

Supplementary Table S6 - Physical health outcomes, fertility and side effects

Study ID	Country	Study design	Study quality	Treated sample	Average age CSH treatment started	Comparator	Intervention	Outcome (measure)	Follow-up specific to outcome	Study results
Physical health outcomes										
Bone health										
Klink 2015	Netherlands	Pre-post	Moderate	34 (15 brm, 19 brf)	Mean age for brm: 16.6 y (SD 1.4 y) Mean age for brf: 16.4 y (SD 2.3 y)	N/A	Hormones	aBMD, BMAD (absolute, z-scores using natal sex)	Single (22 years of age)	No evidence for change in measures (small sample size).
Schagen 2020	Netherlands	Pre-post	Moderate	78 (36 brm, 42 brf)	Mean age for brm: 16.2 y (SD 1.2 y) Mean age for brf: 16.9 y (1.1 y)	N/A	Hormones (with GnRHa)	aBMD, BMAD (absolute, z-scores using natal sex), serum bone markers.	36m	Increase in bone density (both absolute and in comparison to natal sex references) in males and females.
Stoffers 2019	Netherlands	Pre-post	Moderate	62 brf	Median age 17.2 y (range 14.9-18.4 y)	N/A	Testosterone	BMD, BMAD (absolute, z-scores using natal sex)	6m, 12m, 24m	Increase in bone density (both absolute and in comparison to natal sex reference).
Vlot 2017	Netherlands	Pre-post	Moderate	70 (28 brm, 42 brf)	Median age for brm: 16.0 y (range 14.0-18.9 y) Median age for brf: 16.3 y (15.9-19.5)	N/A	Hormones (with GnRHa)	BMAD (absolute, z-scores using natal sex), bone-turnover markers (P1NP, OC and ICTP)	24m	No evidence for change in measures (small sample size).
Body mass index (BMI)/body weight										
van de Grift 2020	Netherlands	Cohort	Moderate	43 (17 brf, 26 brm)	Mean age Tanner 2/3 group: 15 y (SD 0.5 y) Mean age Tanner 4/5 group: 17 y (SD 1.0 y)	157	Hormones	BMI	At initiation of surgery	Adolescents who started hormones earlier had a lower BMI (although not clinically significant) before initiation of surgery than those who started later.
Hannema 2017	Netherlands	Pre-post	Moderate	28 brm	Median age 16.0 y (range 13.9-18.9)	N/A	Oestrogen (with GnRHa)	BMI and BMI SD score (reference to natal sex and affirmed gender)	12m, 24m, 36m	BMI remained constant and BMI SD score decreased over time (for both male and female references).
Jarin 2017	US	Pre-post	Moderate	116 (72 brf, 44 brm)	Mean age brm: 18 y (range 14-25 y) Mean age brf: 16 y (range 13-22 y)	N/A	Hormones (brm plus/minus anti-androgens)	BMI	1-3m, 4-6m, 6m+	No change in BMI.
Klink 2015	Netherlands	Pre-post	Moderate	34 (15 brm , 19 brf)	Mean age brm: 16.6 y (SD 1.4 y) Mean age brf: 16.4 y (SD 2.3 y)	N/A	Hormones	BMI and BMI SD score (reference to natal sex)	Single (22 years of age)	No clinically significant increase in BMI but an increase in BMI SD score.
Laurenzano 2021	US	Pre-post	Moderate	119 brf	Mean age 16.5 (range 13.0-19.9)	N/A	Testosterone	BMI and BMI SD score (reference unspecified)	Single (median 1.9y, range 6m to 5.5y)	No change in BMI and BMI z-score.
Millington 2022	US	Pre-post	Moderate	286 (92 brm, 194 brf)	Median age for brm: 17.3 y (IQR 16.2-18.6 y) Median age for brf: 16.2 y (IQR 15.1-17.5 y)	N/A	Hormones (brm plus/minus anti-androgens)	BMI	6m, 12m	No change in BMI.
Olson 2014	US	Pre-post	Moderate	36 brf	Mean age 18.7 y (SD 2.6 y)	N/A	Testosterone	BMI	6m	No change in BMI.
