysis with Wilcoxon signed-rank tests was conducted with a Bonferroni correction applied, resulting in a significance level at P<0.017. Median (IQR) TSH levels at time of consent, 20 weeks gestation, and 30 weeks gestation were 4.1mIU/L (1.9 to 5.1), 0.3mIU/L (0.1 to 1.0), and 0.3mIU/L (0.03 to 0.9), respectively. There was a significant difference between TSH at time of consent and at 20 weeks gestation (Z=-9.543, P=0.001), as well as at 30 weeks gestation (Z=-9.478, P=0.001). There was also a significant difference between TSH at 20 weeks gestation and 30 weeks gestation (Z=-3.109, P=0.002), even though there was only a slight drop in the median value.
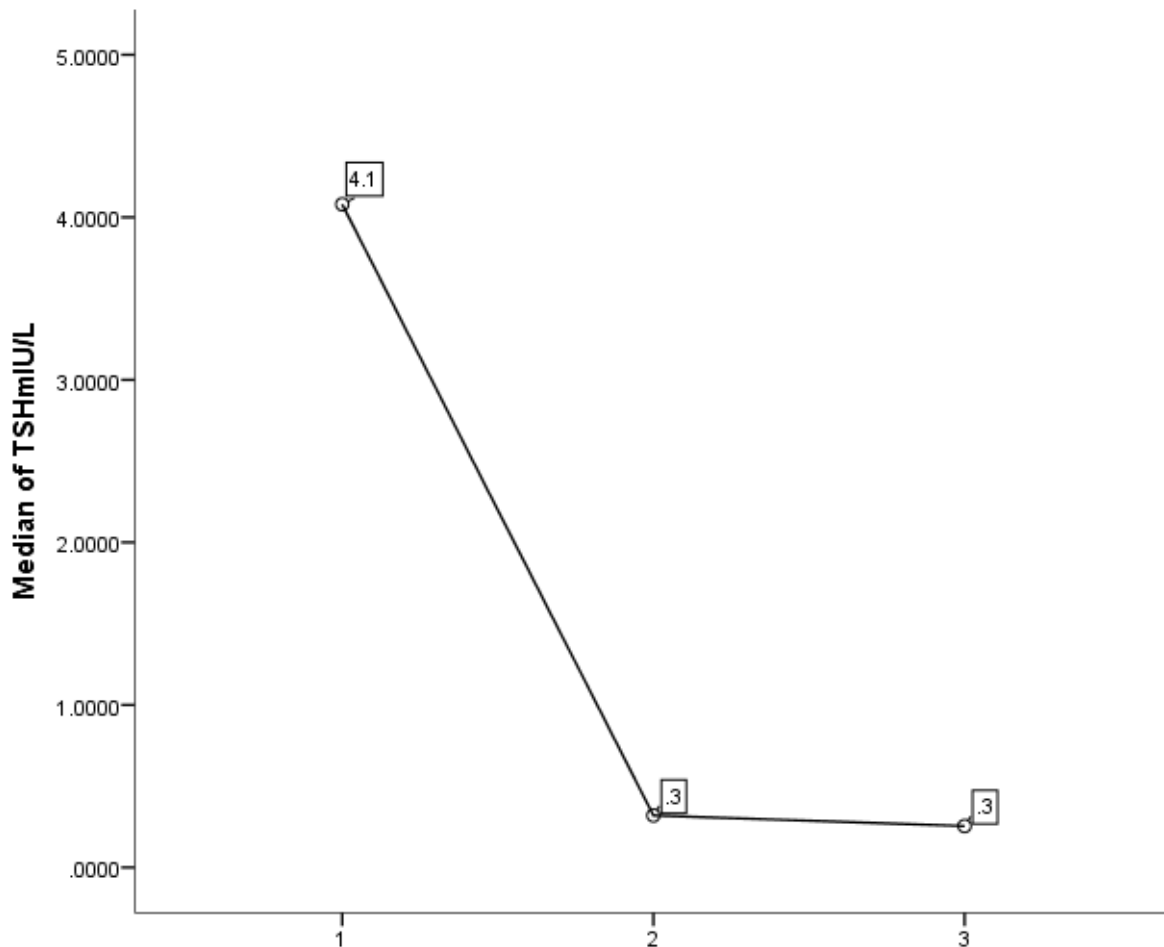
Figure 2 | Median thyroid stimulating hormone (TSH) values of the treated suboptimal gestational thyroid function group taken at: (1) consent into the Controlled Antenatal Thyroid Screening study (median 12 weeks 3 days gestation), (2) 20 weeks (0 days) gestation, and (3) 30 weeks (1 day) gestation. TSH was measured in mIU/L.

