Study details	Population	Interventions	Study outcomes	Appraisal and Funding
			• Internalising score (T0 – T1) 61.00 [±12.21] versus 54.56 [±10.22], *F* (*df, errdf*), *P*: 22.93 (1,52), p<0.001. • Externalising score (T0 – T1) 58.04 [±12.99] versus 53.81 [±11.86], *F* (*df, errdf*), *P*: 12.04 (1,52), p=0.001. There was no statistically significant difference between sex assigned at birth males and sex assigned at birth females for total and internalising CBCL score but there was a significant difference for the externalising score: • Externalising score, *F* (*df, errdf*), *P*: 6.29 (1,52), p=0.015. There was a statistically significant decrease in mean (±SD) total, internalising, and externalising[3] YSR scores between T0 and T1 for all adolescents (n=54): • Total score (T0 – T1) 55.46 [±11.56] versus 50.00 [±10.56], *F* (*df, errdf*), *P*: 16.24 (1,52), p<0.001. • Internalising score (T0 – T1) 56.04 [±12.49] versus 49.78 [±11.63], *F* (*df, errdf*), *P*: 15.05 (1,52), p<0.001. • Externalising score (T0 – T1) 53.30 [±11.87] versus 49.98 [±9.35], *F* (*df, errdf*), *P*: 7.26 (1,52), p=0.009. There was no statistically significant difference between sex assigned at birth males and sex assigned at birth females for total and internalising YSR score but there was a significant difference for the externalising score: • Externalising score, *F* (*df, errdf*), *P*: 9.14 (1,52), p=0.004. There was a statistically significant increase in CGAS mean (±SD) score between T0 and T1 (n=41), 70.24 [±10.12] versus 73.90 [±9.63], *F* (*df, errdf*), *P*: 8.76	

Study details	Population	Interventions	Study outcomes	Appraisal and Funding
			(1,39), p=0.005. There was a statistically significant difference between sex assigned at birth males and sex assigned at birth females, with sex assigned at birth females reporting lower score for global functioning compared with sex assigned at birth males, *F* (*df, errdf*), *P*: 5.77 (1,52), p=0.021. The proportion of adolescents scoring in the clinical range significantly decreased between T0 and T1, on the CBCL total problem scale (44.4% versus 22.2%, X^2[1] = 6.00, p=0.001), and the internalising scale (29.6% versus 11.1%, X^2[1] = 5.71, p=0.017) of the YSR.	

[1] There were statistically significant mean age [±SD] differences between sex assigned at birth males and sex assigned at birth females for age at assessment (13.14 [±1.55] versus 14.10 [±1.99] years, p=0.028), age at start of GnRH analogues (14.25 [±1.79] versus 15.21 [±1.95] years, p=0.036) and age at the start of gender-affirming hormones (16.24 [±1.21] versus 16.99 [±1.09] years, p=0.008). No statistically significant differences were seen for other baseline characteristics, time between GnRH analogue and gender-affirming hormones, full scale IQ, parental marital status, education, and sexual attraction to own, other or both sexes.
[2] Independent t-tests between mean scores on the CBCL, YSR, BDI, TPI, STAI, CGAS, UGS, and BIS of adolescents who completed both assessments and mean scores of adolescents who completed only one of the assessments revealed no significant differences on all used measures, at neither T0 or at T1.
[3] The CBCL/YSR has 2 components: Internalising score which sums the anxious/depressed, withdrawn-depressed, and somatic complaints scores; externalising score which sums rule-breaking and aggressive behaviour. The total problems score is the sum of the scores of all the problem items. The YSR is a child self-report version of the CBCL.
[4] A repeated measures ANOVA (analysis of variance) was used.

Study details	Population	Interventions	Study outcomes	Appraisal and Funding
Joseph T, Ting J, Butler G. (2019) The effect of GnRH analogue treatment on bone mineral density in young adolescents with gender dysphoria: findings from a large national cohort. Journal of pediatric endocrinology & metabolism 32(10): 1077-1081 United Kingdom	Adolescents (12 to 14 years) with gender dysphoria (no diagnostic criteria described), n=70, including 31 transfemales and 39 transmales. All had been seen and assessed by a Gender Identity Development Service multi-disciplinary psychosocial health team for at least 4 assessments over a minimum of 6 months. All participants had entered puberty	Treatment with a GnRH analogue for at least 1 year or ongoing until they reached 16 years. No specific treatment, dose or route of administration reported. No concomitant treatments were reported.	**Critical outcomes** No critical outcomes assessed. **Important outcomes** ***Bone density: lumbar*[1]** **Lumbar spine bone mineral apparent density (BMAD)[2] 0 to 1 year** Transfemales (mean [±SD]): 0.235 (0.030) g/cm3 at baseline, 0.233 g/cm3 (0.029) at 1 year (p=0.459); z-score 0.859 (0.154) at baseline, −0.228 (1.027) at 1 year (p=0.000) Transmales (mean [±SD]):	This study was appraised using the Newcastle-Ottawa quality assessment checklist for cohort studies. **Domain 1: Selection** 1. Somewhat representative of children and adolescents who have gender dysphoria 2. Not applicable 3. Via routine clinical records 4. No

Study details	Population	Interventions	Study outcomes	Appraisal and Funding
Retrospective longitudinal observational single centre study To investigate whether there is any significant loss of bone mineral density (BMD) and bone mineral apparent density (BMAD) for up to 3 years of GnRH analogues. To investigate whether there was a significant drop after 1 year of treatment following abrupt withdrawal. 2011 to 2016	and all but 2 of the transmales were postmenarchal. 57% of the transfemales were in early puberty (G2–3 and testicular volume >4 mL) and 43% were in late puberty (G4–5). Details of the sampling frame were not reported. Further details of how the sample was drawn are not reported.	No comparator.	0.196 (0.035) g/cm3 at baseline, 0.201 (0.033) g/cm3 at 1 year (p=0.074); z-score −0.186 (1.230) at baseline, −0.541 (1.396) at 1 year (p=0.006) **Lumbar spine BMAD 0 to 2 years** Transfemales (mean [±SD]): 0.240 (0.027) g/cm3 at baseline, 0.240 (0.030) g/cm3 at 2 years (p=0.865); z-score 0.486 (0.809) at baseline, −0.279 (0.930) at 2 years (p=0.000) Transmales (mean [±SD]): 0.195 (0.058) g/cm3 at baseline, 0.198 (0.055) at 2 years (p=0.433); z-score −0.361 (1.439) at baseline, −0.913 (1.318) at 2 years (p=0.001) **Lumbar spine bone mineral density (BMD) 0 to 1 year** Transfemales (mean [±SD]): 0.860 (0.154) kg/m2 at baseline, 0.859 (0.129) kg/m2 at 1 year (p=0.962); z-score −0.016 (1.106) at baseline, −0.461 (1.121) at 1 year (p=0.003) Transmales (mean [±SD]): 0.694 (0.149) kg/m2 at baseline, 0.718 (0.124) kg/m2 at 1 year (p=0.006); z-score −0.395 (1.428) at baseline, −1.276 (1.410) at 1 year (p=0.000) **Lumbar spine BMD 0 to 2 years** Transfemales (mean [±SD]): 0.867 (0.141) kg/m2 at baseline, 0.878 (0.130) kg/m2 at 2 years (p=0.395); z-score 0.130 (0.972) at baseline, −0.890 (1.075) at 2 years (p=0.000) Transmales (mean [±SD]): 0.695 (0.220) kg/m2 at baseline, 0.731 (0.209) kg/m2 at 2 years (p=0.058); z-score −0.715 (1.406) at baseline, −2.000 (1.384) at 2 years (p=0.000) ***Bone density: femoral***	**Domain 2: Comparability** 1. No control group **Domain 3: Outcome** 1. Via routine clinical records 2. Yes 3. No statement **Overall quality is assessed as poor**. Other comments: although the evidence is of poor quality, the results suggest a possible association between GnRH analogues and BMAD. However, the results are not reliable and could be due to bias or chance. Further details of how the sample was drawn are not reported. No concomitant treatments were reported. Source of funding: None disclosed

Study details	Population	Interventions	Study outcomes	Appraisal and Funding
			Femoral neck (hip) BMD 0 to 1 year Transfemales (mean [±SD]): 0.894 (0.118) kg/m2 at baseline, 0.905 (0.104) kg/m2 at 1 year (p=0.571); z-score 0.157 (0.905) at baseline, −0.340 (0.816) at 1 year (p=0.002) Transmales (mean [±SD]): 0.772 (0.137) kg/m2 at baseline, 0.785 (0.120) kg/m2 at 1 year (p=0.797); z-score −0.863 (1.215) at baseline, −1.440 (1.075) at 1 year (p=0.000) **Femoral neck (hip) BMD 0 to 2 years** Transfemales (mean [±SD]): 0.920 (0.116) kg/m2 at baseline, 0.910 (0.125) kg/m2 at 2 years (p=0.402); z-score 0.450 (0.781) at baseline, −0.600 (1.059) at 2 years (p=0.002) Transmales (mean [±SD]): 0.766 (0.215) kg/m2 at baseline, 0.773 (0.197) at 2 years (p=0.604); z-score −1.075 (1.145) at baseline, −1.779 (0.816) at 2 years (p=0.001)	

[1] Lumbar spine (L1-L4) BMD was measured by yearly dual energy X-ray absorptiometry (DXA) scans at baseline (n=70), 1 year (n=70), and 2 years (n=31).
[2] BMAD is a size adjusted value of BMD incorporating body size measurements using UK norms in growing adolescents. Reported as g/cm3 and z-scores. Hip BMAD z-scores were not calculated as there were no available reference ranges.

Study details	Population	Interventions	Study outcomes	Appraisal and Funding
Khatchadourian K, Shazhan A, Metzger D. (2014) Clinical management of youth with gender dysphoria in Vancouver. The Journal of Pediatrics 164 (4): 906-11. Canada Retrospective observational chart review single centre study	27 young people with gender dysphoria who started GnRH analogues (at mean age [±SD] 14.7±1.9 years) out of 84 young people seen at the unit between 1998 and 2011. Note: the transmale and transfemale subgroups reported in the paper is discrepant, 15 transmales and 11 transfemales (n=26) reported in the outcomes section rather than the n=27	**Intervention** 84 young people with gender dysphoria were included. For GnRH analogues no specific treatment, dose or route of administration reported. **Comparison** No comparator.	**Critical Outcomes** No critical outcomes assessed. **Important outcomes** ***Stopping treatment*** The authors report that of 15 transmales taking GnRH analogues: • 14 transitioned to testosterone treatment during the observation period • 7 continued taking GnRH analogues after starting testosterone	This study was appraised using the Newcastle-Ottawa tool for cohort studies. **Domain 1: Selection** 1. not reported 2. no non-exposed cohort 3. secure record 4. no **Domain 2: Comparability** 1. not applicable **Domain 3: Outcome**

Study details	Population	Interventions	Study outcomes	Appraisal and Funding
	stated in the paper; complete outcome reporting is also incomplete for the transfemale group. Inclusion criteria were at least Tanner stage 2 pubertal development, previous assessment by a mental health professional and a confirmed diagnosis of gender dysphoria (diagnostic criteria not specified). No exclusion criteria are specified.		• 7 discontinued GnRH analogues after a median of 3.0 years (range 0.2 to 9.2 years), of which: ○ 5 discontinued after hysterectomy and salpingo-oophorectomy ○ 1 discontinued after 2.2 years (transitioned to gender-affirming hormone) ○ 1 discontinued after <2 months due to mood and emotional lability The authors report that of 11 transfemales taking GnRH analogues: • 5 received oestrogen treatment during the observation period • 4 continued taking GnRH analogues during oestrogen treatment • 1 discontinued GnRH analogues during oestrogen treatment (no reason reported) • 1 stopped GnRH analogues after a few months due to emotional lability • 1 stopped GnRH analogues before oestrogen treatment (the following year delayed due to heavy smoking) • 1 discontinued GnRH analogues after 13 months due to choosing not to pursue transition ***Safety*** Of the 27 patients treated with GnRH analogues: • 1 transmale participant developed sterile abscesses; they were switched from leuprolide acetate to triptorelin, and this was well tolerated. • 1 transmale participant developed leg pains and headaches on GnRH analogues, which eventually resolved without treatment.	1. record linkage 2. yes 3. in complete missing data **Overall quality is assessed as poor.** Other comments: mental health comorbidity was reported for all participants but not for the GnRH analogue cohort separately. Concomitant use of other medicines was not reported. Source of funding: No source of funding identified.

Study details	Population	Interventions	Study outcomes	Appraisal and Funding
			• 1 participant gained 19 kg within 9 months of initiating GnRH analogues, although their body mass index was >85 percentile before GnRH analogues.	

Study details	Population	Interventions	Study outcomes	Appraisal and Funding
Klink D, Caris M, Heijboer A et al. (2015) Bone mass in young adulthood following gonadotropin-releasing hormone analog treatment and cross-sex hormone treatment in adolescents with gender dysphoria. The Journal of clinical endocrinology and metabolism 100(2): e270-5 Netherlands Retrospective longitudinal observational single centre study To assess BMD development during GnRH analogues and at age 22 years in adolescents with gender dysphoria who started treatment for gender dysphoria during adolescence. 1998 to 2012	34 adolescents (mean age ±SD 14.9±1.9 for transfemales and 15.0±2.0 for transmales at start of GnRH analogues). Participants were included if they met DSM-IV-TR criteria for gender identity disorder of adolescence and had been treated with GnRH analogues and gender-affirming hormones during their pubertal years. No concomitant treatments were reported.	The intervention was GnRH analogue monotherapy (triptorelin pamoate 3.75 mg subcutaneously every 4 weeks) followed by gender-affirming hormones from 16 years with discontinuation of GnRH analogue after gonadectomy. Median duration of GnRH analogue monotherapy in transfemales was 1.3 years (range, 0.5 to 3.8 years), and in transmales was 1.5 years (range, 0.25 to 5.2 years).	**Critical outcomes** No critical outcomes assessed. **Important outcomes** ***Bone density: lumbar*** **Lumbar spine bone mineral apparent density (BMAD)[1]** Change from starting GnRH analogue (mean age 14.9±1.9) to starting gender-affirming hormones (mean age 16.6±1.4) in transfemales (mean [±SD]): GnRH analogue: 0.22 (0.03) g/cm3, gender-affirming hormones: 0.22 (0.02) g/cm3 (NS); z-score GnRH analogue: −0.44 (1.10), gender-affirming hormones: −0.90 (0.80) (p=NS) Change from starting GnRH analogue (mean age 15.0±2.0) to starting gender-affirming hormones (mean age 16.4±2.3) in transmales (mean [±SD]: GnRH analogue: 0.25 (0.03) g/cm3, gender-affirming hormones: 0.24 (0.02) g/cm3 (NS); z-score GnRH analogue: 0.28 (0.90), gender-affirming hormones: −0.50 (0.81) (p=0.004) **Lumbar spine bone mineral density (BMD)[1]** Change from starting GnRH analogue (mean age 14.9±1.9) to starting gender-affirming hormones (mean age	This study was appraised using the Newcastle-Ottawa quality assessment checklist for cohort studies. **Domain 1: Selection** 1. somewhat representative of children and adolescents who have gender dysphoria 2. not applicable 3. via routine clinical records 4. no **Domain 2: Comparability** 1. no control group **Domain 3: Outcome** 1. via routine clinical records 2. yes 3. follow-up rate variable across timepoints and no description of those lost **Overall quality is assessed as poor**. Other comments: Within person comparison. Small numbers of participants in each subgroup. No concomitant treatments or comorbidities were reported. Source of funding: None disclosed

Study details	Population	Interventions	Study outcomes	Appraisal and Funding
			16.6±1.4) in transfemales (mean [±SD]): GnRH analogue: 0.84 (0.13) g/m2, gender-affirming hormones: 0.84 (0.11) g/m2 (NS); z-score GnRH analogue: −0.77 (0.89), gender-affirming hormones: −1.01 (0.98) (NS) Change from starting GnRH analogue (mean age 15.0±2.0) to starting gender-affirming hormones (mean age 16.4±2.3) in transmales (mean [±SD]): GnRH analogue: 0.95 (0.12) g/m2, gender-affirming hormones: 0.91 (0.10) g/m2 (p=0.006); z-score GnRH analogue: 0.17 (1.18), gender-affirming hormones: −0.72 (0.99) (p<0.001) ***Bone density; femoral*** **Femoral area BMAD[1]** Change from starting GnRH analogue (mean age 14.9±1.9) to starting gender-affirming hormones (mean age 16.6±1.4) in transfemales (mean [±SD]), GnRH analogue: 0.28 (0.04) g/cm3, gender-affirming hormones: 0.26 (0.04) g/cm3 (NS); z-score GnRH analogue: −0.93 (1.22), gender-affirming hormones: −1.57 (1.74) (p=NS) Change from starting GnRH analogue (mean age 15.0±2.0) to starting gender-affirming hormones (mean age 16.4±2.3) in transmales (mean [±SD]), GnRH analogue: 0.32 (0.04) g/cm3, gender-affirming hormones: 0.31 (0.04) (NS); z-score GnRH analogue: 0.01 (0.70), gender-affirming hormones: −0.28 (0.74) (NS)	

Study details	Population	Interventions	Study outcomes	Appraisal and Funding
			Femoral area BMD[1] Change from starting GnRH analogue (mean age 14.9±1.9) to starting gender-affirming hormones (mean age 16.6±1.4) in transfemales (mean [±SD]), GnRH analogue: 0.88 (0.12) g/m2, gender-affirming hormones: 0.87 (0.08) (NS); z-score GnRH analogue: −0.66 (0.77), gender-affirming hormones: −0.95 (0.63) (NS) Change from starting GnRH analogue (mean age 15.0±2.0) to starting gender-affirming hormones (mean age 16.4±2.3) in transmales (mean [±SD]), GnRH analogue: 0.92 (0.10) g/m2, gender-affirming hormones: 0.88 (0.09) (p=0.005); z-score GnRH analogue: 0.36 (0.88), gender-affirming hormones: −0.35 (0.79) (p=0.001)	

[1] BMD and BMAD of the lumbar spine and femoral region (nondominant side) measured by DXA scans at start of GnRH analogues, (n=32), start of gender-affirming hormones (n=34), and at 22 years (n=34).

Study details	Population	Interventions	Study outcomes	Appraisal and Funding
Schagen SEE, Cohen-Kettenis PT, Delemarre-van de Waal HA et al. (2016) Efficacy and Safety of Gonadotropin-Releasing Hormone Agonist Treatment to Suppress Puberty in Gender Dysphoric Adolescents. The journal of sexual medicine 13(7): 1125-32	Adolescents with gender dysphoria (n=116), median age (range) 13.6 years (11.6 to 17.9) in transfemales and 14.2 years (11.1 to 18.6) in transmales during first year of GnRH analogues. Participants were included if they met DSM-IV-TR criteria for gender dysphoria, had lifelong extreme gender dysphoria, were psychologically stable and were living in a supportive environment. No concomitant treatments were	GnRH analogue monotherapy (triptorelin pamoate 3.75 mg at 0, 2 and 4 weeks followed by injections every 4 weeks, route of administration not described) for at least 3 months.	**Critical outcomes** No critical outcomes assessed. **Important outcomes** ***Other safety outcomes: liver function*** Glutamyl transferase was not elevated at baseline or during treatment in any subject. Mild elevations of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) above the reference range were present at baseline but were not more prevalent during treatment than at baseline. Glutamyl transferase, AST, and ALT	This study was appraised using the Newcastle-Ottawa quality assessment checklist for cohort studies. **Domain 1: Selection** 1. somewhat representative of children and adolescents who have gender dysphoria 2. not applicable 3. via routine clinical records 4. no **Domain 2: Comparability** 1. no control group

Study details	Population	Interventions	Study outcomes	Appraisal and Funding
Netherlands Prospective longitudinal study To describe the changes in Tanner stage, testicular volume, gonadotropins, and sex steroids during GnRH analogues of adolescents with gender dysphoria to evaluate the efficacy. To report on liver enzymes, renal function and changes in body composition. 1998 to 2009	reported.		levels did not significantly change from baseline to 12 months of treatment. No values or statistical analyses were reported. ***Other safety outcomes: kidney function*** **Change in serum creatinine between 0 and 1 year** Transfemales (mean [±SD]): 70 (12) micromol/l at baseline, 66 (13) micromol/l at 1 year (p=0.20) Transmales (mean [±SD]): 73 (8) micromol/l at baseline, 68 (13) micromol/l at 1 year (p=0.01)	**Domain 3: Outcome** 1. via routine clinical records 2. yes 3. no statement **Overall quality is assessed as poor**. Other comments: Within person comparison. No concomitant treatments or comorbidities were reported. Source of funding: Ferring pharmaceuticals (triptorelin manufacturer)

Study details	Population	Interventions	Study outcomes	Appraisal and Funding
Staphorsius A, Baudewijntje P, Kreukels P, et al. (2015) Puberty suppression and executive functioning: an fMRI-study in adolescents with gender dysphoria. Psychoneuroendocrinology 565:190-9. Netherlands Cross-sectional (single time point) assessment single centre study	The inclusion criteria were diagnosed with Gender Identity Disorder according to the DSM-IV-TR and at least 12 years old and Tanner stage of at least B2 or G2 to G3 with measurable oestradiol and testosterone levels in girls and boys, respectively. For all group's exclusion criteria were an insufficient command of the Dutch language (how assessed not reported), unadjusted endocrine disorders, neurological or psychiatric disorders that could lead to deviant test results (details not reported) use	**Intervention** GnRH analogues (triptorelin pamoate 3.75 mg every 4 weeks subcutaneously or intramuscularly). **Comparison** The comparison was between adolescents with gender dysphoria receiving GnRH analogues and those without GnRH	**Critical Outcomes** No critical outcomes assessed. **Important outcomes** ***Psychosocial impact*** The Child Behaviour Checklist (CBCL) was used to assess psychosocial impact. The CBCL was administered once during the study. The reported outcomes for each group were (n, mean [±SD]): • Transfemales (all, n=18) 57.8 [±9.2] • Transfemales on GnRH analogues (n=8) 57.4 [±9.8] • Transfemales without GnRH analogues (n=10) 58.2 [±9.3]	This study was appraised using the Newcastle-Ottawa tool for cohort studies. **Domain 1: Selection domain** 1. somewhat representative of children and adolescents who have gender dysphoria 2. drawn from the same community as the exposed cohort 3. via routine clinical records 4. no **Domain 2: Comparability** 1. study controls for age and diagnosis

Study details	Population	Interventions	Study outcomes	Appraisal and Funding
	of psychotropic medication, and contraindications for an MRI scan. Additionally, adolescents receiving puberty delaying medication or any form of hormones besides oral contraceptives were excluded as controls. The sample size was 85 of whom 41 were adolescents (the numbers are discrepant with the number for whom outcomes are reported n=40) with gender dysphoria (20 of whom were being treated with GnRH analogues); 24 girls and 21 boys without gender dysphoria acted as controls (not further reported here). Details of the sampling frame are not reported. The ages at which GnRH analogues were started was not reported. The mean duration of treatment was 1.6 years (SD 1.0) Mean (±SD) Tanner stage for each group was reported: • Transfemales 3.9 [±1.1] • Transfemales on GnRH analogues 4.1 [±1.0] • Transfemales without GnRH analogues 3.8 [±1.1] • Transmales 4.5 [±0.9] • Transmales on GnRH analogues 4.1 [±1.1] Transmales without GnRH analogues 4.9 [±0.3]	analogues.	• Transmales (all, n=22) 60.4 [±10.2] • Transmales on GnRH analogues (n=12) 57.5 [±9.4] • Transmales without GnRH analogues (n=10) 63.9 [±10.5] The analysis of the CBCL data is not discussed, and statistical analysis is unclear. ***Cognitive development or functioning*** **IQ[1]** • Transfemales (mean [±SD]) on GnRH analogues: 94.0 (10.3) • Transfemales (mean [±SD]) without GnRH analogues: 109.4 (21.2) • Transmales (mean [±SD]) on GnRH analogues: 95.8 (15.6) • Transmales (mean [±SD]) without GnRH analogues: 98.5 (15.9) **Reaction time[2]** • Transfemales (mean [±SD]) on GnRH analogues: 10.9 (4.1) • Transfemales (mean [±SD]) without GnRH analogues: 9.9 (3.1) • Transmales (mean [±SD]) on GnRH analogues: 9.9 (3.1) • Transmales (mean [±SD]) without GnRH analogues: 10.0 (2.0) **Accuracy[3]** • Transfemales (mean [±SD]) on GnRH analogues: 73.9 (9.1) • Transfemales (mean [±SD]) without GnRH analogues: 83.4 (9.5) • Transmales (mean [±SD]) on GnRH analogues: 85.7 (10.5) • Transmales (mean [±SD]) without	**Domain 3: Outcome** 1. via clinical assessment 2. yes 3. unclear **Overall quality is assessed as poor.** Other comments: Physical and psychological comorbidity was not reported, concomitant use of other medicines was not reported. Source of funding: This work was supported by an educational grant from the pharmaceutical firm Ferring BV, and by a VICI grant (453-08-003) from the Dutch Science Foundation. The authors state that funding sources did not play a role in any component of this study.

