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**1 CATS II long-term anthropometric and metabolic effects of maternal sub-optimal  
2 thyroid function in offspring and mothers**

3

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## 28 **ABSTRACT**

29 **CONTEXT & OBJECTIVES.** The Controlled Antenatal Thyroid Screening study-I  
30 (CATS-I) was a randomized controlled trial investigating the effects of levothyroxine therapy  
31 for suboptimal gestational thyroid function (SGTF), comparing outcomes in children of  
32 treated (SGTF-T) with untreated (SGTF-U) women during pregnancy. This follow-up study  
33 CATS-II reports the long-term effects on anthropometric, bone and cardiometabolic  
34 outcomes in mothers and offspring, and includes a group with normal gestational thyroid  
35 function (NGTF).

36 **DESIGN & PARTICIPANTS.** 332 mothers (197 NGTF, 56 SGTF-U, 79 SGTF-T) aged  
37  $41.2 \pm 5.3$  years (mean $\pm$ SD) and 326 paired children assessed  $9.3 \pm 1.0$  years after birth for: 1)  
38 body mass index (BMI); 2) lean, fat and bone mass by dual-energy x-ray absorptiometry; 3)  
39 blood pressure, augmentation index and aortic pulse-wave-velocity; 4) thyroid function,  
40 lipids, insulin and adiponectin. The difference between group means was compared using  
41 linear regression.

42 **RESULTS.** Offspring's measurements were similar between groups. Although maternal BMI  
43 was similar between groups at CATS-I, after 9-years (at CATS-II) SGTF-U mothers showed  
44 higher BMI (median[IQR]  $28.3[24.6-32.6]$  kg/m<sup>2</sup>) compared with NGTF ( $25.8[22.9-30.0]$   
45 kg/m<sup>2</sup>,  $p=0.029$ ), driven by fat mass increase. At CATS-II SGTF-U mothers also had higher  
46 TSH values (median[IQR]  $2.45[1.43-3.50]$  mU/L) than NGTF ( $1.54[1.12-2.07]$  mU/L;  
47  $p=0.015$ ), since 64% had never received levothyroxine. At CATS-II SGTF-T mothers had  
48 BMI ( $25.8[23.1-29.8]$  kg/m<sup>2</sup>,  $p=0.672$ ) and TSH ( $1.68[0.89-2.96]$  mU/L;  $p=0.474$ ) values  
49 similar to NGTF mothers.

50 **CONCLUSIONS.** Levothyroxine supplementation of women with SGTF did not affect long-  
51 term offspring anthropometric, bone and cardiometabolic measurements. However, absence

52 of treatment was associated with sustained long-term increase in BMI and fat mass in women  
53 with SGTF.

54

55 **PRÉCIS**

56 Levothyroxine for suboptimal gestational thyroid function did not affect offspring's  
57 anthropometric, bone and cardiometabolic outcomes, but prevented an increase in maternal  
58 fat mass over 9 years.

## 59 INTRODUCTION

60           Pregnancy induces physiological changes in the maternal hypothalamic-pituitary-  
61 thyroid axis, such that interpretation of thyroid function during pregnancy must take into  
62 account trimester-specific reference ranges (1, 2). Suboptimal gestational thyroid function  
63 (SGTF), defined as a low free thyroxine (FT4) concentration and/or raised thyroid-  
64 stimulating hormone (TSH), is common and associated with adverse pregnancy and offspring  
65 outcomes (1, 3, 4).

66           Overt hypothyroidism is well-known to be associated with impaired fetal  
67 neurodevelopment, particularly during the first trimester of pregnancy when the fetus is  
68 entirely dependent on maternal thyroid hormone production for optimal brain development  
69 (1, 2, 5). However, the effects of mild maternal thyroid dysfunction are less clear. Whereas  
70 isolated hypothyroxinemia has been found to be associated with impaired developmental and  
71 neurobehavioral outcomes (1, 6), including verbal delay (7), autism (8, 9) and attention-  
72 deficit/hyperactivity disorder (ADHD) (10-14) in offspring, such effects have not been  
73 described for maternal subclinical hypothyroidism (1, 15-17).

74           Whilst many studies have examined effects on neuro-intellectual and behavioral  
75 outcomes, very little is known about the effects of maternal gestational thyroid dysfunction  
76 on offspring anthropometric, bone and cardiometabolic outcomes. Both high TSH levels and  
77 low FT4 levels during gestation are known to be associated with maternal weight gain and  
78 adverse metabolic pregnancy outcomes (18-20); accordingly, pregnancy-specific reference  
79 ranges for thyroid function assessments are influenced by body mass index (BMI) (21-23). In  
80 turn, maternal overweight and obesity during gestation and an excessive gestational weight  
81 gain, especially during early gestation, are associated with long-term adverse anthropometric  
82 and cardiometabolic outcomes in offspring (24-26). Overt hypothyroidism and  
83 hyperthyroidism negatively affect bone mass maintenance (27, 28); such effects have also

84 been observed in long-term subclinical hyperthyroidism, but not hypothyroidism (29-31),  
85 with only isolated exceptions (32). Thyroid hormones are also a determinant for bone  
86 development and skeletal maturation; it is well known that children with impaired and  
87 untreated thyroid function have short stature (28).

88         The Controlled Antenatal Thyroid Screening (CATS) study I (CATS-I) was the first  
89 randomized controlled trial to investigate the effects of antenatal screening and treatment of  
90 SGTF on offspring cognitive function (15). No effects on intelligence quotient (IQ) of 3 year-  
91 old offspring were found, and a follow-up study (CATS-II) repeating IQ assessment at age 9  
92 (33) confirmed similar findings (17). CATS-II also allowed us to extend the phenotyping of  
93 children to other outcomes known to be influenced by thyroid status. We recently reported  
94 adverse behavioral outcomes in children born to mothers exposed to excess levothyroxine  
95 replacement (34).

96         Here we present for the first time the effects of treatment for SGTF on maternal and  
97 child anthropometric, bone and cardiometabolic outcomes, evaluated 9 years after delivery.  
98 We included the maternal analysis as a reference population for their paired children, and as a  
99 valuable cohort followed long-term to better evaluate the association between  
100 mild/subclinical thyroid dysfunction and overweight/obesity among adult women (35-37), as  
101 well as adverse cardiovascular events (38-41).

## 102 **MATERIALS AND METHODS**

### 103 **STUDY DESIGN AND POPULATION**

104         As previously reported, CATS-II (17, 33, 34) is the follow-up study of CATS-I  
105 (ISRCTN 46178175) (15). Briefly, in CATS-I a total of 21,846 pregnant women (median  
106 gestation of 12 weeks 3 days) were randomized to have their thyroid function measured at

107 recruitment (screening group) or at the end of pregnancy (control group). Those in the  
108 screening group diagnosed with SGTF, defined as FT4 <2.5th percentile and/or TSH >97.5th  
109 percentile of the cohort, were commenced on levothyroxine treatment (SGTF-T) for the  
110 duration of their pregnancy, while the women with SGTF in the control group were left  
111 untreated (SGTF-U) and subsequently referred to their general practitioner for further  
112 management following delivery. In the SGTF-T group, the starting dose of levothyroxine was  
113 150µg daily; TSH and FT4 measurements were repeated after 6 weeks from commencing  
114 treatment and at 30 weeks of gestation, with adjustment of levothyroxine dose where  
115 necessary, aiming to maintain TSH levels in the 0.1-1.0mIU/L range. In CATS-I the IQ of  
116 children of SGTF-T and SGTF-U mothers was measured at age 3 years (15). The follow-up  
117 study (CATS-II) repeated IQ assessment in 9-year-old offspring (17, 33), included UK  
118 participants only and also evaluated children of women with normal gestational thyroid  
119 function (NGTF) during CATS-I. In addition to IQ assessment (17) other outcomes were also  
120 evaluated, including child behavior (34) and the anthropometric, bone and cardiometabolic  
121 measurements presented here. At CATS-II recruitment, clinical data including history of  
122 levothyroxine treatment following CATS-I were collected. The overall study design and  
123 population is summarized in **Figure 1**.

