The Journal of Clinical Endocrinology & Metabolism, 2025, **00**, 1–8
https://doi.org/10.1210/clinem/dgaf619
Advance access publication 15 November 2025
Clinical Research Article

Metabolism and Type 2 Diabetes Across Life Stages in Klinefelter Syndrome: A Population-Based Cohort Study

Simon Chang,[1,2] Lars Pedersen,[3,4] Anne Skakkebæk,[2,4,5] Agnethe Berglund,[1,2,5] and Claus H. Gravholt[1,2,4]

[1]Department of Endocrinology, Aarhus University Hospital, DK-8200 Aarhus N, Denmark
[2]Department of Molecular Medicine, Aarhus University Hospital, DK-8200 Aarhus N, Denmark
[3]Department of Epidemiology, Aarhus University Hospital, DK-8200 Aarhus N, Denmark
[4]Department of Clinical Medicine, Aarhus University, DK-8200 Aarhus N, Denmark
[5]Department of Clinical Genetics, Aarhus University Hospital, DK-8200 Aarhus N, Denmark

Correspondence: Simon Chang, MD, Aarhus Univeristy Hospital, Brendstrupgårdsvej 21A, DK-8200 Aarhus N, Denmark. Email: simon.chang@clin.au.dk.

Abstract

Context: Klinefelter syndrome (KS) is underdiagnosed, undertreated, and associated with metabolic dysfunction.

Objective: We compared incidences of metabolic disorders among men with KS, either undiagnosed (U-KS), diagnosed and untreated (D-KS), or treated with testosterone replacement therapy (T-KS).

Methods: This was a national Danish registry-based study from January 1994 to December 2022. We computed hazard ratios (HR) for incidence and severity of metabolic disorders between risk set matched strata of U-KS, D-KS, and T-KS and male control individuals. We evaluated the effect of parenteral vs transdermal testosterone supplementation on incidence of metabolic disorders in T-KS, applying inverse probability weighting.

Results: We included 508 age-matched strata of U-KS, D-KS, and T-KS, and included 46 241 male controls. Incidence of metabolic conditions was more than 2-fold increased in KS, including type 2 diabetes (HR 2.56 [1.85-3.44]). U-KS presented with the most severe metabolic phenotype, with more obesity and more late-stage diabetes complications compared with T-KS (HR 2.83 [1.33-6.02]). All-cause mortality following diagnosis of type 2 diabetes was increased in D-KS compared with controls (HR 1.77 [1.28-2.45]), but nondifferential for T-KS and controls (HR 1.30 [0.68-2.48]). We saw a pattern of less obesity and type 2 diabetes, but more hypertension and hypercholesterolemia, with parenteral vs transdermal testosterone supplementation in T-KS.

Conclusion: The metabolic profile in men with KS is dependent on diagnosis and treatment status, with pronounced metabolic dysfunction in U-KS. Better diagnosis and treatment of KS are needed to alleviate metabolic dysfunction and improve survival in men with KS.

Key Words: epidemiology, hypogonadism, Klinefelter syndrome, metabolism, sex chromosomes, testosterone replacement therapy, type 2 diabetes

Abbreviations: aDCSI, adapted Diabetes Complications Severity Index; ATC, Anatomical Therapeutic Chemical classification; D-KS, diagnosed Klinefelter syndrome not receiving testosterone; GLP-1, glucagon-like peptide 1; HbA1c, glycated hemoglobin; HR, hazard ratio; ICD, International Classification of Diseases; KS, Klinefelter syndrome; LDL, low-density lipoprotein; T2DM, type 2 diabetes mellitus; T-KS, diagnosed Klinefelter syndrome treated with testosterone; TRT, testosterone replacement therapy; U-KS, undiagnosed Klinefelter syndrome.

Klinefelter syndrome (47,XXY, KS) is challenged by an unfavorable metabolic profile (1). The clinical metabolic phenotype, characterized by increased body fat deposition, dyslipidemia, and insulin resistance, further extends to increased rates of metabolic diseases among men with KS (1, 2). KS is only diagnosed in about 25% to 35% of cases (3), and it has been suggested that metabolic dysfunction could predict the diagnosis of KS. Recently, however, elevated rates of metabolic diseases have also been demonstrated in undiagnosed men with KS, underlining the ubiquitous nature of metabolic dysfunction in KS (4, 5).

Hypogonadism, occurring in virtually all men with nonmosaic KS, has been believed to be the main pathophysiological reason for metabolic dysfunction in KS (1). However, the chromosomal alteration involving an additional X chromosome may induce widespread changes in the global genomic landscape in men with KS that are related to metabolic function independently of hypogonadism, explaining the insufficient rescue of normal metabolism in men with KS receiving testosterone replacement therapy (TRT) (1, 6-9).

Recently, using national Danish health registries, we demonstrated that long-term TRT is associated with an almost 50% reduction in mortality among men with KS (10). Physiologically, it is reasonable to hypothesize that the increased anabolic potential obtained through TRT would be beneficial for the metabolic profile. However, data supporting this hypothesis remain limited, with mostly cross-sectional and short-term longitudinal studies supporting beneficial effects of TRT in male hypogonadism, especially regarding cardiometabolic risk and mortality (11). Conducting large randomized controlled TRT trials has proven difficult, and for example, the recent TRAVERSE study, challenged by short follow-up and a substantial nonadherence in both study arms, only demonstrated noninferiority of TRT regarding cardiovascular outcomes (12). Conducting a large

Received: 12 August 2025. **Accepted:** 7 November 2025. **Corrected and Typeset:** 28 November 2025