Olson-Kennedy 2018	US	Pre-post	Moderate	59 (34 brf, 25 brm)	Mean age 18 y (range 12-23 y)	N/A	Hormones (brm plus/minus progestins)	BMI	24m (range 21-31 months)	No change in BMI.
Perl 2020	Israel	Pre-post	Moderate	9 brf	Mean age 15.1 y (SD 0.9 y)	N/A	Testosterone	BMI and BMI SD score (reference unspecified)	Single (mean 4m, SD 2m)	No change in BMI and BMI SD score.
Perl 2021	Israel	Pre-post	Moderate	15 brm	Mean age 16.1 y (SD 1.5 y)	N/A	Oestrogen (with GnRHa)	BMI and BMI SD score (reference unspecified)	Single (median 18.5m, range 3-63m)	No change in BMI and BMI SD score.
Sequeira 2019	US	Pre-post	Moderate	46 brf	Not reported	N/A	Testosterone	BMI and BMI SD score (reference to natal sex)	6m, 12m	No change in BMI and BMI z-score.
Stoffers 2019	Netherlands	Pre-post	Moderate	62 brf	Median age 17.2 y (range 14.9-18.4 y)	N/A	Testosterone	BMI and BMI SD score (reference unspecified)	6m, 12m, 24m	No change in BMI and BMI SD score
Tack 2016	Belgium	Pre-post	Moderate	38 brf	Mean age 17 y 5 m	N/A	Testosterone (with Lynestrenol)	BMI and BMI SD score (reference to natal sex)	6m, 12m	Increase in BMI and BMI SD score.
Tack 2017	Belgium	Pre-post	Moderate	27 brm	Mean age 17 y 7 m	N/A	Oestrogen (with Cyproterone acetate)	BMI and BMI SD score (reference to natal sex)	6m, 12m	No change in BMI and BMI SD score.
van der Loos 2021	Netherlands	Pre-post	Moderate	322 (106 brm, 216 brf)	Early-puberty group median age 15.7 y (IQR 15.3-16.0 y) Mid-puberty 16.0 y (IQR 15.8-16.6 y) Late-puberty 16.4 y (IQR 16.0-17.4)	N/A	Hormones (with GnRHa)	BMI	Single (> 2y, median 3-4y across groups)	Increase in BMI in early puberty group.
Nokoff 2020	US	Cross-sectional	Moderate	35 (21 brf, 14 brm)	Mean age at data collection for brm 16.3 y (SD 1.4 y, mean treatment duration 12.3 m (9.9m) Mean age at data collection for brf 17.0 y (SD 1.4 y, mean treatment duration T 11.2 m (5.9m)	108 (no GD)	Hormones (plus/minus GnRHa)	BMI percentile	N/A	No evidence of differences in BMI percentile.
Blood pressure										

Hannema 2017	Netherlands	Pre-post	Moderate	28 brm	Median age 16.0 y (range 13.9-18.9)	N/A	Oestrogen (with GnRH α)	Systolic and diastolic blood pressure	12m, 24m, 36m	No change.
Jarin 2017	US	Pre-post	Moderate	116 (72 brf, 44 brm)	Mean age brm: 18 y (range 14-25 y) Mean age brf: 16 y (range 13-22 y)	N/A	Hormones (brm plus/minus anti-androgens)	Systolic and diastolic blood pressure	1-3m, 4-6m, 6m+	No change.
Laurenzano 2021	US	Pre-post	Moderate	119 brf	Mean age 16.5 (range 13.0-19.9)	N/A	Testosterone	Hypertension	Single (median 1.9y, range 6m to 5.5y)	No participants developed hypertension.
Olson 2014	US	Pre-post	Moderate	36 brf	Mean age 18.7 y (SD 2.6 y)	N/A	Testosterone	Systolic and diastolic blood pressure	6m	No change.
Olson-Kennedy 2018	US	Pre-post	Moderate	59 (34 brf, 25 brm)	Mean age 18 y (range 12-23 y)	N/A	Hormones (brm plus/minus progestins)	Systolic and diastolic blood pressure	24m (range 21-31 months)	No change.