Study details	Population	Interventions	Study outcomes	Appraisal and Funding
			GnRH analogues: 88.8 (9.7)	

[1] Estimated with 4 subscales (arithmetic, vocabulary, picture arrangement, and block design) of the Wechsler Intelligence Scale for Children, third edition (WISC-III®, Wechsler 1991) or the Wechsler Adult Intelligence Scale, third edition (WAIS-III®, Wechsler 1997), depending on the participant's age.
[2] Reaction time in seconds in the Tower of London task
[3] Percentage of correct trials in the Tower of London task

Study details	Population	Interventions	Study outcomes	Appraisal and Funding
Vlot, Mariska C, Klink, Daniel T, den Heijer, Martin et al. (2017) Effect of pubertal suppression and cross-sex hormone therapy on bone turnover markers and bone mineral apparent density (BMAD) in transgender adolescents. Bone 95: 11-19 Netherlands Retrospective observational data analysis study To investigate the course of 3 bone turnover markers in relation to bonemineral density, in adolescents with gender dysphoria during GnRH analogue and gender-affirming hormones. 2001 to 2011	Adolescents with gender dysphoria, n=70. Median age (range) 15.1 years (11.7 to 18.6) for transmales and 13.5 years (11.5 to 18.3) for transfemales at start of GnRH analogues. Participants were included if they had a diagnosis of gender dysphoria according to DSM-IV-TR criteria who were treated with GnRH analogues and then gender-affirming hormones. No concomitant treatments were reported. The study categorised participants into a young and old pubertal group, based on their bone age. The young transmales had a bone age of <14 years and the old transmales had a bone age of ≥14 years. The young transfemales group had a bone age of <15 years and the old transfemales group ≥15 years.	GnRH analogues (triptorelin pamoate 3.75 mg every 4 weeks subcutaneously).	**Critical outcomes** No critical outcomes reported **Important outcomes** ***Bone density: lumbar*** **Lumbar spine bone mineral apparent density (BMAD)** Change from starting GnRH analogue to starting gender-affirming hormones in transfemales (bone age of <15 years; median [range]), GnRH analogue: 0.21 (0.17 to 0.25) g/cm3, gender-affirming hormones: 0.20 (0.18 to 0.24) g/cm3 (NS); z-score GnRH analogue: −0.20 (−1.82 to 1.18), gender-affirming hormones: −1.52 (−2.36 to 0.42) (p=0.001) Change from starting GnRH analogue to starting gender-affirming hormones in transfemales (bone age of ≥15; median [range]), GnRH analogue: 0.22 (0.18 to 0.25) g/cm3, gender-affirming hormones: 0.22 (0.19 to 0.24) g/cm3 (NS); z-score GnRH analogue: −1.18 (−1.78 to 1.09), gender-affirming hormones: −1.15 (−2.21 to 0.08) (p≤0.1) Change from starting GnRH analogue to starting gender-affirming hormones in transmales (bone age of <15 years; median [range]), GnRH analogue: 0.23 (0.20 to 0.29) g/cm3, gender-affirming	This study was appraised using the Newcastle-Ottawa quality assessment checklist for cohort studies. **Domain 1: Selection** 1. Somewhat representative of children and adolescents who have gender dysphoria 2. Not applicable 3. Via routine clinical records 4. No **Domain 2: Comparability** 1. No control group **Domain 3: Outcome** 1. Via routine clinical records 2. Yes 3. Follow-up rate variable across outcomes and no description of those lost **Overall quality is assessed as poor**. Other comments: Within person comparison. No concomitant treatments were reported. Source of funding: grant from Abbott diagnostics

Study details	Population	Interventions	Study outcomes	Appraisal and Funding
			hormones: 0.23 (0.19 to 0.28) g/cm3 (NS); z-score GnRH analogue: −0.05 (−0.78 to 2.94), gender-affirming hormones: −0.84 (−2.20 to 0.87) (p=0.003) Change from starting GnRH analogue to starting gender-affirming hormones in transmales (bone age of ≥15; median [range]), GnRH analogue: 0.26 (0.21 to 0.29) g/cm3, gender-affirming hormones: 0.24 (0.20 to 0.28) g/cm3 (p≤0.01); z-score GnRH analogue: 0.27 (−1.60 to 1.80), gender-affirming hormones: −0.29 (−2.28 to 0.90) (p≤ 0.0001) ***Bone density; femoral*** **Femoral neck BMAD** Change from starting GnRH analogue to starting gender-affirming hormones in transfemales (bone age of <15 years; median [range]), GnRH analogue: 0.29 (0.20 to 0.33) g/cm3, gender-affirming hormones: 0.27 (0.20 to 0.33) g/cm3 (p≤0.1); z-score GnRH analogue: −0.71 (−3.35 to 0.37), gender-affirming hormones: −1.32 (−3.39 to 0.21) (p≤0.1) Change from starting GnRH analogue to starting gender-affirming hormones in transfemales (bone age of ≥15; median [range]), GnRH analogue: 0.30 (0.26 to 0.36) g/cm3, gender-affirming hormones: 0.30 (0.26 to 0.34) g/cm3 (NS); z-score GnRH analogue: −0.44 (−1.37 to 0.93), gender-affirming hormones: −0.36 (−1.50 to 0.46) (NS) Change from starting GnRH analogue to starting gender-affirming hormones in transmales (bone age of <15 years; median [range]),	

Study details	Population	Interventions	Study outcomes	Appraisal and Funding
			GnRH analogue: 0.31 (0.26 to 0.36) g/cm3, gender-affirming hormones: 0.30 (0.22 to 0.35) g/cm3 (NS); z-score GnRH analogue: −0.01 (−1.30 to 0.91), gender-affirming hormones: −0.37 (−2.28 to 0.47) (NS) Change from starting GnRH analogue to starting gender-affirming hormones in transmales (bone age of ≥15; median [range]), GnRH analogue: 0.33 (0.25 to 0.39) g/cm3, gender-affirming hormones: 0.30 (0.23 to 0.41) g/cm3 (p≤0.01); z-score GnRH analogue: 0.27 (−1.39 to 1.32), gender-affirming hormones: −0.27 (−1.91 to 1.29) (p=0.002)	

Appendix F Quality appraisal checklists

Newcastle-Ottawa tool for cohort studies

Question	
Domain: Selection	
1. Representativeness of the exposed cohort	Truly representative of the average [describe] in the community Somewhat representative of the average [describe] in the community Selected group of users e.g. nurses, volunteers No description of the derivation of the cohort
2. Selection of the non-exposed cohort	Drawn from the same community as the exposed cohort Drawn from a different source No description of the derivation of the non-exposed cohort
3. Ascertainment of exposure	Secure record (e.g. surgical records) Structured interview Written self-report No description
4. Demonstration that outcome of interest was not present at start of study	Yes / No
Domain: Comparability	
1. Comparability of cohorts on the basis of the design or analysis	Study controls for [select most important factor] Study controls for any additional factor [this criteria could be modified to indicate specific control for a second important factor]
Domain: Outcome	
1. Assessment of outcome	Independent blind assessment Record linkage Self-report No description
2. Was follow-up long enough for outcomes to occur	Yes [select and adequate follow up period for outcome of interest] No
3. Adequacy of follow up of cohorts	Complete follow up (all subjects accounted for) Subjects lost to follow up unlikely to introduce bias (small number lost to follow up [select an adequate %] follow up or description provided of those lost) Follow up rate [select an adequate %] and no description of those lost No statement

Appendix G Grade profiles

Table 2: Question 1. For children and adolescents with gender dysphoria, what is the clinical effectiveness of treatment with GnRH analogues compared with one or a combination of psychological support, social transitioning to the desired gender or no intervention? – gender dysphoria

QUALITY					Summary of findings			IMPORTANCE	CERTAINTY
					No of events/No of patients (n/N%)		Effect		
Study	Risk of bias	Indirectness	Inconsistency	Imprecision	Intervention	Comparator	Result		
Impact on gender dysphoria									
Mean±SD Utrecht Gender Dysphoria Scale[1] (version(s) not reported), time point at baseline (before GnRH analogues) versus follow-up (before gender-affirming hormones, higher scores indicate more gender dysphoria)									
1 cohort study de Vries et al 2011	Serious limitations[2]	No serious indirectness	Not applicable	Not calculable	N=41	None	Baseline: 53.20±7.91 GnRH analogue: 53.9±17.42 *P*=0.333	Critical	VERY LOW

Abbreviations: GnRH, gonadotrophin releasing hormone; *P*, P-value; SD, Standard deviation.

1 The UGDS is a validated screening tool for both adolescents and adults to assess gender dysphoria. It consists of 12 items, to be answered on a 1- to 5-point scale, resulting in a sum score between 12 and 60. The higher the UGDS score the greater the gender dysphoria.
2 Downgraded 1 level - the cohort study by de Vries et al. (2011) was assessed as at high risk of bias (poor quality overall; lack of blinding and no control group).

Table 3: Question 1. For children and adolescents with gender dysphoria, what is the clinical effectiveness of treatment with GnRH analogues compared with one or a combination of psychological support, social transitioning to the desired gender or no intervention? – mental health

QUALITY					Summary of findings			IMPORTANCE	CERTAINTY
					No of events/No of patients (n/N%)		Effect		
Study	Risk of bias	Indirectness	Inconsistency	Imprecision	Intervention	Comparator	Result		
Impact on mental health									

QUALITY					Summary of findings			IMPORTANCE	CERTAINTY
					No of events/No of patients (n/N%)		Effect		
Study	Risk of bias	Indirectness	Inconsistency	Imprecision	Intervention	Comparator	Result		
Mean±SD Beck Depression Inventory-II, time point at baseline (before GnRH analogues) versus follow-up (just before gender-affirming hormones). (Lower scores indicate benefit)									
1 cohort study de Vries et al 2011	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	N=41	None	Baseline: 8.31±7.12 GnRH analogue: 4.95±6.72 *P*=0.004	Critical	VERY LOW
Mean±SD Trait Anger (TPI), time point at baseline (before GnRH analogues) versus follow-up (just before gender-affirming hormones, lower scores indicate benefit)									
1 cohort study de Vries et al 2011	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	N=41	None	Baseline: 18.29±5.54 GnRH analogue: 17.88±5.24 *P*=0.503	Critical	VERY LOW
Mean±SD Trait Anxiety (STAI), time point at baseline (before GnRH analogues) versus follow-up (just before gender-affirming hormones, lower scores indicate benefit)									
1 cohort study de Vries et al 2011	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	N=41	None	Baseline: 39.43±10.07 GnRH analogue: 37.95±9.38 *P*=0.276	Critical	VERY LOW

Abbreviations: GnRH, gonadotrophin releasing hormone; *P*, P-value; SD, Standard deviation.

1 Downgraded 1 level - the cohort study by de Vries et al. (2011) was assessed as at high risk of bias (poor quality overall; lack of blinding and no control group).

Table 4: Question 1. For children and adolescents with gender dysphoria, what is the clinical effectiveness of treatment with GnRH analogues compared with one or a combination of psychological support, social transitioning to the desired gender or no intervention? – body image

QUALITY					Summary of findings			IMPORTANCE	CERTAINTY
					No of events/No of patients (n/N%)		Effect		
Study	Risk of bias	Indirectness	Inconsistency	Imprecision	Intervention	Comparator	Result		
Impact on body image									
Mean±SD Body Image Scale (primary sexual characteristics), time point at baseline (before GnRH analogues) versus follow-up (just before gender-affirming hormones, lower scores indicate benefit)									
1 cohort study de Vries et al 2011	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	N=57	None	Baseline: 4.10±0.56 GnRH analogue: 3.98±0.71 *P*=0.145	Important	VERY LOW
Mean±SD Body Image Scale (secondary sexual characteristics), time point at baseline (before GnRH analogues) versus follow-up (just before gender-affirming hormones, lower scores indicate benefit)									
1 cohort study de Vries et al 2011	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	N=57	None	Baseline: 2.74±0.65 GnRH analogue: 2.82±0.68 *P*=0.569	Important	VERY LOW
Mean±SD Body Image Scale (neutral characteristics), time point at baseline (before GnRH analogues) versus follow-up (just before gender-affirming hormones, lower scores indicate benefit)									
1 cohort study de Vries et al 2011	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	N=57	None	Baseline: 2.41±0.63 GnRH analogue: 2.47±0.56 *P*=0.620	Important	VERY LOW

Abbreviations: GnRH, gonadotrophin releasing hormone; *P*, P-value; SD, Standard deviation.

1 Downgraded 1 level - the cohort study by de Vries et al. (2011) was assessed as at high risk of bias (poor quality overall; lack of blinding and no control group).

Table 5: Question 1. For children and adolescents with gender dysphoria, what is the clinical effectiveness of treatment with GnRH analogues compared with one or a combination of psychological support, social transitioning to the desired gender or no intervention? – psychosocial impact

QUALITY					Summary of findings			IMPORTANCE	CERTAINTY
					No of events/No of patients (n/N%)		Effect		
Study	Risk of bias	Indirectness	Inconsistency	Imprecision	Intervention	Comparator	Result		
Psychosocial impact									
Mean [±SD] Children's Global Assessment Scale score, at baseline, higher scores indicate benefit)									
1 cohort study Costa et al 2015	Serious limitations[1]	No serious indirectness	No serious inconsistency	Not calculable	n=101 58.72 [±11.38]	n=100 56.63 [±13.14]	*P*=0.23	Important	VERY LOW
Mean [±SD] Children's Global Assessment Scale score, at 6 months[2] (higher scores indicate benefit).									
1 cohort study Costa et al 2015	Serious limitations[1]	No serious indirectness	No serious inconsistency	Not calculable	n=101 60.89 [±12.17]	n=100 60.29 [±12.81]	*P*=0.73	Important	VERY LOW
Mean [±SD] Children's Global Assessment Scale score, at 12 months[3] (higher scores indicate benefit).									
1 cohort study Costa et al 2015	Serious limitations[1]	No serious indirectness	No serious inconsistency	Not calculable	n=60 64.70 [±13.34]	n=61 62.97 [±14.10]	*P*=0.49	Important	VERY LOW
Mean [±SD] Children's Global Assessment Scale score, at 18 months[4] (higher scores indicate benefit).									
1 cohort study Costa et al 2015	Serious limitations[1]	No serious indirectness	No serious inconsistency	Not calculable	n=35 67.40 [±13.93]	n=36 62.53 [±13.54]	*P*=0.14	Important	VERY LOW
Mean [±SD] Children's Global Assessment Scale score, participants at 6 months compared to baseline (higher scores indicate benefit).									
1 cohort study Costa et al 2015	Serious limitations[1]	No serious indirectness	No serious inconsistency	Not calculable	N=101 N=101	None	Baseline: 58.72±11.38 6 months: 60.89±12.17 *P*=0.19	Important	VERY LOW
Mean [±SD] Children's Global Assessment Scale score, participants at 12 months compared to baseline (higher scores indicate benefit).									
1 cohort study Costa et al 2015	Serious limitations[1]	No serious indirectness	No serious inconsistency	Not calculable	N=101 N=60	None	Baseline: 58.72±11.38 12 months: 64.70±13.34 *P*=0.003	Important	VERY LOW

QUALITY					Summary of findings			IMPORTANCE	CERTAINTY
					No of events/No of patients (n/N%)		Effect		
Study	Risk of bias	Indirectness	Inconsistency	Imprecision	Intervention	Comparator	Result		
Mean [±SD] Children's Global Assessment Scale score, participants at 18 months compared to baseline (higher scores indicate benefit).									
1 cohort study Costa et al 2015	Serious limitations[1]	No serious indirectness	No serious inconsistency	Not calculable	N=101 N=35	None	Baseline: 58.72±11.38 18 months: 67.40±13.93 *P*<0.001	Important	VERY LOW
Mean [±SD] Children's Global Assessment Scale score, participants at 12 months compared to 6 months (higher scores indicate benefit).									
1 cohort study Costa et al 2015	Serious limitations[1]	No serious indirectness	No serious inconsistency	Not calculable	N=101 N=60	None	6 months: 60.89±12.17 12 months: 64.70±13.34 *P*=0.07	Important	VERY LOW
Mean [±SD] Children's Global Assessment Scale score, participants at 18 months compared to 6 months (higher scores indicate benefit).									
1 cohort study Costa et al 2015	Serious limitations[1]	No serious indirectness	No serious inconsistency	Not calculable	N=101 N=35	None	6 months: 60.89±12.17 18 months: 67.40±13.93 *P*<0.001	Important	VERY LOW
Mean [±SD] Children's Global Assessment Scale score, participants at 18 months compared to 12 months (higher scores indicate benefit).									
1 cohort study Costa et al 2015	Serious limitations[1]	No serious indirectness	No serious inconsistency	Not calculable	N=60 N=35	None	12 months: 64.70±13.34 18 months: 67.40±13.93 *P*=0.35	Important	VERY LOW
Mean [±SD] Children's Global Assessment Scale score, in all participants (including those not treated with GnRH analogues) at 6 months[2] compared to baseline (higher scores indicate benefit).									
1 cohort study Costa et al 2015	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	N=201	None	Baseline: 57.73±12.27 6 months: 60.68±12.47 *P*<0.001	Important	VERY LOW
Mean [±SD] Children's Global Assessment Scale score, in all participants (including those not treated with GnRH analogues) at 12 months[3] compared to baseline (higher scores indicate benefit).									
1 cohort study Costa et al 2015	Serious limitations[1]	No serious indirectness	No serious inconsistency	Not calculable	N=201 N=121	None	Baseline: 57.73±12.27 12 months: 63.31±14.41 *P*<0.001	Important	VERY LOW

QUALITY					Summary of findings			IMPORTANCE	CERTAINTY
					No of events/No of patients (n/N%)		Effect		
Study	Risk of bias	Indirectness	Inconsistency	Imprecision	Intervention	Comparator	Result		
Mean±SD Children's Global Assessment Scale score, in all participants (including those not treated with GnRH analogues) at 18 months[4] compared to baseline (higher scores indicate benefit).									
1 cohort study Costa et al 2015	Serious limitations[1]	No serious indirectness	No serious inconsistency	Not calculable	N=201 N=71	None	Baseline: 57.73±12.27 18 months: 64.93±13.85 *P*<0.001	Important	VERY LOW
Mean±SD Children's Global Assessment Scale score, in all participants (including those not treated with GnRH analogues) at 12 months compared to 6 months (higher scores indicate benefit).									
1 cohort study Costa et al 2015	Serious limitations[1]	No serious indirectness	No serious inconsistency	Not calculable	N=201 N=121	None	6 months: 60.68±12.47 12 months: 63.31±14.41 *P*<0.08	Important	VERY LOW
Mean±SD Children's Global Assessment Scale score, in all participants (including those not treated with GnRH analogues) at 18 months compared to 6 months (higher scores indicate benefit).									
1 cohort study Costa et al 2015	Serious limitations[1]	No serious indirectness	No serious inconsistency	Not calculable	N=201 N=71	None	6 months: 60.68±12.47 18 months: 64.93±13.85 *P*<0.02	Important	VERY LOW
Mean±SD Children's Global Assessment Scale score, in all participants (including those not treated with GnRH analogues) at 18 months compared to 12 months (higher scores indicate benefit).									
1 cohort study Costa et al 2015	Serious limitations[1]	No serious indirectness	No serious inconsistency	Not calculable	N=121 N=71	None	12 months: 63.31±14.41 18 months: 64.93±13.85 *P*<0.45	Important	VERY LOW
Mean±SD Children's Global Assessment Scale score, time point at baseline (before GnRH analogues) versus follow-up (just before gender-affirming hormones, higher scores indicate benefit).									
1 cohort study de Vries et al 2011	Serious limitations[5]	No serious indirectness	Not applicable	Not calculable	N=41	None	Baseline: 70.24±10.12 GnRH analogue: 73.90±9.63 *P*=0.005	Important	VERY LOW

QUALITY					Summary of findings			IMPORTANCE	CERTAINTY
					No of events/No of patients (n/N%)		Effect		
Study	Risk of bias	Indirectness	Inconsistency	Imprecision	Intervention	Comparator	Result		
Mean±SD Child Behaviour Checklist (total T) score, time point at baseline (before GnRH analogues) versus follow-up (just before gender-affirming hormones, lower scores indicate benefit).									
1 cohort study de Vries et al 2011	Serious limitations[5]	No serious indirectness	Not applicable	Not calculable	N=54	None	Baseline: 60.70±12.76 GnRH analogue: 54.46±11.23 *P*<0.001	Important	VERY LOW
Mean±SD Child Behaviour Checklist (internalising T) score, time point at baseline (before GnRH analogues) versus follow-up (just before gender-affirming hormones, lower scores indicate benefit).									
1 cohort study de Vries et al 2011	Serious limitations[5]	No serious indirectness	Not applicable	Not calculable	N=54	None	Baseline: 61.00±12.21 GnRH analogue: 52.1±9.81 *P*<0.001	Important	VERY LOW
Mean±SD Child Behaviour Checklist (externalising T) score, time point at baseline (before GnRH analogues) versus follow-up (just before gender-affirming hormones, lower scores indicate benefit).									
1 cohort study de Vries et al 2011	Serious limitations[5]	No serious indirectness	Not applicable	Not calculable	N=54	None	Baseline: 58.04±12.99 GnRH analogue: 53.81±11.86 *P*=0.001	Important	VERY LOW
Proportion of adolescents scoring in the clinical range Child Behaviour Checklist total problem scale, time point at baseline (before GnRH analogues) versus follow-up (just before gender-affirming hormones, lower scores indicate benefit).									
1 cohort study de Vries et al 2011	Serious limitations[5]	No serious indirectness	Not applicable	Not calculable	N=54	None	Baseline: 44.4% GnRH analogue: 22,2% *P*=0.001	Important	VERY LOW
Mean±SD Youth Self-Report (total T) score, time point at baseline (before GnRH analogues) versus follow-up (just before gender-affirming hormone, lower scores indicate benefit).									
1 cohort study de Vries et al 2011	Serious limitations[5]	No serious indirectness	Not applicable	Not calculable	N=54	None	Baseline: 55.46±11.56 GnRH analogue: 50.00±10.56 *P*<0.001	Important	VERY LOW

QUALITY					Summary of findings			IMPORTANCE	CERTAINTY
					No of events/No of patients (n/N%)		Effect		
Study	Risk of bias	Indirectness	Inconsistency	Imprecision	Intervention	Comparator	Result		
Mean±SD Youth Self-Report (internalising T) score, time point at baseline (before GnRH analogues) versus follow-up (just before gender-affirming hormones, lower scores indicate benefit).									
1 cohort study de Vries et al 2011	Serious limitations[5]	No serious indirectness	Not applicable	Not calculable	N=54	None	Baseline: 56.04±12.49 GnRH analogue: 49.78±11.63 *P*<0.001	Important	VERY LOW
Mean±SD Youth Self-Report (externalising T) score, time point at baseline (before GnRH analogues) versus follow-up (just before gender-affirming hormones, lower scores indicate benefit).									
1 cohort study de Vries et al 2011	Serious limitations[5]	No serious indirectness	Not applicable	Not calculable	N=54	None	Baseline: 53.30±11.87 GnRH analogue: 49.98±9.35 *P*=0.009	Important	VERY LOW
Proportion of adolescents scoring in the clinical range Youth Self-Report (internalising T) score, time point at baseline (before GnRH analogues) versus follow-up (just before gender-affirming hormones, lower scores indicate benefit).									
1 cohort study de Vries et al 2011	Serious limitations[5]	No serious indirectness	Not applicable	Not calculable	N=54	None	Baseline: 29.6% GnRH analogue: 11.1% *P*=0.017	Important	VERY LOW
Mean±SD Child Behaviour Checklist score, transfemales (lower scores indicate benefit									
1 cross-sectional study Staphorsius et al 2015	Serious limitations[6]	No serious indirectness	Not applicable	Not calculable	N=8	N=10	GnRH analogue: 57.4 [±9.8] No GnRH analogue: 58.2 [±9.3]	Important	VERY LOW
Mean±SD Child Behaviour Checklist score, transmales (lower scores indicate benefit)									
1 cross-sectional study Staphorsius et al 2015	Serious limitations[6]	No serious indirectness	Not applicable	Not calculable	N=12	N=10	GnRH analogues: 57.5 [±9.4] No GnRH analogue: 63.9 [±10.5]	Important	VERY LOW

Abbreviations: GnRH, gonadotrophin releasing hormone; *P*, P-value; SD, Standard deviation.

1 Downgraded 1 level - the cohort study by Costa et al. (2015) was assessed as at high risk of bias (poor quality overall; lack of blinding and no control group).

2 6 months from baseline (after 6 months of psychological support – both groups).
3 12 months from baseline (delayed eligible gender dysphoria [GD] adolescents, after 12 months of psychological support; immediately eligible GD adolescents, after 12 months of psychological support + 6 months of puberty suppression).
4 18 months from baseline (delayed eligible gender dysphoria [GD] adolescents, after 12 months of psychological support; immediately eligible GD adolescents, after 12 months of psychological support + 6 months of puberty suppression).
5 Downgraded 1 level - the cohort study by de Vries et al. (2011) was assessed as at high risk of bias (poor quality overall; lack of blinding and no control group).
6 Downgraded 1 level - the cohort study by Staphorsius et al. (2015) was assessed as at high risk of bias (poor quality overall; lack of blinding and no randomisation).

Table 6: Question 1. For children and adolescents with gender dysphoria, what is the clinical effectiveness of treatment with GnRH analogues compared with one or a combination of psychological support, social transitioning to the desired gender or no intervention? – engagement with healthcare services

QUALITY					Summary of findings			IMPORTANCE	CERTAINTY
					No of events/No of patients% (n/N%)		Effect		
Study	Risk of bias	Indirectness	Inconsistency	Imprecision	Intervention	Comparator	Result		
Engagement with healthcare services									
Number (proportion) failing to engage with health care services (did not attend clinic), at (up to) 9 years follow-up									
1 cohort study Brik et al 2018	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	9/214 (4.2%)	None	9 adolescents out of 214 failed to attend clinic and were excluded from the study (4.2%)	Important	VERY LOW
Loss to follow-up									
1 cohort study Costa et al 2015	Serious limitations[2]	No serious indirectness	Not applicable	Not calculable	201	None	The sample size at baseline and 6 months was 201, which dropped by 39.8% to 121 after 12 months and by 64.7% to 71 at 18 months follow-up. No explanation of the reasons for loss to follow-up are reported.	Important	VERY LOW

Abbreviations: GnRH, gonadotrophin releasing hormone.

1 Downgraded 1 level - the cohort study by Brik et al. (2018) was assessed as at high risk of bias (poor quality overall; lack of blinding and no control group).
2 Downgraded 1 level - the cohort study by Costa et al. (2015) was assessed as at high risk of bias (poor quality overall; lack of blinding and no control group).