Supplementary Statistics

All analyses in this section deal with continuous data; therefore are MANCOVAs and include Bonferroni corrections where applicable. The reported statistical test used in the MANCOVAs was Pillai's trace, V. Every analysis was adjusted for the same covariates as in the main article (child gender, mother age at time of consent, whether the mother breastfed for over one month, and social deprivation). Analyses followed the same pattern, three CATS groups compared for the three total questionnaire scores, then a secondary MANCOVA exploring all questionnaire domains with the treated SGTF split into optimal and over-treatment. Exploration of specific maternal FT4 ranges only included the latter MANCOVAs.

Subclinical hypothyroidism

Women identified as having subclinical hypothyroidism during their pregnancies, had a TSH >97.5th and FT4 >2.5th percentiles at time of consent. In the treated SGTF group, 57.26% (n=67) were in this category, and 49.00% (n=49) of the untreated SGTF group. There was no difference between the normal GTF, treated, and untreated subclinical hypothyroid groups for total questionnaire scores (P=0.909). When exploring the effect of treatment to the 17.7pmol/L threshold, there was also no difference in mean scores (P=0.128). See table 2 for descriptive data.

Table 2 | Mean scores for the subclinical hypothyroid and normal GTF groups. Figures are mean values (standard deviation).

Questionnaire domain		Subclinical hypothyroidism			
	Normal GTF N=237	Treated N=67	Untreated N=49	Optimally treated N=41	Over-treated N=26
Strengths and difficulties questionnaire					
Emotion	2.32 (2.24)		2.32 (2.18)	2.22 (1.98)	2.50 (1.73)
Conduct	1.21 (1.50)		1.33 (1.39)	1.44 (1.83)	1.92 (1.83)
Hyperactivity	3.09 (2.52)		3.47 (2.61)	3.10 (2.91)	4.23 (2.55)
Peer problems	1.58 (1.88)		1.10 (1.29)	1.51 (1.90)	1.00 (1.02)
Total difficulties	8.20 (6.01)	8.81 (5.71)	8.22 (5.41)	8.27 (6.13)	9.65 (4.97)
Prosocial	8.78 (1.78)		8.47 (2.09)	8.90 (1.71)	8.69 (1.93)
Child ADHD questionnaire					
Inattention	6.07 (5.67)		5.75 (4.94)	5.88 (5.84)	7.62 (5.73)
Overactivity	2.05 (2.29)		2.37 (2.42)	2.41 (2.88)	3.58 (3.14)
Impulsivity	3.13 (2.79)		3.34 (2.50)	3.68 (3.55)	4.38 (3.28)
Total	11.25 (9.53)	13.37 (11.20)	11.46 (8.52)	11.98 (11.45)	15.58 (10.63)
Social communication questionnaire	4.28 (3.70)	4.59 (4.14)	3.95 (2.96)	4.05 (3.73)	5.43 (4.65)
SGTF=suboptimal gestational thyroid function, ADHD=attention deficit hyperactivity disorder					

Maternal hypothyroxinemia

Women were classified as the having hypothyroxinemia during their pregnancies if their FT4 at consent was <2.5th and their TSH was <97.5th percentiles. There were 30.77% (n=36) of the treated SGTF, and 44.00% (n=44) of the untreated SGTF group in this category. Total questionnaire scores were not significantly different between the normal GTF, treated and untreated hypothyroxinaemia groups P=0.207, see table 3 for scores). There was also no difference when treatment was explored between the groups (P=0.105).

Table 3 | Mean questionnaire scores for the hypothyroxinemic and normal GTF groups. Figures are mean values (standard deviation).