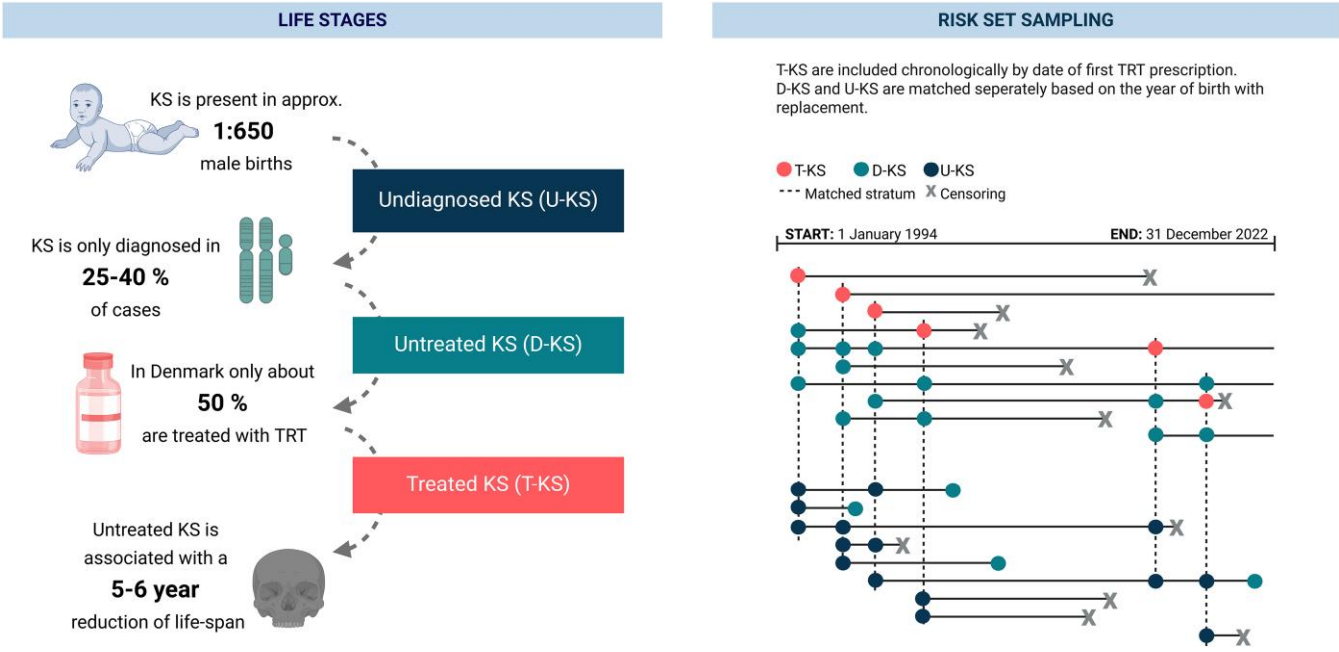

Figure 1. (A) Concept of life stages when living with Klinefelter syndrome. (B) Procedure for risk set matching among men with Klinefelter syndrome across life stages.

randomized TRT trial in men with KS will be virtually impossible. Besides the obvious difficulties of recruiting enough patients, it would also be unethical to withhold TRT from hypogonadal men with KS for extended periods of time (1). The most feasible, cost-effective, and ethical approach for assessing TRT effects in KS seems to be to take advantage of the abundance of real-world evidence available through large health registries.

The aim of the present study was to describe metabolic function in men with KS by utilizing the multitude of Danish health registries. Specifically, for the first time, to address potentially unwarranted effects of delayed diagnosis and insufficient attention to treating hypogonadism in KS, we wanted to compare the severity of the metabolic profile among men with KS across 3 different life stages: (i) undiagnosed (U-KS); (ii) diagnosed and not receiving TRT (D-KS); and (iii) diagnosed and receiving TRT (T-KS) (Fig. 1). We hypothesized that men with KS receiving TRT would have a less severe metabolic profile with fewer incident metabolic disorders.

Methods

Design and Setting

This study forms part of an ongoing series of Danish nationwide registry-based studies examining morbidity patterns in KS (The Danish Data Protection Agency record number 1-16-02-568-15). Findings regarding venous thrombosis, major adverse cardiovascular events, and mortality have been reported previously (2, 10). As described, inpatient and outpatient hospital diagnoses were retrieved from The Danish National Patient Registry (13) according to the International Classification of Diseases (ICD). Use of prescription medicine was collected from the Danish National Prescription Registry (14) according to the Anatomical Therapeutic Chemical classification (ATC) codes. Laboratory analyses were retrieved from the Clinical Laboratory Information Register according to the Nomenclature for Properties and Units (NPU) Laboratory Terminology.

Cohort Identification and TRT Exposure

Men with KS were identified in the Danish Cytogenetic Central Registry (15). KS was defined as any 47,XXY or 46, XY/47,XXY mosaic karyotype. In total, 1325 men with KS were assessed for inclusion in the study cohorts. TRT was assessed by redemption of prescriptions with ATC G03B (16) from January 1, 1994, to December 31, 2022, according to the coverage in the prescription registry.

KS Comparison Cohorts and Risk Set Sampling

We designed a matched cohort study applying risk set sampling with replacement based on timing of TRT exposure in an intention-to-treat analysis (17), as previously described (10). The observation period was from January 1, 1994, until December 31, 2022. We excluded boys with KS who did not reach the age of 15 years before emigrating, dying, or the end of the study. Men with KS older than 15 years at the beginning of the prescription registry could potentially have been receiving TRT prior to this date. We required a grace period of 180 days from January 1, 1994, without any prescriptions to identify an individual as untreated. Those who had TRT prescriptions during the grace period were excluded. We also excluded men with KS receiving TRT before being diagnosed with KS.

Strata were formed according to chronological exposure to TRT (Fig. 1), and each T-KS man was matched on the day of the first prescription to both U-KS and D-KS. Matching was based on year of birth ± 2 years, and up to 5 individuals were selected for each comparison group. Matches for each stratum were sampled randomly among all eligible participants with replacement, meaning that any man with KS could be reselected for different strata, and U-KS/D-KS could

themselves become T-KS at a later stage (17). We further included an age-matched male comparison cohort from the background population, as previously described (10).

Outcomes for Survival Analysis

For all outcomes, the date of event was set as the first occurrence of the outcome. A complete list of ATC, ICD, and NPU codes is given in the Supplement (Table S1 (18)). Some diagnoses were based on combinations of data. As an example, hypertension was defined as the redemption of antihypertensive drugs while hypercholesterolemia was defined as either a prescription for statins, a hospital diagnosis (DE780), or laboratory findings of low-density lipoprotein (LDL) > 3.0 mmol/L, based on previous Danish data indicating increased cardiovascular risk with LDL > 3.0 mmol/L (19).