#### 124 **BMI AND BMI SDS**

125 Standing heights were measured to the nearest 0.1 cm using a Harpenden stadiometer  
126 (Holtain Ltd, Crymych, UK). Participants were weighed to the nearest 0.1 kg in lightweight  
127 clothing without shoes, using the weighing function of a Body Fat Analyzer (TBF-305;  
128 Tanita, Tokyo, Japan). Body mass index (BMI) was calculated as weight (kg) divided by  
129 height (m) squared. Children's BMI standard deviation scores (SDS) were calculated using  
130 the UK reference population established in 1990 (42, 43).

131 **DXA SCAN ANALYSIS**

132 Whole body less head (WBLH), total lumbar spine (L1-L4) and left hip  
133 measurements of bone and/or lean and fat mass were made in 327/332 (98.5%) mothers and  
134 323/326 (99.1%) children using a Hologic QDR Explorer fan-beam dual-energy x-ray  
135 absorptiometry (DXA) scanner (Hologic Inc., Marlborough, USA). Subjects were assessed in  
136 the supine position, in the fasted state and after bladder emptying. Whole body scans were  
137 acquired in explorer (e) mode, equivalent to array mode, while spine and hip scans were  
138 acquired in survey (s) mode, equivalent to fast array. Scans were analyzed using Hologic  
139 software V 13.3.0.1:3 with the auto low-density option being applied to the spine scans where  
140 appropriate.

141 For bone analysis, bone area (cm<sup>2</sup>), bone mineral content (BMC, g) and the BMC per  
142 unit area of bone (bone mineral density [BMD], g/cm<sup>2</sup>) were assessed. In particular, for  
143 mothers the BMD values of femoral neck (FN-BMD) and total lumbar spine (LS-BMD) were  
144 compared across the subject groups. For children DXA bone mineral measurements are  
145 strongly influenced by several factors including sex, chronological and skeletal age, height,  
146 weight, ethnicity and pubertal development (44-48). In order to reduce such biases, the bone  
147 mineral apparent density (BMAD) was also considered; it was calculated for the femoral  
148 neck (FN) using the method of Lu et al. (49), and for the lumbar spine (LS) using the  
149 geometric assumptions made by Carter et al (50), but estimating the volume of each  
150 individual vertebral body (L1–L4) from its bone area and summing the result rather than the  
151 total volume of L2–L4 (51). Depending on the body region studied (LS, FN, WBLH), BMD  
152 and BMAD were expressed as standard deviation scores (SDS) compared to the UK  
153 population (cohorts of the Ward study [W] or the Alphabet study [A]) (51, 52) and the US  
154 population (Hologic manufacturer [H]), for a total of 8 measurements.

155 For body composition, absolute WBLH fat and lean mass (kg), as well as relative fat



156 mass (%), were measured.

## 157 **BIOCHEMICAL ANALYSIS**

158 Fasting blood samples were collected from 294/332 (88.5%) mothers but only 83/326  
159 (25.5%) children, since most of them refused phlebotomy. Serum was prepared by  
160 centrifugation at 4500 rpm for 10 minutes at +4°C, and stored at -80°C until analysis.

161 Thyroid-stimulating hormone (TSH), free-thyroxine (FT4), free-triiodothyronine  
162 (FT3), autoantibodies to thyroid peroxidase (TPOAb), triglyceride, total cholesterol, high-  
163 density lipoprotein (HDL) cholesterol and insulin were measured by Chemiluminescent  
164 Microparticle Immunoassay (Architect® System, Abbott Laboratories, USA). Normal  
165 reference ranges for thyroid function (females aged above 18 years) were 0.30 – 4.4 mIU/L  
166 for TSH, 9.0 – 19.1 pmol/L for FT4 and 2.6 – 5.7 pmol/L for FT3. According to the assay  
167 cut-off, TPOAb values were considered positive if  $\geq 6$  IU/ml and negative if  $< 6$  IU/ml.

168 High molecular weight adiponectin (APN) was measured by ELISA (EMD Millipore,  
169 Billerica, MA, USA).

## 170 **CARDIOVASCULAR FUNCTION**

171 The Vicorder device (Skidmore Medical, UK), a non-invasive cuff-based oscillometric  
172 technique that simultaneously measures the upstroke of femoral and carotid pulsations, was  
173 used to calculate the aortic pulse wave velocity (PWV), a measure of arterial stiffness (53), in  
174 addition to other measurements of peripheral and central blood pressure. This technique is  
175 reproducible (54), validated in both adults (55, 56) and children (57, 58) and agrees closely  
176 with invasive measures of central blood pressure (59). All measurements were performed by  
177 a single trained operator (DS). Measurements were taken with subjects relaxed in a quiet  
178 room, and with the head raised to 30°. Aortic PWV was measured by cuffs placed over the  
179 right carotid and the right thigh, with the length between the two arteries determined using a

180 tape measure placed over the suprasternal notch and the mid-point of the thigh cuff.  
181 Measurements were recorded when the pressure waveforms were reproducible over both  
182 arteries. Additional measurements were undertaken with the cuff placed on the right upper  
183 arm, including the systolic blood pressure (SP), the diastolic blood pressure (DP) and the  
184 aortic augmentation index (AI), a measure of the pulse wave reflection influenced by vessel  
185 stiffness (53).

## 186 **DATA ANALYSIS**

187         Histograms were performed to assess the distribution of variables. Variables were  
188 summarized using the sample mean  $\pm$  standard deviation (SD) if approximately normally  
189 distributed, or using the sample median and interquartile range (IQR) otherwise. In addition,  
190 variables judged to be right-skewed were log-transformed for inclusion in analyses.  
191 Categorical variables (i.e. TPOAb positivity and child sex) were summarized using  
192 percentages, and the statistical significance of associations between them calculated using  
193 Fisher's exact test.

194         The effects of SGTF were first estimated comparing NGTF versus SGTF-U (p1U)  
195 and NGTF versus SGTF-T (p1T) in an unadjusted linear regression model (Model 1) and  
196 then adjusting for age, sex (children analysis only), ethnicity, socioeconomic status (defined  
197 in (17)) and smoking during pregnancy (Model 2). The same analysis was repeated  
198 comparing SGTF-U versus SGTF-T (p2) using linear regression adjusting for all the  
199 variables included in Model 2 and additionally baseline (at CATS-I) values of TSH and FT4  
200 (Model 3).

201         In the CATS-I study the FT4 levels of SGTF-T mothers repeated at either 20 or 30  
202 weeks of gestation were classified as optimal (SGTF-Topt) or suggestive for possible  
203 levothyroxine overtreatment (SGTF-Tover), if respectively below or above the threshold of

204 17.7 pmol/l, calculated as the top 2.5<sup>th</sup> percentile of the entire CATS-I UK population at  
205 recruitment (15, 34). The analysis of Model 3 was then repeated for the SGTF-Topt and  
206 SGTF-Tover subgroups. In the children's analysis, adjustment was additionally made for the  
207 corresponding variable in the mother, where available.

208 **Supplemental Table 1** summarizes the Models used for each analysis (60). The data  
209 were analyzed in STATA, version 12. Obtained p values <0.05 were considered statistically  
210 significant.

## 211 **RESULTS**

### 212 **CHILDREN**

213 The female:male ratio was similar among the NGTF, SGTF-U and SGTF-T groups,  
214 whereas the children of NGTF women were slightly older (by a few months only) than  
215 SGTF-U and SGTF-T as previously reported (17, 34) (**Table 1**); thus age and sex were  
216 included in all regression models.

217 No differences were observed between NGTF, SGTF-U and SGTF-T groups in BMI  
218 SDS or any of the DXA or cardiovascular measurements (**Table 1**). Of note, the BMI SDS  
219 scores were approximately 0.5 SD higher than the UK 1990 reference population (42, 43).

220 All biochemical measurements were similar among groups except for HDL  
221 cholesterol (**Table 1**), which was marginally lower in the SGTF-T group compared with  
222 SGTF-U (p=0.048).