Questionnaire domain		Hypothyroxinemic			
	Normal GTF N=237	Treated N=36	Untreated N=44	Optimally treated N=26	Over-treated N=10
Strengths and difficulties questionnaire					
Emotion	2.32 (2.24)		2.11 (1.94)	2.41 (2.10)	2.90 (2.33)
Conduct	1.21 (1.50)		1.05 (1.40)	1.50 (1.73)	1.70 (1.83)
Hyperactivity	3.09 (2.52)		3.30 (2.79)	3.69 (2.81)	4.30 (4.06)
Peer problems	1.58 (1.88)		1.20 (1.32)	1.31 (1.87)	1.00 (1.33)
Total difficulties	8.20 (6.01)	9.18 (7.17)	7.66 (5.40)	8.91 (6.83)	9.90 (8.35)
Prosocial	8.78 (1.78)		9.02 (1.25)	8.73 (1.93)	9.40 (1.07)
Child ADHD questionnaire					
Inattention	6.07 (5.67)		4.79 (4.63)	6.75 (6.32)	6.90 (7.84)
Overactivity	2.05 (2.29)		2.38 (2.82)	2.31 (2.62)	3.40 (4.14)
Impulsivity	3.13 (2.79)		2.93 (3.04)	3.42 (2.87)	4.10 (4.04)
Total	11.25 (9.53)	13.02 (12.29)	10.10 (9.25)	12.48 (10.99)	14.40 (15.78)
Social communication questionnaire	4.28 (3.70)	5.98 (5.21)	4.26 (2.89)	6.47 (5.44)	4.70 (4.55)
SGTF=suboptimal gestational thyroid function, ADHD=attention deficit hyperactivity disorder					

Overt hypothyroid groups

As the CATS study classified women with FT4 <2.5th and/or TSH >97.5th percentiles for a positive result of SGTF, the secondary analysis examined questionnaire scores by women who had overt hypothyroidism (OH, FT4 <2.5th and TSH >97.5th). OH, though uncommon during pregnancy (3 in 1000(1)), is the most severe underactive thyroid function. There were 8.55% (n=10) of the treated SGTF group, and 9.00% (n=9) of the untreated SGTF group classified as having OH. Comparing these groups with the normal GTF for total

questionnaire scores identified a significant difference at the multivariate level (V=0.063, F(6,496)=2.669, P=0.015, η_p^2=0.031), with a difference found for ADHD Total scores (F(2,249)=4.680, P=0.010, η_p^2=0.036). The untreated OH group had significantly higher scores compared to the normal GTF for ADHD Total (P=0.022 (0.945, 16.876)).

When exploring the effect of treatment to the 17.7pmol/L threshold for maternal FT4, there was a sustained difference at the multivariate level (V=0.203, F(27,726)=1.948, P=0.003 η_p^2=0.068). At the univariate level SDQ Conduct (F(3,248)=4.402, P=0.005, η_p^2=0.051), ADHD Hyperactivity (F(3,248)=5.665, P=0.001, η_p^2=0.064), and ADHD Total (F(3,248)=3.487, P=0.016, η_p^2=0.040) were significantly different. The over-treated OH group had significantly higher scores for SDQ Conduct (P=0.038 (0.072, 4.273)) and ADHD Hyperactivity (P=0.020 (0.357, 6.597)) compared to the normal GTF. Again, for ADHD Total, the untreated OH had significantly higher scores than the normal GTF (P=0.045 (0.117, 7.693).

Children born to mothers who were untreated for OH in pregnancy, had higher scores for the Child ADHD Questionnaire, optimal treatment improved this with some means moving closer to those of the normal GTF (table 4). Over-treatment for OH had a significant effect here on domains found with the entire SGTF sample; SDQ Conduct and ADHD Hyperactivity. Caution is advised as the sample sizes are small for OH.

Table 4 | Questionnaire scores for the normal gestational thyroid function (GTF) group, and treated and untreated overt hypothyroid group. Figures are mean values (standard deviation).

Questionnaire domain	Normal GTF	Treated overt hypothyroid	Untreated overt hypothyroid	Optimally treated	Over-treated
	N=237	N=10	N=9	N=6	N=4
Strengths and difficulties questionnaire					
Emotion	2.32 (2.24)		2.78 (1.64)	2.67 (2.94)	3.25 (3.30)
Conduct	1.21 (1.50)		2.44 (2.07)	0.83 (0.98)	3.25* (2.99)
Hyperactivity	3.09 (2.52)		5.11 (3.37)	4.33 (2.16)	4.25 (4.35)
Peer problems	1.58 (1.88)		1.33 (1.22)	1.33 (1.97)	0.50 (1.00)
Total difficulties	8.20 (6.01)	10.00 (8.51)	11.67 (7.16)	9.17 (7.08)	11.25 (11.41)
Prosocial	8.78 (1.78)		8.44 (1.33)	9.17 (1.33)	8.50 (3.00)
Inattention	6.07 (5.67)		11.44 (6.77)	7.32 (5.44)	9.25 (9.98)
Hyperactivity	2.05 (2.29)		4.11 (3.48)	4.00 (2.61)	5.25* (4.19)
Impulsivity	3.13 (2.79)		5.22 (3.31)	2.17 (1.83)	5.25 (4.50)
Total	11.25 (9.53)	15.99 (12.67)	20.78* (12.10)	13.48 (8.29)	19.75 (18.32)
Social communication questionnaire	4.28 (3.70)	6.70 (8.08)	4.00 (2.78)	6.17 (5.85)	5.72 (8.21)