To define incidences of type 2 diabetes (T2DM), we combined diagnoses, medication use, and laboratory data. ICD-10 codes adhering to "insulin-dependent diabetes" were indicative of type 1 diabetes (T1DM), and codes related to "non-insulin-dependent diabetes" were indicative of T2DM. We considered all prescriptions of glucose-lowering medications (ATC A10) to be indicative of diabetes. Further, 2 measurements of glycated hemoglobin (HbA1c) ≥ 6.5% (48 mmol/mol) more than 30 days apart were considered diagnostic for diabetes. Date of diabetes diagnosis was at first prescription of glucose-lowering medication, first HbA1c ≥ 6.5% (48 mmol/mol), or first registered diabetes diagnosis, whichever came first. We applied a series of criteria to distinguish between T2DM and type 1 diabetes (see Supplement (18)). Nine controls with dual diagnoses could not be assigned as either, and they were excluded.

Individuals with repeated measurements of elevated HbA1c with no diabetes diagnosis and no prescriptions for glucose-lowering medications were considered as lifestyle-intervention-treated T2DM.

Type 2 Diabetes-Only Cohort

We constructed a cohort consisting of all KS cases and controls with T2DM. We stratified men with KS based on life stages as either U-KS, D-KS, or T-KS at the time of T2DM diagnosis. For U-KS, we included individuals whose first T2DM diagnoses occurred prior to coverage in the prescription registry.

Glycemic control was evaluated by levels of HbA1c. Use of glucose-lowering medications was assessed according to prescriptions history. Late-stage diabetes complications were evaluated by the adapted Diabetes Complications Severity Index (aDCSI) based on ICD-10 codes (20, 21). The aDCSI is a validated tool for assessing diabetes severity (22) and has previously been applied to Danish health registry data (23). We updated the algorithm to include ICD E114 for unspecified type of diabetes with complications and to allow inclusion of a neuropathy score = 2 for polyneuropathy (Table S2 (18)).

Statistical Analysis

Survival analysis

Incidences were compared as hazard ratios (HR) from stratified Cox regression with cluster-robust variance estimates and T-KS as the reference. Study entry was at the day of TRT exposure in each stratum, and censoring was at first occurrence of the outcome under investigation, death, emigration, or end of the study. U-KS were censored on the day of KS diagnosis, and D-KS were censored on the day of TRT if treated subsequently. We excluded individuals with the first occurrence of the outcome under investigation prior to study entry from the analysis of that specific outcome.

Supplementary analyses

Cross-sections of continuous variables—for example, levels of biomarkers or age—were compared across groups using one-way ANOVA or Kruskal-Wallis test with Bonferroni correction.

To assess health surveillance in men with KS, we estimated the cumulative HR of undergoing any blood test, using a multiple-failure model to compare across different KS life stages.

Longitudinal changes of continuous variables were assessed across life stages by mixed effects models with pairwise correlations described by an exponential function of the time between measurements to allow for uneven timing of repeated measurements and consideration of individuals progressing to next life stage under observation. Model fit was assessed by Q-Q plots of standardized residuals.

In individuals with T2DM, aDCSI scores at 1 and 10 years of diabetes duration were compared by ordinal logistic regression with adjustment for age at T2DM diagnosis and observation time. We further computed the HR for the combination of prevalent and first incident cases of any late-stage diabetes complication, and adjusting for age at T2DM diagnosis. Similarly, we assessed the cumulative HR of late-stage diabetes complications in a multi-failure setup.

Among T-KS, we computed HRs comparing transdermal and parenteral TRT administration routes. Allocation to either transdermal or parenteral treatment group was based on having more than 80% of prescriptions adhering to either formulation. T-KS, with fewer than 80% of prescriptions adhering to either type of formulation, were excluded. We applied inverse probability of treatment weighting after calculating propensity scores for treatment types based on age of KS diagnosis, age when starting TRT, and year of birth. Balancing of covariates after applying weights was assessed by standardized mean differences.

Analyses were performed using Stata 18 (StataCorp LLC, College Station, TX, USA).

Results

We identified 913 men with KS who were eligible for risk set sampling, including 508 exposed to TRT. From the risk set sampling procedure, we constructed 508 strata matching a total of 2146 U-KS and 2522 D-KS (Table 1). Additionally, we matched 46 241 controls from the background population to the 508 T-KS (Table 1). The median (interquartile range; IQR) year of birth was 1979 (1969-1989) for all groups.

Metabolic Disorders Across KS Life Stages

The incidences of metabolic disorders are shown in Fig. 2. There was a tendency toward higher incidence of obesity, and in particular, extreme obesity in U-KS. Similarly, bariatric surgery was more frequent in U-KS. The incidence of T2DM with multiple complications was higher in U-KS, with a trend toward a higher risk of being treated with insulin. We observed no effect of KS diagnosis or TRT on the incidence of T2DM overall (Fig. 2). Outcomes based either solely or partially on prescription data were commonly less frequent in D-KS, such as glucagon-like peptide 1 (GLP-1) receptor agonist prescriptions, hypercholesterolemia, and hypertension (Fig. 2).

