Metabolic Effects of Estradiol Versus Testosterone in Complete Androgen Insensitivity Syndrome

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Abstract

Purpose

To study differences in metabolic outcomes between testosterone and estradiol replacement in patients with complete androgen insensitivity syndrome (CAIS).

Methods

In this multicentre, double-blind, randomized crossover trial, 26 women with CAIS were included of whom 17 completed the study. After a two-months run in phase with estradiol, patients either received transdermal estradiol followed by crossover to transdermal testosterone or vice versa. After six months, differences in lipids, fasting glucose, insulin, hematocrit, liver parameters and blood pressure between the treatment phases were investigated.

Results

Linear mixed models adjusted for period and sequence did not reveal major group differences according to treatment for the investigated outcomes. In each treatment group, there were however significant uniform changes in BMI and cholesterol. BMI increased significantly, following six months of estradiol (+2.7%; p = 0.036) as well as testosterone treatment (+2.8%; p = 0.036). There was also a significant increase in total (+10.4%; p = 0.001) and LDL-cholesterol (+29.2%; p = 0.049) and a decrease in HDL-cholesterol (-15.8%; p < 0.001) following six months of estradiol as well as six months of testosterone treatment (total cholesterol: +14.6%; p = 0.008; LDL-cholesterol: +39.1%; p = 0.005, HDL-cholesterol: -15.8%; p = 0.004). Other parameters remained unchanged.

Conclusion

Transdermal estradiol as well as testosterone treatment in women with CAIS results in worsening in lipid profiles. Given the relatively small sample size, subtle group differences in other metabolic parameters may have remained undetected.

Introduction

Complete androgen insensitivity syndrome (CAIS) is the most common 46, XY disorder of sexual development (DSD) with an estimated prevalence of 1 in 20 000–90 000 births or 4·1:100 000 girls [1]. It is characterized by complete loss of androgen receptor functioning due to X-linked recessive mutations within the androgen receptor (AR) gene [2] and subsequent development of a complete external female phenotype. Subjects lack Müllerian duct structures and androgen-dependent body hair.

The endocrine profile after puberty is usually characterized by testosterone concentrations in the normal to upper male reference range, while estradiol concentrations are normal to slightly increased relative to normal male references originating primarily from testicular secretion and peripheral aromatization of

androstenedione and testosterone [3]. However, despite aromatization, estradiol concentrations are usually below the normal female reference range [4]. Due to the unknown risk for developing gonadal tumors, women with CAIS usually underwent early gonadectomy until recently [2] and were then depended on sex hormone replacement therapy. So far, the treatment of patients with CAIS after gonadectomy has basically followed the established concepts for the therapy of female hypogonadism. However, this results in the replacement of previously high endogenous androgen concentrations by estrogens [2].

Here we report the results of a secondary metabolic outcome analysis of the first multicentre, randomised, double-dummy, double-blind crossover trial investigating the effects of estradiol in comparison to testosterone replacement therapy in CAIS patients [5]. We could show that androgen replacement seems to be non-inferior to estradiol in terms of quality of life and does result in comparable levels of estrogens. In addition, we could demonstrate that testosterone treatment may have beneficial effects on sexual functioning in CAIS. Although it may seem unlikely that testosterone should exert any distinct effect from that of estradiol in these patients at first glance, hypothetical considerations have underscored the anecdotally reported improvements in general well-being following androgen replacement [6]. With regard to general wellbeing and sexual functioning it has to be kept in mind that testosterone is not only metabolized to estradiol but especially in terms of so-called neurosteroids [7] may also be converted to metabolites that exert their activity neither via estrogen - nor androgen receptors. Furthermore, differences in treatment effects might be plausible assuming that there is a difference between a systemic increase in estradiol in comparison to a local increase depending on the distribution of aromatase expression in the corresponding target tissue [8]. Hence, local estradiol concentrations can significantly differ independent of systemic levels.

Little is known about the metabolic features of CAIS women even though androgens are known to modulate a wide range of cardiometabolic parameters in both sexes [9]. There is evidence that e.g. lack of androgen activity in these patients may have a negative effect on bone mineral density, due to the distinct effects of testosterone and estradiol on bone metabolism [10]. However, most effects on bone by testosterone seem to be primarily mediated by local conversion into estradiol via aromatase enzyme activity [11].

A small study indicated a less favorable cardiometabolic profile in CAIS women in comparison to control women (with gonadal dysgenesis), including lower fat free mass and elevated total and LDL-cholesterol as well as an increase in insulin resistance [12].

We hypothesized that there would be no significant differences in terms of metabolic parameters between CAIS patients receiving testosterone versus estradiol replacement therapy namely, BMI, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglyceride and fasting glucose and insulin-levels.

Subjects And Methods

Study design and participants

The complete study design has been reported elsewhere [5]. In summary the study has been performed at three university medical centers and three specialized treatment institutions in Germany (Lübeck, Berlin, Regensburg, Tübingen, Bochum [Dortmund], and Munich) between November 2011 and January 2016. Diagnosis of CAIS was confirmed by molecular genetic analysis of the AR gene. Gonadectomy had to date back more than 1 year before inclusion in the study.