*Significantly higher (P<0.05) group results (from columns left to right)
SGTF=suboptimal gestational thyroid function, ADHD=attention deficit hyperactivity disorder
Untreated overt hypothyroid (1) Child ADHD questionnaire (ADHD) Inattention, higher than Normal GTF, (2) ADHD Total, higher than Normal GTF, (3) Strengths and difficulties questionnaire Conduct, higher than Normal GTF.
Treated overt hypothyroid ADHD Hyperactivity and SDQ Conduct, higher than Normal GTF. Untreated overt hypothyroid ADHD Total, higher than Normal GTF.

Exploratory analysis; treatment by target range
The following analyses explored potential limits of treatment for minimising specific neurodevelopmental symptoms in the child. Analyses were again MANCOVAs with the same four covariate adjustments.

As can be seen in table 5, the means of the treated group decreased with decreasing FT4 threshold values. This confirms the main conclusions from the article that over-treatment could have an impact for the child's neurodevelopment; as well as the threshold of 17.7pmol/L in pregnancy being the most 'detrimental'. As thresholds decreased, interestingly, those who were treated up to the thresholds increased questionnaire scores. This supports a theory of 'U-curved effects'(2); demonstrating that higher and lower values of maternal FT4 can affect the child. A further possible mechanism could be that those born to mothers with the lowest FT4 had more of an effect to the group mean with decreasing groups with lowering FT4 thresholds. SDQ Peer problems being significantly higher in the normal GTF group were repeatedly found compared to above thresholds for maternal FT4. At the whole group level of the treated SGTF, TSH was positively correlated to SDQ Peer problems at 30 weeks' gestation. As the FT4 levels were high, TSH would have been lowering, and supporting the correlation, the lower the TSH level, the lower SDQ Peer problem scores will have been.

Over 17pmol/L in pregnancy
For the first threshold of 17pmol/L (n=53), there was a significant difference between the questionnaire scores at the multivariate level (V=0.114, $F_{(27,1320)}$=1.922, P=0.003, η_p^2=0.038). Univariate analyses identified difference for SDQ Peer problems ($F_{(3,446)}$=3.942, P=0.009, η_p^2=0.026) and ADHD Overactivity ($F_{(3,446)}$=4.229, P=0.006, η_p^2=0.028). Bonferroni corrected pairwise comparisons evidenced that the normal GTF had a higher group mean than those born to mothers with FT4 values >17pmol/L (P=0.040, 0.019 to 1..363), but the effect was inversed for ADHD Overactivity (P=0.003, 0.324 to 2.340).