## 223 **MOTHERS**

### 224 **Anthropometric, bone and cardiometabolic outcomes**

225 **Table 2** summarizes the results obtained among mothers: SGTF-T were slightly  
226 younger ( $39.7 \pm 4.8$  years) compared with NGTF ( $41.2 \pm 5.5$  years;  $p=0.002$ ), but had a  
227 similar age to SGTF-U ( $40.9 \pm 4.7$  years;  $p=0.144$ ). Untreated mothers (SGTF-U) had a  
228 higher BMI (median: 28.3, IQR: [24.6 - 32.6]  $\text{kg/m}^2$ ) compared with NGTF mothers (25.8  
229 [22.9 - 30.0]  $\text{kg/m}^2$ ;  $p=0.029$ ); in contrast, the BMI of treated mothers (SGTF-T) was similar  
230 to NGTF mothers (25.8 [23.1 - 29.8]  $\text{kg/m}^2$ ;  $p=0.672$ ). Importantly, BMI at CATS-I did not  
231 differ across the three groups (**Figure 2**): NGTF 25.0 [22.4 - 28.3]  $\text{kg/m}^2$ , SGTF-U 26.0 [23.4  
232 - 30.1]  $\text{kg/m}^2$  ( $p=0.111$ ), SGTF-T 25.6 [23.0 - 29.2]  $\text{kg/m}^2$  ( $p=0.112$ ). When additionally  
233 adjusted for BMI at entry into CATS-I, the difference in BMI at CATS-II between SGTF-U  
234 and NGTF mothers remained significant ( $p=0.040$ ). DXA analysis showed that the BMI  
235 increase was attributable to an increase in fat, but not lean mass (**Table 2, Figure 2**). DXA  
236 analysis showed no difference in BMD calculated at the femoral neck, total lumbar spine or  
237 whole body less head.

238 Among the metabolic measurements, SGTF-U mothers had higher triglyceride levels  
239 (median: 1.01, IQR: [0.78-1.40]  $\text{mmol/L}$ ) than NGTF mothers (0.90 [0.70-1.10]  $\text{mmol/L}$ ;  
240  $p=0.041$ ). Fasting insulin levels were also higher in SGTF-U mothers (6.30 [4.40-9.15]  
241  $\mu\text{IU/ml}$ ) than SGTF-T mothers (5.50 [3.85-7.15]  $\mu\text{IU/ml}$ ;  $p=0.046$ ). However, when BMI was  
242 included in the regression models the between-group significance was lost ( $p=0.212$  and  
243  $p=0.169$  respectively).

244 There was no difference in any of the cardiovascular measurements between groups.

### 245 **Thyroid function**

246 As expected, a higher percentage of SGTF-U (20/50, 40%) and SGTF-T (38/71, 53%)  
247 mothers were TPOAb positive, compared with NGTF mothers (21/173, 12%;  $p<0.001$ ; **Table**

248 2). Importantly, SGTF-U mothers at recruitment to CATS-II had significantly higher TSH  
249 levels (median [IQR]: 2.45 [1.43-3.50] mU/L) compared with NGTF mothers (1.54 [1.12-  
250 2.07] mU/L;  $p=0.015$ ); TSH levels were also higher compared with SGTF-T mothers, albeit  
251 not quite reaching statistical significance (1.68 [0.89-2.96] mU/L;  $p=0.070$ ). However, TSH  
252 concentrations were not different between SGTF-U and SGTF-T mothers at CATS-I  
253 recruitment (3.37 [1.22-4.45] mU/L and 4.21 [2.33-5.23] mU/L respectively,  $p=0.098$ ).  
254 SGTF-T mothers had FT4 levels within the normal range ( $15.0 \pm 2.9$ ) in the CATS-II study,  
255 although these were significantly higher than in the NGTF group ( $13.6 \pm 1.7$ ;  $p<0.001$ ).

256 The clinical history revealed that the majority of SGTF-U women had never been  
257 treated with levothyroxine, in contrast to those from the SGTF-T group, half of whom at  
258 recruitment to CATS-II were still on treatment commenced during CATS-I (**Table 3**). Of  
259 note, when TSH values were included in the regression model for BMI, the significance  
260 between SGTF-U and NGTF was lost ( $p=0.131$ ).

261 Furthermore, TSH and BMI correlated positively in mothers in CATS-I ( $p=0.037$ ),  
262 but only when excluding the SGTF-T group in CATS-II ( $p=0.027$ ; **Supplemental Figure 1**)  
263 (60).

## 264 LEVOTHYROXINE OVER-TREATMENT SUBANALYSIS

### 265 Mothers

266 As shown in **Supplemental Table 2** (60), at CATS-II SGTF-T<sub>opt</sub> ( $n= 58$ ) and SGTF-T<sub>over</sub>  
267 ( $n= 21$ ) women had similar thyroid function ( $p=0.962$  for TSH levels), however SGTF-T<sub>over</sub>  
268 had lower BMI ( $p=0.002$ ), absolute fat mass ( $p=0.007$ ), lean mass ( $p<0.001$ ), systolic blood  
269 pressure ( $p=0.036$ ) and higher HDL cholesterol levels ( $p=0.002$ ) compared with SGTF-T<sub>opt</sub>  
270 (60). When including BMI in the regression models, the between-group significance was lost

271 for fat mass ( $p=0.882$ ) and systolic blood pressure ( $p=0.074$ ), but not for HDL cholesterol  
272 ( $p=0.022$ ) and lean mass ( $p=0.003$ ).

273 SGTF-T<sub>over</sub> women had lower BMI ( $p=0.001$ ) and height ( $p=0.032$ ) already at CATS-I,  
274 such that the difference in BMI at CATS-II between SGTF-T<sub>opt</sub> and SGTF-T<sub>over</sub> lost  
275 significance when adjusted for baseline BMI at CATS-I ( $p=0.267$ ).

## 276 **Children**

277 As shown in **Supplemental Table 3** (60), there were no differences in terms of age  
278 ( $p=0.516$ ) and sex ( $p=0.260$ ) between SGTF-T<sub>opt</sub> ( $n= 57$ ) and SGTF-T<sub>over</sub> ( $n= 21$ ) offspring.  
279 However, similarly to their mothers, SGTF-T<sub>over</sub> children had lower height ( $p=0.016$ ), BMI  
280 SDS ( $p=0.001$ ) and lean mass ( $p=0.004$ ) compared with SGTF-T<sub>opt</sub> children, as well as FN-  
281 BMD-H ( $p=0.037$ ), WBLH-BMD-H ( $p=0.002$ ) and WBLH-BMD-A ( $p=0.002$ ). Of note,  
282 when adjusting for the corresponding maternal measurement ( $p^5$  column, **Supplemental**  
283 **Table 3**), only BMI SDS ( $p=0.006$ ) and height ( $p=0.044$ ) remained significantly lower in  
284 SGTF-T<sub>over</sub> children compared with SGTF-T<sub>opt</sub> children (60). When also adjusting for  
285 paternal height, the difference in height between SGTF-T<sub>over</sub> and SGTF-T<sub>opt</sub> children lost  
286 significance ( $p=0.298$ ).

## 287 **DISCUSSION**

288 To our knowledge, the present study is the first to evaluate several long-term  
289 anthropometric, bone and cardiometabolic outcomes in children and mothers from a large  
290 cohort of women with SGTF randomized to receive levothyroxine treatment during  
291 pregnancy.

292 No significant effects were observed on offspring outcomes evaluated at 9 years of age.  
293 Only a slight reduction in HDL cholesterol levels was observed among children of treated  
294 mothers compared with those who were untreated, albeit this was of marginal clinical

295 significance. Considering the additional limitation of the low number of children consenting  
296 to phlebotomy, further studies in larger cohorts are needed before any firm conclusions can  
297 be drawn in this context. It was noteworthy that the children's BMI was higher compared  
298 with the UK children reference population established 30 years ago; this is in line with the  
299 global secular trends in rates of childhood overweight and obesity observed over the last  
300 three decades, as a likely consequence of unhealthy lifestyle (61, 62).