Exclusion criteria were:

- Disorder of Sex Development other than complete androgen insensitivity syndrome
- Steroid medication other than study trial medication
- Gonads in situ
- Disorder of liver function
- Chronic skin disease
- Serious chronic disorders affected by sex steroid medication
- Malignant disorders
- Severe psychiatric disorders

26 women with CAIS aged 18–54 years were included. Secondary analyses included the per-protocol population. Ten patients left the study before completion. Eight left during the run-in, respectively treatment phase. One patient did not attend visit six. As per protocol, data from visit five was used in this case. One patient did not attend the final follow-up visit and was included in the final analysis as well. Two patients were incompliant and had to be excluded. Finally, 12 patients in sequence A and six patients in sequence B were included in our analysis (Figure 1). There were neither differences in demographic, anthropometric nor in medical variables between subjects who completed the study to those who did not (https://doi.org/10.6084/m9.figshare.16944979.v).

Treatment

All participants received standard estradiol 1.5 mg/day during a 2-month run-in phase to accomplish a homogeneous hormonal milieu, as from the initial cohort five patients were not receiving any sex hormone treatment at study inclusion and in one patient it was unclear, if hormone replacement in the preceding month had taken place. The final cohort included four patients without recent hormone replacement. This approach instead of a wash-out period was chosen to avoid leaving the participants without any hormonal replacement, resulting in a completely unphysiological state at the start of the trial.

Participants were randomly assigned (14:12) to receive estradiol (Gynokadin©; Dr Kade Pharmaceuticals, Berlin, Germany) 1.5 mg/day (= 2.5g gel) and a testosterone dummy for 6 months followed by crossover to testosterone (Testogel©; BESINS Healthcare SA, Brussels, Belgium) 50 mg/day (= 5g gel) and estradiol dummy for 6 months (sequence A) or to receive testosterone 50 mg/day and estradiol dummy for 6 months followed by crossover to estradiol 1.5 mg/day and testosterone dummy for 6 months (sequence B; figure 2). The crossover of active component after 6 months was done in a double-blinded manner. The dummy for each study drug was provided by BESINS and Dr Kade Pharmaceuticals. Bioavailability for the testosterone gel is estimated to lie between 9 and 15% [13] and for the estradiol gel around 6%[14].

Hormonal analysis

Testosterone and estradiol were measured by Liquid-chromatography - tandem mass spectrometry LC-MS/MS at the Division of Pediatric Endocrinology and Diabetes, Department of Pediatrics, University Hospital of Schleswig – Holstein, Campus Kiel, Christian-Albrechts University of Kiel with the facilities provided by a previous BMBF-funding [15, 16]. Luteinizing hormone (LH) and follicle stimulating hormone (FSH) were measured in Lübeck by the Roche Elecsys©. The reproducibility for testosterone was lower than 7.1 %, reported in table 2 [15]. For estradiol it was lower than 5% [17]. Four times a year a round robin test was performed for both hormones at the Reference Institute for Bioanalysis.

Laboratory measurements

Cholesterol [total, HDL, LDL], triglycerides, insulin, as well as safety parameters γGT (gamma-glutamyltransferase), GOT (glutamic oxaloacetic transaminase), GPT (alanine transaminase), AP (alcalic phosphatase) were determined centrally in the laboratories of the University-Hospital Schleswig-Holstein, Campus Lübeck. Haematocrit and haemoglobin were analysed locally at the study sites due to instability of the samples. Due to sampling processing issues at one centre 4-5 samples had to be excluded from analysis of AP, fasting glucose and insulin levels. Pathological levels for cholesterol were defined as follows: total cholesterol > 4.9 mmol/l; LDL-cholesterol > 3.0 mmol/l; HDL-cholesterol < 1.2 mmol/l.

Statistical analysis

To compensate for unbalanced dropout between the treatment groups, differences between the two treatments for the secondary endpoints were tested in a mixed linear model analysis for crossover designs, with fixed effects for treatment (estradiol vs testosterone), period (first vs second treatment phase), and sequence (estradiol to testosterone vs testosterone to estradiol) and with a random patient effect. The analysis was based on data at the end of the two treatment phases (visits 4 and 6, respectively). Laboratory parameters were log-transformed to approximate a Gaussian distribution if necessary. In a further exploratory secondary analysis, the Wilcoxon paired difference test was used to compare data from estradiol or testosterone treatments (independent of sequence or period) against the respective baseline values and to compare data from estradiol or testosterone treatments. To exclude the possibility that changes in the laboratory parameters were only due to changes in the BMI, corresponding

correlation analyses and analyses of covariance were performed considering the difference values of the BMI. IBM SPSS Statistics version 24 was used for statistical analyses. A p value less than 0·05 was used to indicate statistical significance.

Results

Results of hormone measures have been reported before [5]. In brief, after run-in treatment with estradiol, median estradiol concentrations were 170pmol/l and this within the lower reference range for women and remained stable during estradiol treatment. Median estradiol concentrations during testosterone treatment phase were 100pmol/l. Median testosterone concentrations during the run-in phase (0.63 nmol/l) and during estradiol treatment (90pmol/l) were in the lower range for adult women. The median testosterone concentration during testosterone treatment (15.6nmol/l) was within the range for young adult men.