Perl 2020	Israel	Pre-post	Moderate	9 brf	Mean age 15.1 y (SD 0.9 y)	N/A	Testosterone	Systolic and diastolic blood pressure	Single (mean 4m, SD 2m)	No change.
Perl 2021	Israel	Pre-post	Moderate	15 brm	Mean age 16.1 y (SD 1.5 y)	N/A	Oestrogen (with GnRH α)	Systolic and diastolic blood pressure	Single (median 18.5m, range 3-63m)	No change.
Stoffers 2019	Netherlands	Pre-post	Moderate	62 brf	Median age 17.2 y (range 14.9-18.4 y)	N/A	Testosterone	Systolic and diastolic blood pressure	6m, 12m, 24m	No clinically significant change.
Nokoff 2020	US	Cross-sectional	Moderate	35 (21 brf, 14 brm)	Mean age at data collection for brm 16.3 y (SD 1.4 y, mean treatment duration 12.3 m (9.9m)) Mean age at data collection for brf 17.0 y (SD 1.4 y, mean treatment duration T 11.2 m (5.9m))	108 (no GD)	Hormones (plus/minus GnRH α)	Systolic and diastolic blood pressure	N/A	Similar blood pressure (no clinically significant differences) in adolescents receiving hormones compared to untreated adolescents not experiencing gender incongruence.
Metabolic measures										
Hannema 2017	Netherlands	Pre-post	Moderate	28 brm	Median age 16.0 y (range 13.9-18.9)	N/A	Oestrogen (with GnRH α)	HbA1c	12m, 24m, 36m	No change
Jarin 2017	US	Pre-post	Moderate	116 (72brf, 44 brm)	Mean age brm: 18 y (range 14-25 y) Mean age brf: 16 y (range 13-22 y)	N/A	Hormones (brm plus/minus anti-androgens)	Total cholesterol, LDL, HDL, triglycerides, triglyceride:HDL ratio, HbA1c	1-3m, 4-6m, 6m+	No evidence for change, except for a decrease in HDL in birth-registered females.
Laurenzano 2021	US	Pre-post	Moderate	119 brf	Mean age 16.5 (range 13.0-19.9)	N/A	Testosterone	Total cholesterol, LDL, HDL, triglycerides.	Single (median 1.9y, range 6m to 5.5y)	No evidence for change, except for a decrease in HDL.
Olson 2014	US	Pre-post	Moderate	36 brf	Mean age 18.7 y (SD 2.6 y)	N/A	Testosterone	Non-fasting total cholesterol.	6m	No evidence for change.
Olson-Kennedy 2018	US	Pre-post	Moderate	59 (34 brf, 25 brm)	Mean age 18 y (range 12-23 y)	N/A	Hormones (brm plus/minus progestins)	Total cholesterol, HDL, triglycerides, glucose	24m (range 21-31 months)	No evidence for change, except for an increase in HDL.
Stoffers 2019	Netherlands	Pre-post	Moderate	62 brf	Median age 17.2 y (range 14.9-18.4 y)	N/A	Testosterone	Total cholesterol, HDL, LDL, triglycerides, HbA1c	6m, 12m, 24m	Evidence for decrease in HDL. Some evidence for a small, early decrease in total cholesterol, no change for other outcomes.
Tack 2016	Belgium	Pre-post	Moderate	38 brf	Mean age 17 y 5 m	N/A	Testosterone (with Lynestrenol)	Total cholesterol, triglycerides, HDL, LDL, fasting insulin, HbA1c, glucose, HOMA-IR	6m, 12m	No evidence for change.
Tack 2017	Belgium	Pre-post	Moderate	27 brm	Mean age 17 y 7 m	N/A	Oestrogen (with Cyproterone acetate)	Triglycerides, total cholesterol, HDL, LDL, HbA1c, glucose, insulin	6m, 12m	No evidence for change.
Nokoff 2020	US	Cross-sectional	Moderate	35 (21 brf, 14 brm)	Mean age at data collection for brm 16.3 y (SD 1.4 y, mean treatment duration 12.3 m (9.9m)) Mean age at data collection for brf 17.0 y (SD 1.4 y, mean treatment duration T 11.2 m (5.9m))	108 (no GD)	Hormones (plus/minus GnRH α)	Fasting insulin, HOMA-IR, fasting glucose, total cholesterol, triglycerides, HDL, LDL, HbA1c	N/A	Brf receiving hormones had lower HDL than controls, whereas brm had higher HDL than controls. Brm had a lower inverse of fasting insulin and higher HOMA-IR than controls.
