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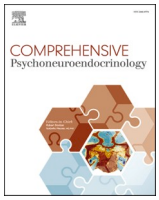

Are steroid hormones and autistic traits affected by metformin? First insights from a pilot

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ABSTRACT

Background: Different lines of evidence imply that metformin could alter steroid hormone homeostasis and thereby improve social impairment. Here, we tried to correlate the impact of metformin treatment on alterations in steroid hormones and autism spectrum traits before versus after treatment with metformin.
Material & methods: Urine steroid hormones were measured using gas chromatography mass spectrometry in 12 male subjects (54.2 ± 9.1 years, 177.3 ± 4.1 cm, 80 ± 10.4 kg) and 7 female subjects (64.14 ± 18.0 years, 162.7 ± 4.1 cm, 76.1 ± 10.4 kg). Furthermore, a questionnaire on autism spectrum traits (Autism Spectrum Questionnaire]) was administered prior to and after metformin treatment.
Results: Overall, a decrease of steroid hormones were detected, which were most pronounced in the metabolites of corticosterone, deoxycortisol, cortisol, as well as androgens. These remained after Bonferroni correction (three classes: glucocorticoid, mineralocorticoid, androgens). No effect on autism spectrum traits (social skills, attention switching skills, attention to detail skills, communication skills, imagination skills), was identified pre versus post metformin treatment.
Discussion: The decreased steroid hormone levels are based on different mechanisms; one effect is likely via mitochondria, another effect via activated protein kinase prior to post treatment. The finding on autistic traits must be taxed as negative and do not directly provide an argument for using metformin in the treatment of autism.

1. Introduction

Metformin is an antidiabetic drug and besides its main usage in the context of lowering blood sugar levels, an off-label use for Poly cystic ovarian syndrome (PCOS), to treat the ingoing hyperandrogenism [1,2]. Autism is also a hyperandrogenic state, thus, elucidating the effects of metformin on steroid hormone and autism spectrum traits seems reasonable [3–6]. It is generally accepted that steroid hormones mediate social behaviour in several forms, whereby one generally accepted role is mediated via hypothalamus-pituitary adrenal axis via the CRH-ACTH system or oxytocin [7]. Thus, the orchestrating role of steroid hormones within the human body is impressive, as they mediate many cascades including blood pressure regulation, the menstrual cycle, (mini-) puberty as well as stress responses and social behaviour [2]. Furthermore, for several illnesses from post-traumatic stress disorders to depression or even Autism a key role for these hormones is also described [8,9]. In a stress-induced mouse model, social impairment was restored with treatment with metformin [10]. As it was shown that metformin showed superior effects as compared to other agents, having an anti-glucocorticoid, anti-androgen and anti-mineralocorticoid effect in this mouse model. However, the mechanism of action of metformin on social behaviour and steroid hormones remains unclear [11]. Two main potential targets for metformin regulating steroid and glucose metabolism are AMP-activated protein kinase (AMPK) signaling and the

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complex I of the mitochondrial respiratory chain [11]. Moreover, other mechanisms of action are described [11]. metformin inhibited androgen production by decreasing beta-Hydroxysteroiddehydrogenase 2 (HSD3B2) expression and 17α-hydroxylase/17,20 lyase (CYP17A1), activities in cell populations [11]. The effect of metformin on androgen production was dose dependent and subject to the presence of organic cation transporters, establishing an important role of organic cation transporters for metformin's action [11]. A role for metformin on the respiratory chain complex I and not the AMPK system is supported by the fact that in AMPK deficient mice an effect of Metformin was identified [12]. Furthermore, metformin seems to reduce oxidative stress [3–5,13–16]. Oxidative stress triggers an increased 17,20-lyase-catalyzing activity of adrenal P450c17, through activation via p38α [13,17]. It was also indicated in a case of a woman with PCOS, her steroid hormone levels were affected by metformin in a dose dependent manner [2]. This is thought to be supported by a subsequent case where the concentration of 18-OH cortisol was decreased after Metformin treatment. As 18-OH Cortisol levels are inversely correlated with glutathione (a main antioxidants) an effect is implied [2,18]. A reduction of all steroid hormone levels were detected after metformin treatment and the effect was stronger after 121 versus 71 days of treatment [2]. Nevertheless, effects of metformin on steroid hormones and autism spectrum traits have not fully been elucidated, specifically for the different metabolite classes (progesterone metabolites, corticosterone metabolites, aldosterone metabolites, androgen metabolites, estrogene metabolites, 11-deoxycortisolmetabolites, cortisol metabolites), as well as a linkage to association with changes in autism spectrum traits (Fig. 1). Thus, our aim was to analyze the effects of metformin on the potential alterations of steroid hormones and autism spectrum traits. We hypothesized that metformin administration would have no influence on urine steroid hormone concentrations or autism spectrum traits [19].