The concentrations of LH and FSH hormone were high before treatment (After run-in: LH 33.9IU/l; FSH 55.7IU/l) and remained high after treatment. No significant difference was found in gonadotrophin concentrations between treatment sequences. Changes according to sequence are presented in figure 3. There was no significant difference at baseline regarding metabolic outcome variables as depicted in Table 1.

Table 1
Baseline characteristics of the whole study sample at baseline

	N	Mean	SD	p*
Age (years)	26	32.7	9.5	0.133
Age at gonadectomy (years)	26	17.2	8.1	0.153
Weight (kg)	26	74.7	12.4	0.950
BMI (kg/m²)	26	25.1	4.0	0.860
Systolic blood pressure (mmHg)	26	120.7	14.4	0.950
Diastolic blood pressure (mmHg)	26	76.1	10.3	0.568
Total cholesterol (mmol/l)	23	4.9	1.0	0.255
HDL-cholesterol (mmol/l)	24	1.8	0.5	0.395
LDL-cholesterol (mmol/l)	24	2.5	1.1	0.989
Triglycerides (mmol/l)	24	1.0	0.5	1.000
Glucose (mmol/l)	24	5.0	1.5	0.635
Insulin (mlU/l)	23	11.6	9.4	0.335
HOMA-IR	23	2.7	2.6	0.228
Hemoglobin (g/dl)	25	13.7	0.7	0.187
Hematocrit (%)	25	40.5	2.1	0.086
GOT (U/l)	24	20.8	6.0	0.897
GPT (U/l)	24	15.4	6.9	0.157
GGT (U/l)	24	14.1	5.8	0.479
Alkaline phosphatase (U/L)	24	62.4	14.9	0.720
LH (IU/l)	25	34.0	15.8	0.979
FSH (IU/l)	25	62.5	31.0	0.687
SHBG (pmol/ml)	25	100.7	50.4	0.434
Estradiol Kiel (pmol/l)	24	89.4	119.9	0.277
Testosteron (Kiel) (nmol/l)	24	0.6	0.4	0.910
** Differences between groups stratified by sequence (Mann-Whitney-Test)*				

With the exception of AP-levels, which were significantly lower in the estradiol (60U/l; 95%CI 52.9-67.2) than in the testosterone group (64.4U/l; 95%CI 57.4-71.4; p = 0.046) no significant differences were found in the effect of estradiol and testosterone on any other of the investigated parameters in the linear mixed model (Table 2). There were no significant changes in the investigate parameters during the 2 months run-in phase (data not shown).

Table 2
Treatment effect, data derived from linear mixed model analysis

	Treatment	Mean	95%-CI		p-value	p-value period	p-value sequence
			Lower	**Upper**			
Weight (kg)	Oestradiol	74.78	66.73	82.38	0.126	0.354	0.421
	Testosterone	75.72	67.68	83.76			
	Difference	-0.94	-2.15	0.27			
BMI (kg/m²)	Oestradiol	24.66	22.39	26.93	0.132	0.330	0.435
	Testosterone	24.95	22.69	27.21			
	Difference	-0.29	-0.67	0.09			
Systolic blood pressure (mmHg)	Oestradiol	120.15	114.45	125.86	0.442	0.847	0.210
	Testosterone	121.96	116.46	127.46			
	Difference	-1.81	-6.49	2.87			
Diastolic blood pressure (mmHg)	Oestradiol	75.48	72.01	78.96	0.724	0.754	**0.012**
	Testosterone	74.91	71.59	78.24			
	Difference	0.57	-2.63	3.76			
Total cholesterol* (mmol/l)	Oestradiol	5.33	4.89	5.77	0.260	**0.006**	0.423
	Testosterone	5.50	5.09	5.92			
	Difference	-0.17	-0.49	0.14			
HDL-cholesterol* (mmol/l)	Oestradiol	1.68	1.47	1.89	0.915	**0.001**	0.093
	Testosterone	1.71	1.51	1.90			
	Difference	-0.03	-0.19	0.12			
LDL-cholesterol* (mmol/l)	Oestradiol	3.01	2.44	3.58	0.221	**0.001**	0.936
	Testosterone	3.13	2.58	3.68			
	Difference	-0.12	-0.48	0.23			
Triglycerides* (mmol/l)	Oestradiol	0.77	0.62	0.92	0.638	0.730	0.532
	Testosterone	0.78	0.63	0.92			
	Difference	-0.004	-0.11	0.10			