Table 7: Question 1. For children and adolescents with gender dysphoria, what is the clinical effectiveness of treatment with GnRH analogues compared with one or a combination of psychological support, social transitioning to the desired gender or no intervention? – stopping treatment

QUALITY					Summary of findings			IMPORTANCE	CERTAINTY
					No of events/No of patients% (n/N%)		Effect		
Study	Risk of bias	Indirectness	Inconsistency	Imprecision	Intervention	Comparator	Result		
Stopping treatment									
Number (proportion) stopping GnRH analogues, at (up to) 9 years follow-up									
1 cohort study Brik et al 2018	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	9/143 (6.2%)	None	9/143 adolescents stopped GnRH analogues (6.2%)[2]	Important	VERY LOW
Number (proportion) stopping from GnRH analogues, at (up to) 13 years follow-up									
1 cohort study Khatchadourian et al 2014	Serious limitations[3]	No serious indirectness	Not applicable	Not calculable	11/27 (42%)	None	11/26 stopped GnRH analogues (42%)[4]	Important	VERY LOW
Number (proportion) stopping GnRH analogues but who wished to continue endocrine treatment, at (up to) 9 years follow-up									
1 cohort study Brik et al 2018	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	4/143 (2.8%)	None	4/143 adolescents stopped GnRH analogues but wished to continue treatment (2.8%)	Important	VERY LOW
Number (proportion) stopping GnRH analogues who no longer wished gender-affirming treatment, at (up to) 9 years follow-up									
1 cohort study Brik et al 2018	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	5/143 (3.5%)	None	5/143 adolescents stopped GnRH analogues and no longer wished to continue gender-affirming treatment (3.5%)	Important	VERY LOW

Abbreviations: GnRH, gonadotrophin releasing hormone.

1 Downgraded 1 level - the cohort study by Brik et al. (2018) was assessed as at high risk of bias (poor quality overall; lack of blinding and no control group).

2 Median duration of 0.8 years (range 0.1 to 3.0). Five adolescents stopped treatment because they no longer wished to receive gender-affirming treatment for various reasons. In 4 adolescents (all transmales), although they wanted to continue treatments for gender dysphoria, GnRH analogues were stopped mainly because of adverse effects (such as mood and emotional lability).
3 Downgraded 1 level - the cohort study by Khatchadourian et al. (2014) was assessed as at high risk of bias (poor quality overall; lack of blinding, no control group and high number of participants lost to follow-up).
4 Because of transitioning to gender-affirming hormones or gender-affirming surgery, adverse effects (such as mood and emotional lability) or no longer wishing to pursue transition.

Table 8. Question 2. For children and adolescents with gender dysphoria, what is the short-term and long-term safety of GnRH analogues compared with one or a combination of psychological support, social transitioning to the desired gender or no intervention? – bone density

QUALITY					Summary of findings			IMPORTANCE	CERTAINTY
					No of events/No of patients% (n/N%)		Effect		
Study	Risk of bias	Indirectness	Inconsistency	Imprecision	Intervention	Comparator	Result		
Bone density: change in lumbar BMAD									
Change in lumbar spine BMAD from baseline to 1 year in transfemales									
1 observational study Joseph et al. (2019)	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	N=31	None	Mean (SD), g/cm^3 Baseline: 0.235 (0.030) 1 year: 0.233 (0.029) p=0.459 z-score Baseline: 0.859 (0.154) 1 year: −0.228 (1.027) p=0.000	IMPORTANT	VERY LOW
Change in lumbar spine BMAD from baseline to 1 year in transmales									
1 observational study Joseph et al. (2019)	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	N=39	None	Mean (SD), g/cm^3 Baseline: 0.196 (0.035) 1 year: 0.201 (0.033) p=0.074 z-score Baseline: −0.186 (1.230) 1 year: −0.541 (1.396) p=0.006	IMPORTANT	VERY LOW

QUALITY					Summary of findings			IMPORTANCE	CERTAINTY
					No of events/No of patients% (n/N%)		Effect		
Study	Risk of bias	Indirectness	Inconsistency	Imprecision	Intervention	Comparator	Result		
Change in lumbar spine BMAD from baseline to 2 years in transfemales									
1 observational study Joseph et al. (2019)	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	N=10	None	Mean (SD), g/cm^3 Baseline: 0.240 (0.027) 2 years: 0.240 (0.030) p=0.865 z-score Baseline: 0.486 (0.809) 2 years: −0.279 (0.930) p=0.000	IMPORTANT	VERY LOW
Change in lumbar spine BMAD from baseline to 2 years in transmales									
1 observational study Joseph et al. (2019)	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	N=21	None	Mean (SD), g/cm^3 Baseline: 0.195 (0.058) 2 years: 0.198 (0.055) p=0.433 z-score Baseline: −0.361 (1.439) 2 years: −0.913 (1.318) p=0.001	IMPORTANT	VERY LOW
Change in lumbar BMAD from starting GnRH analogue (mean age 14.9±1.9) to starting gender-affirming hormones (mean age 16.6±1.4) in transfemales									
1 observational study Klink et al. 2015	Serious limitations[2]	No serious indirectness	Not applicable	Not calculable	N=11 N=12	None	Mean (SD), g/cm^3 GnRH analogue: 0.22 (0.03) Gender-affirming hormones: 0.22 (0.02) NS z-score GnRH analogue: −0.44 (1.10) Gender-affirming hormones: −0.90 (0.80) p-value: NS	IMPORTANT	VERY LOW
Change in lumbar BMAD from starting GnRH analogue (mean age 15.0±2.0) to starting gender-affirming hormones (mean age 16.4±2.3) in transmales									

QUALITY					Summary of findings			IMPORTANCE	CERTAINTY
					No of events/No of patients% (n/N%)		Effect		
Study	Risk of bias	Indirectness	Inconsistency	Imprecision	Intervention	Comparator	Result		
1 observational study Klink et al. 2015	Serious limitations[2]	No serious indirectness	Not applicable	Not calculable	N=18	None	Mean (SD), g/cm^3 GnRH analogue: 0.25 (0.03) Gender-affirming hormones: 0.24 (0.02) NS z-score GnRH analogue: 0.28 (0.90) Gender-affirming hormones: −0.50 (0.81) p-value: 0.004	IMPORTANT	VERY LOW
Change in lumbar BMAD from starting GnRH analogue to starting gender-affirming hormones in transfemales (bone age of <15 years)									
1 observational study Vlot et al. 2017	Serious limitations[3]	No serious indirectness	Not applicable	Not calculable	N=15	None	Median (range), g/cm^3 GnRH analogue: 0.21 (0.17 to 0.25) Gender-affirming hormones: 0.20 (0.18 to 0.24) NS z-score GnRH analogue: −0.20 (−1.82 to 1.18) Gender-affirming hormones: −1.52 (−2.36 to 0.42) p-value: <0.01	IMPORTANT	VERY LOW
Change in lumbar BMAD from starting GnRH analogue to starting gender-affirming hormones in transfemales (bone age of ≥15)									
1 observational study Vlot et al. 2017	Serious limitations[3]	No serious indirectness	Not applicable	Not calculable	N=5	None	Median (range), g/cm^3 GnRH analogue: 0.22 (0.18 to 0.25) Gender-affirming hormones: 0.22 (0.19 to 0.24) NS z-score GnRH analogue: −1.18 (−1.78 to 1.09)	IMPORTANT	VERY LOW

QUALITY					Summary of findings			IMPORTANCE	CERTAINTY
					No of events/No of patients% (n/N%)		Effect		
Study	Risk of bias	Indirectness	Inconsistency	Imprecision	Intervention	Comparator	Result		
							Gender-affirming hormones: −1.15 (−2.21 to 0.08) p-value: p≤0.1		
Change in lumbar BMAD from starting GnRH analogue to starting gender-affirming hormones in transmales (bone age of <14 years)									
1 observational study Vlot et al. 2017	Serious limitations[3]	No serious indirectness	Not applicable	Not calculable	N=11	None	Median (range), g/cm^3 GnRH analogue: 0.23 (0.20 to 0.29) Gender-affirming hormones: 0.23 (0.19 to 0.28) NS z-score GnRH analogue: −0.05 (−0.78 to 2.94) Gender-affirming hormones: −0.84 (−2.20 to 0.87) p-value: ≤0.01	IMPORTANT	VERY LOW
Change in lumbar BMAD from starting GnRH analogue to starting gender-affirming hormones in transmales (bone age of ≥14)									
1 observational study Vlot et al. 2017	Serious limitations[3]	No serious indirectness	Not applicable	Not calculable	N=23	None	Median (range), g/cm3 GnRH analogue: 0.26 (0.21 to 0.29) Gender-affirming hormones: 0.24 (0.20 to 0.28) p≤0.01 z-score GnRH analogue: 0.27 (−1.60 to 1.80) Gender-affirming hormones: −0.29 (−2.28 to 0.90) p-value: p ≤ 0.01)	IMPORTANT	VERY LOW
Bone density: change in lumbar BMD									
Change in lumbar spine BMD from baseline to 1 year in transfemales									

QUALITY					Summary of findings			IMPORTANCE	CERTAINTY
					No of events/No of patients% (n/N%)		Effect		
Study	Risk of bias	Indirectness	Inconsistency	Imprecision	Intervention	Comparator	Result		
1 observational study Joseph et al. (2019)	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	N=31	None	Mean (SD), kg/m2 Baseline: 0.860 (0.154) 1 year: 0.859 (0.129) p=0.962 z-score Baseline: −0.016 (1.106) 1 year: −0.461 (1.121) p=0.003	IMPORTANT	VERY LOW
Change in lumbar spine BMD from baseline to 1 year in transmales									
1 observational study Joseph et al. (2019)	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	N=39	None	Mean (SD), kg/m2 Baseline: 0.694 (0.149) 1 year: 0.718 (0.124) p=0.006 z-score Baseline: −0.395 (1.428) 1 year: −1.276 (1.410) p=0.000	IMPORTANT	VERY LOW
Change in lumbar spine BMD from baseline to 2 years in transfemales									
1 observational study Joseph et al. (2019)	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	N=10	None	Mean (SD), kg/m2 Baseline: 0.867 (0.141) 2 years: 0.878 (0.130) p=0.395 z-score Baseline: 0.130 (0.972) 2 years: −0.890 (1.075) p=0.000	IMPORTANT	VERY LOW
Change in lumbar spine BMD from baseline to 2 years in transmales									
1 observational study	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	N=21	None	Mean (SD), kg/m2 Baseline: 0.695 (0.220) 2 years: 0.731 (0.209) p=0.058	IMPORTANT	VERY LOW

QUALITY					Summary of findings			IMPORTANCE	CERTAINTY
					No of events/No of patients% (n/N%)		Effect		
Study	Risk of bias	Indirectness	Inconsistency	Imprecision	Intervention	Comparator	Result		
Joseph et al. (2019)							z-score Baseline: −0.715 (1.406) 2 years: −2.000 (1.384) p=0.000		
Change in lumbar BMD from starting GnRH analogue (mean age 14.9±1.9) to starting gender-affirming hormones (mean age 16.6±1.4) in transfemales									
1 observational study Klink et al. 2015	Serious limitations[2]	No serious indirectness	Not applicable	Not calculable	N=12 N=11	None	Mean (SD), g/m2 GnRH analogue: 0.84 (0.13) Gender-affirming hormones: 0.84 (0.11) NS z-score GnRH analogue: −0.77 (0.89) Gender-affirming hormones: −1.01 (0.98) NS	IMPORTANT	VERY LOW
Change in lumbar BMD from starting GnRH analogue (mean age 15.0±2.0) to starting gender-affirming hormones (mean age 16.4±2.3) in transmales									
1 observational study Klink et al. 2015	Serious limitations[2]	No serious indirectness	Not applicable	Not calculable	N=18	None	Mean (SD), g/m2 GnRH analogue: 0.95 (0.12) Gender-affirming hormones: 0.91 (0.10) p-value: 0.006 z-score GnRH analogue: 0.17 (1.18) Gender-affirming hormones: −0.72 (0.99) p-value: <0.001	IMPORTANT	VERY LOW
Bone density: change in femoral neck (hip) BMD									
Change in femoral neck BMD from baseline to 1 year in transfemales									
1 observational study	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	N=31	None	Mean (SD), kg/m2 Baseline: 0.894 (0.118) 1 year: 0.905 (0.104) p=0.571	IMPORTANT	VERY LOW

QUALITY					Summary of findings			IMPORTANCE	CERTAINTY
					No of events/No of patients% (n/N%)		Effect		
Study	Risk of bias	Indirectness	Inconsistency	Imprecision	Intervention	Comparator	Result		
Joseph et al. (2019)							z-score Baseline: 0.157 (0.905) 1 year: −0.340 (0.816) p=0.002		
Change from baseline to 1 year in femoral neck BMD in transmales									
1 observational study Joseph et al. (2019)	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	N=39	None	Mean (SD), kg/m2 Baseline: 0.772 (0.137) 1 year: 0.785 (0.120) p=0.797 z-score Baseline: −0.863 (1.215) 1 year: −1.440 (1.075) p=0.000	IMPORTANT	VERY LOW
Change from baseline to 2 years in femoral neck BMD in transfemales									
1 observational study Joseph et al. (2019)	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	N=10	None	Mean (SD), kg/m2 Baseline: 0.920 (0.116) 2 years: 0.910 (0.125) p=0.402 z-score Baseline: 0.450 (0.781) 2 years: −0.600 (1.059) p=0.002	IMPORTANT	VERY LOW
Change from baseline to 2 years in femoral neck BMD in transmales									
1 observational study Joseph et al. (2019)	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	N=21	None	Mean (SD), kg/m2 Baseline: 0.766 (0.215) 2 years: 0.773 (0.197) p=0.604 z-score Baseline: −1.075 (1.145) 2 years: −1.779 (0.816) p=0.001	IMPORTANT	VERY LOW

QUALITY					Summary of findings			IMPORTANCE	CERTAINTY
					No of events/No of patients% (n/N%)		Effect		
Study	Risk of bias	Indirectness	Inconsistency	Imprecision	Intervention	Comparator	Result		
Bone density: change in femoral neck (hip) BMAD									
Change from starting GnRH analogue to starting gender-affirming hormones in femoral neck BMAD in transfemales (bone age of <15 years)									
1 observational study Vlot et al. 2017	Serious limitations[3]	No serious indirectness	Not applicable	Not calculable	N=16	None	Median (range), g/cm3 GnRH analogue: 0.29 (0.20 to 0.33) Gender-affirming hormones: 0.27 (0.20 to 0.33) p≤0.1 z-score GnRH analogue: −0.71 (−3.35 to 0.37) Gender-affirming hormones: −1.32 (−3.39 to 0.21) p≤0.1	IMPORTANT	VERY LOW
Change in femoral neck BMAD from starting GnRH analogue to starting gender-affirming hormones in transfemales (bone age of ≥15)									
1 observational study Vlot et al. 2017	Serious limitations[3]	No serious indirectness	Not applicable	Not calculable	N=6	None	Median (range), g/cm3 GnRH analogue: 0.30 (0.26 to 0.36) Gender-affirming hormones: 0.30 (0.26 to 0.34) NS z-score GnRH analogue: −0.44 (−1.37 to 0.93) Gender-affirming hormones: −0.36 (−1.50 to 0.46) NS	IMPORTANT	VERY LOW
Change in femoral neck BMAD from starting GnRH analogue to starting gender-affirming hormones in transmales (bone age of <14 years)									
1 observational study	Serious limitations[3]	No serious indirectness	Not applicable	Not calculable	N=10	None	Median (range), g/cm3 GnRH analogue: 0.31 (0.26 to 0.36) Gender-affirming hormones: 0.30 (0.22 to 0.35)	IMPORTANT	VERY LOW

QUALITY					Summary of findings			IMPORTANCE	CERTAINTY
					No of events/No of patients% (n/N%)		Effect		
Study	Risk of bias	Indirectness	Inconsistency	Imprecision	Intervention	Comparator	Result		
Vlot et al. 2017							NS z-score GnRH analogue: −0.01 (−1.30 to 0.91) Gender-affirming hormones: −0.37 (−2.28 to 0.47) NS		
Change in femoral neck BMAD from starting GnRH analogue to starting gender-affirming hormones in transmales (bone age of ≥14)									
1 observational study Vlot et al. 2017	Serious limitations[3]	No serious indirectness	Not applicable	Not calculable	N=23	None	Median (range), g/cm3 GnRH analogue: 0.33 (0.25 to 0.39) Gender-affirming hormones: 0.30 (0.23 to 0.41) p-value: ≤0.01 z-score GnRH analogue: 0.27 (−1.39 to 1.32) Gender-affirming hormones: −0.27 (−1.91 to 1.29) p-value: ≤0.01	IMPORTANT	VERY LOW
Bone density: change in femoral area BMD									
Change in femoral BMD from starting GnRH analogue (mean age 14.9±1.9) to starting gender-affirming hormones (mean age 16.6±1.4) in transfemales									
1 observational study Klink et al. 2015	Serious limitations[2]	No serious indirectness	Not applicable	Not calculable	N=14 N=6	None	Mean (SD), g/m2 GnRH analogue: 0.88 (0.12) Gender-affirming hormones: 0.87 (0.08) NS z-score GnRH analogue: −0.66 (0.77) Gender-affirming hormones: −0.95 (0.63) NS	IMPORTANT	VERY LOW

QUALITY					Summary of findings			IMPORTANCE	CERTAINTY
					No of events/No of patients% (n/N%)		Effect		
Study	Risk of bias	Indirectness	Inconsistency	Imprecision	Intervention	Comparator	Result		
Change in femoral BMD from starting GnRH analogue (mean age 15.0±2.0) to starting gender-affirming hormones (mean age 16.4±2.3) in transmales									
1 observational study Klink et al. 2015	Serious limitations[2]	No serious indirectness	Not applicable	Not calculable	N=18 N=13	None	Mean (SD), g/m2 GnRH analogue: 0.92 (0.10) Gender-affirming hormones: 0.88 (0.09) p-value: 0.005 z-score GnRH analogue: 0.36 (0.88) Gender-affirming hormones: −0.35 (0.79) p-value: 0.001	IMPORTANT	VERY LOW
Bone density: change in femoral area BMAD									
Change in femoral BMAD from starting GnRH analogue (mean age 14.9±1.9) to starting gender-affirming hormones (mean age 16.6±1.4) in transfemales									
1 observational study Klink et al. 2015	Serious limitations[2]	No serious indirectness	Not applicable	Not calculable	N=12 N=10	None	Mean (SD), g/cm3 GnRH analogue: 0.28 (0.04) Gender-affirming hormones: 0.26 (0.04) NS z-score GnRH analogue: −0.93 (1.22) Gender-affirming hormones: −1.57 (1.74) p-value: NS	IMPORTANT	VERY LOW
Change in femoral BMAD from starting GnRH analogue (mean age 15.0±2.0) to starting gender-affirming hormones (mean age 16.4±2.3) in transmales									
1 observational study Klink et al. 2015	Serious limitations[2]	No serious indirectness	Not applicable	Not calculable	N=18 N=18	None	Mean (SD), g/cm3 GnRH analogue: 0.32 (0.04) Gender-affirming hormones: 0.31 (0.04) NS z-score	IMPORTANT	VERY LOW

QUALITY					Summary of findings			IMPORTANCE	CERTAINTY
					No of events/No of patients% (n/N%)		Effect		
Study	Risk of bias	Indirectness	Inconsistency	Imprecision	Intervention	Comparator	Result		
							GnRH analogue: 0.01 (0.70) Gender-affirming hormones: −0.28 (0.74) NS		

Abbreviations: BMAD, bone mineral apparent density; BMD, bone mineral density; GnRH, gonadotrophin releasing hormone; NS, not significant; SD, standard deviation.

1 Downgraded 1 level - the cohort study by Joseph et al. (2019) was assessed as at high risk of bias (poor quality overall; lack of blinding and no control group).
2 Downgraded 1 level - the cohort study by Klink et al. (2015) was assessed as at high risk of bias (poor quality overall; lack of blinding, no randomisation, no control group and high number of participants lost to follow-up).
3 Downgraded 1 level - the cohort study by Vlot et al. (2017) was assessed as at high risk of bias (poor quality overall; lack of blinding and no control).

Table 9 Question 2: For children and adolescents with gender dysphoria, what is the short-term and long-term safety of GnRH analogues compared with one or a combination of psychological support, social transitioning to the desired gender or no intervention? – cognitive development or functioning

QUALITY					Summary of findings			IMPORTANCE	CERTAINTY
					No of events/No of patients% (n/N%)		Effect		
Study	Risk of bias	Indirectness	Inconsistency	Imprecision	Intervention	Comparator	Result		
Cognitive development or functioning (1 cross-sectional study)									
IQ (4 subscales: arithmetic, vocabulary, picture arrangement, and block design) at a single time point between GnRH analogue treated and untreated transfemales									
1 Cross-sectional study Staphorsius et al. 2015	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	N=8 Mean (SD) 94.0 (10.3)	N=10 Mean (SD) 109.4 (21.2)	NR	IMPORTANT	VERY LOW
IQ (4 subscales: arithmetic, vocabulary, picture arrangement, and block design) at a single time point between GnRH analogue treated and untreated transmales									

QUALITY					Summary of findings			IMPORTANCE	CERTAINTY
					No of events/No of patients% (n/N%)		Effect		
Study	Risk of bias	Indirectness	Inconsistency	Imprecision	Intervention	Comparator	Result		
1 Cross-sectional study Staphorsius et al. 2015	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	N=12 Mean (SD) 95.8 (15.6)	N=10 Mean (SD) 98.5 (15.9)	NR	IMPORTANT	VERY LOW
Reaction time at a single time point between GnRH analogue treated and untreated transfemales									
1 Cross-sectional study Staphorsius et al. 2015	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	N=8 Mean (SD) 10.9 (4.1)	N=10 Mean (SD) 9.9 (3.1)	NR	IMPORTANT	VERY LOW
Reaction time at a single time point between GnRH analogue treated and untreated transmales									
1 Cross-sectional study Staphorsius et al. 2015	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	N=12 Mean (SD) 9.9 (3.1)	N=10 Mean (SD) 10.0 (2.0)	NR	IMPORTANT	VERY LOW
Accuracy at a single time point between GnRH analogue treated and untreated transfemales									
1 cohort study Staphorsius et al. 2015	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	N=8 Mean (SD) 73.9 (9.1)	N=10 Mean (SD) 83.4 (9.5)	NR	IMPORTANT	VERY LOW
Accuracy at a single time point between GnRH analogue treated and untreated transmales									
1 cohort study Staphorsius et al. 2015	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	N=12 Mean (SD) 85.7 (10.5)	N=10 Mean (SD) 88.8 (9.7)	NR	IMPORTANT	VERY LOW

Abbreviations: GnRH, gonadotrophin releasing hormone; NR, not reported; *P*, P-value; SD, Standard deviation.

1 Downgraded 1 level - the cohort study by Staphorsius et al. (2015) was assessed as at high risk of bias (poor quality overall; lack of blinding and no randomisation).

Table 10: Question 2: In children and adolescents with gender dysphoria, what is the short-term and long-term safety of GnRH analogues compared with one or a combination of psychological support, social transitioning to the desired gender or no intervention? – other safety outcomes

QUALITY					Summary of findings			IMPORTANCE	CERTAINTY
					No of events/No of patients% (n/N%)		Effect		
Study	Risk of bias	Indirectness	Inconsistency	Imprecision	Intervention	Comparator	Result		
Other safety outcomes: change in serum creatinine									
Change in serum creatinine (micromol/l) between baseline and 1 year in transfemales									
1 observational study Schagen et al. 2016	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	N=28	None	Mean (SD) Baseline: 70 (12) 1 year: 66 (13) p-value: 0.20	IMPORTANT	VERY LOW
Change in serum creatinine (µmol/l) between baseline and 1 year in transmales									
1 observational study Schagen et al. 2016	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	N=29	None	Mean (SD) Baseline: 73 (8) 1 year: 68 (13) p-value: 0.01	IMPORTANT	VERY LOW
Other safety outcomes: liver enzymes									
Presence of elevated liver enzymes (AST, ALT, and glutamyl transferase) between baseline and during treatment									
1 observational study Schagen et al. 2016	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	39	None	Glutamyl transferase was not elevated at baseline or during treatment in any subject. Mild elevations of AST and ALT above the reference range were present at baseline but were not more prevalent during treatment than at baseline.	IMPORTANT	VERY LOW

QUALITY					Summary of findings			IMPORTANCE	CERTAINTY
					No of events/No of patients% (n/N%)		Effect		
Study	Risk of bias	Indirectness	Inconsistency	Imprecision	Intervention	Comparator	Result		
							Glutamyl transferase, AST, and ALT levels did not significantly change from baseline to 12 months of treatment.		
Other safety outcomes: adverse effects									
Proportion of patients reporting adverse effects									
1 cohort study Khatchadourian et al 2014	Serious limitations[2]	No serious indirectness	Not applicable	Not calculable[2]	27	None	3/27 adolescents[3]	Important	VERY LOW

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GnRH, gonadotrophin releasing hormone; P, P-value; SD, standard deviation.

1 Downgraded 1 level - the cohort study by Schagen et al. (2016) was assessed as at high risk of bias (poor quality overall; lack of blinding and no control).

2 Downgraded 1 level - the cohort study by Khatchadourian et al. (2014) was assessed as at high risk of bias (poor quality overall; lack of blinding, no control group and high number of participants lost to follow-up).

3 1 transmale developed sterile abscesses; they were switched from leuprolide acetate to triptorelin, and this was well tolerated. 1 transmale developed leg pains and headaches, which eventually resolved without treatment. 1 participant gained 19 kg within 9 months of initiating GnRH analogues.