Over 16pmol/L in pregnancy
The lower threshold 16pmol/L (n=70) also yielded a difference at the multivariate level for questionnaire scores (V=0.127, $F_{(27,1320)}$=2.162, P=0.001, η_p^2=0.042). The domains with significant differences were, SDQ Peer problems ($F_{(3,446)}$=4.755, P=0.003, η_p^2=0.031) and ADHD Overactivity ($F_{(3,446)}$=3.851, P=0.010, η_p^2=0.025). Significance persisted following Bonferroni corrections, and akin to FT4 values >17pmol/L throughout pregnancy, SDQ Peer problems were higher in the normal GTF compared to those mothers with FT4 values >16pmol/L SGTF (P=0.015, 0.088 to 1.289), with those >16pmol/L having higher scores compared to the normal GTF group for ADHD Overactivity (P=0.006, 0.226 to 2.034).
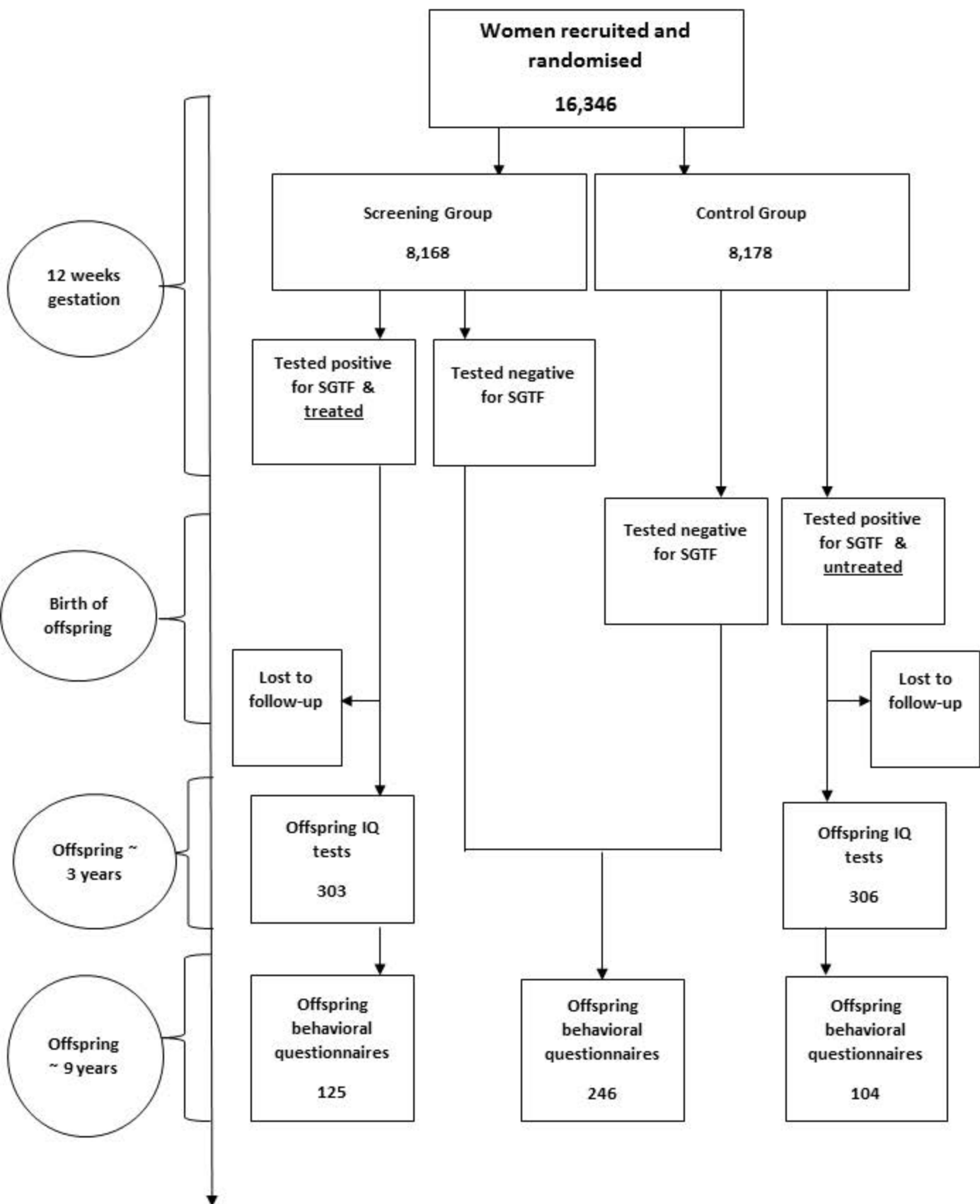
Over 15pmol/L in pregnancy
The lowered threshold of 15pmol/L (n=87) was used for the following analysis. The MANCOVA identified a difference between the groups (V=0.115, $F_{(27,1320)}$=1.941, P=0.003, η_p^2=0.038). Univariate and pairwise comparison analyses identified differences for SDQ Peer problems ($F_{(3,446)}$=5.871, P=0.001, η_p^2=0.038, normal GTF higher than those with maternal FT4 values >15pmol/L SGTF, P=0.009, 0.113 to 1.220), and SCQ ($F_{(3,446)}$=2.872, P=0.036, η_p^2=0.019, children born to mothers with FT4 <14.9pmol/L scored higher than the normal GTF P=0.044, 0.032 to 4.211).