301 Long-term maternal bone and cardiovascular outcomes were also unaffected by SGTF,  
302 whether treated or not. However, BMI was significantly greater at 9 years follow-up only in  
303 the group of mothers with SGTF who had not been randomized during CATS-I to receive  
304 levothyroxine replacement (SGTF-U), with DXA analysis showing that this weight gain was  
305 predominantly attributable to an increase in fat rather than lean mass. On the contrary, the  
306 group of mothers with SGTF who were started on levothyroxine replacement during CATS-I  
307 (SGTF-T), 9 years later had similar BMI and fat mass values to women with NGTF. Of note,  
308 the baseline BMI at enrolment into the CATS-I study was similar among all groups and,  
309 when included in the regression model, did not influence the BMI change observed at CATS-  
310 II. These observations suggest that the BMI increase had occurred in the 9-year time window  
311 from recruitment into CATS-I and CATS-II, and only in the group of women with untreated  
312 SGTF at CATS-I. In line with the higher prevalence of overweight and obesity in the SGTF-  
313 U group, these women also had significantly higher triglyceride and insulin levels; however,  
314 these differences lost significance when adjusted for BMI, suggesting that these metabolic  
315 alterations were driven by overweight/obesity as expected (63-65). Untreated SGTF women  
316 also had current higher TSH concentrations compared with the other two groups, since the  
317 majority of them had not been commenced on levothyroxine treatment during CATS-I or the  
318 following 9 years. On the contrary, nearly half of women with SGTF commencing  
319 levothyroxine at CATS-I (SGTF-T) were still on treatment 9 years later; their higher FT4

320 levels likely reflected the measurement of exogenous T4 (66), especially if blood was  
321 withdrawn after taking levothyroxine (67). The fact that the differences in BMI among  
322 groups lost significance when adjusted for current TSH levels, suggests that the increase in  
323 fat mass was largely driven by untreated suboptimal thyroid function. In this study other  
324 BMI-influencing factors such as physical activity, dietary habits and family history for  
325 obesity were not evaluated, thus their hypothetical influence on observed BMI differences  
326 among groups cannot be excluded. However SGTF-U and SGTF-T women had been  
327 randomised at CATS-I, thus we assume that confounding factors, other than those related to  
328 thyroid function, should not differ between these groups. SGTF-T and NGTF women at  
329 CATS-II presented similar values of thyroid and metabolic outcomes (TSH, BMI, fat mass,  
330 triglyceride, insulin), parameters that were different only in the SGTF-U group. This  
331 difference was statistically significant only when comparing SGTF-U and NGTF women but  
332 not SGTF-U and SGTF-T women, with the sole exception of insulin levels, likely due to the  
333 small size of SGTF-T group (N=79) compared with NGTF group (N=197).

334 Small variation in TSH levels even within the normal reference range is positively  
335 associated with higher BMI and weight gain in other cohorts (68-71), and such a relationship  
336 is bilateral (35-37). Obesity, likely acting via leptin, activates the hypothalamic-pituitary-  
337 thyroid axis and induces a consequent rise in TSH levels (72, 73). On the other hand,  
338 impaired thyroid function favors weight gain due to the consequent myxoedema and  
339 reduction of resting energy expenditure (REE), particularly in overt hypothyroidism (37).  
340 However, even smaller variations of thyroid function, usually considered clinically  
341 insignificant, induce measurable REE modifications, and therefore if sustained have the  
342 potential to affect body weight (74). Accordingly, in our study women with long-term  
343 suboptimal thyroid function, if left untreated experienced increased body weight and fat



344 mass, while those who were treated did not. This suggests that in our cohort thyroid function  
345 determined BMI and not vice versa.

346 A recent meta-analysis did not highlight any benefits on several outcomes, including  
347 BMI, of levothyroxine treatment for subclinical hypothyroidism (75). However, this analysis  
348 included a very large trial evaluating subjects above 65 years of age (76), which represents a  
349 population that may not be comparable with younger individuals such as those analyzed in  
350 our study. With respect to specific effects on BMI, the majority of studies were small-scale  
351 and based on shorter follow-up periods (75), therefore not allowing definitive conclusions.  
352 The study of Zhao et al (77) was one of the largest randomized trials of levothyroxine  
353 replacement, enrolling 369 middle-aged males and females affected with subclinical  
354 hypothyroidism who were followed-up for 15 months. In line with the results of our study,  
355 subjects receiving levothyroxine showed a significant BMI reduction at the end of the follow-  
356 up period, while untreated subjects did not. Clearly, further randomized trials are needed to  
357 clarify the benefits of levothyroxine replacement on BMI in individuals affected with  
358 subclinical hypothyroidism below 65 years of age.

359 Our exploratory sub-analysis showed that women exposed to over-replacement with  
360 levothyroxine during pregnancy (SGTF-T<sub>over</sub>), at CATS-II displayed lower BMI, height,  
361 absolute fat mass, lean mass, SP and higher HDL values compared with those with optimal  
362 gestational FT4 levels (SGTF-T<sub>opt</sub>); fat mass and blood pressure seemed to be driven by BMI.  
363 However, SGTF-T<sub>over</sub> women were noted to have been thinner and shorter from baseline,  
364 before commencing levothyroxine treatment during CATS-I; in fact when including baseline  
365 BMI in the regression model, the BMI change observed at CATS-II lost significance.  
366 Furthermore, levothyroxine doses were promptly reduced during CATS-I to correct the raised  
367 FT4 levels, such that thyroid function of the SGTF-T<sub>over</sub> group at CATS-II was normal and  
368 similar to SGTF-T<sub>opt</sub>. Considering that all women were commenced on a standard 150 µg

369 dose of levothyroxine during pregnancy, it is appropriate to conclude that this likely induced  
370 an excessive increase of FT4 levels in this subgroup of thinner women, since they would  
371 have required a smaller dose. This study further highlights the importance of adjusting  
372 levothyroxine treatment for body weight, especially during pregnancy where high FT4 levels  
373 have been associated with several negative outcomes (1, 2, 6), including a higher prevalence  
374 of behavioral difficulties in the offspring of this cohort (34). Similar to their mothers, SGTF-  
375 T<sub>over</sub> children were thinner and shorter compared to SGTF-T<sub>opt</sub> children. However, correction  
376 for the corresponding maternal and paternal measurements, where available, reduced or  
377 totally eliminated the between-group significance, indicating a genetic component rather than  
378 an effect of levothyroxine overtreatment on anthropometric outcomes.

379 The strengths of our study include the large sample size, baseline randomization, analysis  
380 of several anthropometric, bone and cardiometabolic outcomes, and longitudinal design with  
381 one of the longest available follow-up periods. Our study has limitations, however, including  
382 a lack of detailed information about the levothyroxine doses used and the length of drug  
383 withdrawal periods during the 9 years between CATS-I and CATS-II, as well as a lack of  
384 correction for other BMI-influencing factors, such as dietary habits, physical activity and  
385 family history for obesity.

386 In conclusion, for the first time we evaluated the long-term effects of SGTF and treatment  
387 with levothyroxine during pregnancy on a series of offspring anthropometric, bone and  
388 cardiometabolic measurements, finding no significant evidence for benefit or harm. Women  
389 with long-term untreated mild suboptimal thyroid function persisting after pregnancy showed  
390 a significant increase in BMI, fat mass, triglyceride and insulin levels, that were absent in the  
391 group of women treated with levothyroxine. Our study also emphasizes the need for careful  
392 adjustment of levothyroxine dose for bodyweight to avoid overtreatment, especially during  
393 pregnancy. Our findings thus highlight the need for dedicated large-scale randomized trials to

394 investigate the long-term benefits of levothyroxine treatment in young and middle-aged  
395 individuals with suboptimal thyroid function, whether in relation to pregnancy or not. If our  
396 observations were confirmed, the current indications to such treatment may need to be  
397 revised.

398

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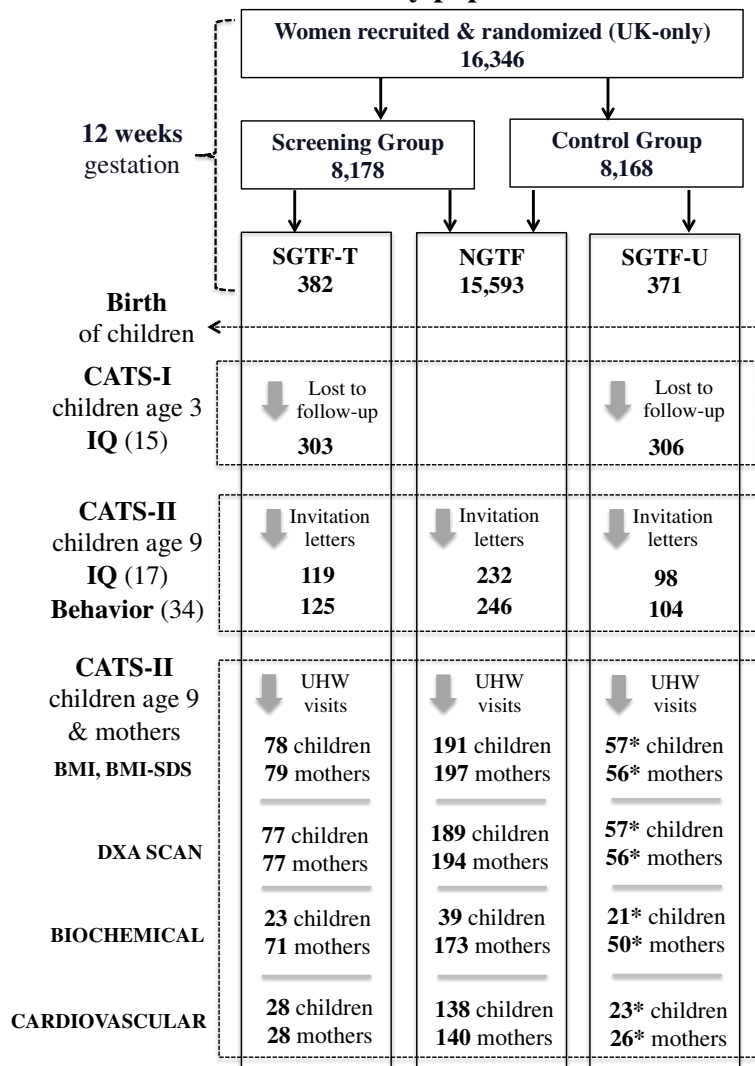
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609 **FIGURE 1. Flow chart of study population**