Table 1. Study cohorts

Risk set cohort	U-KS	D-KS	T-KS	Controls	
No of participants, n[a]	2146	2522	508	46 241	
Total observation time, years	11 621	24 366	6460	595 268	
Observation time per participant, years	4.0 (1.6-7.7)	8.2 (3.2-15.0)	11.7 (6.7-17.9)	11.7 (6.6-18.2)	
Age at entry, years	28.4 (16.8-36.9)	30.5 (17.3-40.0)	30.3 (17.1-39.5)	30.3 (17.1-39.6)	
T2DM cohort	**U-KS**	**D-KS**	**T-KS**	**Controls**	***P***
T2DM, yes	**n = 25**	**n = 106**	**n = 86**	**n = 10 678**	
No of diagnostic criteria met, n (%)					
1	4 (16)	17 (16)	18 (21)	2753 (26)	
2	< 3 (< 12)	31 (29)	29 (34)	3821 (36)	
3	18 (72)	58 (55)	39 (45)	4104 (38)	
Age at T2DM diagnosis, years	42.4 ± 14.9	54.2 ± 12.9	47.6 ± 11.4	57.9 ± 13.0	<.0001[b]
Age at KS diagnosis	48.2 ± 14.2	26.8 ± 10.3	27.0 ± 13.1	—	<.0001[c]
Age at eventual TRT, years (n)	50.0 ± 11.2 (12)	43.4 ± 10.4 (11)	35.2 ± 13.1		.0004[d]
Delay of TRT from T2DM diagnosis, years	7.7 ± 6.8	6.9 ± 5.9	−12.4 ± 7.4		
Late-stage diabetes complications					
Single failure any late-stage T2DM complications, hazard ratio	2.28 (1.35-3.86)	0.94 (0.60-1.46)	1 (reference)	0.65 (0.46-0.92)	
Multiple failure any late-stage T2DM complications, hazard ratio	2.83 (1.33-6.02)	0.95 (0.53-1.72)	1 (reference)	0.69 (43-1.10)	

Abbreviations: D-KS, diagnosed Klinefelter syndrome; T2DM, type 2 diabetes mellitus; T-KS, testosterone-treated Klinefelter syndrome; TRT, testosterone replacement therapy; U-KS, undiagnosed Klinefelter syndrome.
Data are median (interquartile range).
[a]The matched strata were constructed through matching and resampling among a total cohort of 913 individuals with KS.
[b]One-way ANOVA: D-KS > U-KS ($P < .001$), D-KS > T-KS ($P = .002$), Controls < all KS groups ($P < .001$ to .03).
[c]One-way ANOVA: U-KS > D-KS and T-KS ($P < .001$).
[d]Younger in T-KS compared with other KS groups.

For most metabolic conditions, the incidence in T-KS was more than twice as high as in controls (Fig. 2).

We found an indication of overall reduced health surveillance in both D-KS, and in particular U-KS. Compared with T-KS, the HR for blood testing in D-KS was halved (HR 0.51 [0.43-0.61]) and more than 90% reduced in U-KS (HR 0.06 [0.05-0.08]).

We assessed glycemic control by evaluating HbA1c. In those with no history of diabetes, we saw no difference in overall levels of HbA1c between groups (771 KS and 68 160 controls with available measurements). We then assessed the longitudinal effects of TRT on HbA1c in T-KS only. We compared levels of HbA1c measured before TRT (−1 year) with levels after TRT (0.5-1.5 years). We applied a mixed model with adjustment for age at sampling and T2DM (yes/no). Samples taken after receipt of a prescription for any glucose-lowering medications were not considered. TRT was associated with a nonsignificant small reduction in HbA1c (β [95 CI], −0.3 [−0.9; 0.3], $P = .30$). We then applied a similar approach for evaluation of LDL, with adjustment for statin use at the time of sampling. TRT was associated with a reduction in LDL levels (β [95 CI], −0.5 [−0.9; 0.1], $P = .02$).

Evaluation of Type 2 Diabetes in KS Life Stages and Controls

In total, we identified 217 men with KS diagnosed with T2DM (Table 1). At the time of T2DM diagnosis, 25 were U-KS, 106 were D-KS, and 86 were T-KS. We additionally identified 10 678 controls diagnosed with T2DM (Table 1). The majority of T2DM cases fulfilled more than 1 of 3 criteria for diabetes: hospital diagnosis, elevated HbA1c, or a prescription for glucose-lowering medication (Table 1). Among U-KS, there was a delay of almost 6 years from the diagnosis of T2DM until diagnosis of KS. For both U-KS and D-KS, TRT was ever initiated in only a subset of patients, with a 7-year delay following T2DM diagnosis (Table 1). Additionally, among KS men with T2DM and no history of TRT, 13 of 15 with available laboratory assessments, had at least one measurement of testosterone below the lower reference limit.

Glycemic Control

In the T2DM cohort, < 3 U-KS, 39 D-KS, 51 T-KS, and 5461 controls had available measures of HbA1c around the time of T2DM diagnosis. The median level of HbA1c ± 90 days of T2DM diagnosis was not different among the groups ($P = .8$), and no difference was seen regarding the median lowest values of HbA1c obtained within 2 years of T2DM diagnosis ($P = .13$).

Combined, 199 men with KS and T2DM and 9906 controls with T2DM had more than one measurement of HbA1c available at any point. Due to large inter-individual variability in sampling frequency and timing of sampling relative to T2DM duration, we computed the area under the curve (AUC) for HbA1c. We then applied a mixed model with the log-transformed AUC as the dependent variable, with grouping (U-KS, D-KS, T-KS, and controls) as fixed effect, adjusting for the log-transformed timespan between the first and last sample for each participant, as well as the age at first sampling. By this approach, we did not observe any difference in HbA1c levels between groups over the duration of T2DM.