2. Material & Methods

2.1. Participants

12 male subjects (54.2 ± 9.1 years, 177.3 ± 4.1 cm, 80 ± 10.4 kg) and 7 female subjects (64.14 ± 18.0 years, 162.7 ± 4.1 cm, 76.1 ± 10.4 kg) were analyzed for measurements of the most relevant steroid metabolites. Subjects were enrolled if having an indication of metformin for example due to a prediabetes or a PCOS.

2.2. Trial design

For this study, 24-h urinary samples were collected prior to a first intake (start in the morning for 24 h) and after around 40 days (for details see results section) of metformin treatment. Furthermore, to assess autism spectrum traits, we captured potential changes before metformin treatment and equally after around 40 days (for details see results section) of metformin treatment via an autism questionnaire (Autism-Spectrum Quotient) [20–22]. Besides a total score, there are items for social skills, attention switching items, attention to detail items, communication items and imagination items [23].

2.3. Steroid measurement procedures

Analysis of urinary steroids was conducted via gas chromatography-mass spectrometry (GC-MS). Urine samples were collected prior to treatment and after intake of metformin. Urine sample preparation comprises pre-extraction, enzymatic hydrolysis, extraction from the hydrolysis mixture, derivatization and gel filtration. The recovery standard were prepared by adding 2.5 µg of medroxyprogesterone to 1.5 mL of urine. The sample was extracted on a Sep-Pak C18 column (Waters Corp., Milford, MA, USA), dried, reconstituted in a 0.1 M acetate buffer, pH 4.6, and hydrolyzed with a powdered Helix pomatia enzyme (12.5 mg; Sigma Chemical Co., St. Louis, MI, USA) and 12.5 µL of β-glucuronidase/arylsulfatase liquid enzyme (Roche Diagnostics, Rotkreuz, Switzerland). The resulting free steroids were extracted on a Sep-Pak C18 cartridge. A mixture of internal standards (2.5 µg each of 5α-androstane-3α, 17α-diol, stigmasterol, and cholesterol butyrate, and 0.15 µg of 3β5β-tetrahydroaldosterone) was added to this extract, and the sample was derivatized to form the methyloxime-trimethylsilylethers. Analyzes were performed on a Hewlett Packard gas chromatograph 6890 (Hewlett Packard, Palo Alto, CA, USA) with a mass selective detector 5973 by selective ion monitoring (SIM). One characteristic ion was chosen for each compound measured. The derivatized samples were analyzed during a temperature-programmed run (210–265 °C) over a 35-min period. The calibration standard consists of a steroid mixture containing known quantities of all the steroid metabolites to be measured. Responses and retention times were recorded regularly. In each case, the ion peak was quantified against the internal stigmasterol standard. All procedures were performed as described several times by us and others [3,4,24–26].