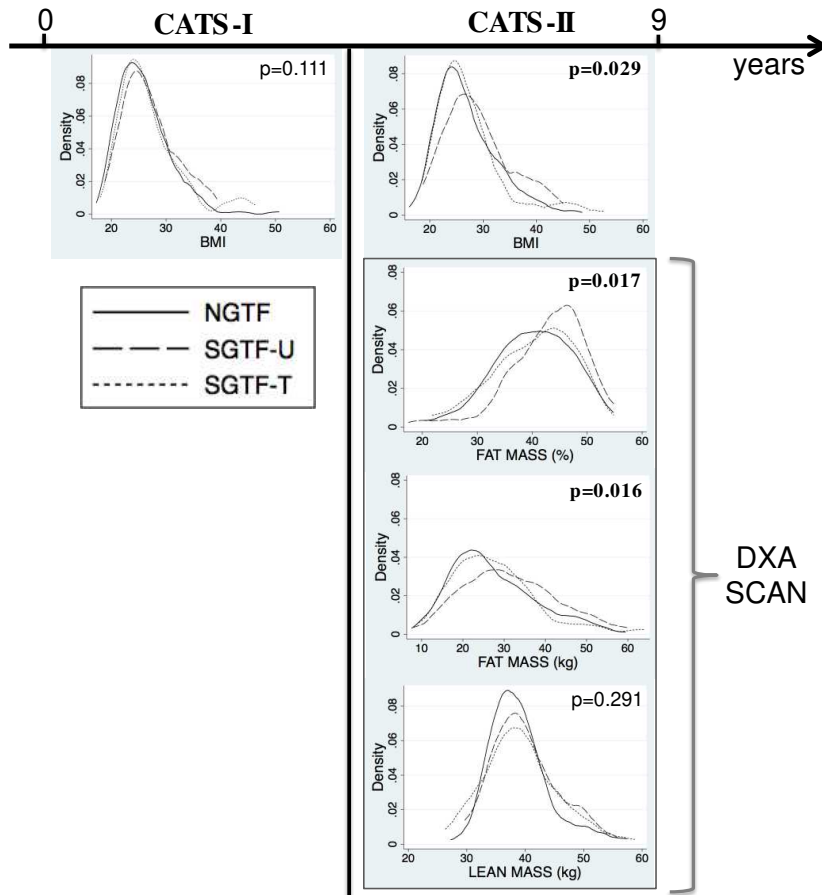
610  
 611 The recruitment of study participants initially started with the CATS-I study, assessing child  
 612 cognition at 3 years of age (15). A UK-only subset of the original population and an  
 613 additional third group (NGTF) were involved in the subsequent follow-up CATS-II study,  
 614 assessing child cognition (17) and behavior (34) at 9 years of age. Only CATS-II children  
 615 and paired mothers attending visits at the research center were included in the present study  
 616 and assessed for cardiovascular, metabolic and bone measurements.

617 \* = Children tended to participate less in this study compared with their mothers, except for  
 618 one mother in the SGTF-U group, refusing to be included in the study but agreeing for her  
 619 son to participate.

620 **BMI** = body mass index. **CATS** = Controlled Antenatal Thyroid Screening study. **DXA** =  
 621 dual-energy x-ray absorptiometry. **IQ** = intelligence quotient. **NGTF** = normal gestational  
 622 thyroid function. **SDS** = standard deviation scores. **SGTF-T** = suboptimal gestational thyroid  
 623 function treated with levothyroxine during pregnancy (Treated). **SGTF-U** = suboptimal  
 624 gestational thyroid function not treated with levothyroxine during pregnancy (Untreated).  
 625 **UHW** = University Hospital of Wales (research center).



626 **FIGURE 2. Comparison of body composition at CATS-I and CATS-II studies among**  
 627 **women**



628  
 629 **BMI** = body mass index. **CATS** = Controlled Antenatal Thyroid Screening study. **DXA** =  
 630 dual-energy x-ray absorptiometry. **NGTF** = normal gestational thyroid function. **SGTF-T** =  
 631 suboptimal gestational thyroid function treated with levothyroxine during pregnancy  
 632 (Treated). **SGTF-U** = suboptimal gestational thyroid function not treated with levothyroxine  
 633 during pregnancy (Untreated).  
 634 Reported p values refer to the comparisons between NGTF and SGTF-U.

635  
 636

**Table 1. Anthropometric, bone and cardiometabolic outcomes: children**

	<b>TOT</b> N = 326	<b>NGTF</b> N = 191	<b>SGTF-U</b> N = 57	<b>SGTF-T</b> N = 78	<b>p1U</b>	<b>p1T</b>	<b>p2</b>
Age (years)	9.3 ± 1.0	9.6 ± 0.7	9.0 ± 1.1	8.8 ± 1.1	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.485
Female children N (%)	158 (48.5%)	88 (46.1%)	32 (56.1%)	38 (48.7%)	0.227	0.788	0.486
Height (cm)	136.7 ± 7.9	138.5 ± 7.3	133.5 ± 8.7	134.8 ± 7.4	0.051	0.779	0.089
BMI (kg/m <sup>2</sup> )	17.2 [15.8 - 19.2]	17.3 [15.9 - 19.5]	17.0 [15.8 - 19.3]	16.8 [15.5 - 18.7]	0.587	0.822	0.464
BMI-SDS UK1990	0.49 ± 1.15	0.48 ± 1.15	0.57 ± 1.15	0.46 ± 1.15	0.646	0.763	0.481
<b><i>DXA SCAN<sup>1</sup></i></b>							
WBLH Lean Mass (kg)	19.7 ± 3.5	20.3 ± 3.3	18.6 ± 3.6	19.0 ± 3.6	0.395	0.808	0.454
WBLH Fat Mass (kg)	8.36 [6.12 - 11.89]	8.67 [6.27 - 12.90]	8.71 [6.26 - 10.85]	7.46 [5.84 - 10.46]	0.979	0.573	0.553
WBLH Fat Mass (%)	31.8 ± 7.9	31.9 ± 7.8	32.6 ± 8.1	30.8 ± 7.9	0.617	0.475	0.279
LS-BMD-H (SDS)	0.33 ± 1.00	0.28 ± 0.97	0.28 ± 1.00	0.50 ± 1.08	0.721	0.302	0.424
LS-BMD-A (SDS)	0.23 ± 1.01	0.16 ± 0.97	0.19 ± 0.99	0.43 ± 1.10	0.751	0.261	0.403
LS-BMAD-A (SDS)	0.35 ± 1.08	0.30 ± 1.08	0.29 ± 1.04	0.53 ± 1.12	0.950	0.214	0.384
LS-BMAD-W (SDS)	0.27 ± 1.07	0.22 ± 1.07	0.22 ± 1.03	0.43 ± 1.1	0.948	0.223	0.471
FN-BMD-H (SDS)	0.49 ± 1.04	0.00 ± 0.95	0.02 ± 1.17	0.19 ± 1.14	0.900	0.358	0.732
FN-BMAD-W (SDS)	0.21 ± 0.99	0.16 ± 0.88	0.27 ± 1.26	0.29 ± 1.01	0.255	0.314	0.755
WBLH-BMD-H (SDS)	-0.83 ± 0.86	-0.80 ± 0.80	-0.93 ± 0.87	-0.85 ± 0.99	0.459	0.837	0.973
WBLH-BMD-A (SDS)	-0.87 ± 0.96	-0.87 ± 0.90	-0.98 ± 0.95	-0.79 ± 1.10	0.339	0.850	0.546
<b><i>BIOCHEMICAL<sup>2</sup></i></b>							
TSH (mU/L)	1.77 [1.42 - 2.52]	1.64 [1.38 - 2.31]	1.80 [1.41 - 2.29]	1.81 [1.66 - 2.76]	0.511	0.305	0.764
FT4 (pmol/L)	14.69 ± 1.48	14.20 ± 1.38	15.39 ± 1.65	14.82 ± 1.25	0.098	0.597	0.335
FT3 (pmol/L)	5.60 ± 0.64	5.43 ± 0.63	5.87 ± 0.45	5.65 ± 0.73	0.158	0.938	0.290
TPOAb Positive/Total (%)	3/83 (3.6)	1/39 (2.6)	1/21 (4.8)	1/23 (4.3)	1.000	1.000	1.000
Cholesterol TOT (mmol/L)	4.33 ± 0.60	4.35 ± 0.63	4.41 ± 0.62	4.22 ± 0.54	0.398	0.887	0.403
Cholesterol HDL (mmol/L)	1.20 ± 0.26	1.22 ± 0.27	1.26 ± 0.29	1.10 ± 0.21	0.178	0.566	<b>0.048</b>
Triglyceride (mmol/L)	0.71 [0.60 - 0.90]	0.70 [0.50 - 1.00]	0.80 [0.62 - 0.88]	0.68 [0.60 - 0.89]	0.789	0.509	0.374
Insulin (µIU/mL)	4.45 [3.30 - 5.95]	4.90 [3.60 - 6.90]	4.40 [3.30 - 5.70]	3.85 [3.3 - 5.1]	0.385	0.272	0.950
Adiponectin (ng/mL)	13.49 ± 5.19	12.36 ± 4.44	14.75 ± 5.41	14.25 ± 5.96	0.368	0.554	0.703
<b><i>CARDIOVASCULAR<sup>3</sup></i></b>							
SP (mmHg)	125.4 ± 12.9	124.8 ± 12.9	126.9 ± 10.4	127.2 ± 14.8	0.330	0.840	0.284
DP (mmHg)	63.0 ± 7.9	63.0 ± 7.7	61.8 ± 9.0	64.1 ± 8.0	0.497	0.682	0.228
AI (%)	7.33 [3.67 - 11.67]	7.33 [3.42 - 11.17]	10.67 [4.00 - 14.00]	7.00 [5.00 - 11.33]	0.363	0.911	0.454
PWV (m/s)	6.22 ± 0.65	6.22 ± 0.68	6.22 ± 0.51	6.17 ± 0.61	0.919	0.903	0.452