Other physical parameters										
Hannema 2017	Netherlands	Pre-post	Moderate	28 brm	Median age 16.0 y (range 13.9-18.9)	N/A	Oestrogen (with GnRH α)	Prolactin, ALT, AST, ALP, γ -glutamyl transferase, creatinine, haematocrit, haemoglobin	12m, 24m, 36m	Alkaline phosphatase decreased, no other changes were observed.
Jarin 2017	US	Pre-post	Moderate	116 (72brf, 44 brm)	Mean age brm: 18 y (range 14-25 y) Mean age brf: 16 y (range 13-22 y)	N/A	Hormones (brm plus/minus anti-androgens)	AST, ALT, haemoglobin, haematocrit, prolactin, serum urea nitrogen, creatinine	1-3m, 4-6m, 6m+	No evidence for change.
Laurenzano 2021	US	Pre-post	Moderate	119 brf	Mean age 16.5 (range 13.0-19.9)	N/A	Testosterone	Haematocrit, AST, ALT	Single (median 1.9y, range 6m to 5.5y)	Evidence for an increase in haematocrit.
Madsen 2021	Netherlands	Pre-post	Moderate	1073 brf	Median age 22.5 (IQR 18.4-31.8)	N/A	Testosterone	Erythrocytosis (haematocrit levels)	Annually up to 20 years (>12m)	Haematocrit levels of >0.50L/L occurred in 24% of brf. Levels of >0.52L/L and >0.54L/L occurred 7.6% and 2.2% respectively.
Millington 2022	US	Pre-post	Moderate	286 (92 brm, 194 brf)	Median age for brm: 17.3 y (IQR 16.2-18.6 y) Median age for brf: 16.2 y (IQR 15.1-17.5 y)	N/A	Hormones (brm plus/minus anti-androgens)	Kidney function (serum creatinine, estimated glomerular filtration rate (eGFR))	6m, 12m, 18m, 24m	Serum creatinine increased in brf and decreased in brm. eGFR increased initially in brm and decreased in brf, and then returned to baseline levels.

Mullins 2021	US	Pre-post	Moderate	611 (428 brf, 183 brm)	Not reported	N/A	Hormones	Occurrence of thrombosis	Single (median 554/7d brm/f, range 283-1037)	No thrombosis occurred whilst receiving hormones.
Olson 2014	US	Pre-post	Moderate	36 brf	Mean age 18.7 y (SD 2.6 y)	N/A	Testosterone	Haemoglobin, ALT, AST	6m	There was an increase in haemoglobin levels.
Olson-Kennedy 2018	US	Pre-post	Moderate	59 (34 brf, 25 brm)	Mean age 18 y (range 12-23 y)	N/A	Hormones (brm plus/minus progestins)	AST, ALT, potassium, prolactin, haemoglobin	24m (range 21-31m)	No change except for a decrease in AST.
Schagen 2018	Netherlands	Pre-post	Moderate	127 (73 brf, 54 brm)	Mean age for brm: 16.3 y (SD 1.2y) Mean age for brf: 16.8 (SD 1.1)	N/A	Hormones (with GnRHa)	DHEAS and A4	12m, 24m	DHEAS remained constant. A4 remained constant in brm and increased in brf.
Stoffers 2019	Netherlands	Pre-post	Moderate	62 brf	Median age 17.2 y (range 14.9-18.4 y)	N/A	Testosterone	SHBG, TSH, prolactin, free thyroxine, DHEAS, A4, haemoglobin, haematocrit, creatinine, ALP, vitamin D, ureum	6m, 12m, 24m	Decrease in SHBG and increase in both haemoglobin and haematocrit. No change in others.