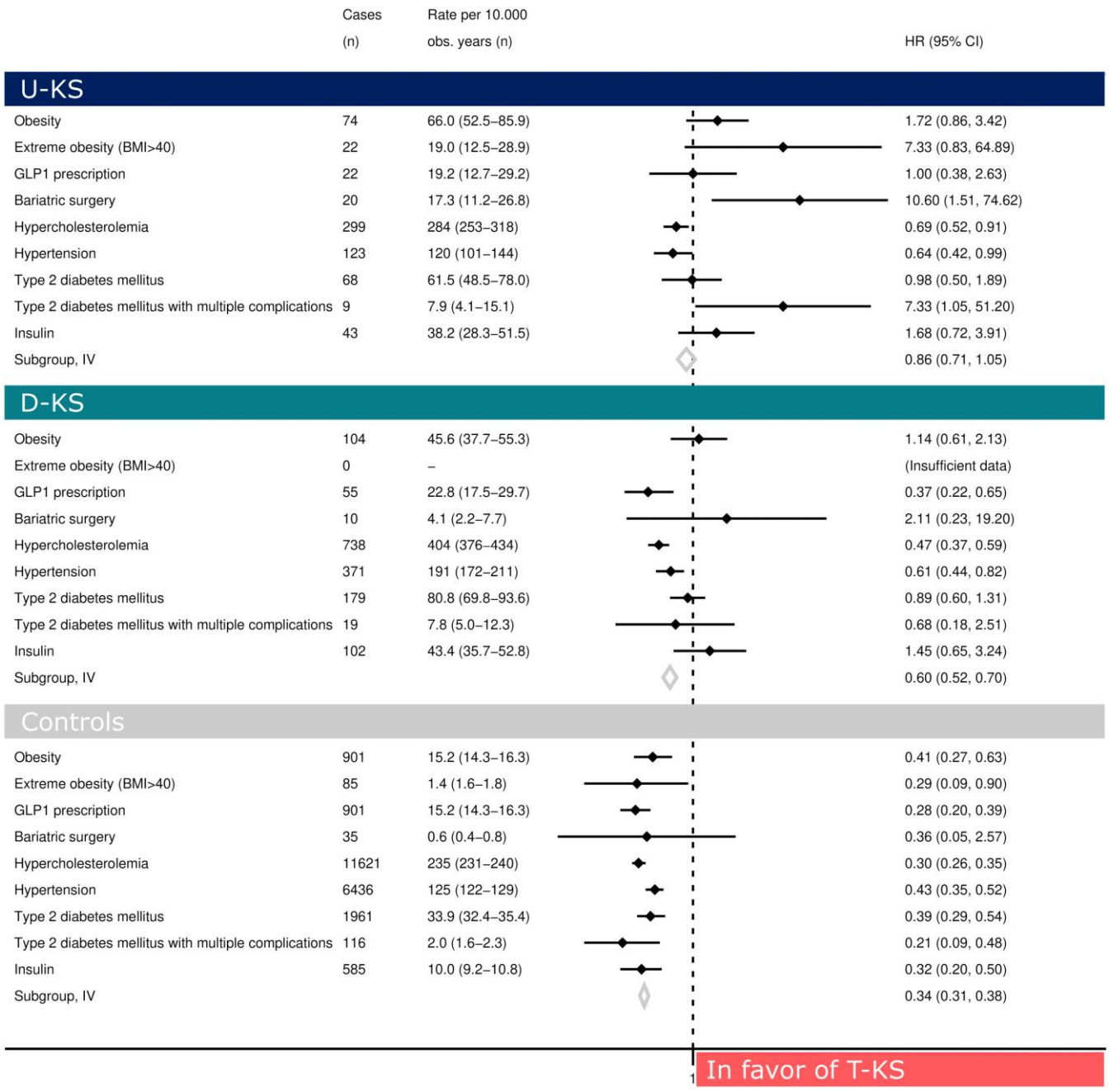

Figure 2. Hazard ratios for occurrence of metabolic disorders across Klinefelter syndrome (KS) life stages with testosterone-treated KS (T-KS) as the reference and comparing with undiagnosed KS (U-KS), diagnosed but untreated KS (D-KS) and male controls from the background population. Hazard ratios > 1 are in favor of T-KS. Abbreviations: BMI, body mass index; GLP-1, glucagon-like peptide 1 analogue; HR; hazard ratio.

Glucose-Lowering Medications

Ever-use of insulin, GLP-1, sulfonylurea, and metformin was higher in KS (Pearson chi-square, $P < .03$ for all, see Fig. S1 (18)). Fewer men with KS than controls were ever treated with 3 or fewer different drugs (Pearson Chi-square, $P < .001$). The number of glucose-lowering medications was not different comparing D-KS and T-KS.

T2DM Complication Status

The HR for first late-stage diabetes complication in the T2DM cohort was more than doubled in U-KS compared with T-KS, and further reduced by one-third in controls compared with T-KS (Table 1), reflecting a 3.5-fold increased incidence of any first late-stage diabetes complication in U-KS compared with controls (HR 3.48 [2.32-5.22]). Odds ratios (OR) for a higher aDCSI at 1 year and 10 years T2DM duration, were numerically increased in U-KS compared with T-KS but failed to reach statistical significance (OR 2.11 [0.70-6.40] and 1.57 [0.62-3.98], respectively).

The HR for all-cause mortality following a T2DM diagnosis was increased in D-KS compared with controls (HR 1.77 [1.28-2.45]) but not significantly different for T-KS and controls (HR 1.30 [0.68-2.48]).

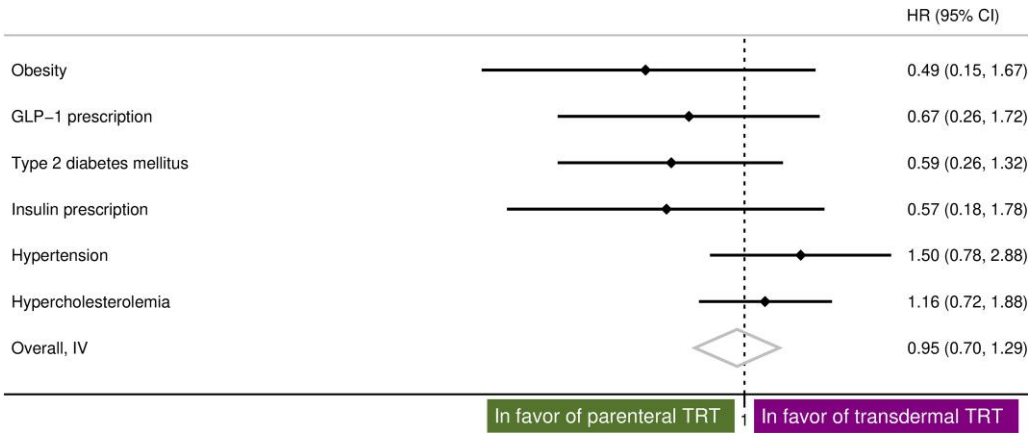

Figure 3. Hazard ratios for metabolic traits, comparing parenteral and transdermal administration of testosterone replacement therapy in testosterone treated Klinefelter syndrome. Hazard ratios > 1 are in favor of transdermal administration. Abbreviations: GLP-1, glucagon-like peptide 1 analogue; HR, hazard ratio; TRT, testosterone replacement therapy.