Table 11: Question 4. From the evidence selected, are there any subgroups of children and adolescents with gender dysphoria that may derive more (or less) advantage from treatment with GnRH analogues than the wider population of children and adolescents with gender dysphoria? – critical outcomes

QUALITY					Summary of findings			IMPORTANCE	CERTAINTY
					No of events/No of patients (n/N%)		Effect		
Study	Risk of bias	Indirectness	Inconsistency	Imprecision	Sex assigned at birth males	Sex assigned at birth females	Result		
Subgroups: sex assigned at birth males compared with sex assigned at birth females									

QUALITY					Summary of findings			IMPORTANCE	CERTAINTY
					No of events/No of patients (n/N%)		Effect		
Study	Risk of bias	Indirectness	Inconsistency	Imprecision	Sex assigned at birth males	Sex assigned at birth females	Result		
Impact on gender dysphoria									
Mean [±SD] Utrecht Gender Dysphoria Scale (version(s) not reported), time point at baseline (before GnRHa) versus follow-up (just before gender-affirming hormones).									
1 cohort study de Vries et al 2011	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	n-NR[2] score at T0 47.95 [±9.70] score at T1 49.67 [±9.47]	n-NR[2] score at T0 56.57 [±3.89] score at T1 56.62 [±4.0]	*F*-ratio 15.98 (*df, errdf*: 1,39), *P*<0.001	Critical	VERY LOW
Impact on mental health									
Mean [±SD] Beck Depression Inventory-II, time point at baseline (T0 before GnRH analogues) versus follow-up (T1 just before gender-affirming hormones).									
1 cohort study de Vries et al 2011	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	n-NR[2] score at T0 5.71 [±4.31] score at T1 3.50 [±4.58]	n-NR[2] score at T0 10.34 [±8.24] score at T1 6.09 [±7.93]	*F*-ratio 3.85 (*df, errdf*: 1,39), *P*=0.057	Critical	VERY LOW
Mean [±SD] Trait Anger (TPI), time point at baseline (T0 before GnRH analogues) versus follow-up (T1 just before gender-affirming hormones).									
1 cohort study de Vries et al 2011	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	n-NR[2] score at T0 5.22 [±2.76] score at T1 5.00 [±3.07]	n-NR[2] score at T0 6.43 [±2.78] score at T1 6.39 [±2.59]	*F*-ratio 5.70 (*df, errdf*: 1,39), *P*=0.022	Critical	VERY LOW

QUALITY					Summary of findings			IMPORTANCE	CERTAINTY
					No of events/No of patients (n/N%)		Effect		
Study	Risk of bias	Indirectness	Inconsistency	Imprecision	Sex assigned at birth males	Sex assigned at birth females	Result		
Mean [±SD] Trait Anxiety (STAI), time point at baseline (T0 before GnRH analogues) versus follow-up (T1 just before gender-affirming hormones).									
1 cohort study de Vries et al 2011	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	n-NR[2] score at T0 4.33 [±2.68] score at T1 4.39 [±2.64]	n-NR[2] score at T0 7.00 [±2.36] score at T1 6.17 [±2.69]	*F*-ratio 16.07 (*df, errdf*: 1,39), *P*<0.001	Critical	VERY LOW

Abbreviations: GnRH, gonadotrophin releasing hormone; NR, not reported; *P*, P-value; SD, Standard deviation.

1 Downgraded 1 level - the cohort study by de Vries et al. (2011) was assessed as at high risk of bias (poor quality overall; lack of blinding and no control group).
2 The overall sample size completing the outcome at both time points was 41.

Table 11: Question: 4. From the evidence selected, are there any subgroups of children and adolescents with gender dysphoria that may derive more (or less) advantage from treatment with GnRH analogues than the wider population of children and adolescents with gender dysphoria? – important outcomes

QUALITY					Summary of findings			IMPORTANCE	CERTAINTY
					No of events/No of patients (n/N%)		Effect		
Study	Risk of bias	Indirectness	Inconsistency	Imprecision	Sex assigned at birth males	Sex assigned at birth females	Result		
Subgroups: sex assigned at birth males compared with sex assigned at birth females									
Impact on body image									
Mean [±SD] Body Image Scale (primary sexual characteristics), time point at baseline (T0 before GnRH analogues) versus follow-up (T1 just before gender-affirming hormones).									

QUALITY					Summary of findings			IMPORTANCE	CERTAINTY
					No of events/No of patients (n/N%)		Effect		
Study	Risk of bias	Indirectness	Inconsistency	Imprecision	Sex assigned at birth males	Sex assigned at birth females	Result		
1 cohort study de Vries et al 2011	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	n-NR[2] score at T0 4.02 [±0.16] score at T1 3.74 [±0.78]	n-NR[2] score at T0 4.16 [±0.52] score at T1 4.17 [±0.58]	*F*-ratio 4.11 (*df, errdf*: 1,55), *P*=0.047	Important	VERY LOW
Mean [±SD] Body Image Scale (secondary sexual characteristics), time point at baseline (T0 before GnRH analogues) versus follow-up (T1 just before gender-affirming hormones).									
1 cohort study de Vries et al 2011	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	n-NR[2] score at T0 2.66 [±0.50] score at T1 2.39 [±0.69]	n-NR[2] score at T0 2.81 [±0.76] score at T1 3.18 [±0.42]	*F*-ratio 11.57 (*df, errdf*: 1,55), *P*=0.001[3]	Important	VERY LOW
Mean [±SD] Body Image Scale (neutral characteristics), time point at baseline (T0 before GnRH analogues) versus follow-up (T1 just before gender-affirming hormones).									
1 cohort study de Vries et al 2011	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	n-NR[2] score at T0 2.60 [±0.58] score at T1 2.32 [±0.59]	n-NR[2] score at T0 2.24 [±0.62] score at T1 2.61 [±0.50]	*F*-ratio 0.081 (*df, errdf*: 1,55), *P*=0.777[3]	Important	VERY LOW
Psychosocial impact									
Mean [±SD] Children's Global Assessment Scale score, at baseline.									
1 cohort study Costa et al 2015	Serious limitations[4]	No serious indirectness	No serious inconsistency	Not calculable	n=not reported	n=not reported	*t*-test 2.15; *P*=0.03[5]	Important	VERY LOW

QUALITY					Summary of findings			IMPORTANCE	CERTAINTY
					No of events/No of patients (n/N%)		Effect		
Study	Risk of bias	Indirectness	Inconsistency	Imprecision	Sex assigned at birth males	Sex assigned at birth females	Result		
					55.4 [±12.7]	59.2 [±11.8]			
Mean [±SD] Children's Global Assessment Scale score, time point at baseline (T0 before GnRH analogues) versus follow-up (T1 just before gender-affirming hormones).									
1 cohort study de Vries et al 2011	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	n-NR[6] score at T0 73.10 [±8.84] score at T1 77.33 [±8.69]	n-NR[6] score at T0 67.25 [±11.06] score at T1 70.30 [±9.44]	*F*-ratio 5.77 (*df, errdf*: 1,39), *P*=0.021	Important	VERY LOW
Mean [±SD] Child Behaviour Checklist (total T) score, time point at baseline (T0 before GnRH analogues) versus follow-up (T1 just before gender-affirming hormones).									
1 cohort study de Vries et al 2011	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	n-NR[7] score at T0 59.42 [±11.78] score at T1 50.38 [±10.57]	n-NR[7] score at T0 61.73 [±13.60] score at T1 57.73 [±10.82]	*F*-ratio 2.64 (*df, errdf*: 1,52), *P=0.110*	Important	VERY LOW
Mean [±SD] Child Behaviour Checklist (internalising T) score, time point at baseline (T0 before GnRH analogues) versus follow-up (T1 just before gender-affirming hormones).									
1 cohort study de Vries et al 2011	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	n-NR[7] score at T0 60.00 [±9.51] score at T1 52.17 [±9.81]	n-NR[7] score at T0 61.80 [±14.12] score at T1 56.30 [±10.33]	*F*-ratio 1.16 (*df, errdf*: 1,52), *P*=0.286	Important	VERY LOW

QUALITY					Summary of findings			IMPORTANCE	CERTAINTY
					No of events/No of patients (n/N%)		Effect		
Study	Risk of bias	Indirectness	Inconsistency	Imprecision	Sex assigned at birth males	Sex assigned at birth females	Result		
Mean [±SD] Child Behaviour Checklist (externalising T) score, time point at baseline (T0 before GnRH analogues) versus follow-up (T1 just before gender-affirming hormones).									
1 cohort study de Vries et al 2011	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	n-NR[7] score at T0 54.71 [±12.91] score at T1 48.75 [±10.22]	n-NR[7] score at T0 60.70 [±12.64] score at T1 57.87 [±11.66]	*F*-ratio 6.29 (*df, errdf*: 1,52), *P*=0.015	Important	VERY LOW
Mean [±SD] Youth Self-Report (total T) score, time point at baseline (T0 before GnRH analogues) versus follow-up (T1 just before gender-affirming hormones).									
1 cohort study de Vries et al 2011	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	n-NR[7] score at T0 53.56 [±12.26] score at T1 47.84 [±10.86]	n-NR[7] score at T0 57.10 [±10.87] score at T1 51.86 [±10.11]	*F*-ratio 1.99 (*df, errdf*: 1,52), *P*=0.164	Important	VERY LOW
Mean [±SD] Youth Self-Report (internalising T) score, time point at baseline (T0 before GnRH analogues) versus follow-up (T1 just before gender-affirming hormones).									
1 cohort study de Vries et al 2011	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	n-NR[7] score at T0 55.88 [±11.81] score at T1 49.24 [±12.24]	n-NR[7] score at T0 56.17 [±13.25] score at T1 50.24 [±11.28]	*F*-ratio 0.049 (*df, errdf*: 1,52), *P*=0.825	Important	VERY LOW
Mean [±SD] Youth Self-Report (externalising T) score, time point at baseline (T0 before GnRHa) versus follow-up (T1 just before gender-affirming hormones).									

QUALITY					Summary of findings			IMPORTANCE	CERTAINTY
					No of events/No of patients (n/N%)		Effect		
Study	Risk of bias	Indirectness	Inconsistency	Imprecision	Sex assigned at birth males	Sex assigned at birth females	Result		
1 cohort study de Vries et al 2011	Serious limitations[1]	No serious indirectness	Not applicable	Not calculable	n-NR[7] score at T0 48.72 [±11.83] score at T1 46.52 [±9.23]	n-NR[7] score at T0 57.24 [±10.59] score at T1 52.97 [±8.51]	*F*-ratio 9.14 (*df, errdf*: 1,52), *P*=0.004	Important	VERY LOW

Abbreviations: GnRH, gonadotrophin releasing hormone; NR, not reported; *P*, P-value; SD, Standard deviation.

1 Downgraded 1 level - the cohort study by de Vries et al. (2011) was assessed as at high risk of bias (poor quality overall; lack of blinding and no control group).
2 The overall sample size completing the outcome at both time points was 57.
3 There was a significant interaction effect between sex assigned at birth and BDI between T0 and T1; sex assigned at birth females became more dissatisfied with their secondary F (df, errdf), P: 14.59 (1,55), P<0.001) and neutral F (df, errdf), P: 15.26 (1,55), P<0.001) sex characteristics compared with sex assigned at birth males.
4 Serious limitations – the cohort study by Costa et al. 2015 was assessed as at high risk of bias (poor quality).
5 At baseline, CGAS scores were not associated with any demographic variable, in both sex assigned at birth males and females. There were no statistically significant differences in CGAS scores between gender dysphoric sex assigned at birth males and females in all follow-up evaluations (P>0.1; full data not reported).
6 The overall sample size completing the outcome at both time points was 41
7 The overall sample size completing the outcome at both time points was 54.

Glossary

Beck Depression Inventory-II (BDI-II)	The BDI-II is a tool for assessing depressive symptoms. There are no specific scores to categorise depression severity, but it is suggested that 0 to 13 is minimal symptoms, 14 to 19 is mild depression, 20 to 28 is moderate depression, and severe depression is 29 to 63.
Body Image Scale (BIS)	The BIS is used to measure body satisfaction. The scale consists of 30 body features, which the person rates on a 5-point scale. Each of the 30 items falls into one of 3 basic groups based on its relative importance as a gender-defining body feature: primary sex characteristics, secondary sex characteristics, and neutral body characteristics. A higher score indicates more dissatisfaction.
Bone mineral apparent density (BMAD)	BMAD is a size adjusted value of bone mineral density (BMD) incorporating body size measurements using UK norms in growing adolescents.
Child Behaviour Checklist (CBCL)	CBCL is a checklist parents complete to detect emotional and behavioural problems in children and adolescents.
Children's Global Assessment Scale (CGAS)	The CGAS tool is a validated measure of global functioning on a single rating scale from 1 to 100. Lower scores indicate poorer functioning.
Gender	The roles, behaviours, activities, attributes, and opportunities that any society considers appropriate for girls and boys, and women and men.
Gender dysphoria	Discomfort or distress that is caused by a discrepancy between a person's gender identity (how they see themselves regarding their gender) and that person's sex assigned at birth (and the associated gender role, and/or primary and secondary sex characteristics).
Gonadotrophin releasing hormone (GnRH) analogues	GnRH analogues competitively block GnRH receptors to prevent the spontaneous release of 2 gonadotropin hormones, Follicular Stimulating Hormone (FSH) and Luteinising Hormone (LH) from the pituitary gland. The reduction in FSH and LH secretion reduces oestradiol secretion from the ovaries in those whose sex assigned at birth was female and testosterone secretion from the testes in those whose sex assigned at birth was male.
Sex assigned at birth	Sex assigned at birth (male or female) is a biological term and is based on genes and how external and internal sex and reproductive organs work and respond to hormones. Sex is the label that is recorded when a baby's birth is registered.
Tanner stage	Tanner staging is a scale of physical development.
Trait Anger Spielberger scales of the State-Trait Personality Inventory (TPI)	The TPI is a validated 20-item inventory tool which measures the intensity of anger as the disposition to experience angry feelings as a personality trait. Higher scores indicate greater anger.
Transgender (including transmale and transfemale)	Transgender is a term for someone whose gender identity is not congruent with their birth-registered sex. A transmale is a person who identifies as male and a transfemale is a person who identifies as female.

Utrecht Gender Dysphoria Scale (UGDS)	The UGDS is a validated screening tool for both adolescents and adults to assess gender dysphoria. It consists of 12 items, to be answered on a 1- to 5-point scale, resulting in a sum score between 12 and 60. The higher the UGDS score the greater the impact on gender dysphoria.
Youth Self-Report (YSR)	The self-administered YSR is a checklist to detect emotional and behavioural problems in children and adolescents. It is self-completed by the child or adolescent. The scales consist of a Total problems score, which is the sum of the scores of all the problem items. An internalising problem scale sums the anxious/depressed, withdrawn-depressed, and somatic complaints scores while the externalising problem scale combines rule-breaking and aggressive behaviour.

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Evidence review: Gender-affirming hormones for children and adolescents with gender dysphoria

This document will help inform Dr Hilary Cass' independent review into gender identity services for children and young people. It was commissioned by NHS England and Improvement who commissioned the Cass review. It aims to assess the evidence for the clinical effectiveness, safety and cost-effectiveness of gender-affirming hormones for children and adolescents aged 18 years or under with gender dysphoria.

The document was prepared by NICE in October 2020.

The content of this evidence review was up to date on 21 October 2020. See summaries of product characteristics (SPCs), British National Formulary (BNF) or the Medicines and Healthcare products Regulatory Agency (MHRA) or NICE websites for up-to-date information.

Contents

1. Introduction

This review aims to assess the evidence for the clinical effectiveness, safety and cost-effectiveness of gender-affirming hormones for children and adolescents aged 18 years or under with gender dysphoria. The review follows the NHS England Specialised Commissioning process and template and is based on the criteria outlined in the PICO framework (see appendix A). This document will help inform Dr Hilary Cass' independent review into gender identity services for children and young people.

Gender dysphoria in children, also known as gender identity disorder or gender incongruence of childhood (World Health Organisation 2020), refers to discomfort or distress that is caused by a discrepancy between a person's gender identity (how they see themselves[1] regarding their gender) and that person's sex assigned at birth and the associated gender role, and/or primary and secondary sex characteristics (Diagnostic and Statistical Manual of Mental Disorders 2013).

Gender-affirming hormones are oestradiol for sex assigned at birth males (transfemales) and testosterone for sex assigned at birth females (transmales). The aim of gender-affirming hormones is to induce the development of the physical sex characteristics congruent with the individual's gender expression while aiming to improve mental health and quality of life outcomes.

No oestradiol-containing products are licensed for gender dysphoria and therefore any use for children and adolescents with gender dysphoria is off-label.

The only testosterone-containing product licensed for gender dysphoria is Sustanon 250 mg/ml solution for injection, which is indicated as supportive therapy for transmales, use of all other testosterone-containing products for children and adolescents with gender dysphoria is off-label.

For children and adolescents with gender dysphoria it is recommended that management plans are tailored to the needs of the individual and aim to ameliorate the potentially negative impact of gender dysphoria on general developmental processes, to support young people and their families in managing the uncertainties inherent in gender identity development and to provide ongoing opportunities for exploration of gender identity. The plans may also include psychological support and exploration and, for some individuals, the use of gonadotrophin releasing hormone (GnRH) analogues in adolescence to suppress puberty; this may be followed later with gender-affirming hormones of the desired sex (NHS England 2013).

Currently NHS England, as part of the Gender Identity Development Service for Children and Adolescents, routinely commissions gender-affirming hormones for young people with continuing gender dysphoria from around their 16th birthday subject to individuals meeting the eligibility and readiness criteria (Clinical Commissioning Policy 2016).

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[1] Gender refers to the roles, behaviours, activities, attributes and opportunities that any society considers appropriate for girls and boys, and women and men (World Health Organisation, Health Topics: Gender).

2. Executive summary of the review

Ten observational studies were included in the evidence review. Seven studies were retrospective observational studies (Allen et al. 2019, Kaltiala et al. 2020, Khatchadourian et al. 2014, Klaver et Al. 2020, Klink et al. 2015, Stoffers et al. 2019, Vlot et al. 2017) and 3 studies were prospective longitudinal observational studies (Achille et al. 2020, Kuper et al. 2020, Lopez de Lara et al. 2020). No studies directly compared gender-affirming hormones to a control group (either placebo or active comparator). Follow-up was relatively short across all studies, with an average duration of treatment with gender-affirming hormones between around 1 year and 5.8 years.

The terminology used in this topic area is continually evolving and is different depending on stakeholder perspectives. In this evidence review we have used the phrase 'people's assigned sex at birth' rather than saying natal or biological sex and 'cross sex hormones' are now referred to as 'gender-affirming hormones'. The research studies may use historical terms which are no longer considered appropriate.

In children and adolescents with gender dysphoria, what is the clinical effectiveness of treatment with gender-affirming hormones compared with one or a combination of psychological support, social transitioning to the desired gender or no intervention?

Critical outcomes

The critical outcomes for decision making are impact on gender dysphoria, impact on mental health and quality of life. The quality of evidence for all these outcomes was assessed as very low certainty using modified GRADE.

Impact on gender dysphoria
The study by Lopez de Lara et al. 2020 in 23 adolescents with gender dysphoria found that during treatment with gender-affirming hormones, gender dysphoria (measured using the Utrecht Gender Dysphoria Scale [UGDS]) was statistically significantly reduced (improved) from a mean [±SD] score of 57.1 (±4.1) points at baseline to 14.7 (±3.2) points at 12 months, which is below the threshold (40 points) for gender dysphoria ($p<0.001$).

Impact on mental health
Depression
The study by Lopez de Lara et al. 2020 in 23 adolescents with gender dysphoria found that during treatment with gender-affirming hormones, depression (measured using the Beck Depression Inventory-II [BDI-II]) was statistically significantly reduced from a mean [±SD] score of 19.3 (±5.5) points at baseline to 9.7 (±3.9) points at 12 months ($p<0.001$).

The study by Achille et al. 2020 in 50 adolescents with gender dysphoria found that during treatment with gender-affirming hormones, depression was statistically significantly reduced from baseline to about 12 months follow-up:

- The Center for Epidemiologic Studies Depression (CESD-R) improved from a mean score of 21.4 points at baseline to 13.9 points ($p<0.001$).
- The Patient Health Questionnaire (PHQ 9) Modified for Teens improved, although absolute scores were not reported numerically ($p<0.001$).

The study by Kuper et al. 2020 in 148 adolescents with gender dysphoria (of whom 123 received gender-affirming hormones) found that during treatment with gender-affirming hormones for an average of 10.9 months, the impact on depression (measured using the Quick Inventory of Depressive Symptoms [QIDS]) was unclear as no statistical analysis was reported. The mean (±SD) self-reported score was 9.6 points (±5.0) at baseline and 7.4 (±4.5) at follow-up. The mean (±SD) clinician-reported score was 5.9 points (±4.1) at baseline and 6.0 (±3.8).

The study by Kaltiala et al. 2020 in 52 adolescents with gender dysphoria found that during treatment with gender-affirming hormones, statistically significantly fewer participants needed treatment for depression (54% at initial assessment compared with 15% at 12-month follow-up, p<0.001). No details of the treatments for depression are reported.

Anxiety

The study by Lopez de Lara et al. 2020 in 23 adolescents with gender dysphoria found that during treatment with gender-affirming hormones, state anxiety (measured using the State-Trait Anxiety Inventory [STAI] – State subscale) was statistically significantly reduced from a mean (±SD) score of 33.3 points (±9.1) at baseline to 16.8 points (±8.1) at 12 months (p<0.001). Trait anxiety (measured using STAI – Trait subscale) was also statistically significantly reduced from a mean (±SD) score of 33.0 (±7.2) points at baseline to 18.5 (±8.4) points at 12 months (p<0.001).

The study by Kuper et al. 2020 in 148 adolescents with gender dysphoria found that during treatment with gender-affirming hormones, small reductions were seen in anxiety, panic, generalised anxiety, social anxiety and separation anxiety symptoms and school avoidance (measured using the Screen for Child Anxiety Related Emotional Disorders [SCARED] questionnaire) from baseline to follow-up (mean duration of treatment 10.9 months). The statistical significance of these findings are unknown as no statistical analyses were reported.

The study by Kaltiala et al. 2020 in 52 adolescents with gender dysphoria found that during treatment with gender-affirming hormones, statistically significantly fewer participants needed treatment for anxiety (48% at initial assessment compared with 15% at 12-month follow-up, p<0.001). No details of treatments for anxiety are reported.

Suicidality and self-injury

The study by Allen et al. 2019 in 47 adolescents with gender dysphoria found that during treatment with gender-affirming hormones, suicide risk (measured using the Ask Suicide-Screening Questions [ASQ]) was statistically significantly reduced from an adjusted mean (±SE) score of 1.11 points (±0.22) at baseline to 0.27 points (±0.12) after about 12 months (p<0.001).

The study by Achille et al. 2020 in 50 adolescents with gender dysphoria (of whom 35 received gender-affirming hormones at follow-up) found that during treatment with gender-affirming hormones, the impact on suicidal ideation was unclear (measured using the PHQ 9_Modified for Teens with additional questions for suicidal ideation). At baseline 10%of participants had suicidal ideation and 6% had suicidal ideation after about 12 months, but it is unclear if these participants received gender-affirming hormones. No statistical analyses were reported.

The study by Kuper et al. 2020 in 148 adolescents with gender dysphoria reported the impact on suicidal ideation, suicide attempts and non-suicidal self-injury during treatment with gender-affirming hormones, after mean 10.9 months follow-up . The statistical significance of these findings are unknown as no statistical analyses were reported:

- Suicidal ideation was reported in 25% of participants 1 month before the initial assessment and in 38% of participants during follow-up.
- Suicide attempts were reported in 2% of participants at 3 months before the initial assessment and in 5% during follow-up.
- Self-injury was reported in 10% of participants at 3 months before the initial assessment and in 17% during follow-up.

The study by Kaltiala et al. 2020 in 52 adolescents with gender dysphoria reported that during treatment with gender-affirming hormones, statistically significantly fewer participants needed treatment for suicidal ideation or self-harm (35% at initial assessment compared with 4% at 12-month follow-up, p<0.001). No details of treatments for suicidal ideation or self-harm are reported.

Other related symptoms

The study by Kaltiala et al. 2020 in 52 adolescents with gender dysphoria found that during treatment with gender-affirming hormones, there was no statistically significant difference in the number of people needing treatment for either psychotic symptoms or psychosis, conduct problems or antisocial behaviour, substance abuse, autism, attention deficit hyperactivity disorder (ADHD) or eating disorders during the 12-month 'real life' phase compared with before or during the assessment. No details of the treatments received are reported.

Impact on quality of life

The study by Achille et al. 2020 in 50 adolescents with gender dysphoria (of whom 35 were receiving gender-affirming hormones at follow-up) found that during treatment with gender-affirming hormones, quality of life (measured using the Quality of Life Enjoyment and Satisfaction Questionnaire [QLES-Q-SF]) was statistically significantly improved from baseline to about 12 months, but absolute scores were not reported numerically (p<0.001).

The study by Allen et al. 2019 in 47 adolescents with gender dysphoria found that during treatment with gender-affirming hormones, quality of life (measured using the General Well-Being Scale [GWBS] of the Paediatric Quality of Life Inventory) was statistically significantly improved from an adjusted mean (±SE) score of 61.70 (±2.43) points at baseline to 70.23 (±2.15) points at about 12 months (p<0.002).

Important outcomes

The important outcomes for decision making are impact on body image, psychosocial impact, engagement with healthcare services, impact on extent of and satisfaction with surgery and de-transition. The quality of evidence for all these outcomes was assessed as very low certainty using modified GRADE.

Impact on body image

The study by Kuper et al. 2020 in 148 adolescents with gender dysphoria found that during treatment with gender-affirming hormones, the impact on body image is unclear (measured using the Body Image Scale [BIS]). The mean (±SD) BIS score was 70.7 points (±15.2) at baseline and 51.4 points (±18.3) at follow-up (mean duration of treatment 10.9 months; no statistical analysis was reported).

Psychosocial impact
The study by Lopez de Lara et al. 2020 in 23 adolescents with gender dysphoria found that during treatment with gender affirming hormones, family functioning is unchanged (measured using the Family Adaptability, Partnership, Growth, Affection and Resolve [APGAR] test). The mean score was 17.9 points at baseline and 18.0 points at 12-month follow-up (no statistical analysis was reported).

The study by Lopez de Lara et al. 2020 in 23 adolescents with gender dysphoria found that during treatment with gender affirming hormones, behavioural problems (measured using the Strengths and Difficulties Questionnaire [SDQ]) were statistically significantly improved from a mean (±SD) of 14.7 (±3.3) points at baseline to 10.3 points (±2.9) at 12-month follow-up ($p<0.001$).

The study by Kaltiala et al. 2020 in 52 adolescents with gender dysphoria found that about 12-months after starting treatment with gender-affirming hormones:

- Statistically significantly fewer participants were living with parents or guardians (73% versus 40%, p=0.001) and statistically significantly fewer participants had normal peer contacts (89% versus 81%, $p<0.001$).
- There were no statistically significant differences in:
    - progress in school or work (64% versus 60%, p=0.69),
    - the number of participants who had been dating or in steady relationships (62% versus 58%, p=0.51)
    - the ability to cope with matters outside of the home (for example, shopping and travelling alone on local public transport; 81% versus 81%, p=1.0)

Engagement with health care services
No evidence was identified.