**log transformed*

Glucose (mmol/l)	Oestradiol	4.86	4.28	5.45	0.434	0.702	0.313
	Testosterone	5.02	4.45	5.58			
	Difference	-0.15	-0.54	0.24			
Insulin (mlU/l)	Oestradiol	5.64	3.73	7.55	0.416	0.828	0.731
	Testosterone	6.19	4.35	8.04			
	Difference	-0.55	-1.89	0.80			
HOMA-IR	Oestradiol	1.75	0.50	2.99	0.578	0.448	0.310
	Testosterone	1.35	0.18	2.52			
	Difference	0.40	0.32	0.47			
Hematocrit* (%)	Oestradiol	39.92	38.64	41.19	0.538	0.263	0.464
	Testosterone	40.13	38.89	41.38			
	Difference	-0.21	-0.96	0.53			
Hemoglobin* (g/dl)	Oestradiol	13.65	13.21	14.10	0.752	0.214	0.332
	Testosterone	13.61	13.18	14.04			
	Difference	0.05	-0.24	0.33			
GOT* (U/l)	Oestradiol	21.56	19.07	24.04	0.144	0.543	0.143
	Testosterone	23.33	21.06	25.60			
	Difference	-1.77	-4.21	0.66			
GPT* (U/l)	Oestradiol	17.17	14.24	20.11	0.858	0.889	0.224
	Testosterone	17.12	14.44	19.82			
	Difference	0.05	-2.74	2.84			
GGT* (U/l)	Oestradiol	15.01	12.04	17.97	0.641	0.263	0.695
	Testosterone	15.12	12.30	17.94			
	Difference	-0.11	-2.19	1.96			
AP* (U/l)	Oestradiol	60.04	52.88	67.20	**0.046**	0.999	0.666
	Testosterone	64.36	57.36	71.36			
	Difference	-4.33	-8.96	0.31			
**log transformed*							

There was a significant increase in BMI following six months of estradiol (+2.7%, z = 2.107; p = 0.036) as well as testosterone treatment (+2.8%, z = -2.101; p = 0.036) in comparison to visit 2 after the run-in phase. There was also a significant increase in total (+10.4%, z = -3.409; p = 0.001) and LDL-cholesterol (+29.2%, z = 3.510; p < 0.001) and a decrease in HDL-cholesterol (-15.8%, z = -1.965; p < 0.049) during six months of treatment with estradiol (Table 3). A similar pattern was seen following the testosterone sequence (total cholesterol: +14.6%, z = -2.636; p = 0.008; LDL-cholesterol: +39.1%, z = -2.832; p = 0.005, HDL-cholesterol: -15.8%, z = -2.912; p = 0.004) (Table 4). There was no correlation between the changes in lipid levels and those in the BMI and no effect of BMI in the ANCOVA, suggesting that the differences in lipid levels were treatment specific (data not shown).

Table 3
Treatment with Oestradiol

	Visit 2			**Visit 4/6**				
	N	**Mean**	**SD**	**N**	**Mean**	**SD**	**%**	**p***
Weight (kg)	17	74.4	15.7	17	76.5	18.4	2.9	**0.035**
BMI (kg/m²)	17	24.6	4.4	17	25.3	5.1	2.7	**0.036**
Systolic blood pressure (mmHg)	17	122.9	14.0	18	120.3	13.4		0.381
Diastolic blood pressure (mmHg)	16	74.8	9.9	17	76.8	8.6		0.569
Total cholesterol (mmol/l)	17	4.8	0.9	17	5.3	1.0	11.4	**0.001**
HDL-Cholesterol (mmol/l)	17	1.9	0.4	17	1.6	0.5	-13.0	**0.049**
LDL-Cholesterol (mmol/l)	17	2.4	0.9	17	3.1	1.2	28.1	**<0.001**
Triglycerides (mmol/l)	17	0.7	0.3	17	0.9	0.4		0.184
Glucose (mmol/l)	14	5.1	0.7	14	5.0	1.0		0.900
Insulin (mlU/l)	12	4.4	3.2	12	5.7	4.3		0.477
HOMA-IR	12	2.2	3.5	12	2.5	5.4		0.804
Hemoglobin (g/dl)	14	13.7	0.9	15	13.6	1.0		0.916
Hematocrit (%)	13	40.2	2.7	14	39.8	3.2		0.686
GOT (U/l)	17	23.2	7.9	17	21.9	4.4		0.798
GPT (U/l)	17	16.9	5.4	17	17.2	7.5		0.887
GGT (U/l)	17	14.6	4.5	17	16.3	9.2		0.328
Alkaline phosphatase (U/L)	13	63.6	15.7	14	62.7	18.0		0.780
** Between visit 2 and 4/6*								

Table 4
Treatment with Testosterone

	Visit 2			Visit 4/6				
	N	**Mean**	**SD**	**N**	**Mean**	**SD**	**%**	**p***
Weight (kg)	18	73.9	15.4	18	75.8	17.0	2.6	0.056
BMI (kg/m²)	18	24.4	4.4	18	25.0	4.7	2.4	**0.036**
Systolic blood pressure (mmHg)	17	122.9	14.0	18	120.3	13.4		0.381
Diastolic blood pressure (mmHg)	16	74.9	10.0	17	76.9	9.3		0.477
Total cholesterol (mmol/l)	18	4.8	0.9	18	5.5	1.0	14.9	**0.008**
HDL-Cholesterol (mmol/l)	17	1.9	0.4	17	1.6	0.6	-16.2	**0.004**
LDL-Cholesterol (mmol/l)	18	2.3	0.9	18	3.2	1.4	38.7	**0.005**
Triglycerides (mmol/l)	18	0.7	0.3	18	0.8	0.4	15.4	0.459
Glucose (mmol/l)	14	5.1	0.7	14	5.4	0.9		0.173
Insulin (mlU/l)	13	5.4	4.7	14	6.2	4.8		0.184
HOMA-IR	13	1.2	1.2	14	1.5	1.3		0.151
Hemoglobin (g/dl)	16	13.8	1.0	17	13.8	0.8		0.864
Hematocrit (%)	16	40.8	2.6	17	40.6	2.4		0.894
GOT (U/l)	18	23.0	7.7	18	24.2	7.5		0.538
GPT (U/l)	18	16.6	5.4	18	16.8	5.8		0.931
GGT (U/l)	18	14.7	4.4	18	15.0	5.9		0.593
Alkaline phosphatase (U/L)	14	62.5	15.3	63.5	45.0	106.0		0.084
** Between visit 2 and 4/6*								