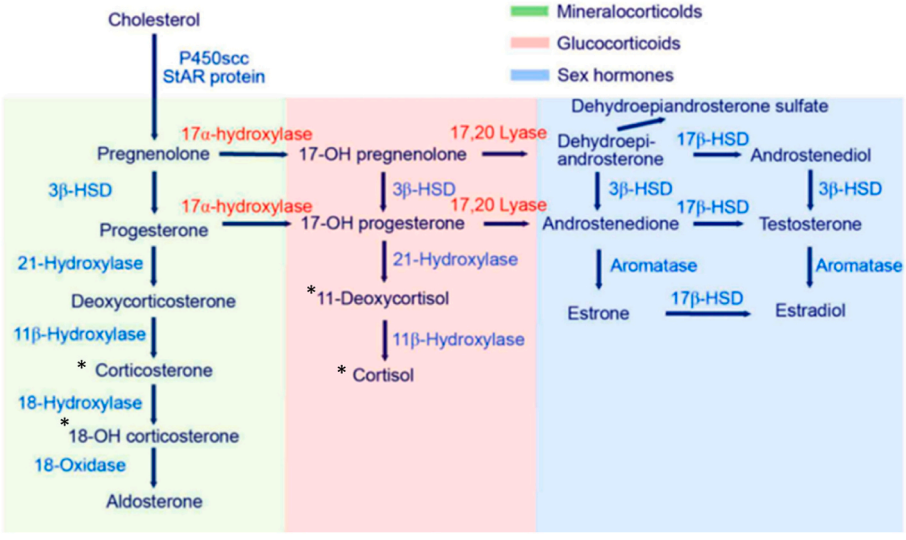

Fig. 1. Steroidogenesis by metabolite class in a simplified form (for details see Ackermann et al. [48]) Starting from the precursor Cholesterol main metabolites are shown. The color code is not totally strict, implying that for example a glucocorticoid metabolite can bind on an androgen receptor as well as glucocorticoid receptor. Thereby, a respective metabolite can have for example a similar effect to a smaller extent or even the effect of a competitive inhibitor. The analyses performed have to be therefore considered to be descriptive. Metabolites with an asterix* showed significant differences ($p < 0.05$) prior-to post treatment with Metformin. The problem however remains, we do not really know the cascades targeted by Metformin, descriptions are only parts of the big picture but an encompassing mechanistic or intuitive understanding of all metabolites, enzymes and receptors involved in steroidogenesis in the different tissues is out of view. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2.4. Autism spectrum traits - measurement methods

To measure the potential effect of metformin on autism spectrum traits changes, an autism questionnaire was conducted before and after metformin treatment. The Autism Spectrum Quotient (AQ) is a measure respectively a questionnaire developed by the Cambridge research group, guided by Simon Baron-Cohen [20–22]. Later, in addition to other themes, research by this group, focused on steroid hormones. This questionnaire was chosen due to its ease of use. It comprises 50 questions, made up of 10 questions, assessing 5 different areas: social skill; attention switching; attention to detail; communication; and imagination. Each of the items listed above scores 1 point if the respondent records the abnormal or autistic-like behaviour, either mildly or strongly. Approximately half the items were worded to produce a 'disagree response, and half an 'agree response, in a high scoring person with autism, which was to avoid a response bias either way [20–22].

2.5. Statistical analysis

Mean and SEM (standard error of mean) of all metabolites were calculated pre-versus post metformin treatment. As normal distribution was identified in nearly all subsamples of metabolite concentrations with Jarque Bera tests, two-sided t-tests were performed to analyze differences pre versus post metformin treatment. Mean and SD (Standard Deviation) was calculated for the total score and all subclasses of the administered questionnaire (social skills, attention switching skills, attention to detail skills, communication skills, imagination skills). The differences between pre-versus post intervention were analyzed with t-tests. Furthermore, it is to mention that if following Morin-Papunen et al., 2003, the most prominent serum metabolite testosterone decreased by 2.7 ± 0.3 to 2.0 ± 0.2 nmol/L in patients receiving metformin (500 mg twice daily for 3 months or longer, then 1000 mg twice daily for 3 months or longer) [27]. By taking an alpha of 0.05% corrected by Bonferoni (0.005/44 (number of metabolites intended to analyze) = 0.001136) and a target power of 80% we would get an n = 7 patients [27–30]. Concerning the Autism questionnaire, it is to mention that an average score in students of 17.6 ± 6.4 was detected compared to healthy controls with an average score of 16.4 ± 6.3 [20.21.22]. When taking an alpha of 0.05% and a target power of 80% this would yield to a large sample size of 894 subjects [20.21.22]. When performing a comparison with students participating in the Math Olympiad scoring 24.5 ± 6.4 versus students with the already mentioned score of 17.6 ± 6.4, a sample size of 28 results [20, 21, 22]. Calculations were performed with Microsoft Excel and SPSS (Microsoft Inc., Redmond, WA, USA).