Impact on extent of and satisfaction with surgery
No evidence was identified.

De-transition
No evidence was identified.

In children and adolescents with gender dysphoria, what is the short-term and long-term safety of gender-affirming hormones compared with one or a combination of psychological support, social transitioning to the desired gender or no intervention?

Important outcomes

The important outcomes for decision making are short- and long-term safety outcomes and adverse effects. The quality of evidence for all these outcomes was assessed as very low certainty using modified GRADE.

Bone density

The study by Klink et al. 2015 in 34 adolescents with gender dysphoria (who were previously treated with a GnRH analogue) found that gender-affirming hormones may increase lumbar spine and femoral neck bone density. However, not all results are statistically significant (particularly in transfemales). Z-scores suggest the average bone density at the end of follow-up was generally lower than in the equivalent cisgender population (transfemales compared with cis-males and transmales compared with cis-females). From starting gender-affirming hormones to age 22 years:

- There was no statistically significant difference in lumbar spine bone mineral apparent density (BMAD) z-score in transfemales, but this was statistically significantly higher in transmales (z-score [±SD]: start of hormones -0.50 [±0.81], age 22 years -0.033 [±0.95], p=0.002).
- There was no statistically significant difference in lumbar spine bone mineral density (BMD) z-score in transfemales or transmales.
- Actual lumbar spine BMAD and BMD values were statistically significantly higher in transfemales and transmales.
- There was no statistically significant difference in femoral neck BMD z-score in transfemales, but this was statistically significantly higher in transmales (z-score [SD]: start of hormones -0.35 [0.79], age 22 years -0.35 [0.74], p=0.006).
- There was no statistically significant difference in actual femoral neck BMAD values in transfemales, but this was statistically significantly higher in transmales.
- Actual femoral neck BMD values were statistically significantly higher in transfemales and transmales.

The study by Vlot et al. 2017 in 70 adolescents with gender dysphoria (who were previously treated with a GnRH analogue) found that gender-affirming hormones may increase lumbar spine and femoral neck bone density. However, not all results are statistically significant. Z-scores suggest the average bone density at the end of follow-up was generally lower than the equivalent cisgender population (transfemales compared with cis-males and transmales compared with cis-females). From starting gender-affirming hormones to 24-month follow-up:

- The z-score for lumbar spine BMAD was statistically significantly higher in transfemales with a bone age of less than 15 years (z-score [range]: start of hormones -1.52 [-2.36 to 0.42], 24-month follow-up -1.10 [-2.44 to 0.69], p≤ 0.05) and 15 years and older (z-score [range]: start of hormones -1.15 [-2.21 to 0.08], 24-month follow-up -0.66 [-1.66 to 0.54], p≤ 0.05).
- The z-score for lumbar spine BMAD was statistically significantly higher in transmales with a bone age of less than 14 years (z-score [range]: start of hormones -0.84 [-2.2 to 0.87], 24-month follow-up -0.15 [-1.38 to 0.94], p≤ 0.01) and 14 years and older (z-score [range]: start of hormones -0.29 [-2.28 to 0.90], 24-month follow-up -0.06 [-1.75 to 1.61], p≤ 0.01).
- Actual lumbar spine BMAD values were statistically significantly higher in transfemales and transmales of all bone ages.
- There was no statistically significant difference in femoral neck BMAD z-score in transfemales (all bone ages).
- The z-score for femoral neck BMAD was statistically significantly higher in transmales with a bone age of less than 14 years (z-score [range]: start of hormones

-0.37 [-2.28 to 0.47], 24-month follow-up -0.37 [-2.03 to 0.85], p≤ 0.01) and 14 years and older (z-score [range]: start of hormones -0.27 [-1.91 to 1.29], 24-month follow-up 0.02 [-2.1 to 1.35], p≤0.05).
- There was no statistically significant difference in actual femoral neck BMAD values in transfemales (all bone ages), but this was statistically significantly higher in transmales (all bone ages).

The study by Stoffers et al. 2019 in 62 sex assigned at birth females (transmales) with gender dysphoria (who were previously treated with a GnRH analogue) found that during treatment with gender-affirming hormones there was no statistically significant difference in lumbar spine or femoral neck bone density (measured as BMD z-scores or actual values) from starting gender-affirming hormones to any timepoint (6, 12 and 24 months).

Change in clinical parameters

The study by Klaver et al. 2020 in 192 adolescents with gender dysphoria found that during treatment with gender-affirming hormones, from starting treatment to age 22 years:

- Glucose levels, insulin levels and insulin resistance were largely unchanged in transfemales and transmales.
- Total cholesterol, HDL cholesterol and LDL cholesterol levels were unchanged in transfemales, and there was a statistically significant improvement in triglyceride levels.
- Total cholesterol, HDL cholesterol, LDL cholesterol and triglyceride levels significantly worsened in transmales, but mean levels were within the UK reference range at the end of treatment.
- Diastolic blood pressure was statistically significantly increased in transfemales and transmales. Systolic blood pressure was also statistically significantly increased in transmales, but not in transfemales. The absolute increases in blood pressure were small.
- Body mass index was statistically significantly increased in transfemales and transmales, although most participants were within the healthy weight range (18.5 to 24.9 kg/m).

The study by Stoffers et al. 2019 in 62 sex assigned at birth females (transmales) with gender dysphoria found that during treatment with gender affirming hormones, from starting treatment to 24-month follow-up:

- There was no statistically significant change in glycosylated haemoglobin (HbA1c).
- There was no statistically significant change in aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyltransferase (GCT).
- There was a statistically significant increase in alkaline phosphatase (ALP) at some timepoints, but the difference was not statistically significant by 24-months.
- There was a statistically significant increase in serum creatinine levels at all timepoints up to 24 months, but these were within the UK reference range. Serum urea levels were unchanged (follow-up duration not reported).

Treatment discontinuation and adverse effects

The study by Khatchadourian et al. 2014 in 63 adolescents (24 transfemales and 39 transmales) with gender dysphoria found that during treatment with gender affirming hormones (duration of treatment not reported):

- No participants permanently discontinued treatment.
- No transfemales temporarily discontinued treatment, but 3 transmales temporarily discontinued treatment due to mental health comorbidities (n=2) and androgenic alopecia (n=1). All 3 participants eventually resumed treatment, although timescales were not reported
- No severe complications were reported.
- No transfemales reported minor complications, but 12 transmales developed minor complications which were: severe acne (n=7), androgenic alopecia (n=1), mild dyslipidaemia (n=3) and significant mood swings (n=1).

In children and adolescents with gender dysphoria, what is the cost-effectiveness of gender-affirming hormones compared to one or a combination of psychological support, social transitioning to the desired gender or no intervention?

No cost-effectiveness evidence was found for gender-affirming hormones for children and adolescents with gender dysphoria.

From the evidence selected, are there particular sub-groups of children and adolescents with gender dysphoria that derive comparatively more (or less) benefit from treatment with gender-affirming hormones than the wider population of children and adolescents with gender dysphoria?

Some studies reported data separately for the following subgroups of children and adolescents with gender dysphoria:

- Sex assigned at birth males (transfemales).
- Sex assigned at birth females (transmales).
- Tanner stage at which GnRH analogue or gender-affirming hormones started.
- Diagnosis of a mental health condition.

Some direct comparisons of transfemales and transmales were included. No evidence was found for other specified subgroups.

Sex assigned at birth males (transfemales)
Impact on mental health
In the study by Kuper et al. 2020 in 33 to 45 (number varies by outcome) sex assigned at birth males (transfemales) with gender dysphoria found that during treatment with gender-affirming hormones changes were seen in depression, anxiety and anxiety-related symptoms from baseline to follow-up (mean duration of treatment 10.9 months). The authors did not report any statistical analyses, so it is unclear if any changes were statistically significant.

The study by Allen et al. 2019 in 47 adolescents with gender dysphoria found that during treatment with gender-affirming hormones, suicide risk (measured using the ASQ) is not statistically significant different in transfemales compared with transmales, between baseline and the final assessment at about 12 months (p=0.79).

The study by Achille et al. 2020 in 17 transfemales with gender dysphoria found that during treatment with gender-affirming hormones, suicidal ideation (measured using the PHQ 9_Modified for Teens with additional questions for suicidal ideation) was reported in 11.8%

(2/17) of transfemales at baseline compared with 5.9% (1/17) at about 12-months follow-up (no statistical analysis was reported).

Impact on quality of life
The study by Allen et al. 2019 in 47 adolescents with gender dysphoria found that during treatment with gender-affirming hormones, quality of life (measured using the GWBS of the Paediatric Quality of Life Inventory) was not statistically significant different in transfemales compared with transmales, between baseline and the final assessment at about 12 months (p=0.32).

Bone density
The studies by Klink et al. 2015 and Vlot et al. 2017 provided evidence on bone density in transfemales; see above for details.

Change in clinical parameters
The study by Klaver et al. 2020 provided evidence on the following clinical parameters in transfemales:

- Glucose levels, insulin levels and insulin resistance.
- Total cholesterol, HDL cholesterol and LDL cholesterol and triglycerides.
- Blood pressure.
- Body mass index.

See above for details.

Treatment discontinuation and adverse effects
The study by Khatchadourian et al. 2014 provided evidence on treatment discontinuation and adverse effects in transfemales; see above for details.

Sex assigned at birth females (transmales)
Impact on mental health
In the study by Kuper et al. 2020 in 65 to 78 (number varies by outcome) sex assigned at birth females (transmales) with gender dysphoria found that during treatment with gender-affirming hormones, changes were seen in depression, anxiety and anxiety-related symptoms from baseline to 10.9 month follow-up. The authors did not report any statistical analyses, so it is unclear if any changes were statistically significant.

The study by Allen et al. 2019 in 47 adolescents with gender dysphoria found that during treatment with gender-affirming hormones, suicide risk (measured using the ASQ) is not statistically significantly different in transmales compared with transfemales, between baseline and the final assessment (p=0.79).

The study by Achille et al. 2020 in 33 transmales with gender dysphoria found that during treatment with gender-affirming hormones, suicidal ideation (measured using the PHQ 9_Modified for Teens with additional questions for suicidal ideation) was reported in 9.1% (3/33) of transmales at baseline compared with 6.1% (2/33) at about 12-months follow-up (no statistical analysis reported).

Impact on quality of life
The study by Allen et al. 2019 in 47 adolescents with gender dysphoria found that during treatment with gender-affirming hormones, quality of life (measured using the GWBS of the

Paediatric Quality of Life Inventory) was not statistically significantly different in transmales compared with transfemales, between baseline and the final assessment at about 12 months (p=0.32).

Bone density
The studies by Klink et al. 2015, Stoffers et al. 2019 and Vlot et al. 2017 provided evidence on bone density in transmales; see above for details.

Change in clinical parameters
The study by Klaver et al. 2020 provided evidence on the following clinical parameters in transmales:

- Glucose levels, insulin levels and insulin resistance.
- Total cholesterol, HDL cholesterol and LDL cholesterol and triglycerides.
- Blood pressure.
- Body mass index.

See above for details.

The study by Stoffers et al. 2019 provided evidence on HbA1c, liver enzymes and renal function in transmales; see above for details.

Treatment discontinuation and adverse effects
The study by Khatchadourian et al. 2014 provided evidence on treatment discontinuation and adverse effects in transmales; see above for details.

Tanner stage at which GnRH analogues or gender-affirming hormones started
The study by Kuper et al. 2020 stated that the impact of Tanner stage on outcomes was considered, but it is unclear if this refers to Tanner stage at the initial assessment, at the start of GnRH analogue treatment or another timepoint. No results were reported.

Diagnosis of a mental health condition
Impact on mental health
The study by Achille et al. 2020 in 50 adolescents with gender dysphoria found that during treatment with gender-affirming hormones, there was no statistically significant difference in depression (measured using the CESD-R and PHQ 9_Modified for Teens) when the results were adjusted for engagement in counselling and medicines for mental health problems, from baseline to about 12-months follow-up.

Impact on quality of life
The study by Achille et al. 2020 in 50 adolescents with gender dysphoria found that during treatment with gender-affirming hormones, there was no statistically significant difference in quality of life (measured using the QLES-Q-SF) when the results were adjusted for engagement in counselling and medicines for mental health problems, from baseline to about 12-months follow-up.

From the evidence selected,
(a) what are the criteria used by the research studies to define gender dysphoria, gender identity disorder and gender incongruence of childhood?
(b) what were the ages at which participants commenced treatment with gender-affirming hormones?

(c) what was the duration of treatment with GnRH analogues?

The most commonly reported diagnostic criteria for gender dysphoria was the DSM criteria in use at the time (5/10 studies). In 3 studies (Klaver et al. 2020, Klink et al. 2015 and Vlot et al. 2017) DSM-IV-TR criteria was used. In 2 studies (Kuper et al. 2020 and Stoffers et al. 2019) DSM-V criteria was used. One study from Finland (Kaltiala et al. 2020) used the ICD-10 diagnosis of 'transexualism'. It was not reported how gender dysphoria was defined in the remaining 4 studies.

In the studies, treatment with gender-affirming hormones started at about 16 to 17 years, with a range of about 14 to 19 years. Most studies did not report the duration of treatment with GnRH analogues, but where this was reported there was a wide variation ranging from a few months up to about 5 years (Klaver et al. 2020, Klink et al. 2015 and Stoffers et al. 2019).

Discussion

The key limitation to identifying the effectiveness and safety of gender-affirming hormones for children and adolescents with gender dysphoria is the lack of reliable comparative studies.

All the studies included in the evidence review are uncontrolled observational studies, which are subject to bias and confounding and were of very low certainty using modified GRADE. A fundamental limitation of all the uncontrolled studies included in this review is that any changes in scores from baseline to follow-up could be attributed to a regression-to-the-mean.

The included studies have relatively short follow-up, with an average duration of treatment with gender-affirming hormones between around 1 year and 5.8 years. Further studies with a longer follow-up are needed to determine the long-term effect of gender-affirming hormones for children and adolescents with gender dysphoria.

Most studies included in this review did not report comorbidities (physical or mental health) and no study reported concomitant treatments in detail. Because of this it is not clear whether any changes seen were due to gender-affirming hormones or other treatments the participants may have received.

There is a degree of indirectness in some studies, with some participants included that fall outside of the population of this evidence review. Furthermore, participant numbers are poorly reported in some studies, with high numbers lost to follow-up or outcomes not reported for some participants. The authors provide no explanation for this incomplete reporting.

Details of the gender-affirming hormone treatment regimen are poorly reported in most of the included studies, with limited information provided about the medicines, doses and routes of administration used. It is not clear whether the interventions used in the studies are reflective of current UK practice for children and adolescents with gender dysphoria.

It is difficult to draw firm conclusions for many of the effectiveness and safety outcomes reported in the included studies because many different scoring tools and methods were used to assess the same outcome, often with conflicting results. In addition to this, most

outcomes reported across the included studies do not have an accepted minimal clinically important difference (MCID), making it difficult the determine whether any statistically significant changes seen are clinically meaningful. However, the authors of some studies report thresholds to interpret the results of the scoring tools (for example, by linking scores to symptom severity), so some conclusions can be made.

Conclusion

Any potential benefits of gender-affirming hormones must be weighed against the largely unknown long-term safety profile of these treatments in children and adolescents with gender dysphoria.

Results from 5 uncontrolled, observational studies suggest that, in children and adolescents with gender dysphoria, gender-affirming hormones are likely to improve symptoms of gender dysphoria, and may also improve depression, anxiety, quality of life, suicidality, and psychosocial functioning. The impact of treatment on body image is unclear. All results were of very low certainty using modified GRADE.

Safety outcomes were reported in 5 observational studies. Statistically significant increases in some measures of bone density were seen following treatment with gender-affirming hormones, although results varied by bone region (lumber spine versus femoral neck) and by population (transfemales versus transmales). However, z-scores suggest that bone density remained lower in transfemales and transmales compared with an equivalent cisgender population. Results from 1 study of gender-affirming hormones started during adolescence reported statistically significant increases in blood pressure and body mass index, and worsening of the lipid profile (in transmales) at age 22 years, although longer term studies that report on cardiovascular event rates are required. Adverse events and discontinuation rates associated with gender-affirming hormones were only reported in 1 study, and no conclusions can be made on these outcomes.

This review did not identify sub-groups of patients who may benefit more from gender-affirming hormones.

No cost-effectiveness evidence was found to determine whether gender-affirming hormones are a cost-effective treatment for children and adolescents with gender dysphoria.

3. Methodology

Review questions

The review question(s) for this evidence review are:

1. For children and adolescents with gender dysphoria, what is the clinical effectiveness of treatment with gender-affirming hormones compared with one or a combination of psychological support, social transitioning to the desired gender or no intervention?
2. For children and adolescents with gender dysphoria, what is the short-term and long-term safety of gender-affirming hormones compared with one or a combination of psychological support, social transitioning to the desired gender or no intervention?

3. For children and adolescents with gender dysphoria, what is the cost-effectiveness of gender-affirming hormones compared to one or a combination of psychological support, social transitioning to the desired gender or no intervention?
4. From the evidence selected, are there particular sub-groups of children and adolescents with gender dysphoria that derive comparatively more (or less) benefit from treatment with gender-affirming hormones than the wider population of children and adolescents with gender dysphoria?
5. From the evidence selected,
   (a) what are the criteria used by the research studies to define gender dysphoria, gender identity disorder and gender incongruence of childhood?
   (b) what were the ages at which participants commenced treatment with gender-affirming hormones?
   (c) what was the duration of GnRH analogues treatment?

See appendix A for the full review protocol.

Review process

The methodology to undertake this review is specified by NHS England in their 'Guidance on conducting evidence reviews for Specialised Services Commissioning Products' (2020).

The searches for evidence were informed by the PICO and were conducted on 21 July 2020.

See appendix B for details of the search strategy.

Results from the literature searches were screened using their titles and abstracts for relevance against the criteria in the PICO framework. Full text references of potentially relevant evidence were obtained and reviewed to determine whether they met the inclusion criteria for this evidence review.

See appendix C for evidence selection details and appendix D for the list of studies excluded from the review and the reasons for their exclusion.

Relevant details and outcomes were extracted from the included studies and were critically appraised using a checklist appropriate to the study design. See appendix E and appendix F for individual study and checklist details.

The available evidence was assessed by outcome for certainty using modified GRADE. See appendix G for GRADE Profiles.

4. Summary of included studies

Ten observational studies were included in the evidence review. Seven studies were retrospective observational studies (Allen et al. 2019, Kaltiala et al. 2020, Khatchadourian et al. 2014, Klaver et Al. 2020, Klink et al. 2015, Stoffers et al. 2019, Vlot et al. 2017) and three

studies were prospective longitudinal observational studies (Achille et al. 2020, Kuper et al. 2020, Lopez de Lara et al. 2020).

The terminology used in this topic area is continually evolving and is different depending on stakeholder perspectives. In this evidence review we have used the phrase 'people's assigned sex at birth' rather than saying natal or biological sex and 'cross sex hormones' are now referred to as 'gender-affirming hormones'. The research studies may use historical terms which are no longer considered appropriate.

Table 1 provides a summary of these included studies and full details are given in appendix E.

Table 1 Summary of included studies

Study	Population	Intervention and comparison	Outcomes reported
Achille et al. 2020 Prospective longitudinal study Single centre, New York, United States	50 children, adolescents and young adults with gender dysphoria; 17 transfemales and 33 transmales Mean age at baseline was 16.2 years (SD 2.2)	**Intervention** Endocrine interventions (the collective term used for puberty suppression and gender-affirming hormones) were introduced as per Endocrine Society and the World Professional Association for Transgender Health (WPATH) guidelines Puberty suppression was: • GnRH analogue and/or anti-androgens (transfemales) • GnRH analogue or medroxyprogesterone (transmales) Once eligible, gender-affirming hormones were offered, these were: • Oestradiol (transfemales) • Testosterone (transmales) Doses and formulations not reported	**Critical Outcomes** *Impact on mental health* • Depression- The Center for Epidemiologic Studies Depression Scale (CESD-R) • Depression- The Patient Health Questionnaire Modified for Teens (PHQ 9_Modified for Teens) *Impact on quality of life* • Quality of Life Enjoyment and Satisfaction Questionnaire (QLES-Q-SF) **Important Outcomes** *None reported*

Study	Population	Intervention and comparison	Outcomes reported
		After about 12-months treatment ('wave 3'): • 24 people (48%) were on gender-affirming hormones alone • 12 people (24%) were on puberty suppression alone • 11 people (22%) were on both gender-affirming hormones and puberty suppression • 3 people (6%) were on no endocrine intervention **Comparison** No comparison group. Change over time reported	
Allen et al. 2019 Retrospective longitudinal study Single centre, Kansas City, USA	47 adolescents and young adults with gender dysphoria: 14 transfemales and 33 transmales Mean age at administration (start of treatment) 16.5 years	**Intervention** 39 participants received gender-affirming hormones only 8 participants received hormones and a GnRH analogue Mean duration of treatment with gender-affirming hormones was 349 days (range 113 to 1,016) **Comparison** No comparison group. Comparison over time reported	**Critical Outcomes** *Impact on mental health* • Suicidality- Ask Suicide-Screening Questions (ASQ) instrument *Impact on quality of life* • General Well-Being Scale (GWBS) of the Pediatric Quality of Life Inventory **Important Outcomes** *None reported*
Kaltiala et al. 2020 Retrospective chart review Single centre, Tampere, Finland	52 adolescents with gender dysphoria: 11 transfemales and 41 transmales. Mean age at diagnosis 18.1 years (range 15.2 to 19.9)	**Intervention** Hormonal sex assignment treatment – details of intervention not reported, although all patients received gender-affirming hormones.	**Critical Outcomes** *Impact on mental health* • Need for mental health treatment **Important Outcomes** *Psychosocial Impact* Measure of functioning in different domains of

Study	Population	Intervention and comparison	Outcomes reported
		Comparison No comparison group. Comparison over time reported	adolescent development, which were: • Living with parent(s)/ guardians • Normative peer contacts • Progresses normatively in school/ work • Has been dating or had steady relationships • Is age-appropriately able to deal with matters outside of the home
Khatchadourian et al. 2014 Retrospective chart review Single centre, Vancouver, Canada	84 young people with gender dysphoria, of whom 63 received gender-affirming hormones. Median age at start of gender-affirming hormones was: • 17.3 years (range 13.7-19.8) for testosterone • 17.9 years (range 13.3-22.3) for oestrogen	**Intervention** Transfemales: Oestrogen (oral micronized 17β-oestradiol) Transmales: Testosterone (injectable testosterone enanthate and/or cypionate) 19 participants (30%) had previously received a GnRH analogue **Comparison** No comparison group. Comparison over time reported.	**Critical Outcomes** *None reported* **Important Outcomes** *Safety:* • Adverse events • Discontinuation rates
Klaver et al. 2020 Retrospective chart review Single centre, Amsterdam, Netherlands	192 people with gender dysphoria who started GnRH analogues before the age of 18 years, and started gender-affirming hormones within 1.5 years of their 22nd birthday. Mean age at start of gender-affirming hormones: • Transfemale – 16.4 years (SD 1.1) • Transmale – 16.9 years (SD 1.9)	**Intervention** Oral oestrogen or intramuscular (IM) testosterone **Comparison** No comparison group. Comparison over time reported	**Critical Outcomes** *None reported* **Important Outcomes** *Safety* • Body mass index (BMI) • Systolic blood pressure • Diastolic blood pressure • Glucose • Insulin • HOMA-IR

Study	Population	Intervention and comparison	Outcomes reported
			• Total cholesterol • HDL cholesterol • LDL cholesterol • Triglycerides
Klink et al. 2015 Retrospective longitudinal study Single centre, Amsterdam, Netherlands	34 young people with gender dysphoria who had received GnRH analogues, gender-affirming hormones and gonadectomy. The study included 15 transfemales and 19 transmales; mean age at start of gender-affirming hormones was 16.6 years (SD 1.4) and 16.4 years (SD 2.3) respectively. At the start of gender-affirming hormone treatment, in the transfemale subgroup the median Tanner P was 4 (IQR 2) and the median Tanner G was 12 (IQR 11) In the transmale subgroup the median Tanner B was 5 (IQR 2) and the median Tanner P was 5 (IQR 0)	**Intervention** Transfemales – oral 17-β oestradiol (incremental dosing) Transmales – IM testosterone (Sustanon 250 mg/ml; incremental dosing) Median duration of treatment with gender-affirming hormones for transfemales was 5.8 years (range 3.0 to 8.0) and for transmales was 5.4 years (range 2.8 to 7.8) The GnRH analogue was subcutaneous (SC) triptorelin 3.75 mg every 4 weeks No details of gonadectomy reported **Comparison** No comparison group. Comparison over time reported.	**Critical Outcomes** None **Important Outcomes** *Safety* • Bone mineral apparent density (BMAD) • Bone mineral density (BMD) Measures reported at 3 timepoints: start of GnRH analogue treatment, start of gender-affirming hormone treatment and age 22 years.
Kuper et al. 2020 Prospective longitudinal study Single centre, Texas, USA	Children and adolescents with gender dysphoria (9 to18 years), n=148, of whom: • 25 received puberty suppression only • 93 received gender-affirming hormone therapy only • 30 received both Mean age 14.9 years	**Intervention** Gender-affirming hormones, guided by Endocrine Society Clinical Practice Guidelines **Comparison** No comparison group. Comparison over time reported.	**Critical Outcomes** *Impact on mental health* • Depression- Quick Inventory of Depressive Symptoms (QIDS), self-reported • Depression- QIDS, clinician-reported • Anxiety- Screen for Child Anxiety Related Emotional Disorders (SCARED)

Study	Population	Intervention and comparison	Outcomes reported
			• Panic- specific questions from SCARED • Generalised anxiety-specific questions from SCARED • Social anxiety - specific questions from SCARED • Separation anxiety-specific questions from SCARED • School avoidance-specific questions from SCARED **Important Outcomes** *Impact on body image* • Body Image Scale (BIS)
Lopez de Lara et al. 2020 Prospective analytical study Single centre, Madrid, Spain	23 adolescents with gender dysphoria: 7 transfemales and 16 transmales. Mean age at baseline was 16 years (range 14 to 18)	**Intervention** Gender-affirming hormones: • Oral oestradiol • Intramuscular testosterone Participants had previously received GnRH analogues in the intermediate pubertal stages (Tanner 2 to 3). Participants were assessed twice: • pre-treatment (T0), • after 12 months treatment with gender-affirming hormones (T1) **Comparison** No comparison group. Comparison over time reported.	**Critical Outcomes** *Impact on gender dysphoria* • Utrecht Gender Dysphoria Scale (UGDS) *Impact on mental health* • Depression- Beck Depression Inventory II (BDI-II) • Anxiety- State-Trait Anxiety Inventory **Important Outcomes** *Psychosocial Impact* • Family functioning- Family APGAR test • Patient strengths and difficulties- Strengths and Difficulties Questionnaire, Spanish Version (SDQ-Cas).
Stoffers et al. 2019 Retrospective chart review	62 transmales with gender dysphoria. Patients had received a GnRH analogue and more than 6 months of testosterone treatment.	**Intervention** Testosterone intramuscular injections (Sustanon 250 mg). Dose was titrated to a	**Critical Outcomes** None **Important Outcomes** *Safety*

Study	Population	Intervention and comparison	Outcomes reported
Single centre, Leiden, Netherlands	Median age at start of testosterone was 17.23 years (range 14.9 to 18.4) Median treatment duration was 12 months (range 5 to 33) Change over time	maintenance dose of 125 mg every 2 weeks. Participants who started GnRH analogues at 16 years or older had their dose increased more rapidly. Some participants chose to receive testosterone every 3-4 weeks, and participants could switch to transdermal preparations if needed. **Comparison** No comparison group. Comparison over time reported.	• Body mass index (BMI) • Blood pressure • BMD • Acne • Liver enzymes • Creatinine • Urea • HbA1c
Vlot et al. 2017 Retrospective chart review Single centre, Amsterdam, Netherlands	70 children and adolescents with gender dysphoria Median age at baseline – • 13.5 years (11.5-18.3) for transfemales • 15.1 years (range 11.7-18.6) for transmales Comparison is change over time. 24 month follow-up.	**Intervention** Oestrogen or testosterone (had previously received triptorelin for puberty suppression) **Comparison** No comparison group. Comparison over time reported.	**Critical Outcomes** None **Important Outcomes** *Safety* • Bone mineral apparent density (BMAD)

5. Results

In children and adolescents with gender dysphoria, what is the clinical effectiveness of gender-affirming hormones compared with one or a combination of psychological support, social transitioning to the desired gender or no intervention?