At visit two, nine patients (52.9%) in the estradiol arm had pathologically elevated total cholesterol levels. Three patients with initially normal cholesterol levels developed pathological levels following six months of E2 treatment. Before randomization, all patients had HDL-cholesterol levels within the normal range of whom four developed pathologically low levels during treatment. Regarding LDL-cholesterol, from twelve patients (70.6%) with initially normal levels at visit two, five developed pathological levels during treatment.

In the testosterone arm, nine patients (50%) had normal cholesterol levels, while the other half had pathologically elevated cholesterol levels. Among these, following six months of T treatment, four developed elevated levels. Total cholesterol normalized in one patient with initially elevated levels. HDL-

cholesterol levels were normal in all patients at visit two. Among these, in five decreased levels into the pathological range during treatment. LDL-cholesterol was normal in 13 patients and elevated in five patients. In four patients, LDL-cholesterol levels rose into the pathological range while in one patient LDL-cholesterol normalized in the course of treatment.

There were no differences in blood pressure, hemoglobin/hematocrit, triglycerides, liver parameters or insulin/glucose following any treatment sequence. There was no significant change in either parameter between visit 1 and 2 (= run-in-phase, data not shown).

Discussion

This is the first study investigating two different treatment options in CAIS individuals with regard to metabolic effects in a randomised controlled fashion. Both treatments resulted in a less favorable lipid profile, as there was a significant increase in total and LDL-cholesterol and a significant decrease in HDL-cholesterol. In addition, there was a slight but significant increase in BMI in both groups. Changes in cholesterol levels were however independent of changes in weight. Although the observed changes may be due to a simple time effect this is unlikely in view of the relatively short time period of six months in each treatment phase. Changes might be clinically significant as in almost half of patients with initially normal LDL-cholesterol levels in both treatment arms, levels rose into the pathological range. There were no significant differences between both treatments in terms of metabolic and safety parameters. Although the study might be underpowered regarding the detection of more subtle changes in the investigated outcomes, the results do not indicate that there are major differences between both treatments.

Despite the fact that there was no significant change in lipid parameters in our study within the 2-months run-in phase, it might have played a role that not all patients had received hormonal treatment in the last months before inclusion in the study and also in those who did, steroid exposure might have been different from that provided in a controlled fashion during participation in the trial where medication use was well monitored.

As being evident by the sexually dimorphic fat distribution pattern emerging with onset of puberty [18], sex steroids and in particular estradiol have significant implications in fat and lipid metabolism and regulation [19]. Ovariectomy in rodent models as well as natural menopause in women results in increases in adipose tissue [20] preferably in the abdominal region. These changes can be reversed by estrogen replacement [21, 22]. Also, in men, aromatization of testosterone to estradiol seems to be crucial in terms of energy homeostasis and lipid distribution [23]. Men with aromatase deficiency [24] as well as estrogen receptor defects [25] e.g., present with features of the metabolic syndrome despite normal testosterone levels. In men with aromatase deficiency estradiol substitution results in a significant increase in HDL- and decrease in LDL- cholesterol [24].

That estradiol as well as testosterone treatment in our study resulted in worsening of lipid parameters was unexpected and resemble the effects seen in hyperandrogenism in women [26]. There is further

evidence that there might be a U-shaped relationship between androgen levels and cardiometabolic risk in women [27]. However, his is not a uniform finding [28] and it cannot conclusively explain the detrimental effects of both treatments in our study.

High concentrations of testosterone e.g., as seen in gender-affirming hormone treatment (GAHT) in transgender men (=male gender identity) result in a decrease in subcutaneous fat with unchanged visceral fat depots [9, 29]. In addition, GAHT usually results in an unfavorable lipid profile with a decrease in HDL- and an increase in LDL-cholesterol [9]. In contrast, aside from CAIS, a clear human model for isolated hypoandrogenism without estradiol deficiency is missing. We can therefore only speculate on the cause of the changes observed in our study.

Unfortunately, there is a paucity of studies on metabolic characteristic in women with CAIS in general and on the effects of hormone replacement in particular that could help us to classify our results. An unfavorable metabolic profile in women with CAIS receiving estrogen replacement was also documented in a small cross-sectional study by Dati and colleagues [12]. CAIS women presented with lower fat free mass and elevated total and LDL-cholesterol levels and increased insulin resistance in comparison to control women. While overall mean BMI was not significantly increased, prevalence of obesity was higher in women with CAIS than expected for the corresponding Italian reference population. The comparability with our study is however limited by the fact that patients in the aforementioned study received various regimens of estrogen replacement and 23% of patients included had not undergone gonadectomy and therefore preserved testosterone secretion.