The results are presented as mean  $\pm$  SD or median [IQR], if Normally- or non-Normally distributed, respectively.

<sup>1</sup> = Data available for 323 subjects; <sup>2</sup> = Data available for 83 subjects; <sup>3</sup> = Data available for 189 subjects

**A** = Alphabet study reference cohort. **AI** = augmentation index. **BMAD** = bone mineral apparent density. **BMD** = bone mineral density. **BMI** = body mass index. **BMI-SDS** = standard deviation score of body mass index. **DP** = diastolic blood pressure. **DXA** = dual-energy x-ray absorptiometry. **FN** = femoral neck. **FT3** = free-triiodothyronine. **FT4** = free-thyroxine. **H** = Hologic manufacturer reference cohort. **LS** = lumbar spine (from L1 to L4). **NGTF** = children of women with normal gestational thyroid function. **PWV** = aortic pulse wave velocity. **SGTF-T** = children of women with suboptimal gestational thyroid function treated with levothyroxine during pregnancy (Treated). **SGTF-U** = children of women with suboptimal gestational thyroid function not treated with levothyroxine during pregnancy (Untreated). **SDS** = standard deviation score. **SP** = systolic blood pressure. **TPOAb** = autoantibodies to thyroid peroxidase. **TSH** = thyrotropin. **W** = Ward study reference cohort. **WBLH** = whole body less head.  
**p1U** = NGTF vs SGTF-U. **p1T** = NGTF vs SGTF-T. **p2** = SGTF-U vs SGTF-T.

**Table 2. Anthropometric, bone and cardiometabolic outcomes: mothers**

	<b>TOT</b> N = 332	<b>NGTF</b> N = 197	<b>SGTF-U</b> N = 56	<b>SGTF-T</b> N = 79	<b>p1U</b>	<b>p1T</b>	<b>p2</b>
Age (years)	41.2 ± 5.3	41.8 ± 5.5	40.9 ± 4.7	39.7 ± 4.8	0.252	<b>0.002</b>	0.144
Height (cm)	164.0 ± 6.4	164.3 ± 6.1	163.2 ± 5.7	164.1 ± 7.6	0.213	0.580	0.402
BMI CATS-I (kg/m <sup>2</sup> )	25.4 [22.7 - 28.7]	25.0 [22.4 - 28.3]	26.0 [23.4 - 30.1]	25.6 [23.0 - 29.2]	0.111	0.112	0.806
BMI (kg/m <sup>2</sup> )	26.1 [23.1 - 30.3]	25.8 [22.9 - 30.0]	28.3 [24.6 - 32.6]	25.8 [23.1 - 29.8]	<b>0.029</b>	0.672	0.139
<b>DXA SCAN<sup>1</sup></b>							
WBLH Lean Mass (kg)	39.2 ± 5.6	38.9 ± 5.2	40.0 ± 5.7	39.3 ± 6.4	0.291	0.698	0.661
WBLH Fat Mass (kg)	28.3 ± 10.7	27.5 ± 10.3	31.5 ± 11.4	27.8 ± 10.7	<b>0.016</b>	0.791	0.084
WBLH Fat Mass (%)	40.7 ± 7.3	40.2 ± 7.2	42.8 ± 7.2	40.4 ± 7.4	<b>0.017</b>	0.784	0.072
LS-BMD (g/cm <sup>2</sup> )	1.07 ± 0.12	1.06 ± 0.11	1.06 ± 0.12	1.08 ± 0.12	0.834	0.150	0.541
FN-BMD (g/cm <sup>2</sup> )	0.84 ± 0.11	0.83 ± 0.11	0.85 ± 1.24	0.83 ± 0.11	0.297	0.949	0.321
<b>BIOCHEMICAL<sup>2</sup></b>							
TSH CATS-I (mU/L)	1.66 [0.94 - 3.37]	1.22 [0.77 - 1.79]	3.37 [1.22 - 4.45]	4.21 [2.33 - 5.23]	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.098
TSH (mU/L)	1.64 [1.10 - 2.52]	1.54 [1.12 - 2.07]	2.45 [1.43 - 3.50]	1.68 [0.89 - 2.96]	<b>0.015</b>	0.474	0.070
FT4 (pmol/L)	14.0 ± 2.3	13.6 ± 1.7	13.9 ± 2.8	15.0 ± 2.9	0.471	<b>&lt;0.001</b>	0.200
FT3 (pmol/L)	4.13 ± 0.51	4.15 ± 0.45	4.07 ± 0.53	4.11 ± 0.63	0.239	0.299	0.428
TPOAb Positive/Total (%)	79/294 (26.9)	21/173 (12.1)	20/50 (40.0)	38/71 (53.5)	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.196
Cholesterol TOT (mmol/L)	4.93 ± 0.87	4.91 ± 0.83	5.07 ± 0.93	4.88 ± 0.94	0.224	0.708	0.506
Cholesterol HDL (mmol/L)	1.29 ± 0.32	1.32 ± 0.31	1.24 ± 0.33	1.24 ± 0.34	0.125	0.101	0.785
Triglyceride (mmol/L)	0.90 [0.70 - 1.20]	0.90 [0.70 - 1.10]	1.01 [0.78 - 1.40]	0.80 [0.64 - 1.29]	<b>0.041</b>	0.730	0.193
Insulin (μIU/mL)	5.90 [4.40 - 7.80]	5.90 [4.60 - 7.80]	6.30 [4.40 - 9.15]	5.50 [3.85 - 7.15]	0.231	0.073	<b>0.046</b>
Adiponectin (ng/mL)	10.59 ± 4.80	10.63 ± 4.71	10.54 ± 4.65	10.52 ± 5.19	0.964	0.988	0.872
<b>CARDIOVASCULAR<sup>3</sup></b>							
SP (mmHg)	125.7 ± 12.8	126.6 ± 12.9	124.6 ± 9.3	122.1 ± 14.4	0.569	0.155	0.220
DP (mmHg)	70.3 ± 8.3	70.6 ± 8.4	70.8 ± 8.0	68.7 ± 7.9	0.966	0.409	0.578
AI (%)	17.5 ± 7.2	18.1 ± 7.5	15.6 ± 4.8	16.5 ± 7.00	0.186	0.588	0.494
PWV (m/s)	8.80 [7.50 - 10.95]	9.28 [7.66 - 11.17]	7.87 [7.37 - 9.47]	7.93 [6.83 - 9.77]	0.491	0.101	0.605

The results are presented as mean ± SD or median [IQR], if Normally- or non-Normally distributed, respectively. All values refer to the Controlled Antenatal Thyroid Screening (CATS) study II analysis, except for BMI CATS-I and TSH CATS-I, relative to CATS study I analysis.