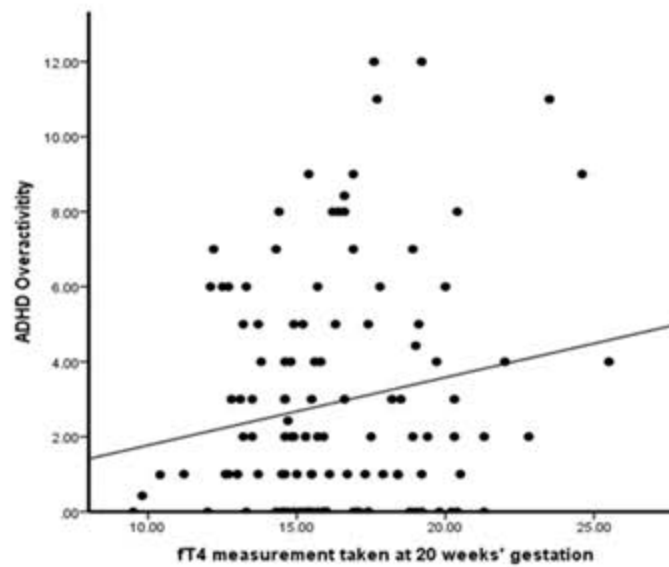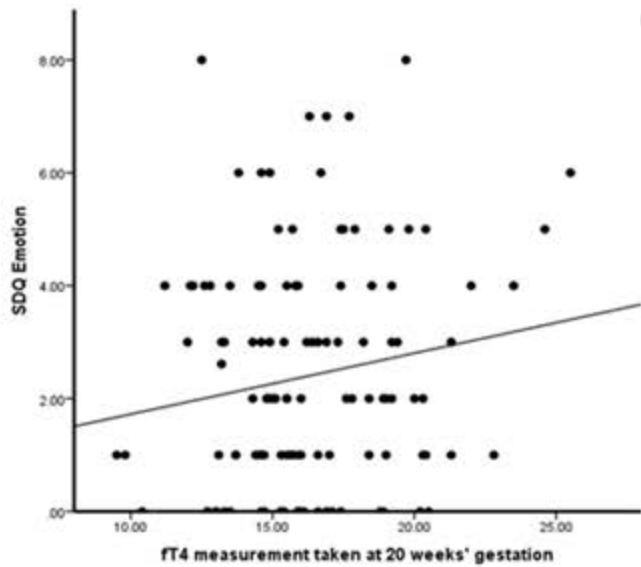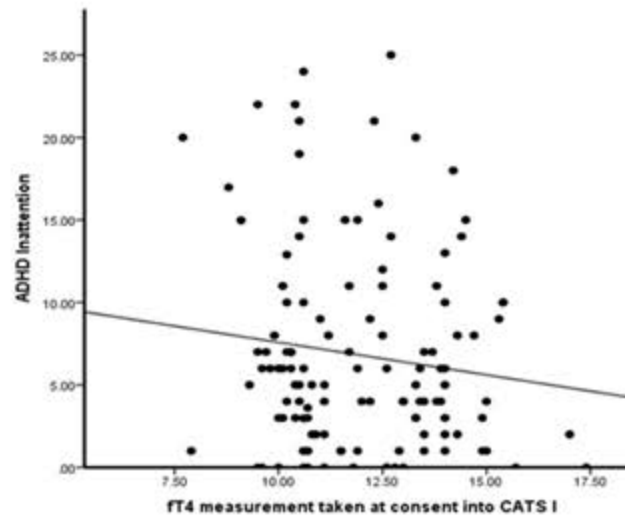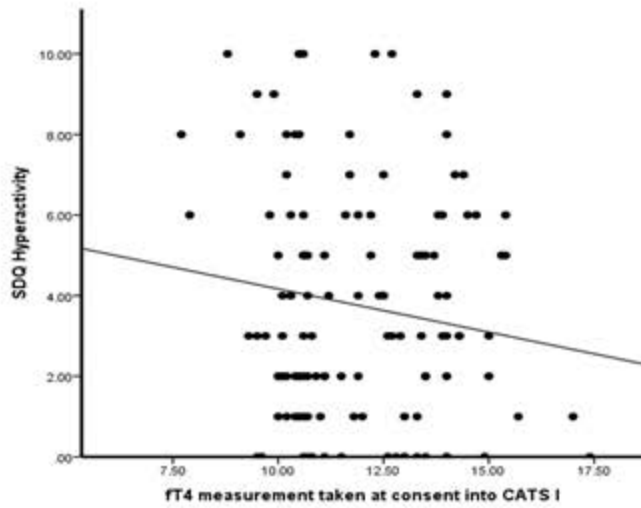**Table 5| Mean questionnaire scores for thresholds of FT4 in pregnancy. Figures are mean values (standard deviation).**