In another study by Tsimaris and colleagues [30] the effects of six months sex hormone replacement on cardiometabolic parameters were investigated in 23 patients with 46,XY DSD. Hormone therapy was initiated either after gonadectomy, or after a 3-month wash-out period of prior HT. In contrast to our study, the authors could show that hormone replacement therapy resulted in a raise in HDL-cholesterol and a decrease in triglyceride levels, while there were no changes in total and LDL-cholesterol. It has however to be highlighted that this study also included eight patients with XY gonadal dysgenesis and results were not reported separately for the remaining CAIS patients. Furthermore, all patients received 1mg norethisterone acetate in addition to 2mg of 17b- estradiol orally. It is known that progestins can have independent effects on metabolism [31, 32] and similar effects should be expected for women with CAIS with an intact progesterone receptor (PR). But progestins usually result in a decrease in HDL-cholesterol in the general population.

In addition, it has been demonstrated that there is a difference in the effects of estrogens on lipid levels depending on the application route [33]. While in postmenopausal women, both oral and transdermal application have beneficial effects on the HDL/LDL-cholesterol-ratios, this effect is more pronounced when taken orally [33, 34], while transdermal application is considered to have more favorable effects on triglyceride levels [34].

Finally, as expected, there were no significant differences in any other investigated parameters, including hemoglobin levels. Hemoglobin production is highly sensitive to testosterone [35] and its effects are also

preserved on a female genetic background, as having been demonstrated by the fact that a few months of treatment in transmen are usually sufficient to increase its levels to those of the general male population [36]. That the effects of testosterone on hematopoiesis do not depend on aromatization [37] is in line with unchanged hemoglobin levels found in our study.

A strength of our study is that it is the first of its kind investigating two treatment options in a molecularly well-defined cohort of CAIS patients in a randomized controlled fashion. A limitation of our study is that the groups were rather small due to the relatively high drop-out rates and that power-analysis was not performed for secondary outcomes. This might have resulted in a bias. However, subjects who dropped out of the study did not differ in the investigate baseline characteristics in comparisons to those who successfully completed the study.

While the effects on cholesterol levels were quite strong, the study may be underpowered for the detection of more subtle group effects in other parameters. In addition, the subjects in our study were put on a fixed dosage of transdermal sex steroids. Although the corresponding dosage was selected on the basis of providing according to the manufacturer's average serum levels within the reference range of men and women from the general population, there is a high inter-individual variance due to differences in transdermal absorption. A dose titration scheme-based protocol might therefore have helped to average out these differences.

Nevertheless, given the rarity of the disease and the paucity of studies on metabolic effects of hormone treatment in CAIS in general, in our opinion, this study adds valuable information to the understanding of the action of sex steroids on metabolism in this unique condition.

In addition, anthropometric measures such as body composition and waist/hip circumference were not recorded, therefore we cannot determine if the observed changes in BMI were due to an increase in fat or lean mass or simple water retention.

Conclusion

In summary we could show both treatments seem to result in a worsened lipid profile while we did not detect major changes or group differences in other parameters. The exact mechanisms of this finding still must be determined. As we have shown before that testosterone might have beneficial effects in terms of sexual desire [5] in comparison to estradiol treatment in women with CAIS, its use may have additional value without any trade-off in terms of safety. As there is evidence that androgen resistance in women with CAIS has negative effect on bone mineral density [10] and androgens may have postive effects on bone in women [38], further studies addressing this so far unanswered question would be appreciated.

Declarations

Data Availability

The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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Competing Interests

The authors report no conflicts of interest in this work.

Author Contributions

WB, LM and OH planned the study and coordinated the study centers. WB, LM, KR, BK, AR-U, MKA, and OH were in charge of individual study centers, recruited patients, and did the study. RW did the molecular genetic studies. AK, P-MH, MFH, and SAW did the metabolic studies. AL and SK were in charge of statistical analysis. All authors contributed to the writing of the report.

Ethics approval

This study was done in accordance with the Declaration of Helsinki and Ethical approval was obtained from all participating study sites, with Lübeck's Ethics Committee being the leading institution (reference 11-066). This trial is registered with the German Clinical Trials Register, number DRKS00003136, and with the European Clinical Trials Database, number 2010-021790-37.

Consent to participate

All eligible participants gave written informed consent.

Consent to publish

The authors affirm that research participants provided informed consent for publication of their anonymized data for this study.