3. Results

The average treatment duration with metformin was in males: 39.8 ± 13.4 days (mean ± SEM), with the average dosage during this treatment period being 1 ± 0.11 g (mean ± SEM) metformin per day. The dosage per kg was 0.0125 ± 0.037 (mean ± SEM), in males. For females, the average treatment duration was 42.8 ± 16.9 days (mean ± SEM), with the average dosage being 0.97 ± 0.23 g metformin per day. The dosage per kg was 0.013 ± 0.004 (mean ± SEM) in females. No significant differences between males and females concerning days of treatment (p = 0.74) and total dosage (p = 0.83), as well as dosage per kg (p = 0.54) were detected. Table 1 shows (both male and female subjects), the average concentrations pre-versus post treatment. To summarize, metabolite concentrations of steroid hormones significantly differed in both male and female subjects after metformin treatment. Most of these significant results held after Bonferroni correction was applied (for biological plausibility for the three main classes in adrenal gland layer zona glomerulosa - mineralocorticoid, zona fasciculata – glucocorticoid and zona reticularis – androgens). Specifcaly, some androgens (androsteron, etiocholanolon, 11b-OH-androsteron), the glucocorticoids (cortison, TH-cortison, 20a-dihydrocortison, 20b-dihydrocortison, cortisol, TH-cortisol, b-cortol, 20b-cihydrocortisol) and the corticosteronmetabolites (TH-corticosteron, 5a-TH-corticosteron) were all significantly altered (Table 1). In the subsequent metabolite classes, the percentage decrease was substantial, but the very broad reference intervals have to also be considered (Table 1, Fig. 2) [48].

Concerning the administration of the autism questionnaire, the total sample the score was 13.8 ± 7 prior-treatment and 14.2 ± 8.6 post-treatment, showing no statistically significant differences (p = 0.91; Fig. 3). When analyzing subclasses (social skills, attention switching skills, attention to detail skills, communication skills, imagination skills), in additions no significant differences were detected (see Fig. 3 and its legend for details).

4. Discussion

The aim of this study was to analyze the relationship between a potential effect of metformin on steroid hormones and to correlate potential changes, with an additional possible influence on autism spectrum traits. In short, a substantial effect was detected on steroid hormone levels, but no change on autism spectrum traits. To consider, evidence implies a beneficial effect of metformin on several diseases, (e.g. depression) [12,27,28]. For depression, an altered cortisol levels have been described several times in line with the suggestion of an influence of metformin on steroid hormones [12,27,28]. Furthermore, a beneficial effect was implied for neurodevelopmental disorders (e.g. autism), neurodegenerative disorders, (e.g. Alzheimer's disease) and genetic diseases with neurodevelopmental consequences (e.g. fragile X syndrome) [12,27,28]. For all of these, steroid hormones might be relevant and the findings could be of interest to develop rational pharmacotherapies. In our study, the average age was 54.2 years in males and 64.1 years in females for first usage of metformin as antidiabetic drug. The average time between the pre and post measurement was around 40 days which should be a sufficient half-life time and to have an effect on different cascades measured here.

A decrease in mainly metabolites of corticosterone, deoxycortisol, cortisol as well as androgens were detected (Fig. 2). These findings suggest that metformin has lowering effects on steroid hormone levels as already shown in the context of PCOS, where it is broadly used to lower the associated hyperandrogenism. Thereby the higher effect of Metformin in female subjects might be due to the fact that two subjects with a PCOS were included having steroid hormone levels prior to treatment in the higher area of the range as compared to the male sample with all subjects simply having an indication of Metformin for a pre-diabetic state but not due to a PCOS ingoing with higher levels of steroid hormones.