Questionnaire domain			Treatment thresholds					
	Normal GTF N=237	Untreated SGTF N=100	>17pmol/L N=53	<16.9pmol/L N=64	>16pmol/L N=70	<15.9pmol/L N=47	>15pmol/L N=87	<14.9pmol/L N=30
Strengths and difficulties questionnaire								
Emotion	2.32 (2.24)	2.24 (2.01)	2.58 (1.96)	2.28 (2.13)	2.46 (2.08)	2.35 (2.02)	2.34 (2.02)	2.62 (2.16)
Conduct	1.21 (1.50)	1.27 (1.42)	1.60 (1.84)	1.59 (1.81)	1.43 (1.73)	1.84 (1.93)	1.44 (1.70)	2.03 (2.09)
Hyperactivity	3.09 (2.52)	3.51 (2.74)	3.91 (3.03)	3.48 (2.80)	3.73 (3.06)	3.60 (2.67)	3.61 (3.00)	3.87 (2.62)
Peer problems	1.58 (1.88)	1.16 (1.28)	0.92 (1.03)	1.48 (1.96)	0.91 (1.21)	1.70 (2.02)	0.93 (1.33)	2.10 (2.07)
Total difficulties	8.20 (6.01)	8.18 (5.42)	9.02 (6.15)	8.83 (6.65)	8.53 (6.28)	9.49 (6.61)	8.33 (6.25)	10.62 (6.64)
Prosocial	8.78 (1.78)	8.73 (1.72)	8.89 (1.70)	8.72 (1.81)	9.09 (1.55)	8.47 (2.00)	9.05 (1.52)	8.23 (2.25)
Child ADHD questionnaire								
Inattention	6.07 (5.67)	5.83 (5.25)	7.21 (6.51)	6.24 (5.76)	6.79 (6.44)	6.52 (5.62)	6.34 (6.19)	7.65 (5.83)
Overactivity	2.05 (2.29)	2.53 (2.73)	3.34 (3.45)	2.41 (2.62)	3.17 (3.41)	2.32 (2.35)	2.79 (3.25)	2.93 (2.42)
Impulsivity	3.13 (2.79)	3.32 (2.85)	3.92 (3.67)	3.55 (2.98)	3.91 (3.56)	3.43 (2.89)	3.69 (3.39)	3.80 (3.08)
Total	11.25 (9.53)	11.67 (9.50)	14.47 (12.78)	12.20 (10.30)	13.87 (12.66)	12.27 (9.56)	12.83 (12.03)	14.38 (9.89)
Social communication questionnaire	4.28 (3.70)	4.09 (2.89)	4.84 (5.06)	5.41 (4.81)	4.72 (4.75)	5.80 (5.13)	4.66 (4.82)	6.57 (4.99)
SGTF=suboptimal gestational thyroid function, ADHD=attention deficit hyperactivity disorder								

1.	Tunbridge W, Evered D, Hall R, Appleton D, Brewis M, Clark F, et al. The spectrum of thyroid disease in a community: the Whickham survey. Clin Endocrinol. 1977;7(6):481-93.

2.	Korevaar T, Muetzel R, Medici M, Chaker L, Jaddoe VWV, de Rijke YB, et al. Association of maternal thyroid function during early pregnancy with offspring IQ and brain morphology in childhood: a population-based prospective cohort study. The Lancet Diabetes & Endocrinology. 2016;4(1):35-43.

Women recruited and randomised

16,346

12 weeks gestation

Screening Group

8,168

Control Group

8,178

Tested positive for SGTF & <u>treated</u>

Tested negative for SGTF

Tested negative for SGTF

Tested positive for SGTF & <u>untreated</u>

Birth of offspring

Lost to follow-up

Lost to follow-up

Offspring ~ 3 years

Offspring IQ tests

303

Offspring IQ tests

306

Offspring ~ 9 years

Offspring behavioral questionnaires

125

Offspring behavioral questionnaires

246

Offspring behavioral questionnaires

104

Table 1 | Characteristics of the cohort. Values are means (standard deviations) unless stated otherwise.