Tack 2016	Belgium	Pre-post	Moderate	38 brf	Mean age 17 y 5 m	N/A	Testosterone (with Lynestrenol)	Haemoglobin, haematocrit, creatinine, AST, ALT, TSH, free thyroxine, anti-Mullerian hormone, SHBG	6m, 12m	Increases in haemoglobin, haematocrit and creatinine. Decrease in free thyroxin.
Tack 2017	Belgium	Pre-post	Moderate	27 brm	Mean age 17 y 7 m	N/A	Oestrogen (with Cyproterone acetate)	DHEAS, haemoglobin, haematocrit, creatinine, AST, ALT, prolactin, TSH, free thyroxin, SHBG	6m, 12m	Evidence for an increase in SHBG and prolactin. No change in others.
Nokoff 2020	US	Cross-sectional	Moderate	35 (21 brf, 14 brm)	Mean age at data collection for brm 16.3 y (SD 1.4 y, mean treatment duration 12.3 m (9.9m) Mean age at data collection for brf 17.0 y (SD 1.4 y, mean treatment duration T 11.2 m (5.9m)	108 (no GD)	Hormones (plus/minus GnRHa)	AST, ALT, SHBG, free androgen index	N/A	No evidence of differences, except for higher AST, lower SHBG and higher free androgen index compared to controls (brf only).
Fertility										
de Nie 2022	Netherlands	Cohort	Moderate	78 brm	Not reported	136	Hormones	Germ cell type, Johnsen's score, hyalinisation and lumen status	Single (at time of surgery when mean age 29.6 y (SD 12.4))	Mature spermatozoa only observed in those starting treatment at Tanner stage 4 or higher. Immature germ cells present in all treated in early puberty.
Side effects										
Jensen 2019	US	Cohort	High	17 (11 brf, 6 brm)	Median age of GnRHa group: 14.9 y (range 14.1-15.7 y) Median age of no GnRHa group: 16.7 y (14.4-18.2 y)	66*	Hormones (with or without GnRHa)	Side-effects recorded by clinicians and in electronic medical record notes	Single (range 6.4-53.0m)	In brm, breast tenderness common; increased liver enzymes, oestradiol levels above limit less common. In brf, acne, mood changes, elevated red blood markers, increased appetite common. Headaches, hot flashes, fatigue, hair loss less common. Similar in GnRHa / no GnRHa.
Laurenzano 2021	US	Pre-post	Moderate	119 brf	Mean age 16.5 (range 13.0-19.9)	N/A	Testosterone	Acne	Single (median 1.9y, range 6m to 5.5y)	Acne severity progressed in ~65% of participants, advanced acne management documented in ~20%.
Tack 2016	Belgium	Pre-post	Moderate	38 brf	Mean age 17 y 5 m	N/A	Testosterone (with Lynestrenol)	Patient-reported side-effects	6m, 12m	Acne increased during testosterone treatment, and metrorrhagia increased slightly.
Tack 2017	Belgium	Pre-post	Moderate	27 brm	Mean age 17 y 7 m	N/A	Oestrogen (with Cyproterone acetate)	Patient-reported side-effects	6m, 12m	Breast tenderness, mood swings and hunger were frequently reported during oestradiol (no figures given).
Abbreviations: A4 – androstenedione; ALP – alkaline phosphatase; ALT - alanine aminotransferase; AST – aspartate aminotransferase; aBMD – areal bone mineral density; BMAD – bone mineral apparent density; BMD – bone mineral density; BMI – body mass index; brf – birth-registered females; brm – birth-registered males; CSH – cross-sex hormones; d – days; DHEAS – dehydroepiandrosterone sulfate; GnRHa – Gonadotropin-Releasing Hormone analogues; m – months; HbA1c – glycated haemoglobin; HDL – high-density lipoprotein; HOMA-IR – Homeostatic Model Assessment for Insulin Resistance; ICTP – Type I collagen degradation product; IQR – inter-quartile range; LDL – low-density lipoprotein; OC – osteocalcin; P1NP – procollagen type I N-terminal propeptide; QTc – heart-rate corrected QT interval; SD – standard deviation; SHBG - sex hormone binding globulin; TSH – thyroid stimulating hormone, y – years.										
* This study compared the effect of hormones taken concurrently with or without GnRHa. Comparator group received no GnRHa.										