Moreover, autism is now clearly associated with Hyperandrogenism, thus the rationale for elucidating the effects of metformin treatment might be promising [10,28–31]. In studies conducted on women with PCOS, a treatment duration of 6 weeks metformin at a standard target dosage of 1500 mg daily was sufficient for a decreasing effect on hormonal levels [33–38,38,39].

The effect of metformin on androgens has also been previously implied [32]. Direct studies report an effect of metformin on adrenal glands [40], with both free and bioavailability testosterone being significantly reduced by about 20% after 5 days of treatment [41]; this concurs with other findings [42,43]. Supporting the findings, after 3-month treatment, the change of total and bioavailable testosterone levels in the metformin group was still significantly decreased [44]. In addition, median oestradiol levels decreased by around 20% between baseline and 6 months on metformin vs placebo levels [45]. Larger reductions were detected by between 6.1% (TH-aldosterone) and 51.8% (corticosterones) for the total sample. Based on the broad reference intervals for normal subjects (see Ackermann et al. [48]), it is still very likely that the measurements have a high validity. The findings are further supported by analyses on receptor level, whereby after 3 months of metformin treatment, the expression of androgen receptor was

Table 1
Values of measured concentrations of the 24-h urine collection of the different steroid hormones for the total sample, the female subsample and the male subsample. Color coded with dark green <0.1, light green with 0.1 < p-value<0.5 and red with >0.5.