Effects of TRT Administration Routes

We identified 133 transdermally treated T-KS (tdT-KS) and 265 parenterally treated T-KS (pT-KS). After applying inverse probability of treatment weighting, standardized mean differences for covariates associated with treatment choice were < 0.08. We then assessed the incidences of metabolic disorders and cardiometabolic risk factors, observing a pattern suggesting a benefit of parenteral vs transdermal TRT regarding obesity, GLP-1 use, T2DM and insulin prescription use, and the opposite for hypertension and hypercholesterolemia (Fig. 3). Compared with controls, the HR for T2DM was still more than 2-fold increased regardless of TRT formulation (HR pT-KS vs controls 2.33 [1.54-3.51]); HR tdT-KS v controls (2.66 [1.48-4.97]).

TRT formulation did not affect the change in either HbA1c or LDL before and after TRT ($P = .77$ and $P = .80$, respectively).

Discussion

This study aimed to describe the metabolic consequences of KS across different life stages. We present the most comprehensive mapping of metabolic traits in KS to date in a nationwide real-world setting. To our knowledge, this is the first study to directly highlight the potential consequences of delayed diagnosis and treatment of hypogonadism on metabolic health in KS. We demonstrate that TRT in KS cannot fully alleviate the negative impact of hypogonadism on metabolism. Compared with controls, T-KS had a 2- to 3-fold increased risk of obesity and other metabolic diseases, including hypercholesterolemia and hypertension. Furthermore, the early onset and persistently increased risk of T2DM in KS across life stages underline the severity of the metabolic phenotype in KS, likely contributing significantly to the reduced life expectancy among these patients (1, 24).

The effects of TRT on T2DM risk and metabolism has previously been assessed in randomized settings among non-KS male populations (12, 25-29). However, transferring these results to KS is challenging, as those studies provided relatively short-term TRT to men with late-onset testosterone deficiency. In the T4DM study, testosterone undecanoate was provided for 2 years in a double-blind, placebo-controlled trial that included 1007 men with a high risk of T2DM (25). The study found improved insulin sensitivity and reduced risk of T2DM with TRT. More recently, a small randomized controlled trial applying testosterone undecanoate injections in men with metabolic syndrome and hypogonadism observed improved insulin sensitivity with 2 years of TRT (26). The T-Trials applied transdermal testosterone or placebo for 1 year in older men with obesity (70%), among whom 37% were diagnosed with diabetes (27). The study only demonstrated a small improvement in insulin sensitivity with TRT, and no impact on HbA1c levels (28). The TRAVERSE study evaluated the cardiovascular safety of transdermal TRT in men with hypogonadal symptoms and low testosterone levels (12). In a sub-study including 5204 randomized participants, 1175 with prediabetes and 3880 with diabetes, TRT had no effect on the risk of either progression from prediabetes to diabetes, or normalization of HbA1c levels in those with diabetes (29). The testosterone levels obtained in the T4DM study were higher than in both the T-Trials and TRAVERSE. This is potentially explained by the choice of TRT formulation, with a more pronounced effect of TRT on diabetes-related outcomes in studies opting for parenteral vs transdermal testosterone. We demonstrate, for the first time, a comparable pattern of a differential metabolic effect of TRT formulation among men with KS. We find a tendency toward less obesity and better glucose metabolism among those with parenteral vs transdermal TRT in KS. Our data also demonstrate that neither parenteral nor transdermal TRT was superior in reducing the massively increased risk of T2DM in KS. Hypogonadism has direct negative effect on metabolism (30) that, if not attended to, could invoke irreversible long-term changes to metabolic function, so-called metabolic memory (31). Prolonged hypogonadism in KS could impede the efficacy of TRT and the correction of metabolic dysfunction, but the inability of TRT to abolish metabolic dysfunction also indicates that metabolic dysfunction in KS is not attributable to hypogonadism alone. Future identification of genetic alterations induced by the presence of a supernumerary X chromosome and associated with the metabolic profile of KS appears increasingly important for advancing our understanding of disease mechanisms in KS. Still, TRT should be the cornerstone of treatment in KS, as demonstrated here by the lower mortality among T-KS vs D-KS with T2DM, and the previously demonstrated overall lowered mortality following TRT in KS (10).

Interpretation of the combined data collected for this study is challenging. Nondiagnosis of comorbidities could affect our estimates. Based on the frequency of blood sampling, the level of health surveillance seems highly dependent on KS life stage, with very little attention given to U-KS. The reason for this could, of course, be a less morbid phenotype among U-KS, but we tend to believe that the low number of blood samples in U-KS reflects a true deficit of medical attention. Poor socioeconomic status could further hinder active self-care and attention to medical issues in U-KS (1, 4). We have no exact knowledge of the factors leading to the diagnosis of KS or subsequent adherence to monitoring and treatment of hypogonadism with TRT. Our data indicate that for some men with KS, the threshold for seeking medical attention and ultimately being diagnosed with KS is quite high, resulting in higher incidences of the more severe phenotypes in U-KS. We further believe that this lack of attention to health among U-KS explains the counterintuitive finding of higher rates of hypercholesterolemia and hypertension in T-KS compared with other KS groups. We suspect the higher rates of these conditions among T-KS reflect a positive effect of increased surveillance. This is also supported by the actual reduction in LDL observed with TRT. Lower detection rates in U-KS could even cause our estimates to underestimate a true beneficial effect of TRT in KS on the incidence of metabolic disease. A similar pattern is observed among D-KS, and our findings further highlight the challenges related to the care of KS males, with some U-KS being subjected to bariatric surgery before diagnosis of KS and several D-KS never being offered TRT despite presenting with low adult testosterone levels. We believe, as healthcare is easily accessible in Denmark, that the challenge of procuring sufficient treatment could even be more pronounced in other parts of the world. We speculate that those not receiving treatment are being cared for in nonspecialized settings, such as by primary care physicians, stressing the need for an international guideline on care for KS males (32).