Outcome	Evidence statement
Clinical Effectiveness	
Critical outcomes	
Impact on gender dysphoria **Certainty of evidence: very low**	This is a critical outcome because gender dysphoria in children and adolescents is associated with significant distress and problems with functioning. One uncontrolled, prospective, observational study (Lopez de Lara et al. 2020) provided evidence relating to the impact on gender dysphoria, measured using the Utrecht Gender Dysphoria Scale (UGDS) score

	during the first year of treatment with gender-affirming hormones. The UGDS is a validated, screening tool for both adolescents and adults, used to assess gender dysphoria. It consists of 12 items, to be answered on a 1- to 5-point scale, resulting in a sum score between 12 and 60. The authors state that the cut-off point to identify gender dysphoria is 40 points. The higher the UGDS score the greater the gender dysphoria. In this study (n=23), the mean (±SD) UGDS score was statistically significantly reduced (improved) from 57.1 (±4.1) points at baseline to 14.7 points (±3.2) at 12 months (p<0.001). A UGDS score below 40 suggests an absence of gender dysphoria **(VERY LOW)**. **This study provides very low certainty evidence that gender-affirming hormones statistically significantly improve gender dysphoria from baseline to 12 months follow-up. The mean UGDS score was below the threshold for gender dysphoria at follow-up.**
Impact on mental health: depression **Certainty of evidence: very low**	This is a critical outcome because depression may impact on social, occupational, or other areas of functioning in children and adolescents. Four observational studies (Achille et al. 2020; Kaltiala et al. 2020; Kuper et al. 2020; Lopez de Lara et al. 2020) provided evidence relating to the impact on depression in children and adolescents with gender dysphoria, with follow-up of around 12 months. Five different outcome measures for depression were reported. **Beck Depression Inventory (BDI-II)** One uncontrolled, prospective, analytical study (Lopez de Lara et al. 2020) reported the change in BDI-II. The BDI-II is a valid, reliable, and widely used tool for assessing depressive symptoms. There are no specific scores to categorise depression severity, but it is suggested that 0 to 13 is minimal symptoms, 14 to 19 is mild depression, 20 to 28 is moderate depression, and severe depression is 29 to 63. In Lopez de Lara et al. 2020 (n=23) the mean (±SD) BDI-II score was statistically significantly reduced (improved) from 19.3 (±5.5) points at baseline to 9.7 (±3.9) points at 12 months (p<0.001) **(VERY LOW)**. **Center for Epidemiologic Studies Depression (CESD-R)** One uncontrolled, prospective, longitudinal study (Achille et al. 2020) reported the change in CESD-R scale. The CESD-R is a valid, widely used tool to assess depressive symptoms. Total score ranges from 0 to 60, with higher scores indicating more depressive symptoms. There are no specific scores to categorise depression severity, although the authors of the study suggest that a total CESD-R score less than 16 suggests no clinical depression. In Achille et al. 2020 (n=50), the mean CESD-R score statistically significantly reduced (improved) from 21.4 points at baseline to 13.9 points at about 12 months follow-up (p<0.001; standard deviation not reported) **(VERY LOW)**. **Patient Health Questionnaire (PHQ 9) Modified for Teens** One uncontrolled, prospective, longitudinal study (Achille et al. 2020) reported the change in PHQ 9_Modified for Teens score. The PHQ

	9_Modified for Teens is a validated tool to assess depression, dysthymia and suicide risk. The tool consists of 9 questions scored from 0 to 3 (total score 0 to 27), plus an additional 4 questions that are not scored. A score of 0 to 4 suggests no or minimal depressive symptoms, 5 to 9 mild, 10 to 14 moderate, 15 to 19 moderately severe, and 20-27 severe symptoms. In Achille et al. 2020 (n=50), the mean PHQ 9_Modified for Teens score statistically significantly reduced (improved) from baseline to around 12 months follow-up, although absolute scores were not reported numerically (p<0.001). From the visual representation of results, the PHQ-9_Modified for Teens score is about 9 at baseline and about 5 at final follow-up **(VERY LOW)**. **Quick Inventory of Depressive Symptoms (QIDS)** One uncontrolled, prospective, longitudinal study (Kuper et al. 2020) reported the change in QIDS, clinician-reported and self-reported. Both the clinician-reported and self-reported QIDS are validated tools to assess depressive symptoms. The tool consists of 16 items, with the highest score for 9 domains (sleep, weight, psychomotor changes, depressed mood, decreased interest, fatigue, guilt, concentration, and suicidal ideation) added to give a total score ranging from 0 to 27. A score of 0 to 5 suggests no depression, 6 to 10 mild symptoms, 11 to 15 moderate symptoms, 16 to 20 severe symptoms, and 21 to 27 very severe symptoms. In Kuper et al. 2020 (n=105), the mean (±SD) QIDS self-reported score was 9.6 points (±5.0) at baseline and 7.4 (±4.5) after 10.9 months of treatment with gender-affirming hormones (no statistical analysis reported). The mean (±SD) QIDS clinician-reported score was 5.9 points (±4.1) at baseline and 6.0 (±3.8) after 10.9 months of treatment with gender-affirming hormones (no statistical analysis was reported) **(VERY LOW)**. **Participants needing treatment for depression** One observational study (Kaltiala et al. 2020) reported the proportion of participants needing treatment for depression before or during the initial assessment and during the 12-month follow-up period after starting gender-affirming hormones. In Kaltiala et al. 2020 (n=52), statistically significantly fewer participants needed treatment for depression during the 12-month 'real life' phase (15%, 8/52) compared with before or during the assessment (54%, 28/52; p<0.001). No details of what treatments for depression the participants received are reported **(VERY LOW)**. **These studies provide very low certainty evidence that during treatment with gender-affirming hormones depression is reduced from baseline to about 12 months follow-up. However, most participants had mild symptoms at the start of treatment.**
Impact on mental health: anxiety	This is a critical outcome because anxiety may impact on social, occupational, or other areas of functioning in children and adolescents.

Certainty of evidence: very low	Three observational studies (Kaltiala et al. 2020; Kuper et al. 2020; Lopez de Lara et al. 2020) provided evidence relating to the impact on anxiety in children and adolescents with gender dysphoria. **State-Trait Anxiety Inventory (STAI)** One uncontrolled, prospective, analytical study (Lopez de Lara et al. 2020) reported the change in STAI scores. STAI is a validated and commonly used measure of trait and state anxiety. It has 20 items and can be used in clinical settings to diagnose anxiety and to distinguish it from depressive illness. Higher scores indicate greater anxiety. In Lopez de Lara et al. 2020 (n=23), the mean (±SD) STAI-State subscale was statistically significantly reduced (improved) with gender-affirming hormones from 33.3 points (±9.1) at baseline to 16.8 points (±8.1) at 12 months (p<0.001). The mean STAI-Trait subscale scores also statistically significantly reduced (improved) from 33.0 points (±7.2) at baseline to 18.5 points (±8.4) at 12 months (p<0.001) **(VERY LOW)**. **Screen for Child Anxiety Related Emotional Disorders (SCARED)** One uncontrolled, prospective, longitudinal study (Kuper et al. 2020) reported anxiety symptoms using the SCARED questionnaire. Other anxiety-related symptoms using specific questions from the SCARED questionnaire were also reported: panic, generalised anxiety, social anxiety, separation anxiety and school avoidance. SCARED is a validated, 41-point questionnaire, with each item scored 0 to 2. A total score of 25 or more is suggestive of anxiety disorder, with scores above 30 being more specific. Certain scores for specific questions may indicate the presence of other anxiety-related disorders: • A score of 7 or more in questions related to panic disorder or significant somatic symptoms may indicate the presence of these. • A score of 9 or more in questions related to generalised anxiety disorder may indicate the presence of this. • A score of 5 or more in questions related to separation anxiety may indicate the presence of this. • A score of 8 or more in questions related to social anxiety disorder may indicate the presence of this. • A score of 3 or more in questions related to significant school avoidance may indicate the presence of this. In Kuper et al. 2020 (n=80 to 82, varies by outcome), small reductions were seen in anxiety, panic, generalised anxiety, social anxiety and separation anxiety and school avoidance symptoms (measured using the SCARED questionnaire) from baseline to follow-up (mean duration of treatment 10.9 months). The statistical significance of these findings are unknown as no statistical analyses were reported **(VERY LOW)**. **Participants needing treatment for anxiety** One observational study (Kaltiala et al. 2020) reported the proportion of participants needing treatment for anxiety before or during initial assessment and during the 12-month follow-up period after starting gender-affirming hormones.

	In Kaltiala et al. 2020 (n=52), statistically significantly fewer participants needed treatment for anxiety during the 12-month 'real life' phase (15%, 8/52) compared with before or during the assessment (48%, 25/52; p<0.001). No details of what treatments for anxiety the participants received are reported **(VERY LOW)**. **These studies provide very low certainty evidence that during treatment with gender-affirming hormones anxiety symptoms may be reduced from baseline to around 12 months follow-up.**
Impact on mental health: suicidality and self-injury **Certainty of evidence: very low**	These are critical outcomes because self-harm and thoughts of suicide have the potential to result in significant physical harm and, for completed suicides, the death of the young person. Four observational studies (Achille et al. 2020; Allen et al. 2019; Kaltiala et al. 2020; Kuper et al. 2020) provided evidence relating to suicidal ideation in children and adolescents with gender dysphoria, with an average follow-up of around 12 months. **Ask Suicide-Screening Questions (ASQ)** One uncontrolled, retrospective, longitudinal study (Allen et al. 2019) reported the change in ASQ. This is a 4-item dichotomous (yes/no) response measure designed to identify risk of suicide. The authors of Allen et al. 2019 amended 1 question in the ASQ ("*Have you ever tried to kill yourself?*") by prefacing it with "*In the past few weeks . . .*" as they were not investigating lifetime incidence. A response of 'no' is scored as 0 and a response of 'yes' is scored as 1; each item is summed to give an overall score for suicidal ideation ranging from 0 to 4. A person is considered to have screened positive if they answer 'yes' to any item with higher scores indicating higher levels of suicidal ideation. In Allen et al. 2019 (n=39), the adjusted mean (±SE) ASQ score statistically significantly reduced from 1.11 points (±0.22) at baseline to 0.27 points (±0.12) after a mean duration of treatment of about 12 months (p<0.001) **(VERY LOW)**. **PHQ 9_Modified for Teens (additional questions for suicidal ideation)** One uncontrolled, prospective, longitudinal study (Achille et al. 2020) reported the change in suicidal ideation measured using additional questions from the PHQ 9_Modified for Teens. This is a validated tool to assess depression, dysthymia and suicide risk (see above for detailed description). In addition to the 9 scored questions, the PHQ 9_Modified Teens asked 4 additional questions relating to suicidal ideation and difficulty dealing with problems of life. Responses to the PHQ 9_Modified for Teens were used to determine if the participant had suicidal ideation or not, but specific details of how this was determined are not reported. In Achille et al. 2020 (n=50), 10% (5/50) of participants had suicidal ideation at baseline and 6% (3/50) had suicidal ideation after about 12 months treatment with gender-affirming hormones (no statistical analysis reported) **(VERY LOW)**. **Suicidality and non-suicidal self-injury**

	One uncontrolled, prospective, longitudinal study (Kuper et al. 2020) reported on suicidal ideation, suicide attempts and non-suicidal self-injury, although it was unclear how and when this outcome was measured. In Kuper et al. 2020 (n=130), 25% of participants reported suicidal ideation 1 month before the initial assessment and 38% reported this during the follow-up period (no statistical analysis reported). Suicide attempts were reported in 2% of participants at 3 months before the initial assessment and 5% during follow-up. Self-injury was reported in 10% of participants at 3 months before the initial assessment and 17% during follow-up. No statistical analysis was reported for any outcomes. Mean duration of gender-affirming hormone treatment was 10.9 months **(VERY LOW)**. **Participants needing treatment for suicidality or self-harm** One observational study (Kaltiala et al. 2020) reported the proportion of participants requiring treatment for suicidality or self-harm before or during initial assessment and during the 12-month follow-up period after starting gender-affirming hormones. In Kaltiala et al. 2020 (n=52) statistically significantly fewer participants needed treatment for suicidality or self-harm during the 12-month 'real life' phase (4%, 2/52) compared with before or during the assessment (35%, 18/52; p<0.001). No details of what treatments for suicidal ideation or self-harm the participants received are reported **(VERY LOW)**. **These studies provide very low certainty evidence that gender-affirming hormones may reduce suicidality from baseline to about 12 months follow-up. However, results are inconsistent and it is difficult to draw conclusions.**
Impact on mental health: other **Certainty of evidence: very low**	This is a critical outcome because mental health problems may impact on social, occupational, or other areas of functioning in children and adolescents. One observational study (Kaltiala et al. 2020) reported the proportion of participants needing treatment for either psychotic symptoms or psychosis, substance abuse, autism, attention deficit hyperactivity disorder (ADHD) or eating disorders before or during initial assessment and during the 12-month follow-up period after starting gender-affirming hormones. In Kaltiala et al. 2020 (n=52) there was no statistically significant difference in the number of people needing treatment for either psychotic symptoms / psychosis, substance abuse, autism, attention deficit hyperactivity disorder (ADHD) or eating disorders during the 12-month 'real life' phase compared with before or during the assessment. No details of which specific treatments the participants received are reported **(VERY LOW)**. **This study provides very low certainty evidence on the need for treatment for either psychotic symptoms or psychosis, conduct problems or antisocial behaviour, substance abuse, autism, attention deficit hyperactivity disorder (ADHD) or eating disorders**

	during treatment with gender-affirming hormones. No conclusions could be drawn.
Impact on quality of life score **Certainty of evidence: very low**	This is a critical outcome because gender dysphoria in children and adolescents may be associated with a significant reduction in health-related quality of life. Two uncontrolled longitudinal studies Achille et al. 2020; Allen et al. 2019) provided evidence relating to quality of life in children and adolescents with gender dysphoria. **Quality of Life Enjoyment and Satisfaction Questionnaire (QLES-Q-SF)** One uncontrolled, prospective, longitudinal study (Achille et al. 2020) reported the change in QLES-Q-SF scores from baseline to about 12 months of treatment with gender-affirming hormones. QLES-Q-SF is a validated questionnaire, consisting of 15 questions that rate quality of life on a scale of 1 (poor) to 5 (very good). In Achille et al. 2020 (n=50), the mean QLES-Q-SF score was statistically significantly reduced from baseline to about 12 months ($p<0.001$). However, absolute scores are not reported numerically **(VERY LOW)**. **General Well-Being Scale (GWBS) of the Paediatric Quality of Life Inventory** One uncontrolled, retrospective, longitudinal study (Allen et al. 2019) reported the change in adjusted mean GWBS of the Paediatric Quality of Life Inventory score from baseline to about 12 months of treatment with gender-affirming hormones. The GWBS of the Paediatric Quality of Life Inventory contains 7 items that measure two dimensions: general wellbeing (6 items) and general health (1 item). Each item is scored from 0 to 4, and the total score is linearly transformed to a 0 to 100 scale. Higher scores reflect fewer perceived problems and greater well-being. In Allen et al. 2019 (n=47), the adjusted mean (±SE) GWBS of the Paediatric Quality of Life Inventory score was statistically significantly increased (improved) from 61.70 (±2.43) points at baseline to 70.23 (±2.15) points at about 12 months ($p<0.002$) **(VERY LOW)**. **This study provides very low certainty evidence that gender-affirming hormones statistically significantly improve quality of life and well-being from baseline to 12 months follow-up.**
Important outcomes	
Impact on body image **Certainty of evidence: very low**	This is an important outcome because some children and adolescents with gender dysphoria may want to take steps to suppress features of their physical appearance associated with their sex assigned at birth or accentuate physical features of their desired gender. One uncontrolled, prospective, longitudinal study (Kuper et al. 2020) provided evidence relating to the impact on body image in children and adolescents with gender dysphoria who started treatment with gender-affirming hormones (median duration 10.9 months; range 1 to 18), measured by the change in Body Image Scale (BIS) score. BIS is a

	validated 30-item scale covering 3 aspects: primary, secondary and neutral body characteristics. Higher scores represent a higher degree of body dissatisfaction. In Kuper et al. 2020 (n=86), the mean (±SD) BIS score was 70.7 points (±15.2) at baseline and 51.4 points (±18.3) at follow-up (no statistical analysis reported) **(VERY LOW)**. **This study provides very low certainty evidence on the effects of gender-affirming hormones on body image during treatment with gender-affirming hormones (mean duration of treatment 10.9 months). No conclusions could be drawn.**
Psychosocial impact **Certainty of evidence: very low**	This is an important outcome because gender dysphoria in children and adolescents is associated with internalising and externalising behaviours, and emotional and behavioural problems which may impact on social and occupational functioning. Two uncontrolled, observational studies (Kaltiala et al. 2020; Lopez de Lara et al. 2020) provided evidence related to psychosocial impact in children and adolescents with gender dysphoria. **Family APGAR (Adaptability, Partnership, Growth, Affection and Resolve) test** One uncontrolled, prospective, analytical study (Lopez de Lara et al. 2020) reported the Family APGAR test. The Family APGAR test is a 5-item questionnaire, with higher scores indicating better family functioning. The authors reported the following interpretation of the test: functional, 17 to 20 points; mildly dysfunctional, 16 to 13 points; moderately dysfunctional, 12 to 10 points; severely dysfunctional, <9 points. In Lopez de Lara et al. 2020 (n=23), the mean Family APGAR test score was unchanged from baseline (17.9 points) to 12-month follow-up (18.0 points; no statistical analysis or standard deviations reported) **(VERY LOW)**. **Strengths and Difficulties Questionnaire (SDQ)** One uncontrolled, prospective, analytical study (Lopez de Lara et al. 2020) reported on behaviour using the Strengths and Difficulties Questionnaire (SDQ, Spanish version). The SDQ includes 25-items covering emotional symptoms, conduct problems, hyperactivity/ inattention, peer relationship problems and prosocial behaviour. The authors state that a score of more than 20 suggests having a behavioural disorder (normal 0 to 15, borderline 16 to 19, abnormal 20 to 40). In Lopez de Lara et al. 2020 (n=23), the mean (±SD) SDQ score was statistically significantly reduced (improved) from 14.7 points (±3.3) at baseline to 10.3 points (±2.9) at 12-month follow-up (p<0.001) **(VERY LOW)**. **Psychosocial functioning** One uncontrolled, retrospective chart review (Kaltiala et al. 2020) reported various markers of functioning in adolescent development, covering living arrangements, peer contacts, school or work progress,

	relationships, and ability to cope with matters outside the home. These measures were reported during the gender identity assessment and at about 12 months after starting gender-affirming hormones (referred to as the 'real-life phase'). In Kaltiala et al. 2020 (n=52), from the gender identity assessment to the 12-month follow-up period: • statistically significantly fewer participants were living with parents or guardians (73% versus 40%, p=0.001) • statistically significantly fewer participants had normal peer contacts (89% versus 81%, p<0.001) • there was no statistically significant difference in progress in school or work (64% versus 60%, p=0.69) • there was no statistically significant difference in the number of participants who had been dating or in steady relationships (62% versus 58%, p=0.51) • there was no statistically significant difference in the participant's ability to cope with matters outside of the home (81% versus 81%, p=1.00) **(VERY LOW)**. **These studies provide very low certainty evidence that gender-affirming hormones statistically significantly improve behavioural problems (measured by SDQ score). However, the SDQ score was in the 'normal' range at baseline and at 12-month follow up. There was no significant impact on other measures of psychosocial functioning.**
Engagement with health care services	This is an important outcome because patient engagement with health care services will impact on their clinical outcomes. No evidence was identified.
Impact on extent of and satisfaction with surgery	This is an important outcome because some children and adolescents with gender dysphoria may proceed to transitioning surgery. No evidence was identified.
De-transition	This is an important outcome because there is uncertainty about the short- and long-term safety and adverse effects of gender-affirming hormones in children and adolescents with gender dysphoria No evidence was identified.

Abbreviations: APGAR: Adaptability, Partnership, Growth, Affection and Resolve; ASQ: Ask Suicide-Screening Questions; BDI-II: Beck Depression Inventory II; BIS: Body Image Scale; CESD-R: Center for Epidemiologic Studies Depression; GWBS: General Well-Being Scale; p: p-value; PHQ 9_Modified for Teens: Patient Health Questionnaire Modified for Teens; QIDS: Quick Inventory of Depressive Symptoms; QLES-Q-SF: Quality of Life Enjoyment and Satisfaction Questionnaire; SCARED: Screen for Child Anxiety Related Emotional Disorders; SD: standard deviation; SE: standard error; SDQ: Strengths and Difficulties Questionnaire; STAI: State-Trait Anxiety Inventory; UGDS: Utrecht Gender Dysphoria Scale.

In children and adolescents with gender dysphoria, what is the short-term and long-term safety of gender-affirming hormones compared with one or a combination of psychological support, social transitioning to the desired gender or no intervention?