<sup>1</sup> = Data available for 327 subjects; <sup>2</sup> = Data available for 294 subjects; <sup>3</sup> = Data available for 194 subjects.

**AI** = augmentation index. **BMD** = bone mineral density. **BMI** = body mass index. **DP** = diastolic blood pressure. **DXA** = dual-energy x-ray absorptiometry. **FN** = femoral neck. **FT3** = free-triiodothyronine. **FT4** = free-thyroxine. **LS** = lumbar spine (from L1 to L4). **NGTF** = normal gestational thyroid function. **PWV** = aortic pulse wave velocity. **SGTF-T** = suboptimal gestational thyroid function treated with levothyroxine during pregnancy (Treated). **SGTF-U** = suboptimal gestational thyroid function not treated with levothyroxine during pregnancy (Untreated). **SP** = systolic blood pressure. **TPOAb** = autoantibodies to thyroid peroxidase. **TSH** = thyrotropin. **WBLH** = whole body less head. **p1U** = NGTF vs SGTF-U. **p1T** = NGTF vs SGTF-T. **p2** = SGTF-U vs SGTF-T.

**Table 3. Levothyroxine treatment**

	<b>TOT</b> N = 332	<b>NGTF</b> N = 197	<b>SGTF-U</b> N = 57	<b>SGTF-T</b> N = 79	<b>p</b>
Never	176 (53.0%)	139 (70.6%)	38 (66.7%)	0 (0.0%)	<b>&lt;0.001</b>
Yes stopped	36 (10.8%)	3 (1.5%)	0 (0.0%)	33 (41.8%)	<b>&lt;0.001</b>
Yes current	65 (19.6%)	8 (4.1%)	16 (28.1%)	41 (51.9%)	<b>0.008</b>
Unknown	55 (16.6%)	47 (23.8%)	3 (5.2%)	5 (6.3%)	1.000

History of women's levothyroxine treatment collected at recruitment into the Controlled Antenatal Thyroid Screening study II (CATS-II).

**NGTF** = normal gestational thyroid function. **SGTF-T** = suboptimal gestational thyroid function treated with levothyroxine during pregnancy (Treated). **SGTF-U** = suboptimal gestational thyroid function not treated with levothyroxine during pregnancy (Untreated). **Yes stopped** = taken in the past but not currently. **Yes current** = started in the past and currently still on treatment.

Reported p values refer to the comparisons between SGTF-U and SGTF-T

**Supplemental Table 1. Models used for analysis**

<b>MODEL</b>	<b>INCLUDED VARIABLES</b>	<b>PARAMETERS EVALUATED</b>	<b>COMPARISON</b>
<b>1</b>	None (unadjusted)	Age	All
<b>2</b>	Age, ethnicity, social class, smoking during pregnancy	All numerical parameters except age	NGTF vs SGTF-U (p1U) NGTF vs SGTF-T (p1T)
<b>3</b>	Age, ethnicity, social class, smoking during pregnancy, baseline (CATS-I) TSH, baseline (CATS-I) FT4	All numerical parameters except age	SGTF-U vs SGTF-T (p2) SGTF-TOpt vs SGTF-TOver (p)

**CATS-I** = Controlled Antenatal Thyroid Screening study I. **FT4** = free-thyroxine. **NGTF** = normal gestational thyroid function. **SGTF-T** = suboptimal gestational thyroid function treated with levothyroxine during pregnancy (Treated). **SGTF-TOpt** = SGTF-T optimally treated with levothyroxine. **SGTF-TOver** = SGTF-T over-treated with levothyroxine. **SGTF-U** = suboptimal gestational thyroid function not treated with levothyroxine during pregnancy (Untreated). **TSH** = thyrotropin.

**Supplemental Table 2. Levothyroxine over-treatment sub-analysis: mothers**

	<b>SGTF-T<sub>opt</sub></b> <b>N = 58</b>	<b>SGTF-T<sub>over</sub></b> <b>N = 21</b>	<b>P</b>
Age (years)	40.3 ± 4.8	38.1 ± 4.7	0.069
Height (cm)	164.9 ± 7.4	161.8 ± 7.7	<b>0.032</b>
BMI CATS-I (kg/m <sup>2</sup> )	26.4 [23.8 - 32.0]	23.2 [21.3-25.7]	<b>0.001</b>
BMI (kg/m <sup>2</sup> )	26.7 [23.9 - 30.9]	23.5 [22.2 - 25.3]	<b>0.002</b>
<b><i>DXA SCAN</i><sup>1</sup></b>			
WBLH Lean Mass (kg)	40.9 ± 6.3	34.8 ± 4.3	<b>&lt;0.001</b>
WBLH Fat Mass (kg)	30.1 ± 11.1	21.9 ± 7.0	<b>0.007</b>
WBLH Fat Mass (%)	41.3 ± 7.2	37.9 ± 7.6	0.180
LS-BMD (g/cm <sup>2</sup> )	1.10 ± 0.12	1.05 ± 0.11	0.198
FN-BMD (g/cm <sup>2</sup> )	0.85 ± 0.12	0.80 ± 0.73	0.111
<b><i>BIOCHEMICAL</i><sup>2</sup></b>			
TSH CATS-I (mU/L)	4.09 [1.94 - 5.30]	4.40 [3.75 - 4.62]	0.379
TSH (mU/L)	1.52 [0.86 - 2.96]	1.76 [1.22 - 3.32]	0.962
FT4 (pmol/L)	15.06 ± 2.79	14.73 ± 3.31	0.762
FT3 (pmol/L)	4.13 ± 0.66	4.05 ± 0.56	0.867
TPOAb Positive/Total (%)	23/51	10/20	0.794
Cholesterol TOT (mmol/L)	4.81 ± 0.85	5.07 ± 1.16	0.073
Cholesterol HDL (mmol/L)	1.15 ± 0.33	1.44 ± 0.27	<b>0.002</b>
Triglyceride (mmol/L)	0.91 [0.68 - 1.29]	0.80 [0.60 - 1.21]	0.562
Insulin (μIU/mL)	5.50 [3.90 - 7.50]	5.65 [3.75 - 6.85]	0.772
Adiponectin (ng/mL)	10.16 ± 5.70	11.44 ± 3.53	0.727
<b><i>CARDIOVASCULAR</i><sup>3</sup></b>			
SP (mmHg)	127.6 ± 13.5	113.7 ± 11.7	<b>0.036</b>
DP (mmHg)	70.4 ± 8.0	66.0 ± 7.3	0.767
AI (%)	17.8 ± 6.3	14.6 ± 7.9	0.746
PWV (m/s)	8.05 [6.83 - 13.03]	7.13 [6.80 - 8.17]	0.855

The results are presented as mean ± SD or median [IQR], if having a normal or non-normal distribution, respectively. All values refer to the Controlled Antenatal Thyroid Screening (CATS) study II analysis, except for BMI CATS-I and TSH CATS-I, relative to CATS study I analysis.

<sup>1</sup> = Data available for 56 SGTF-T<sub>opt</sub> and 21 SGTF-T<sub>over</sub> subjects;

<sup>2</sup> = Data available for 51 SGTF-T<sub>opt</sub> and 20 SGTF-T<sub>over</sub> subjects;

<sup>3</sup> = Data available for 17 SGTF-T<sub>opt</sub> and 11 SGTF-T<sub>over</sub> subjects.