The overall quality of Danish health registries is high (13), but diagnoses directly indicating obesity are insufficiently assigned. In the UK Biobank study, the mean body mass index (BMI) was 29.7 ± 5.7 kg/m^2 among mostly undiagnosed KS (4), indicating that obesity is likely present in as many as half of all men with KS. National data from 2023 have demonstrated that 53% of Danes have a BMI > 25 (33), contrary to our finding of a diagnosis of obesity in only 2% of the background controls. Additionally, there are no readily available sources for obtaining actual measures of weight, BMI, or blood pressure, or to assess nonpharmacological interventions against obesity in the primary care sector. As such, our study most likely underestimates the true prevalence of obesity and hypertension across all participants. Lastly, because obesity develops over time, some individuals diagnosed with obesity at a given life stage would likely also have had obesity at an earlier stage.

This study comprised a large, nationwide cohort of males with KS with a long follow-up duration. There will likely never be a randomized controlled trial for evaluating the health effects of KS life stages with a similar number of participants and follow-up, making observational data the best possible research scenario. Studies based on observational data have traditionally suffered from the inability to assess causal inference. However, emerging statistical approaches allow for the evaluation of causality from observational studies under specific conditions (34). The current study applies a risk set matched design and uses propensity score-based inverse probability of treatment weighting to approach an evaluation of the true causal effect of progressing through KS life stages, in many ways mimicking steps applied for modern epidemiological methods, such as Target Trial Emulation (35). However, our study could still be subject to an uneven distribution of unmeasured confounders, and as discussed above, differences in surveillance would likely represent such an issue. We cannot exclude the possibility that other unknown factors influencing TRT might independently affect the risk of the evaluated outcomes. The risk set sampling procedure was implemented to mitigate some of this potential bias, although the direction of any remaining bias remains unpredictable. Consequently, we cannot conclude on causality based on the present study alone. There is a dire need for similar studies coming from other settings around the world.

In conclusion, we demonstrate an increased incidence and earlier onset of obesity and obesity-related disorders in KS irrespective of life stage and TRT, with a persistent 2- to 3-fold increased risk of T2DM. U-KS presented with a more severe metabolic profile, underlining the need for earlier diagnosis to prevent the long-term effects of hypogonadism. Furthermore, T-KS with T2DM had reduced mortality compared with D-KS with T2DM, which underlines the continued importance of ensuring optimal treatment of hypogonadism in KS, which again can only be achieved by ensuring early diagnosis. Also, the choice of TRT formulation could impact some aspects of metabolism among men with KS, but we cannot recommend either parenteral or transdermal formulations based on the current study.

Acknowledgments

The authors would like to thank retired associate professor Mogens Erlandsen for providing statistical guidance.

Funding

This work was supported by unrestricted research grants from The A.P. Moller Foundation, Fonden af 17-12-1981, the Danish Diabetes and Endocrine Academy which is funded by the Novo Nordisk Foundation, grant number NNF22SA 0079901, the Novo Nordisk Foundation (NNF15OC001 6474, NNF20OC0060610); the Independent Research Fund Denmark (2096-00165A); the Sygesikringen Danmark (2022-0189).

Author Contributions

S.C. conceptualized the study, provided funding, collected and curated data, performed the analysis, and drafted the manuscript. L.P. conceptualized the study, supervised the analysis, and reviewed the manuscript. A.S. collected and curated data and reviewed the manuscript. A.B. collected and curated data and reviewed the manuscript. C.H.G. conceptualized the study, provided funding and supervision, and reviewed the manuscript.

All authors approved the final version of the manuscript.

Disclosures

In addition to the sources of funding mentioned directly supporting the current work, within the past 36 months, A.B. has received an unrestricted grant from the fund "Bedre vilkår til Forskning," Aarhus University Hospital, A.S. has received unrestricted grants from the Novo Nordisk Foundation (NNF23OC0082718) and the Independent Research Fund

Denmark (0134-00130B), and C.G. has received honoraria for lectures by Novo Nordisk, Merck, and Sandoz. None of these sources of separate funding, which are unrelated to the current manuscript, has played any role for the current manuscript.

Data Availability

Public registry data used for this study is available by request to the Danish Health Data Authority.