Outcome	Evidence statement
Safety	
Change in bone density: lumbar spine **Certainty of evidence: very low**	This is an important outcome because childhood and adolescence is a key time for bone development and gender-affirming hormones may affect bone development, as shown by changes in lumbar spine bone density. Three uncontrolled, observational studies (2 retrospective and 1 prospective) provided evidence related to bone density: lumbar spine in children and adolescents with gender dysphoria. This was reported as either bone mineral density (BMD), bone mineral apparent density (BMAD), or both. One study reported change in bone density from start of treatment with gender-affirming hormones to age 22 years (Klink et al. 2015). Two studies reported change in bone density from start of gender-affirming hormones up to 24-month follow-up (Stoffers et al. 2019 and Vlot et al. 2017). All participants had previously been treated with a GnRH analogue. All outcomes were reported separately for transfemales and transmales; also see subgroups table below. **Bone mineral apparent density (BMAD)** Two uncontrolled, observational studies reported change in lumbar BMAD (Klink et al. 2015; Vlot et al. 2017). BMAD is a size adjusted value of BMD, incorporating bone size measurements using a UK reference population of growing cis-gender adolescents (up to age 17 years). BMAD is used to correct for height and height gain and may provide a more accurate estimate of bone density in growing adolescents. BMAD was reported as g/cm^3 and as z-scores. Z-scores report how many standard deviations from the mean a measurement sits. A z-score of 0 is equal to the mean, a z-score of -1 is equal to 1 standard deviation below the mean, and a z-score of +1 is equal to 1 standard deviation above the mean. A cis-gender population was used to calculate the bone density z-score, meaning transfemales were compared with cis-males and transmales were compared with cis-females. In Klink et al. 2015 (n=34): • There was no statistically significant difference in lumbar spine BMAD z-score from starting gender-affirming hormones to age 22 years in transfemales. • The z-score for lumbar spine BMAD was statistically significantly higher at age 22 years compared with the start of gender-affirming hormones in transmales (z-score [±SD]: start of hormones -0.50 [±0.81], age 22 years -0.033 [±0.95], p=0.002). • Actual lumbar spine BMAD values in g/cm^3 were statistically significantly higher at age 22 years compared with the start of gender-affirming hormones in transfemales and transmales **(VERY LOW)**. In Vlot et al. 2017 (n=70): • The z-score for lumbar spine BMAD in transfemales with a bone age of <15 years was statistically significantly higher at 24-month follow-up compared with start of gender-affirming hormones (z-score [range]: start of hormones -1.52 [-2.36 to

	0.42], 24-month follow-up -1.10 [-2.44 to 0.69], p≤ 0.05). Statistically significant improvements in z-score for lumbar spine BMAD in transfemales with a bone age of ≥15 years were also seen (z-score [range]: start of hormones -1.15 [-2.21 to 0.08], 24-month follow-up -0.66 [-1.66 to 0.54], p≤ 0.05). • The z-score for lumbar spine BMAD in transmales with a bone age of <14 years was statistically significantly higher at 24-month follow-up compared with start of gender-affirming hormones (z-score [range]: start of hormones -0.84 [-2.2 to 0.87], 24-month follow-up -0.15 [-1.38 to 0.94], p≤ 0.01). Statistically significant improvements in z-score for lumbar spine BMAD in transmales with a bone age of ≥14 years were also seen (z-score [range]: start of hormones -0.29 [-2.28 to 0.90], 24-month follow-up -0.06 [-1.75 to 1.61], p≤ 0.01). • Actual lumbar spine BMAD values in g/cm^3 were statistically significantly higher at 24-month follow-up compared with start of gender-affirming hormones in transfemales and transmales of all bone ages **(VERY LOW)**. **Bone mineral density (BMD)** Two uncontrolled, observational studies reported change in lumbar BMD (Klink et al. 2015; Stoffers et al. 2019). BMD was determined using dual energy x-ray absorptiometry (DXA-scan; HologicQDR4500, Hologic). BMD was reported as g/cm^2 and as z-scores – see BMAD above for more details). In Klink et al. 2015 (n=34): • There was no statistically significant difference in lumbar spine BMD z-score from starting gender-affirming hormones to age 22 years in transfemales or transmales. • Actual lumbar spine BMD values in g/cm^2 were statistically significantly higher at age 22 years compared with the start of gender-affirming hormones in transfemales and transmales **(VERY LOW)**. In Stoffers et al. 2019 (n=62 at 6-month follow-up; n=15 at 24-month follow-up): • There was no statistically significant difference in lumbar spine BMD z-score in transmales from starting gender-affirming hormones to any timepoint (6, 12 and 24 months). • There was also no statistically significant difference in actual lumbar spine BMD values in g/cm^2 from starting gender-affirming hormones to any timepoint (6, 12 and 24 months) **(VERY LOW)**. **These studies provide very low certainty evidence that lumber spine bone density (measured by BMAD) increases during treatment with gender-affirming hormones (from baseline to follow-up of 2 to 5 years). Z-scores at the end of follow-up suggest the average lumbar spine bone density was generally lower than the equivalent cisgender population (transfemales compared with cis-males and transmales compared with cis-females). The results for bone density (measured by BMD) were inconsistent.**

Change in bone density: femoral neck **Certainty of evidence: very low**	This is an important outcome because childhood and adolescence is a key time for bone development and gender-affirming hormones may affect bone development, as shown by changes in femoral neck bone density. Three uncontrolled, observational studies (2 retrospective and 1 prospective) provided evidence related to bone density: femoral neck in children and adolescents with gender dysphoria. This was reported as either bone mineral density (BMD), bone mineral apparent density (BMAD), or both. One study reported change in bone density from start of gender-affirming hormones to age 22 years (Klink et al. 2015). Two studies reported change in bone density from start of gender-affirming hormones up to 24-month follow-up (Stoffers et al. 2019 and Vlot et al. 2017). All participants had previously been treated with a GnRH analogue. All outcomes were reported separately for transfemales and transmales; also see subgroups table below. **Bone mineral apparent density (BMAD)** Two uncontrolled, observational studies reported change in femoral neck BMAD (Klink et al. 2015; Vlot et al. 2017). See above for more details on BMAD. In Klink et al. 2015 (n=34): • The z-score for femoral neck BMAD was reported for the start of gender-affirming hormones but not at age 22 years in transfemales or transmales. No statistical analysis reported. • In transfemales there was no statistically significant difference in actual femoral neck BMAD values in g/cm^3 at age 22 years compared with start of gender-affirming hormones. In transmales actual lumbar spine BMAD values in g/cm^3 were statistically significantly higher at age 22 years compared with start of gender-affirming hormones (mean [±SD]: start of hormones 0.31 [±0.04], age 22 years 0.33 [±0.05], p=0.010) **(VERY LOW)**. In Vlot et al. 2017 (n=70): • In transfemales (all bone ages), there was no statistically significant difference in femoral neck BMAD z-score from start of gender-affirming hormones to 24-month follow-up. • The z-score for femoral neck BMAD in transmales with a bone age of <14 years was statistically significantly higher at 24-month follow-up compared with start of gender-affirming hormones (z-score [range]: start of hormones -0.37 [-2.28 to 0.47], 24-month follow-up -0.37 [-2.03 to 0.85], p≤0.01). Statistically significant improvements in z-score for lumbar spine BMAD in transmales with a bone age of ≥14 years were also seen (z-score [range]: start of hormones -0.27 [-1.91 to 1.29], 24-month follow-up 0.02 [-2.1 to 1.35], p≤0.05). • In transfemales of all bone ages, there was no statistically significant change in actual femoral neck BMAD values in g/cm^3 from start of gender-affirming hormones to 24-month follow-up. In transmales of all bone ages, actual femoral neck BMAD values in g/cm^3 were statistically significantly higher at 24-month follow-up compared with start of gender-affirming hormones **(VERY LOW)**.

	Bone mineral density (BMD) Two uncontrolled, observational studies reported change in femoral neck BMD (Klink et al. 2015; Stoffers et al. 2019). See above for more details on BMD. In Klink et al. 2015 (n=34): • In transfemales, there was no statistically significant difference in femoral neck BMD z-score from start of gender-affirming hormones to age 22 years. In transmales, femoral neck BMD z-score was statistically significantly higher at age 22 years compared with start of gender-affirming hormones (z-score [SD]: start of hormones -0.35 [0.79], age 22 years -0.35 [0.74], p=0.006). • Actual femoral neck BMD values in g/cm^2 were statistically significantly higher at age 22 years compared with start of gender-affirming hormones in transfemales and transmales **(VERY LOW)**. In Stoffers et al. 2019 (n=62 at 6-month follow-up; n=15 at 24-month follow-up): • there was no statistically significant difference in right or left femoral neck BMD z-score in transmales, from the start of gender-affirming hormones to any timepoint (6, 12 and 24 months). • There was also no statistically significant difference in transmales in right or left actual femoral neck BMD values in g/cm^2 from start of gender-affirming hormones to any timepoint (6, 12 and 24 months) **(VERY LOW)**. **These studies provide very low certainty evidence that during treatment with gender-affirming hormones from baseline to follow-up of 2 to 5 years, femoral neck bone density (measured by BMAD) was unchanged in transfemales but was statistically significantly increased in transmales (although the absolute change was small). Z-scores at the end of follow-up suggest that average femoral neck bone density was lower in both transfemales and transmales than in the equivalent cisgender population (transfemales compared with cis-males and transmales compared with cis-females). The results for bone density (measured by BMD) were inconsistent.**
Change in clinical parameters: glucose, insulin and HbA1c **Certainty of evidence: very low**	This is an important outcome because the effect of gender-affirming hormones on insulin sensitivity and cardiovascular risk in children and adolescents with gender dysphoria is unknown. Two uncontrolled, retrospective chart reviews (Klaver et al. 2020; Stoffers et al. 2019) provided evidence on glucose, insulin and HbA1c. All outcomes were reported separately for transfemales and transmales; also see subgroups table below. **Glucose levels, insulin levels and insulin resistance** One retrospective chart review (Klaver et al. 2020) reported non-comparative evidence on the change in glucose levels, insulin levels and insulin resistance (measured using Homeostatic Model

	Assessment of Insulin Resistance [HOMA-IR]) between starting gender-affirming hormones and age 22 years. In Klaver et al. 2020 (n=192): • There was no statistically significant change in glucose levels, insulin levels and insulin resistance in transfemales. • There was no statistically significant change in glucose levels in transmales. • There was a statistically significant decrease in insulin levels in transmales (mean change [95% CI] -2.1 mU/L [-3.9 to -0.3], p<0.05; mean insulin level at 22 years [95% CI] 8.6 mU/L [6.9 to 10.2]). • There was a statistically significant decrease in insulin resistance in transmales (HOMA-IR; mean change [95% CI] -0.5 [-1.0 to -0.1], p<0.05; mean HOMA-IR at 22 years [95% CI] 1.8 [1.4 to 2.2]) **(VERY LOW)**. **HbA1c** One retrospective chart review (Stoffers et al. 2019; n=62) reported non-comparative evidence on the change in HbA1c in transmales between starting gender-affirming hormones and 24-month follow-up. There was no statistically significant change in HbA1c **(VERY LOW)**. **These studies provide very low certainty evidence that gender-affirming hormones do not affect HbA1c, glucose levels, insulin levels and insulin resistance.**
Change in clinical parameters: lipids **Certainty of evidence: very low**	This is an important outcome because the effect of gender-affirming hormones on lipid profiles and cardiovascular risk in children and adolescents with gender dysphoria is unknown. One retrospective chart review (Klaver et al. 2020) provided non-comparative evidence on the change in lipids (total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides) between starting gender-affirming hormones and age 22 years. All outcomes were reported separately for transfemales and transmales; also see subgroups table below. In Klaver et al. 2020 (n=192): • There was no statistically significant change in total cholesterol, HDL cholesterol and LDL cholesterol in transfemales. • There was a statistically significant decrease (improvement) in triglycerides in transfemales (mean change [95% CI] +0.2 mmol/L [0.0 to 0.5], p<0.05; mean triglyceride level at 22 years [95% CI] 1.1 mmol/L [0.9 to 1.4]). • There was a statistically significant increase in total cholesterol in transmales (mean change [95% CI] +0.4 mmol/L [0.2 to 0.6], p<0.001; mean total cholesterol at 22 years [95% CI] 4.6 mmol/L [4.3 to 4.8]). • There was a statistically significant decrease (worsening) in HDL cholesterol (mean change in transmales [95% CI] -0.3 mmol/L [-0.4 to -0.1], p<0.001; mean HDL cholesterol at 22 years [95% CI] 1.3 mmol/L [1.2 to 1.3]). • There was a statistically significant increase (worsening) in LDL cholesterol in transmales (mean change [95% CI]

	+0.4 mmol/L [0.2 to 0.6], p<0.001; mean LDL cholesterol at 22 years [95% CI] 2.6 mmol/L [2.4 to 2.8]). • There was a statistically significant increase (worsening) in triglycerides in transmales (mean change [95% CI] +0.5 mmol/L [0.3 to 0.7], p<0.001; mean triglyceride level at 22 years [95% CI] 1.3 mmol/L [1.1 to 1.5]) **(VERY LOW)**. **This study provides very low certainty evidence that gender-affirming hormones do not affect lipid profiles in transfemales. In transmales, there was a small but statistically significant worsening in cholesterol levels from start of gender-affirming hormone treatment to age 22 years, but mean cholesterol and triglyceride levels were within the UK reference range at the end of treatment.**
Change in clinical parameters: blood pressure **Certainty of evidence: very low**	This is an important outcome because the effect of gender-affirming hormones on blood pressure and cardiovascular risk in children and adolescents with gender dysphoria is unknown. One retrospective chart review (Klaver et al. 2020) provided non-comparative evidence on the change in blood pressure between starting gender-affirming hormones and at age 22 years. All outcomes were reported separately for transfemales and transmales; also see subgroups table below. In Klaver et al. 2020 (n=192): • There was no statistically significant change in systolic blood pressure (SBP) in transfemales. However, there was a statistically significant increase in diastolic blood pressure (DBP) in transfemales (mean change [95% CI] +6 mmHg [3 to 10], p<0.001; mean DBP at 22 years [95% CI] 75 [72 to 78]). • In transmales, there was a statistically significant increase in SBP (mean change [95% CI] +5 mmHg [1 to 9], p<0.05; mean SBP at 22 years [95% CI] 126 [122 to 130]), and DBP (mean change [95% CI] +6 mmHg [4 to 9], p<0.001; mean DBP at 22 years [95% CI] 74 [72 to 77]) **(VERY LOW)**. **This study provides very low certainty evidence that gender-affirming hormones statistically significantly increase blood pressure from start of treatment to age 22 years, although the absolute increase was small.**
Change in clinical parameters: body mass index (BMI) **Certainty of evidence: very low**	This is an important outcome because the effect of gender-affirming hormones on weight gain and cardiovascular risk in children and adolescents with gender dysphoria is unknown. One retrospective chart review (Klaver et al. 2020) provided non-comparative evidence on the change in body mass index (BMI) between starting gender-affirming hormones and age 22 years. All outcomes were reported separately for transfemales and transmales; also see subgroups table below. In Klaver et al. 2020 (n=192): • There was a statistically significant increase in BMI in transfemales from the start of gender-affirming hormones to age 22 years (mean change [95% CI] +1.9 [0.6 to 3.2], p<0.005; mean BMI at 22 years [95% CI] 23.2 [21.6 to 24.8]. At age 22

	years, 9.9% of transfemales were obese, compared with 3.0% in a reference population of cisgender men. • There was a statistically significant increase in BMI in transmales from the start of gender-affirming hormones to age 22 years (mean change [95% CI] +1.4 [0.8 to 2.0], p<0.005; mean BMI at 22 years [95% CI] 23.9 [23.0 to 24.7]). At age 22 years, 6.6% of transmales were obese, compared with 2.2% in a reference population of cisgender women **(VERY LOW)**. **This study provides very low certainty evidence that gender-affirming hormones statistically significantly increase BMI from start of treatment to age 22 years, although most participants were within the healthy weight range.**
Change in clinical parameters: liver function **Certainty of evidence: very low**	This is an important outcome because if treatment-induced liver injury (raised liver enzymes are a marker of this) is suspected, gender-affirming hormones may need to be stopped. One retrospective chart review (Stoffers et al. 2019) provided non-comparative evidence on the change in liver enzymes in transmales between starting gender-affirming hormones and up to 24-months follow-up. In Stoffers et al. 2019 (n=62): • There was no statistically significant change in aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyltransferase (GCT) in transmales. • There was a statistically significant increase in alkaline phosphatase (ALP) levels from starting gender-affirming hormones to 6- and 12-months follow-up, although by 24-months the difference was not statistically significant (median [IQR]: start of hormones 102 [78 to 136], 6-month follow-up 115 [102 to 147] p<0.001, 12-month follow-up 112 [88 to 143] p<0.001) **(VERY LOW)**. **This study provides very low certainty evidence that gender-affirming hormones do not affect liver function in transmales from baseline to 24 months follow-up.**
Change in clinical parameters: kidney function **Certainty of evidence: very low**	This is an important outcome because if renal damage (raised serum creatinine and urea are markers of this) is suspected, treatment with gender-affirming hormones may need to be stopped. One retrospective chart review (Stoffers et al. 2019) provided non-comparative evidence on the change in serum creatinine and serum urea levels in transmales between starting gender-affirming hormones and up to 24-months follow-up. In Stoffers et al. 2019 (n=62): • There was a statistically significant increase in creatinine levels in transmales at all timepoints up to 24 months (mean [SD]: start of hormones 62 umol/L [7], 6 months 70 umol/L [9] , 12 months 74 umol/L [10], 24 months 81 umol/L [10], p<0.001). • There was no statistically significant change in urea in transmales (follow-up duration not reported) **(VERY LOW)**.

	This study provides very low certainty evidence on the effects of gender-affirming hormones on kidney function in transmales from baseline to 24 months follow-up. A statistically significant increase in creatinine levels was seen, but these were within the UK reference range. Urea levels were unchanged.

Treatment discontinuation
 	
Certainty of evidence: very low
	This is an important outcome because there is uncertainty about the short- and long-term impact of stopping treatment with gender-affirming hormones in children and adolescents with gender dysphoria.
	
	One uncontrolled, retrospective chart review (Khatchadourian et al. 2014) provided evidence relating to permanent or temporary treatment discontinuation in children and adolescents with gender dysphoria.
	
	Khatchadourian et al. 2014 narratively reported treatment discontinuation in a cohort of 63 adolescents (24 transfemales and 39 transmales) who received gender-affirming hormones:
	• No participants permanently discontinued gender-affirming hormones.
	• No transfemales temporarily discontinued gender-affirming hormones.
	• Three transmales temporarily discontinued gender-affirming hormones due to:
	  ○ mental health comorbidities (n=2)
	  ○ androgenic alopecia (n=1).
	All 3 participants eventually resumed treatment, although timescales were not reported **(VERY LOW)**.
	
	This study provides very low certainty evidence that the rates of discontinuation during treatment with gender-affirming hormones are low (duration of treatment not reported).

Adverse effects
 	
Certainty of evidence: very low
	This is an important outcome because if there are adverse effects, gender-affirming hormones may need to be stopped.
	
	One uncontrolled, retrospective chart review (Khatchadourian et al. 2014) provided evidence relating to adverse effects from gender-affirming hormones in children and adolescents with gender dysphoria.
	
	Khatchadourian et al. 2014 narratively reported adverse effects in a cohort of 63 adolescents (24 transfemales and 39 transmales) receiving treatment with gender-affirming hormones:
	• No severe complications were reported.
	• No transfemales reported minor complications.
	• Twelve transmales developed minor complications, which were:
	  ○ severe acne, requiring isotretinoin treatment (n=7)
	  ○ androgenic alopecia (n=1)
	  ○ mild dyslipidaemia (further details not provided; n=3)
	  ○ significant mood swings (n=1) **(VERY LOW)**.
	
	This study provides very low certainty evidence about the potential adverse effects of gender-affirming hormones (duration of treatment not reported). No conclusions could be drawn.

Abbreviations: ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMAD: bone mineral apparent density; BMD: bone mineral density; BMI: body mass index; DBP: diastolic blood pressure; GGT: gamma-glutamyl transferase; HbA1c:

glycated haemoglobin; HDL: high-density lipoproteins; HOMA-IR: Homeostatic Model Assessment of Insulin Resistance; IQR: interquartile range; LDL: low-density lipoproteins; p: p-value; SBP: systolic blood pressure; SD: standard deviation.

In children and adolescents with gender dysphoria, what is the cost-effectiveness of gender-affirming hormones compared to one or a combination of psychological support, social transitioning to the desired gender or no intervention?

Outcome	Evidence statement
Cost-effectiveness	No studies were identified to assess the cost-effectiveness of gender-affirming hormones for children and adolescents with gender dysphoria.

From the evidence selected, are there any subgroups of children and adolescents with gender dysphoria that may benefit from gender-affirming hormones more than the wider population of interest?

Subgroup	Evidence statement
Sex assigned at birth males (transfemales) **Certainty of evidence: Very low**	Some studies reported data separately for sex assigned at birth males (transfemales). This included some direct comparisons with sex assigned at birth females (transmales). **Impact on mental health: depression and anxiety** One uncontrolled, prospective, longitudinal study (Kuper et al. 2020) reported the change in depression (measured using QIDS clinician-reported and self-reported), anxiety and anxiety-related symptoms (measured using SCARED) in transfemales. See the clinical effectiveness results above for full details. In Kuper et al. 2020 (n=33 to 45, varies by outcome), changes were seen in depression, anxiety and anxiety-related symptoms from baseline to follow-up but the authors did not report any statistical analyses, so it is unclear if was any changes were statistically significant **(VERY LOW)**. **This study provides very low certainty evidence on the effects of gender-affirming hormones on depression, anxiety and anxiety-related symptoms over time in sex assigned at birth males (transfemales; mean duration of treatment 10.9 months). No conclusions could be drawn.** **Impact on mental health: suicidality** One uncontrolled, retrospective, longitudinal study (Allen et al. 2019) reported the change in Ask Suicide-Screening Questions (ASQ) in transfemales compared with transmales. See the clinical effectiveness results above for full details. Between baseline and the final assessment, there was no statistically significant difference in change in ASQ score for transfemales compared with transmales (p=0.79; n=47) **(VERY LOW)**.

	One uncontrolled, prospective, longitudinal study (Achille et al. 2020) reported the change in suicidal ideation in transfemales measured using additional questions from the PHQ 9_Modified for Teens. See the clinical effectiveness results above for full details. At baseline, 11.8% (2/17) of transfemales had suicidal ideation, compared with 5.9% (1/17) at about 12-months follow-up (no statistical analysis reported) **(VERY LOW)**. **These studies provide very low certainty evidence that any change in suicidal ideation is not different between sex assigned at birth males (transfemales) and sex assigned at birth females (transmales) from baseline to follow-up of about 12 months.** **Impact on quality of life** One uncontrolled, retrospective, longitudinal study (Allen et al. 2019) reported the change in the GWBS of the Paediatric Quality of Life Inventory in transfemales compared with transmales. See the clinical effectiveness results above for full details. Between baseline and final assessment, there was no statistically significant difference in change in GWBS of the Paediatric Quality of Life Inventory for transfemales compared with transmales (p=0.32; n=47) **(VERY LOW)**. **This study provides very low certainty evidence that any change in general wellbeing is not different between sex assigned at birth males (transfemales) and sex assigned at birth females (transmales) from baseline to follow-up of about 12 months.** **Impact on body image** One uncontrolled, prospective, longitudinal study (Kuper et al. 2020) reported change in Body Image Scale (BIS) in transfemales. See the clinical effectiveness results above for full details. In Kuper et al. 2020 (n=30), the mean (±SD) BIS score was 67.5 points (±19.5) at baseline and 49.0 points (±21.6) at follow-up (no statistical analysis reported) **(VERY LOW).** **This study provides very low certainty evidence on the effects of gender-affirming hormones on body image over time in transfemales (mean duration of treatment 10.9 months). No conclusions could be drawn.** **Change in bone density: lumbar spine** Two uncontrolled, observational, retrospective studies provided evidence relating to the effect of gender-affirming hormones on lumber spine bone density in transfemales (Klink et al. 2015 and Vlot et al. 2017). See the safety results table above for a full description of the results.

	These studies provide very low certainty evidence that lumbar spine bone density (measured by BMAD) increases during treatment with gender-affirming hormones in sex assigned at birth males (transfemales). Z-scores at the end of follow-up suggest average lumbar spine bone density was generally lower than in the equivalent cisgender population. The results for lumbar spine bone density (measured by BMD) were inconsistent. **Change in bone density: femoral neck** Two uncontrolled, observational, retrospective studies provided evidence relating to the effect of gender-affirming hormones on femoral neck bone density in transfemales (Klink et al. 2015 and Vlot et al. 2017). See the safety results table above for a full description of the results. **These studies provide very low certainty evidence that femoral neck bone density (measured by BMAD) was unchanged in sex assigned at birth males (transfemales) during treatment with gender-affirming hormones (follow-up between 2 and 5 years). Z-scores at the end of follow-up suggest and the average femoral neck bone density was lower than in the equivalent cisgender population. The results for femoral neck bone density (measured by BMD) were inconsistent.** **Change in clinical parameters: glucose, insulin and HbA1c** One uncontrolled, retrospective chart review (Klaver et al. 2020) provided evidence on glucose, insulin and HbA1c in transfemales. See the safety results table above for a full description of the results. **This study provided very low certainty evidence that gender-affirming hormones do not affect HbA1c, glucose levels, insulin levels and insulin resistance in sex assigned at birth males (transfemales) from the start of treatment to age 22 years.** **Change in clinical parameters: lipids** One retrospective chart review (Klaver et al. 2020) provided evidence on the change in lipids (total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides) in transfemales. See the safety results table above for a full description of the results. **This study provides very low certainty evidence that gender-affirming hormones do not affect lipid profiles in sex assigned at birth males (transfemales) from the start of treatment to age 22 years.** **Change in clinical parameters: blood pressure** One retrospective chart review (Klaver et al. 2020) provided evidence on the change in blood pressure in transfemales. See the safety results table above for a full description of the results. **This study provides very low certainty evidence that gender-affirming hormones statistically significantly increase blood pressure in sex assigned at birth males (transfemales),**

	although the absolute increase was small from the start of treatment to age 22 years. **Change in clinical parameters: body mass index (BMI)** One retrospective chart review (Klaver et al. 2020) provided evidence on the change in BMI in transfemales. See the safety results table above for a full description of the results. **This study provides very low certainty evidence that gender-affirming hormones statistically significantly increase BMI in sex assigned at birth males (transfemales), although most participants were within the healthy weight range from the start of treatment to age 22 years.** **Treatment discontinuation** One uncontrolled, retrospective chart review provided evidence relating to permanent or temporary discontinuation of gender-affirming hormones in transfemales (Khatchadourian et al. 2014). **This study provides very low certainty evidence that the rates of discontinuation during treatment with gender-affirming hormones in sex assigned at birth males (transfemales) are low. Duration of treatment with gender-affirming hormones was not reported.** **Adverse effects** One uncontrolled, retrospective chart review provided evidence relating to adverse effects from gender-affirming hormones in transfemales (Khatchadourian et al. 2014). **This study provides very low certainty evidence about the potential adverse effects of gender-affirming hormones in sex assigned at birth males (transfemales). No conclusions could be drawn. Duration of treatment with gender-affirming hormones was not reported.**
Sex assigned at birth females (transmales) **Certainty of evidence: Very low**	Some studies reported data separately for sex assigned at birth females (transmales). This included some direct comparisons with sex assigned at birth males (transfemales). **Impact on mental health: depression and anxiety** One uncontrolled, prospective, longitudinal study (Kuper et al. 2020) reported the change in depression (measured using QIDS clinician-reported and self-reported), anxiety and anxiety-related symptoms (measured using SCARED) in transmales. See the clinical effectiveness results above for full details. In Kuper et al. 2020 (n=65 to 78, varies by outcome), changes were seen in depression, anxiety and anxiety-related symptoms from baseline to follow-up but the authors did not report any statistical analysis, so it is unclear if any changes are statistically significant **(VERY LOW)**.

	This study provides very low certainty evidence on the effects of gender-affirming hormones on depression, anxiety and anxiety-related symptoms over 10.9 months in transmales. No conclusions could be drawn. **Impact on mental health: suicidality** One uncontrolled, retrospective, longitudinal study (Allen et al. 2019) reported the change in Ask Suicide-Screening Questions (ASQ) in transmales compared with transfemales. See the sex assigned at birth males (transfemales) row above for full details of the results. One uncontrolled, prospective, longitudinal study (Achille et al. 2020) reported the change in suicidal ideation in transmales measured using additional questions from the PHQ 9_Modified for Teens. See the clinical effectiveness results above for full details. At baseline, 9.1% (3/33) of transmales had suicidal ideation, compared with 6.1% (2/33) at about 12-months follow-up (no statistical analysis reported) **(VERY LOW)**. **These studies provide very low certainty evidence that any change in suicidal ideation is not different between sex assigned at birth females (transmales) and sex assigned at birth males (transfemales). Mean duration of treatment about 12 months.** **Impact on quality of life** One uncontrolled, retrospective, longitudinal study (Allen et al. 2019) reported the change in the GWBS of the Paediatric Quality of Life Inventory in transmales compared with transfemales. See the sex assigned at birth males (transfemales) row above for full details of the results. **This study provides very low certainty evidence that any change in general wellbeing is not different between sex assigned at birth females (transmales) and sex assigned at birth males (transfemales). Mean duration of treatment about 12 months.** **Impact on body image** One uncontrolled, prospective, longitudinal study (Kuper et al. 2020) reported change in Body Image Scale (BIS) in transmales. See the clinical effectiveness results above for full details. In Kuper et al. 2020 (n=66), the mean (±SD) BIS score was 71.1 points (±13.4) at baseline and 52.9 points (±16.8) at follow-up (no statistical analysis reported) **(VERY LOW)**. **This study provides very low certainty evidence on the effects of gender-affirming hormones on body image over 10.9 months in transmales. No conclusions could be drawn.** **Change in bone density: lumbar spine** Three uncontrolled, observational, retrospective studies provided evidence relating to the effect of gender-affirming hormones on lumber spine bone density in transmales (Klink et al. 2015, Stoffers et al. 2019 and Vlot et al. 2017). See the safety results table above for a full details of the results.