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Figures

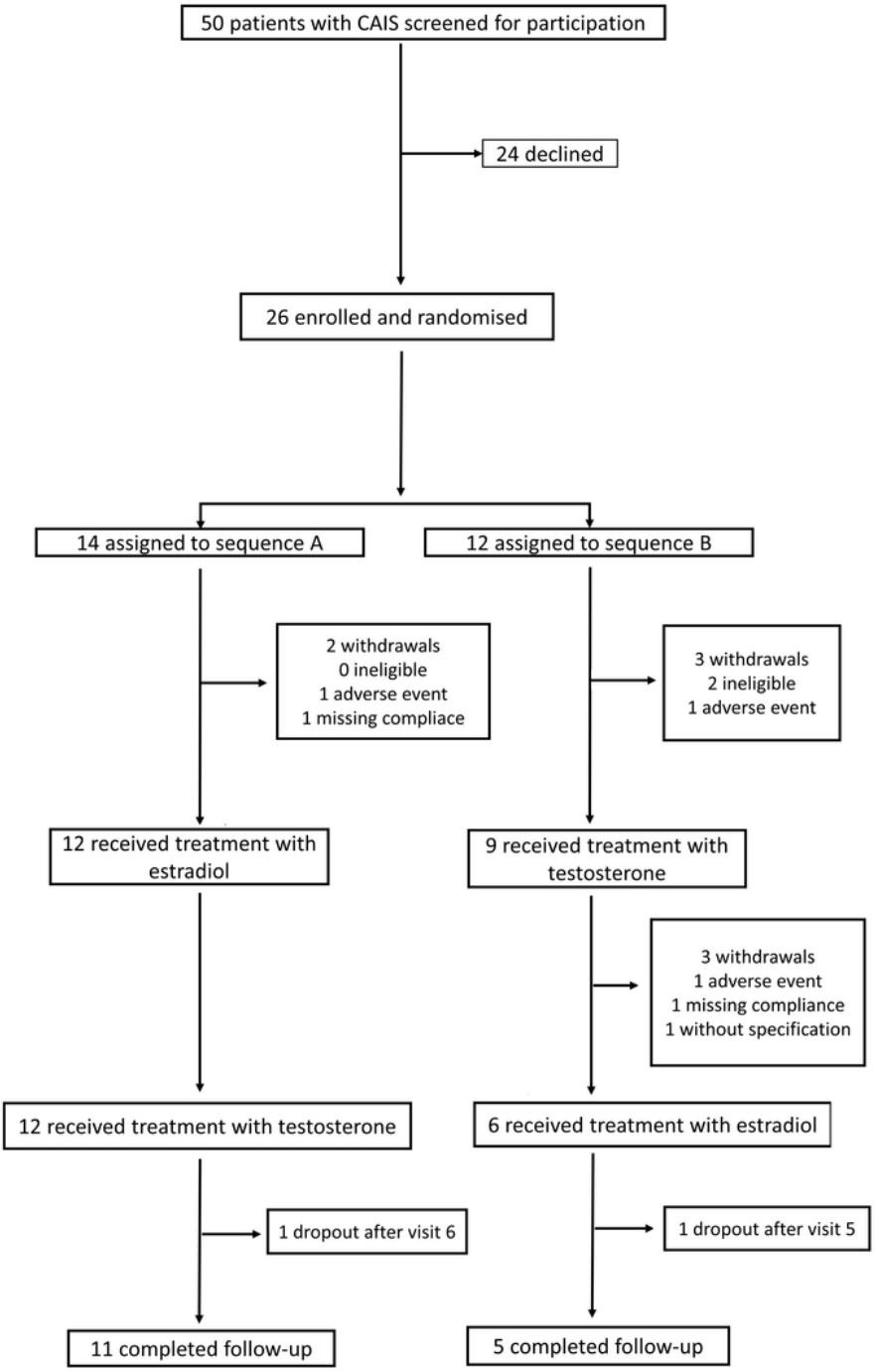

Figure 1

Trial profile

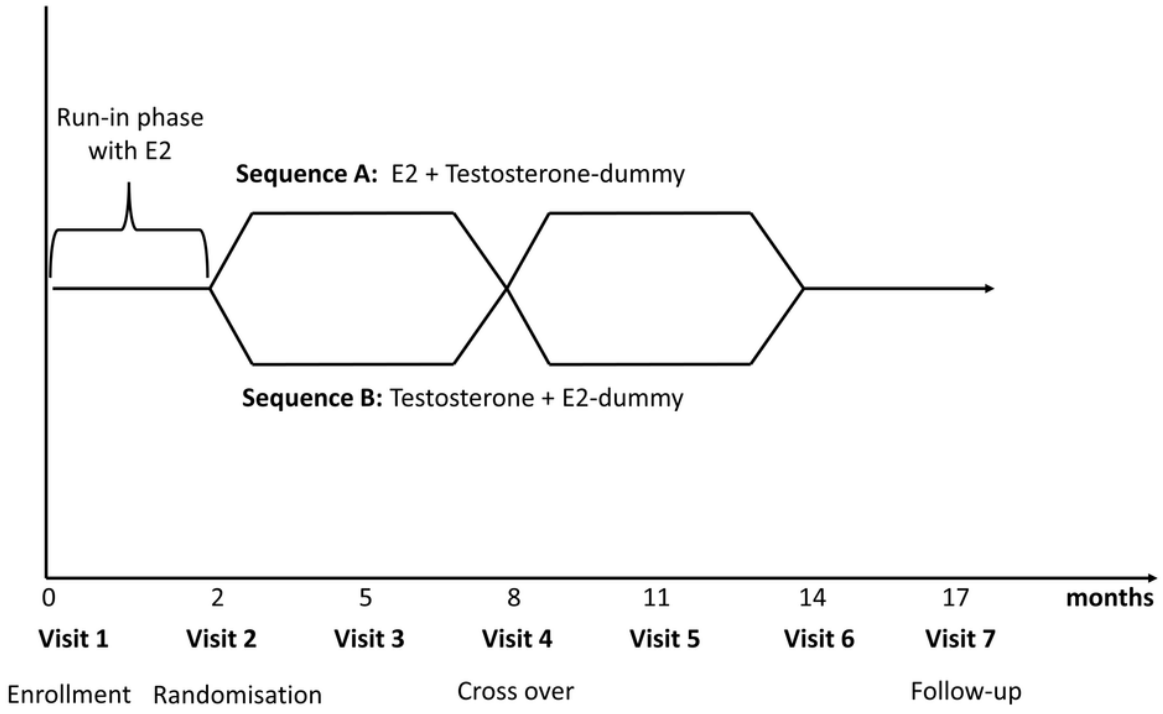

Figure 2

Study design

All participants received standard estradiol 1.5 mg/day during a 2-month run-in phase to accomplish a homogeneous hormonal milieu. Participants were randomly assigned (14:12) to receive estradiol 1.5 mg/day and a testosterone dummy for 6 months followed by crossover to testosterone 50 mg/day and estradiol dummy for 6 months (sequence A) or