Characteristics	Normal-GTF	Treated SGTF	Untreated SGTF	Normal-GTF vs. Treated SGTF (P)	Normal-GTF vs. Untreated SGTF (P)	Treated SGTF vs Untreated SGTF (P)
	N=246	N=125	N=104			
TSH at study entry (mlU/L)†	1..2 (0.7-1.8)	4.1 (1.9-5.1)	3..6 (1.1-4..5)	0.001	0.001	0.003
FT4 at study entry (pmol/L)	14.1 (1..7)	12.0 (1.9)	11.7 (1.9)	0.001 (95% CI 1.675 to 2.633)	0.001 (95% CI 1.867 to 2.887)	1.000
Age of mother at study entry (years)	31.7 (5.2)	30.2 (5.1)	30.9 (4.8)	0.025 (0.138, 2.824)	0.588	0.879
Social deprivation† quintile (mean)	4 (3-5) (3.7)	5 (3-5) (3.8)	4 (2-5) (3.4)	0.687	0.491	0.162
Child breastfed for over one month (%)*	65.3	58.9	56.3	0.226	0.113	0.697
Male children (%)	50.0	52.0	48.1	0.716	0.742	0.554
Age of children†	9..8 (9.0-10.3)	9.6 (9.1-9.8)	9..4 (9.0-9..9)	0.003	0.017	0.996

†Median and interquartile ranges presented.
*missing per group, n=1
Confidence intervals presented for continuous data only. Current free thyroxine reference ranges for adults during pregnancy per trimester are as follows: first trimester 10.5-18.3pmol/L, second trimester 9.5-15.9pmol/L, third trimester 8.6-13.7pmol/L (serum TSH and FT4 were measured with the use of Immunochemiluminescence, ADVIA Centaur, Siemens Healthcare Diagnostics).
SGTF= suboptimal gestational thyroid function, TSH=thyroid stimulating hormone, FT4=free thyroxine

**Table 2 | Group total scores for the questionnaires. p
Figures are mean values (standard deviation)**

Questionnaire total scores	Normal-GTF	Treated SGTF	Untreated SGTF	
	N=237	N=117	N=100	
Strengths and difficulties questionnaire*	8.20 (6.01)	8.92 (6.40)	8.17 (5.42)	0.53
Child ADHD questionnaire	11.24 (9.53)	13.23 (11.49)	11.67 (9.50)	0.22
Social communication questionnaire	4.28 (3.70)	5.15 (4.91)	4.09 (2.89)	0.08
*Total difficulties score				

Table 3 | High questionnaire scores from the normal gestational thyroid function (GTF), treated, and untreated suboptimal GTF groups. Figures are numbers (percentages).

Questionnaire domain	Normal GTF	Treated SGTF	Untreated SGTF	Optimally treated	Over-treated
	N=237	N=117	N=100	N=77	N=40
Strengths and difficulties questionnaire					
Emotion					
High*/very high	39 (16.4)	19 (16.3)	13 (13.0)	11 (14.3)	8 (20.0)
Conduct					
High/very high	20 (8.5)	18 (15.4)^	7 (7.0)	9 (11.7)	9 (22.5)^^^
Hyperactivity					
High/very high	15 (6.3)	16 (13.7)^	10 (10.0)	10 (13.0)	5 (12.5)
Peer problems					
High/very high	39 (16.4)	13 (11.1)	7 (7.0)	12 (15.6)	1 (2.5)
Total difficulties					
High/very high	28 (11.8)	16 (13.7)	8 (8.0)	10 (13.0)	6 (15.0)
Prosocial					
Low/very low	27 (11.4)	13 (11.1)	10 (10.0)	8 (10.4)	5 (12.5)
Child ADHD questionnaire					
Inattention					
>2SD†	11 (4.8)	9 (7.8)	3 (3.0)	5 (6.5)	4 (10.0)
Hyperactivity					
> 2SD	9 (3.9)	12 (10.2)^	5 (5.0)	5 (6.5)	7 (17.5)^^
Impulsivity					
>2SD	6 (2.5)	7 (6.1)	3 (3.0)	3 (3.9)	4 (10.0)
Social communication questionnaire					
Total >15	4 (1.7)	7 (6.0)^	1 (1.0)	4 (5.2)	3 (7.5)^^

SGTF=suboptimal gestational thyroid function normal-GTF = normal gestational thyroid function

*high = 90-95th percentile, as calculated by the SDQ (scores >90th percentile are clinically significant); very high>95th percentile, as calculated by the SDQ

†SDs calculated by Thapar and colleagues

^p<0.05 treated versus normal-GTF

^^p<0.05 over-treated versus untreated SGTF

^^^ p< 0.01 over-treated versus untreated SGTF