References

1. Gravholt CH, Chang S, Wallentin M, Fedder J, Moore P, Skakkebaek A. Klinefelter syndrome—integrating genetics, neuropsychology and endocrinology. *Endocr Rev*. 2018;39(4):389-423.
2. Chang S, Christiansen CF, Bojesen A, Juul S, Munster AB, Gravholt CH. Klinefelter syndrome and testosterone treatment: a national cohort study on thrombosis risk. *Endocr Connect*. 2020;9(1):34-43.
3. Berglund A, Viuff MH, Skakkebaek A, Chang S, Stochholm K, Gravholt CH. Changes in the cohort composition of turner syndrome and severe non-diagnosis of Klinefelter, 47,XXX and 47, XYY syndrome: a nationwide cohort study. *Orphanet J Rare Dis*. 2019;14(1):16.
4. Zhao Y, Gardner EJ, Tuke MA, *et al*. Detection and characterization of male sex chromosome abnormalities in the UK Biobank study. *Genet Med*. 2022;24(9):1909-1919.
5. Davis SM, Teerlink C, Lynch JA, *et al*. Prevalence, morbidity, and mortality of men with sex chromosome aneuploidy in the million veteran program cohort. *JAMA Netw Open*. 2024;7(3):e244113.
6. Chang S, Just J, Skakkebaek A, *et al*. Testosterone replacement therapy in klinefelter syndrome-follow-up study associating hemostasis and RNA expression. *J Clin Endocrinol Metab*. 2024;109(4):978-991.
7. Host C, Bojesen A, Erlandsen M, *et al*. A placebo-controlled randomized study with testosterone in Klinefelter syndrome: beneficial effects on body composition. *Endocr Connect*. 2019;8(9):1250-1261.
8. Chang S, Skakkeaek A, Trolle C, *et al*. Anthropometry in klinefelter syndrome—multifactorial influences due to CAG length, testosterone treatment and possibly intrauterine hypogonadism. *J Clin Endocrinol Metab*. 2015;100(3):E508-E517.
9. Bojesen A, Kristensen K, Birkebaek NH, *et al*. The metabolic syndrome is frequent in Klinefelter's syndrome and is associated with abdominal obesity and hypogonadism. *Diabetes care*. 2006;29(7):1591-1598.
10. Chang S, Pedersen L, Skakkebæk A, Berglund A, Gravholt CH. Cardiovascular risk and mortality in men receiving testosterone replacement therapy for Klinefelter syndrome in Denmark: a retrospective cohort study. *The Lancet Regional Health—Europe*. 2025;51:101230.
11. Bhasin S, Brito JP, Cunningham GR, *et al*. Testosterone therapy in men with hypogonadism: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab*. 2018;103(5):1715-1744.
12. Lincoff AM, Bhasin S, Flevaris P, *et al*. Cardiovascular safety of testosterone-replacement therapy. *N Engl J Med*. 2023;389(2):107-117.
13. Schmidt M, Schmidt SA, Sandegaard JL, Ehrenstein V, Pedersen L, Sorensen HT. The Danish national patient registry: a review of content, data quality, and research potential. *Clin Epidemiol*. 2015;7:449-490.
14. Kildemoes HW, Sorensen HT, Hallas J. The Danish national prescription registry. *Scand J Public Health*. 2011;39(7_suppl):38-41.
15. Videbech P, Nielsen J. Electronic data processing in the Danish cytogenetic central register and EDP problems of registers in general. *Clin Genet*. 1979;15(2):137-146.
16. Methodology WCCfDS. WHO ATC/DDD index. 2025. https://www.whocc.no/
17. Heide-Jørgensen U, Adelborg K, Kahlert J, Sørensen HT, Pedersen L. Sampling strategies for selecting general population comparison cohorts. *Clin Epidemiol*. 2018;10:1325-1337.
18. Chang S. Supplementum for "Metabolism and type 2 diabetes across life stages in Klinefelter syndrome: a population-based cohort study". *OSF*. 2025. https://doi.org/10.17605/OSF.IO/SHR8K
19. Varbo A, Freiberg JJ, Nordestgaard BG. Extreme nonfasting remnant cholesterol vs extreme LDL cholesterol as contributors to cardiovascular disease and all-cause mortality in 90000 individuals from the general population. *Clin Chem*. 2015;61(3):533-543.
20. Wicke FS, Glushan A, Schubert I, *et al*. Performance of the adapted diabetes complications severity Index translated to ICD-10. *Am J Manag Care*. 2019;25(2):e45-e49.
21. Glasheen WP, Renda A, Dong Y. Diabetes complications severity Index (DCSI)-update and ICD-10 translation. *J Diabetes Complications*. 2017;31(6):1007-1013.
22. Hu YW, Yeh CM, Liu CJ, Chen TJ, Huang N, Chou YJ. Adapted diabetes complications severity index and charlson comorbidity Index in predicting all-cause and cause-specific mortality among patients with type 2 diabetes. *BMJ Open Diabetes Res Care*. 2023;11(2):e003262.
23. Østergaard L, Mogensen UM, Bundgaard JS, *et al*. Duration and complications of diabetes mellitus and the associated risk of infective endocarditis. *Int J Cardiol*. 2019;278:280-284.
24. Barker MM, Davies MJ, Sargeant JA, *et al*. Age at type 2 diabetes diagnosis and cause-specific mortality: observational study of primary care patients in England. *Diabetes care*. 2023;46(11):1965-1972.
25. Wittert G, Bracken K, Robledo KP, *et al*. Testosterone treatment to prevent or revert type 2 diabetes in men enrolled in a lifestyle programme (T4DM): a randomised, double-blind, placebo-controlled, 2-year, phase 3b trial. *Lancet Diabetes Endocrinol*. 2021;9(1):32-45.
26. Tishova Y, Kalinchenko S, Mskhalaya G, *et al*. Testosterone therapy reduces insulin resistance in men with adult-onset testosterone deficiency and metabolic syndrome. Results from the Moscow study, a randomized controlled trial with an open-label phase. *Diabetes Obes Metab*. 2024;26(6):2147-2157.
27. Snyder PJ, Bhasin S, Cunningham GR, *et al*. Effects of testosterone treatment in older men. *N Engl J Med*. 2016;374(7):611-624.
28. Mohler ER, 3rd, Ellenberg SS, Lewis CE, *et al*. The effect of testosterone on cardiovascular biomarkers in the testosterone trials. *J Clin Endocrinol Metab*. 2018;103(2):681-688.
29. Bhasin S, Lincoff AM, Nissen SE, *et al*. Effect of testosterone on progression from prediabetes to diabetes in men with hypogonadism: a substudy of the TRAVERSE randomized clinical trial. *JAMA Intern Med*. 2024;184(4):353-362.
30. Ornstrup MJ, Høst C, Rittig N, Gravholt CH. Acute effects of testosterone on whole body protein metabolism in hypogonadal and eugonadal conditions: a randomized, placebo-controlled, crossover study. *J Appl Physiol (1985)*. 2024;136(6):1460-1467.
31. Dong H, Sun Y, Nie L, *et al*. Metabolic memory: mechanisms and diseases. *Signal Transduct Target Ther*. 2024;9(1):38.
32. Gravholt CH, Ferlin A, Gromoll J, *et al*. New developments and future trajectories in supernumerary sex chromosome abnormalities: a summary of the 2022 3rd international workshop on Klinefelter syndrome, trisomy X, and XYY. *Endocr Connect*. 2023;12(3):e220500.
33. Den Nationale Sundhedsprofl. *Midtvejsundersøgelsen 2023—centrale Udfordringer*. The Danish Health Authority; 2024.
34. Hernán MA, Wang W, Leaf DE. Target trial emulation: a framework for causal inference from observational data. *JAMA*. 2022;328(24):2446-2447.
35. Hernán MA, Robins JM. Using big data to emulate a target trial when a randomized trial is not available. *Am J Epidemiol*. 2016;183(8):758-764.