to receive testosterone 50 mg/day and estradiol dummy for 6 months followed by crossover to estradiol 1.5 mg/day and testosterone dummy for 6 months (sequence B). The crossover of active component after 6 months was done in a double-blind fashion.

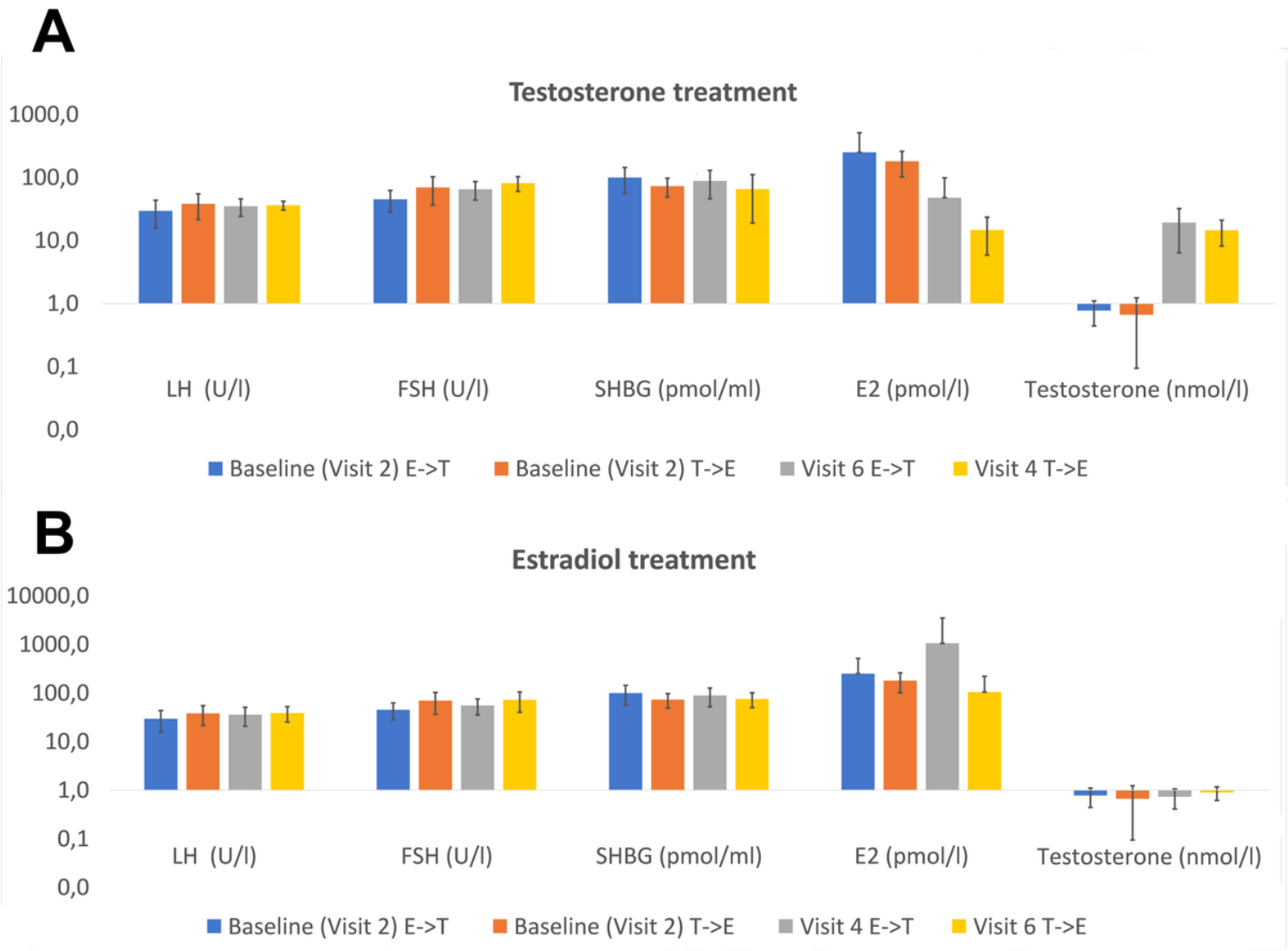

Figure 3

Changes in hormonal parameters separated by sequence

Changes in hormonal parameters under estradiol (A) and testosterone (B) treatment. Y-axis is log-transformed. E: estradiol; T: testosterone; ->: sequence. Means and SD are depicted.