**AI** = augmentation index. **BMD** = bone mineral density. **BMI** = body mass index. **DP** = diastolic blood pressure. **DXA** = dual-energy x-ray absorptiometry. **FN** = femoral neck. **FT3** = free-triiodothyronine. **FT4** = free-thyroxine. **LS** = lumbar spine (from L1 to L4). **PWV** = aortic pulse wave velocity. **SGTF-T<sub>opt</sub>** = suboptimal gestational thyroid function optimally treated with levothyroxine during pregnancy. **SGTF-T<sub>over</sub>** = suboptimal gestational thyroid function over-treated with levothyroxine during pregnancy. **SP** = systolic blood pressure. **TPOAb** = autoantibodies to thyroid peroxidase. **TSH** = thyrotropin. **WBLH** = Whole body less head.

**Supplemental Table 3. Levothyroxine over-treatment sub-analysis: children**

	<b>SGTF-Topt</b> N = 57	<b>SGTF-Tover</b> N = 21	<b>p</b>	<b>p<sup>4</sup></b>	<b>p<sup>5</sup></b>
Age (years)	8.8 ± 1.2	9.0 ± 0.9	0.516	NA	NA
Female children N (%)	30 (52.6%)	8 (38.1%)	0.260	NA	NA
Height (cm)	135.1 ± 7.7	132.9 ± 8.1	<b>0.016</b>	NA	<b>0.044</b>
BMI (kg/m <sup>2</sup> )	17.6 [16.0 - 19.1]	15.2 [14.9 - 16.7]	<b>0.005</b>	NA	<b>0.041</b>
BMI-SDS UK1990 (SDS)	0.70 ± 1.07	-0.19 ± 1.14	<b>0.001</b>	NA	<b>0.006</b>
<b>DXA SCAN<sup>1</sup></b>					
WBLH Lean Mass (kg)	19.6 ± 3.3	17.6 ± 3.9	<b>0.004</b>	NA	0.109
WBLH Fat Mass (kg)	9.66 ± 4.64	7.22 ± 4.19	0.087	NA	0.298
WBLH Fat Mass (%)	31.9 ± 8.0	28.0 ± 7.1	0.234	NA	0.511
LS-BMD-H (SDS)	0.59 ± 1.00	0.26 ± 1.24	0.174	0.394	0.603
LS-BMD-A (SDS)	0.51 ± 1.07	0.23 ± 1.21	0.217	0.449	0.669
LS-BMAD-A (SDS)	0.57 ± 1.07	0.42 ± 1.25	0.405	0.423	0.679
LS-BMAD-W (SDS)	0.46 ± 1.05	0.33 ± 1.27	0.455	0.467	0.702
FN-BMD-H (SDS)	0.33 ± 1.05	-0.19 ± 1.30	<b>0.037</b>	0.164	0.272
FN-BMAD-W (SDS)	0.31 ± 1.03	0.23 ± 0.99	0.952	0.803	0.988
WBLH-BMD-H (SDS)	-0.62 ± 0.80	-1.44 ± 1.19	<b>0.002</b>	<b>0.036</b>	0.216
WBLH-BMD-A (SDS)	-0.57 ± 0.96	-1.39 ± 1.26	<b>0.002</b>	<b>0.042</b>	0.193
<b>BIOCHEMICAL<sup>2</sup></b>					
TSH (mU/L)	1.81 [1.66 - 2.76]	1.82 [1.55 - 2.41]	0.540	NA	0.526
FT4 (pmol/L)	14.91 ± 1.24	14.40 ± 1.40	0.251	NA	0.280
FT3 (pmol/L)	5.83 ± 0.55	4.77 ± 0.92	<b>0.036</b>	NA	0.051
TPOAb Positive/Total (%)	1/19 (5.3)	0/4 (0.0)	1.000	NA	NA
Cholesterol TOT (mmol/L)	4.24 ± 0.53	4.13 ± 0.68	0.197	NA	0.218
Cholesterol HDL (mmol/L)	1.08 ± 0.21	1.20 ± 0.14	0.717	NA	0.332
Triglyceride (mmol/L)	0.68 [0.60 - 0.90]	0.65 [0.55 - 0.75]	0.484	NA	0.505
Insulin (µIU/mL)	3.70 [3.30 - 5.60]	4.00 [2.80 - 4.60]	0.824	NA	0.544
Adiponectin (ng/mL)	13.91 ± 5.92	15.87 ± 6.76	0.292	NA	0.298
<b>CARDIOVASCULAR<sup>3</sup></b>					
SP (mmHg)	125.3 ± 16.9	130.5 ± 10.2	0.768	NA	0.260
DP (mmHg)	64.2 ± 8.4	63.9 ± 7.8	0.586	NA	0.637
AI (%)	6.67 [3.67 - 11.33]	7.12 [7.00 - 9.00]	0.367	NA	0.412
PWV (m/s)	6.03 ± 0.46	6.37 ± 0.77	<b>0.041</b>	NA	<b>0.035</b>

The results are presented as mean ± SD or median [IQR], if having a normal or non-normal distribution, respectively.

<sup>1</sup> = Data available for 56 SGTF-T<sub>opt</sub> and 21 SGTF-T<sub>over</sub> subjects;

<sup>2</sup> = Data available for 19 SGTF-T<sub>opt</sub> and 4 SGTF-T<sub>over</sub> subjects;

<sup>3</sup> = Data available for 18 SGTF-T<sub>opt</sub> and 10 SGTF-T<sub>over</sub> subjects;

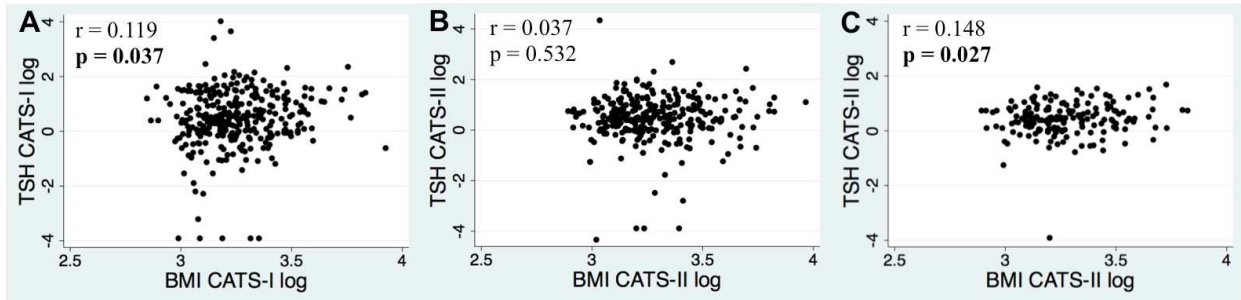
<sup>4</sup> = linear regression adjusted also for child's height;

<sup>5</sup> = linear regression adjusted also for the corresponding maternal parameter. For BMI SDS used mother's BMI. For bone DXA scan standard deviation scores used the maternal BMD of the same area.

**A** = Alphabet study reference cohort. **AI** = augmentation index. **BMAD** = bone mineral apparent density. **BMD** = bone mineral density. **BMI** = body mass index. **BMI-SDS** = standard deviation score of body mass index. **DP** = diastolic blood pressure. **DXA** = dual-energy x-ray absorptiometry. **FN** = femoral neck. **FT3** = free-triiodothyronine. **FT4** = free-thyroxine. **LS** = lumbar spine (from L1 to L4). **NA** = Not Applicable. **PWV** = aortic pulse wave velocity. **SGTF-Topt** = children of women with suboptimal gestational thyroid function optimally treated with levothyroxine during pregnancy. **SGTF-Tover** = children of women with suboptimal gestational thyroid function over-treated with levothyroxine during pregnancy. **SDS** = standard deviation score. **SP** = systolic blood pressure. **TPOAb** = autoantibodies to thyroid peroxidase. **TSH** = thyrotropin. **W** = Ward study reference cohort. **WBLH** = whole body less head.



## Supplemental Figure 1. Correlation between BMI and TSH: mothers



**Panel A:** entire women cohort at the Controlled Antenatal Thyroid Screening study I (CATS-I). **Panel B:** entire women cohort at the Controlled Antenatal Thyroid Screening study II (CATS-II). **Panel C:** women cohort at CATS-II excluding those with suboptimal gestational thyroid function treated with levothyroxine during pregnancy (SGTF-T).