	These studies provide very low certainty evidence that lumbar spine bone density (measured by BMAD) increases during 2 to 5 years treatment with gender-affirming hormones in sex assigned at birth females (transmales). Z-scores at the end of follow-up suggest the average lumbar spine bone density was generally lower than in the equivalent cisgender population. The results for lumbar spine bone density (measured by BMD) were inconsistent. **Change in bone density: femoral neck** Three uncontrolled, observational, retrospective studies provided evidence relating to the effect of gender-affirming hormones on femoral neck bone density in transmales (Klink et al. 2015, Stoffers et al. 2019 and Vlot et al. 2017). See the safety results table above for a full details of the results. **These studies provide very low certainty evidence that femoral neck bone density (measured by BMAD) statistically significantly increased in sex assigned at birth females (transmales) during 2 to 5 years treatment with gender-affirming hormones. Z-scores at the end of follow-up suggest the average femoral neck bone density was generally lower than in the equivalent cisgender population. The results for femoral neck bone density (measured by BMD) were inconsistent.** **Change in clinical parameters: glucose, insulin and HbA1c** Two uncontrolled, retrospective chart reviews (Klaver et al. 2020; Stoffers et al. 2019) provided evidence on glucose, insulin and HbA1c in transmales. See the safety results table above for full details of the results. **This study provided very low certainty evidence that gender-affirming hormones do not affect HbA1c, glucose levels, insulin levels and insulin resistance in sex assigned at birth females (transmales). Reported from start of treatment to age 22 years.** **Change in clinical parameters: lipids** One retrospective chart review (Klaver et al. 2020) provided evidence on the change in lipids (total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides) in transmales. See the safety results table above for full details of the results. **This study provides very low certainty evidence that treatment with gender-affirming hormones is associated with a small but statistically significant worsening of cholesterol levels in sex assigned at birth females (transmales), but mean cholesterol and triglyceride levels were within the UK reference range at end of treatment, from start of treatment to age 22 years.** **Change in clinical parameters: blood pressure** One retrospective chart review (Klaver et al. 2020) provided evidence on the change in blood pressure in transmales. See the safety results table above for full details of the results.

	This study provides very low certainty evidence that gender-affirming hormones statistically significantly increase blood pressure in sex assigned at birth females (transmales), although the absolute increase was small, from start of treatment to age 22 years. **Change in clinical parameters: body mass index (BMI)** One retrospective chart review (Klaver et al. 2020) provided evidence on the change in body mass index (BMI) in transmales. See the safety results table above for full details of the results. **This study provides very low certainty evidence that gender-affirming hormones statistically significantly increase BMI in sex assigned at birth females (transmales), although most participants were within the healthy weight range, from start of treatment to age 22 years.** **Change in clinical parameters: liver function** One retrospective chart review (Stoffers et al. 2019) provided non-comparative evidence on the change in liver enzymes in transmales between starting gender-affirming hormones and up to 24-months follow-up. See the safety results table above for full details of the results. **This study provides very low certainty evidence that gender-affirming hormones for about 12 months do not affect liver function in sex assigned at birth females (transmales).** **Change in clinical parameters: kidney function** One retrospective chart review (Stoffers et al. 2019) provided non-comparative evidence on the change in serum creatinine and serum urea levels in transmales between starting gender-affirming hormones and up to 24-months follow-up. See the safety results table above for full details of the results. **This study provides very low certainty evidence on the effects of gender-affirming hormones on kidney function in sex assigned at birth females (transmales). A statistically significant increase in creatinine levels was seen at about 12 months follow-up, but these were within the UK reference range. Urea levels were unchanged.** **Treatment discontinuation** One uncontrolled, retrospective chart review provided evidence relating to permanent or temporary discontinuation of gender-affirming hormones in transmales (Khatchadourian et al. 2014). See the safety results table above for full details of the results. **This study provides very low certainty evidence that the rates of treatment discontinuation with gender-affirming hormones in sex assigned at birth females (transmales) is low. Duration of gender-affirming hormones not reported.** **Adverse effects**

	One uncontrolled, retrospective chart review provided evidence for adverse effects from gender-affirming hormones in transmales (Khatchadourian et al. 2014). See the safety results table above for full details of the results. **This study provides very low certainty evidence about the potential adverse effects of gender-affirming hormones in sex assigned at birth females (transmales). No conclusions could be drawn. Duration of gender-affirming hormones not reported.**
Duration of gender dysphoria	No evidence was identified.
Age at onset of gender dysphoria	No evidence was identified.
Age at onset of puberty	No evidence was identified.
Tanner stage at which GnRH analogue or gender-affirming hormones started	One uncontrolled, prospective, longitudinal study (Kuper et al. 2020) reported the impact of Tanner stage on outcomes, although it is not clear whether this is referring to Tanner stage at initial assessment, at the start of GnRH analogues or at another timepoint.
Diagnosis of autistic spectrum disorder	No evidence was identified.
Diagnosis of a mental health condition	One uncontrolled, prospective, longitudinal study (Achille et al. 2020) reported outcomes that were adjusted for engagement in counselling and medicines for mental health problems. Information about diagnoses and treatment were not provided. Rates of mental health issues appear to be high in the cohort. **Impact on mental health** Achille et al. 2020 reported the change in depression scores, controlled for engagement in counselling and medicines for mental health problems (measured using the Center for Epidemiologic Studies Depression [CESD-R] scale and Patient Health Questionnaire Modified for Teens [PHQ 9_Modified for Teens] score: • There was no statistically significant change in CESD-R from baseline to about 12-months follow-up. • There was no statistically significant change in PHQ 9_Modified for Teens score from baseline to about 12-months follow-up **(VERY LOW)**. **Impact on quality of life** Achille et al. 2020 reported the change in quality of life scores, controlled for engagement in counselling and medicines for mental health problems (measured using the Quality of Life Enjoyment and Satisfaction Questionnaire [QLES-Q-SF] score: • There was no statistically significant change in QLES-Q-SF score from baseline to about 12-months follow-up **(VERY LOW)**. **This study provides very low certainty evidence about outcomes that were adjusted for engagement in counselling and medicines for mental health problems. No conclusions could be drawn.**

Abbreviations: ASQ: Ask Suicide-Screening Questions; CESD-R: Center for Epidemiologic Studies Depression; GnRH: Gonadotrophin releasing hormone; GWBS: General Well-Being

Scale; HDL: high-density lipoproteins; LDL: low-density lipoproteins; p: p-value; PHQ 9_Modified for Teens: Patient Health Questionnaire Modified for Teens; QLES-Q-SF: Quality of Life Enjoyment and Satisfaction Questionnaire.

From the evidence selected,

(a) what are the criteria used by the research studies to define gender dysphoria, gender identity disorder and gender incongruence of childhood?

(b) what were the ages at which participants commenced treatment with gender-affirming hormones?

(c) what was the duration of treatment with GnRH analogues?

<table>
<tr><th>Outcome</th><th>Evidence statement</th></tr>
<tr><td>Diagnostic criteria</td><td>The DSM-IV-TR criteria was used in 3 studies (Klaver et al. 2020, Klink et al. 2015 and Vlot et al. 2017).

The DSM-V criteria was used in 2 studies (Kuper et al. 2020 and Stoffers et al. 2019). The DSM-V has one overarching definition of gender dysphoria with separate specific criteria for children and for adolescents and adults. The general definition describes a conflict associated with significant distress and/or problems functioning associated with this conflict between the way they feel and think of themselves which must have lasted at least 6 months.

The ICD-10 diagnosis of ‘transsexualism’ was used in 1 study (Kaltiala et al. 2020). The authors state that this is the corresponding diagnosis to ‘gender dysphoria’ in the DSM-V, and that diagnostic assessments in the study location (Finland) take place according to ICD-10.

It was not reported how gender dysphoria was defined in the remaining 4 studies (VERY LOW).

From the evidence selected, the most commonly reported diagnostic criteria for gender dysphoria (5/10 studies) was the DSM criteria in use at the time the study was conducted.</td></tr>
<tr><td>Age when gender-affirming hormones started</td><td>8/10 studies reported the age at which participants started treatment with gender-affirming hormones, either as the mean age (with SD) or median age (with the range):

<table>
<tr><th>Study</th><th>Mean age (± SD)</th></tr>
<tr><td>Allen et al. 2019</td><td>16.7 years (not reported)</td></tr>
<tr><td>Khatchadourian et al. 2014</td><td>17.4 years (1.9)</td></tr>
<tr><td>Klaver et al. 2020</td><td>16.4 years (1.1) in transfemales
16.9 years (0.9) in transmales</td></tr>
<tr><td>Kuper et al. 2020</td><td>16.2 (1.2)</td></tr>
<tr><td>Klink et al. 2015</td><td>16.6 years (1.4) in transfemales
16.4 years (2.3) in transmales</td></tr>
</table>

<table>
<tr><th>Study</th><th>Median age (range)</th></tr>
<tr><td>Stoffers et al. 2019</td><td>17.2 years (15 to 19.5)</td></tr>
<tr><td>Vlot et al. 2017</td><td>16.3 years (15.9 to 19.5) in transfemales
16.0 years (14.0 to 18.9) in transmales</td></tr>
</table>
</td></tr>
</table>

<table>
<tr><td></td><td>Age at the start of treatment was not reported in 3 studies:
In Achille et al. 2020 the mean age at initial assessment (baseline) was 16.2 years (SD ±2.2)
In Kaltiala et al. 2020 the mean age at diagnosis was 18.1 years (range 15.2 to 19.9)
In Lopez de Lara et al. 2020 the mean age of participants was 16 years (range 14 to 18), although it is not clear if this is at the initial assessment or at the start of gender-affirming hormones.
The evidence included showed that most children and adolescents started treatment with gender-affirming hormones at about 16 to 17 years, with a range of about 14 to 19 years.</td></tr>
<tr><td>Duration of treatment with GnRH analogues</td><td>The duration of treatment with GnRH analogues was reported in 3/10 studies:<table><tr><th>Study</th><th>Median duration</th></tr><tr><td>Klaver et al. 2020</td><td>2.1 years (IQR 1.0 to 2.7) in transfemales
1.0 years (IQR 0.5 to 2.9) in transmales</td></tr><tr><td>Klink et al. 2015</td><td>1.3 years (range 0.5 to 3.8) in transfemales
1.5 years (range 0.25 to 5.2) in transmales
(GnRH analogue monotherapy)</td></tr><tr><td>Stoffers et al. 2019</td><td>8 months (range 3 to 39)</td></tr></table>The evidence included showed wide variation in the duration of treatment with gender-affirming hormones, but most studies did not report this information. Treatment duration ranged from a few months up to about 5 years.</td></tr>
</table>

Abbreviations: DSM, Diagnostic and Statistical Manual of Mental Disorders criteria; GnRH, Gonadotrophin-releasing hormone; ICD, International Statistical Classification of Diseases and Related Health Problems; IQR, interquartile range; SD, standard deviation.

6. Discussion

A key limitation to identifying the effectiveness and safety of gender-affirming hormones for children and adolescents with gender dysphoria is the lack of reliable comparative studies. All the studies included in this evidence review are uncontrolled observational studies, which are subject to bias and confounding and were of very low certainty using modified GRADE. The size of the population with gender dysphoria means conducting a prospective trial may be unrealistic, at least on a single centre basis. There may also be ethical issues with a 'no treatment arm' in comparative trials of gender-affirming hormones, where there may be poor mental health outcomes if treatment is withheld. However, the use of an active comparator such as close psychological support may reduce ethical concerns in future trials. A fundamental limitation of all the uncontrolled studies included in this review is that any changes in scores from baseline to follow-up could be attributed to a regression-to-the-mean.

The included studies have relatively short follow-up, with an average duration of treatment with gender-affirming hormones between around 1 year and 5.8 years. Further studies with a

longer follow-up are needed to determine the long-term effect of gender-affirming hormones for children and adolescents with gender dysphoria.

Most studies included in this review did not report comorbidities (physical or mental health) and no study reported concomitant treatments in detail. Because of this it is not clear whether any changes observed were due to gender-affirming hormones or other treatments the participants may have received. For example, we do not know if any improvement in depression symptom score over time was the result of gender-affirming hormones or the mental health support the person may be receiving (including medicines or counselling). This may be of particular importance for the mental health outcomes discussed in this review, since depression, anxiety and other related symptoms are common in children and adolescents with gender dysphoria. In Achille et al. 2020, at baseline around one-third of participants were taking medicines for mental health problems and around two-thirds reported being depressed in the past year. In Kaltiala et al. 2020, half the participants needed mental health treatment during and before gender identity assessment, with the most common reasons for treatment being depression, anxiety and suicidality. Only 1 study reported outcomes adjusted for engagement in counselling and medicines for mental health problems (Achille et al. 2020). This study found that gender-affirming hormones had no significant impact on depression and quality of life when adjusted for mental health care, despite significant approvements reported for the unadjusted results. However, it is not possible to draw conclusions on the impact of concurrent mental health treatment on the effect of gender-affirming hormones based on this study alone. Details of the mental health care provided are not reported in the study and results are presented for transfemales and transmales separately, resulting in small patient numbers and possible underpowering.

In most of the included studies, details of the gender-affirming hormone treatment regimens are poorly reported, with limited information provided about the medicines, doses and routes of administration used. It is not clear whether the interventions used in the studies are reflective of current UK practice for children and adolescents with gender dysphoria. There is also the suggestion that the hormone dose used in 1 study may have been too low; the authors of Klink et al. 2015 suggest that the relatively low initial dose of oestrogen for transfemales may be the reason for the observed lack of effect on lumber spine bone density. Duration of treatment with a GnRH analogue is also poorly reported and is only stated in 3/10 studies.

There is a degree of indirectness in some studies, with some participants included that fall outside of the population of this evidence review. For example, in Kuper et al. 2020 17% of participants received puberty suppression alone, and in Achille et al. 2020, 30% of participants received no treatment or puberty suppression alone. Some results and statistical analyses are only reported for the whole cohort in these studies and not the subgroup of participants who received gender-affirming hormones.

Participant numbers are poorly reported in some of the included studies. In Achille et al. 2020, 47% (45/95) of the people who entered the study did not have follow-up data and were excluded from the analyses, with no explanation or description of those people lost to follow-up. In Kuper et al. 2020, the number of participants varied by outcome, with less than two-thirds of participants providing data for some outcomes. The authors provide no explanation for this incomplete reporting.

It is not clear whether some outcome measures, specifically those related to psychosocial functioning, are relevant to the UK population. In Kaltiala et al. 2020, an observational study conducted in Finland, the proportion of participants living with parents or guardians is reported as marker of appropriate functioning. The authors state that in Finnish culture young people tend to leave the parental home early, with only around one-quarter of 20 to 24 year olds still living at home. This is lower than in the UK, where around half of 20 to 24 year olds live with their parents or guardians (ONS: Why are more young people living with their parents?).

It is difficult to draw firm conclusions for many of the effectiveness and safety outcomes reported in the included studies because many different scoring tools and methods were used to assess the same outcome, often with conflicting results. For example, bone density is reported as bone mineral density (BMD) and bone mineral apparent density (BMAD) in the same study, the latter being a size-adjusted measure often useful for people whose bones are still growing. For some populations (transfemale versus transmale) and bone regions (lumber spine versus femoral neck), statistically significant differences in BMD are reported but not for BMAD, and vice versa.

In addition to this, most outcomes reported across the included studies do not have an accepted minimal clinically important difference (MCID), making it difficult the determine whether any observed statistically significant changes are clinically meaningful. However, the authors of some studies report thresholds to interpret the results of the scoring tools, so some conclusions can be made. For example, the mean Utrecht Gender Dysphoria Scale (UGDS) score (a measure of gender dysphoria symptoms) reduced to about 15 points after treatment with gender-affirming hormones (Lopez de Lara et al. 2020). The authors state that scores of 40 points or above signify gender dysphoria, suggesting that after about 12 months of treatment with gender-affirming hormones, the majority of participants did not have symptoms of gender dysphoria.

The impact of gender-affirming hormones on bone density was reported in 3 studies (Klink et al. 2015, Stoffers et al. 2019 and Vlot et al. 2017). Although these studies did not include a control group, comparisons to a reference population are reported using z-scores. Comparisons were made to a cisgender population, meaning for example that bone density in transfemales was compared with bone density in cisgender males. The authors of Klink et al. 2015 note that this may not be the ideal comparison, because androgens and oestrogens affect bone differently, and that bone properties in a trans population differ from their age- and sex assigned at birth-matched controls. Beyond this, a major limitation when trying to determine the impact of gender-affirming hormones on the short- and long-term bone health of children and adolescents is the lack of data on fracture rates and other patient-orientated outcomes, including rates of osteoporosis. Studies of GnRH analogues in children and adolescents with gender dysphoria suggest that GnRH analogue treatment may reduce the expected increase in bone density (which is expected during puberty). Although improvements in bone density were reported following treatment with gender-affirming hormones, Z-scores suggest that bone density remained lower in transfemales and transmales compared with an equivalent cisgender population.

One study reported on cardiovascular risk factors at age 22 years in people who started gender-affirming hormones for gender dysphoria as adolescents. While glucose levels, insulin levels and insulin resistance were broadly unchanged at 22 years, statistically

significant increases in blood pressure and body mass index were seen. A small but statistically significant worsening of the lipid profile in transmales who received testosterone was also seen at age 22 years. However, further studies with a considerably longer follow-up and a focus on patient-oriented outcomes, including cardiovascular events and mortality are needed to determine the long-term impact on cardiovascular health of starting gender-affirming hormones during childhood and adolescence.

Only 1 study reported adverse events and discontinuation rates with gender-affirming hormones in children and adolescents. Conclusions on these outcomes cannot be made based on this study alone.

This review did not identify sub-groups of people who may benefit more from gender-affirming hormones. Limited evidence from 2 studies suggests there was no difference in response to treatment between transfemales and transmales for mental health and quality of life (Achille et al. 2020 and Allen et al. 2019).

7. Conclusion

This evidence review found limited evidence for the effectiveness and safety of gender-affirming hormones in children and adolescents with gender dysphoria, with all studies being uncontrolled, observational studies, and all outcomes of very low certainty. Any potential benefits of treatment must be weighed against the largely unknown long-term safety profile of these treatments.

The results from 5 uncontrolled, observational studies (Achille et al. 2020, Allen et al. 2019, Kaltiala et al. 2020. Kuper et al. 2020, Lopez de Lara et al. 2020) suggest that, in children and adolescents with gender dysphoria, gender-affirming hormones are likely to improve symptoms of gender dysphoria, and may also improve depression, anxiety, quality of life, suicidality, and psychosocial functioning. The impact of treatment on body image is unclear. All results were of very low certainty. The clinical relevance of any improvements to the person is difficult to determine because most outcomes do not have a recognised minimal clinically important difference, and the authors do not present statistical analysis for some outcomes.

A further 5 uncontrolled, observational studies (Khatchadourian et al. 2014, Klaver et al. 2020, Klink et al. 2015, Stoffers et al. 2019 and Vlot et al. 2017) reported on safety outcomes, all of which provided very low certainty evidence. Statistically significant increases in some measures of bone density were seen following treatment with gender-affirming hormones, although results varied by bone region (lumber spine versus femoral neck) and by population (transfemales versus transmales). However, z-scores suggest that bone density remained lower in transfemales and transmales compared with an equivalent cisgender population. Results from 1 study of gender-affirming hormones started during adolescence reported statistically significant increases in blood pressure and body mass index, and worsening of the lipid profile (in transmales) at age 22 years, although longer term studies that report on cardiovascular event rates are needed. Adverse events and discontinuation rates associated with gender-affirming hormones were only reported in 1 study, and no conclusions can be made on these outcomes.

This review did not identify sub-groups of people who may benefit more from gender-affirming hormones. Limited evidence from 2 studies suggests there was no difference in

response to treatment between transfemales and transmales for mental health and quality of life (Achille et al. 2020 and Allen et al. 2019).

No cost-effectiveness evidence was found to determine whether gender-affirming hormones are a cost-effective treatment for children and adolescents with gender dysphoria.

Appendix A PICO

The review questions for this evidence review are:

1. For children and adolescents with gender dysphoria, what is the clinical effectiveness of treatment with gender-affirming hormones compared with one or a combination of psychological support, social transitioning to the desired gender or no intervention?
2. For children and adolescents with gender dysphoria, what is the short-term and long-term safety of gender-affirming hormones compared with one or a combination of psychological support, social transitioning to the desired gender or no intervention?
3. For children and adolescents with gender dysphoria, what is the cost-effectiveness of gender-affirming hormones compared to one or a combination of psychological support, social transitioning to the desired gender or no intervention?
4. From the evidence selected, are there particular sub-groups of children and adolescents with gender dysphoria that derive comparatively more (or less) benefit from treatment with gender-affirming hormones than the wider population of children and adolescents with gender dysphoria?
5. From the evidence selected,
   (a) what are the criteria used by the research studies to define gender dysphoria, gender identity disorder and gender incongruence of childhood?
   (b) what were the ages at which participants commenced treatment with gender-affirming hormones?
   (c) what was the duration of GnRH analogues treatment?

PICO table

P –Population and Indication	Children and adolescents aged 18 years or less who have gender dysphoria, gender identity disorder or gender incongruence of childhood as defined by the study. The following subgroups of children and adolescents with gender dysphoria, gender identity disorder or gender incongruence of childhood need to be considered: • Sex assigned at birth males • Sex assigned at birth females

	• The duration of gender dysphoria: less than 6 months, 6-24 months, and more than 24 months) • The age at which treatment was initiated with GnRH analogues and with gender-affirming hormones. • The age of onset of gender dysphoria • The age of onset of puberty • Adolescents with gender dysphoria who have a pre-existing diagnosis of autistic spectrum disorder. • Adolescents with gender dysphoria who had a significant mental health symptom load at diagnosis including anxiety, depression (with or without a history of self-harm and suicidality), psychosis, personality disorder, Attention Deficit Hyperactivity Disorder and eating disorders.
I – Intervention	Gender-affirming hormone treatments: • A testosterone preparation for sex assigned at birth female patients which may include testosterone in the form of Sustanon injections*; testosterone enantate injections; Tostran gel*; Testogel; Testim gel; oral testosterone capsules in the form of testosterone undecanoate (Restandol); Andriol testocaps; Nebido • An oestradiol preparation** for sex assigned at birth male patients which may include: oral estradiol valerate*; oestrogen patches (7β-oestradiol patches e.g. Evorel or Estradem); Estradot patches; ethinyloestradiol *** *These are the used by Leeds Hospital, England. ** Be aware that the American spelling is oestrogen without the 'o'. ***Ethinyloestradiol is rarely used.
C – Comparator(s)	One or a combination of: • Psychological support • Social transitioning to the gender with which the individual identifies. No intervention
O – Outcomes	There are no known minimal clinically important differences and there are no preferred timepoints for the outcome measures selected. **All outcomes should be stratified by:** • The age at which treatment with gender-affirming hormones was initiated • The length of treatment with GnRH analogues where possible. **A: Clinical Effectiveness** *Critical to decision making* • **Impact on gender dysphoria** This outcome is critical because gender dysphoria in adolescents and children is associated with significant distress and problems functioning. Impact on gender dysphoria may be measured by the Utrecht Gender

	Dysphoria Scale. Other measures as reported in studies may be used as an alternative to the stated measure. • **Impact on mental health** Examples of mental health problems include self-harm, thoughts of suicide, suicide attempts, suicide, eating disorders, depression/low mood and anxiety. These outcomes are critical because self-harm and thoughts of suicide have the potential to result in significant physical harm and for completed suicides the death of the young person. Disordered eating habits may cause significant morbidity in young people. Depression and anxiety are also critical outcomes because they may impact on social, occupational, or other areas of functioning of children and adolescents. The Child and Adolescent Psychiatric Assessment (CAPA) may be used to measure depression and anxiety. The impact on self-harm and suicidality (ideation and behaviour) may be measured using the Suicide Ideation Questionnaire Junior. Other measures may be used as an alternative to the stated measure. • **Impact on Quality of Life** This outcome is critical because gender dysphoria in children and adolescents may be associated with a significant reduction in health-related quality of life. Quality of Life may be measured by the KINDL questionnaire, Kidscreen 52. Other measures as reported in studies may be used as an alternative to the stated measures. *Important to decision making* • **Impact on body image** This outcome is important because some young people with gender dysphoria may desire to take steps to suppress features of their physical appearance associated with their sex assigned at birth or accentuate physical features of their experienced gender. The Body Image Scale could be used as a measure. Other measures as reported in studies may also be used as an alternative to the stated measure. • **Psychosocial Impact** Examples of psychosocial impact are: coping mechanisms which may impact on substance misuse; family relationships; peer relationships. This outcome is important because gender dysphoria in adolescents and children is associated with internalising and externalising behaviours and emotional and behavioural problems which may impact on social and occupational functioning. The child behavioural check list (CBCL) may be used to measure the impact on psychosocial functioning. Other measures as reported in studies may be used as an alternative to the stated measure. • **Engagement with health care services** This outcome is important because patient engagement with healthcare services will impact on their clinical outcomes. Engagement with health care services may be measured using the Youth Health Care measure-satisfaction, utilization, and needs (YHC-SUN) questionnaire. Loss to follow up and should also be ascertained as part of this outcome.