	total sample	sem	post mean	sem	p	female sample	sem	post mean	sem	p	male sample	sem	post mean	sem	p
	prior mean					prior mean					prior mean				
PROGESTERONMETABOLITE															
17-Hydroxypregnanolon	193.1	32.9	146.7	30.3	0.114	170.7	59.9	94.3	36.6	0.265	209.1	42.1	184.1	41.4	0.042
Pregnanediol	709.3	372.3	557.3	227.6	0.704	1151.9	908.3	907.9	541.2	0.821	393.1	48.8	306.8	48.1	0.292
Pregnanetriol	762.4	176.1	586.8	110.1	0.061	726.8	323.3	464.2	165.8	0.199	787.9	151.9	674.4	149.6	0.182
11-Oxo-Pregnanetriol	19	7.4	14.8	4.4	0.293	14.6	4.6	8.3	2.6	0.074	22.1	7.2	19.4	7.1	0.686
CORTICOSTERONMETABOLITE															
TH-Deoxycorticost.	20.1	6.4	13.4	1.3	0.277	30	14.9	10.8	2.1	0.254	13.1	1.5	15.2	1.5	0.407
TH-11-DH-corticost.	229.7	52	143	22	0.039	329.7	100.8	176.2	32.2	0.136	158.3	29.1	119.3	28.7	0.172
TH-corticosteron	190.5	30.4	120.4	17.4	0.031	286.8	37.6	137.6	39	0.061	121.7	14	108.2	13.8	0.337
5a-TH-corticosteron	409.5	66.1	282.9	51	0.018	491.6	79.8	227.6	61.9	0.072	350.8	77.3	322.4	76.2	0.676
ALDOSTERONMETABOLIT															
TH-Aldosteron	25.9	3.9	24.4	5.4	0.576	32.8	8.1	19.6	5	0.111	20.9	8.9	27.9	8.7	0.34
ANDROGENMETABOLITE															
Androsteron	2019.4	405	1542.4	379.8	0.049	1607.2	427.4	860.1	150.8	0.056	2313.9	599.2	2029.8	590.1	0.291
Etiocholanolon	2215.3	538.9	1544	354.9	0.032	2317.2	1086.7	1268.3	564	0.117	2142.5	491	1741	483.6	0.054
Dihydroandrosteron	79.2	16.5	62.4	12.6	0.136	55.6	22.7	33.7	8.3	0.225	96.1	17.7	82.8	17.4	0.209
11-Oxo-Etiocholanolon	484.5	145.2	362.3	91.9	0.081	471.9	162.9	280.3	81.8	0.092	493.5	151	420.8	148.8	0.46
11b-OH-Androsteron	1140.7	209.2	890.1	173	0.017	1075	176.3	687.1	75.4	0.086	1187.6	292.8	1035.1	288.3	0.064
11b-OH-Etiocholanolon	425.5	102.1	371.3	107.1	0.357	527.5	185.1	362.2	220.3	0.046	352.6	118.6	377.8	116.8	0.78
Dehydroepiandrosteron	850.5	522	254.1	117.8	0.222	1418.8	1245.2	184.3	141.2	0.319	444.7	185.7	304	182.9	0.172
Androstenediol	142.4	59.2	72.9	17.6	0.202	197.9	139.1	48.2	23.6	0.259	102.8	24.8	90.5	24.4	0.526
16a-OH-DHEA	455.1	143.5	409.7	118.6	0.625	390.9	256.6	269.4	159.7	0.295	500.9	171.2	510	168.6	0.912
Androstenetriol	271.3	52.9	234.4	44.9	0.153	188.2	62	158.4	55	0.296	330.7	62.2	288.7	61.3	0.211
Pregnenetriol	164.6	58.4	66.2	23.3	0.027	249.2	122.6	74.8	45.7	0.092	104.2	27.5	60.1	27	0.102
Testosteron	57.9	13.8	48	11.2	0.472	29.1	9	19	6.4	0.286	78.5	14.4	68.7	14.2	0.563
5a-Dihydrotestosteron	45.6	12	34.6	8.5	0.123	35.8	15.1	19.1	2.3	0.257	52.5	13.4	45.8	13.2	0.312
ESTROGENMETABOLITE															
Estriol	7	1.2	5.8	1	0.537	8.2	2.1	6.7	0.9	0.529	6.2	1.7	5.2	1.7	0.646
17b-Estradiol	6.5	0.9	5.2	0.6	0.197	7.5	2.1	4.2	0.8	0.081	5.8	0.8	6	0.8	0.881
11-DEOXYCORTISOLMETABOLIT															
TH-11-deoxycortisol	121.2	18.3	81.8	14.2	0.051	143.3	27.4	67.5	26.3	0.149	105.5	16.7	92.1	16.4	0.401
CORTISOLMETABOLITE															
Cortison	177.3	18.9	130.8	18.6	0.009	177.9	31.2	88.4	15.6	0.04	176.8	25.1	161	24.7	0.358
TH-cortison	4848.8	960.8	3431.1	699.8	0.02	5038.8	747.4	2880	520.3	0.108	4713.1	1181.9	3824.7	1163.9	0.125
a-Cortolon	611.1	181.2	337.4	61.4	0.055	921.8	398.7	401.8	135.5	0.151	389.2	50.7	291.4	50	0.224
b-Cortolon	767	170	522.9	104.2	0.037	769.3	104.8	448.1	98.8	0.12	765.4	171.5	576.3	168.9	0.198
20a-Dihydrocortison	29.6	4.5	19.7	3.4	0.019	26.8	4	12.8	3.8	0.065	31.6	4.6	24.6	4.6	0.185
20b-Dihydrocortison	93.9	16.4	58	10.4	0.003	91.2	20	39.5	14.4	0.028	95.9	13.5	71.2	13.3	0.117
Cortisol	111.6	17.7	72.7	11.1	0.018	133.1	28.3	59.8	11.6	0.066	96.3	17.3	82	17	0.113
TH-cortisol	2577.4	327.7	1775.1	270.4	0.025	2694.9	303.3	1547.2	484.4	0.194	2493.4	338.1	1937.9	333	0.082
5a-THcortisol	1718	430.7	1322.4	287.8	0.048	1445.8	330.1	841.2	211.7	0.146	1912.4	445.6	1666.1	438.9	0.541
a-Cortol	1318.9	345.8	875	204.6	0.034	1109.3	445.1	653.1	328.3	0.17	1468.5	272.1	1033.6	268	0.159
b-Cortol	551.1	95.2	454.8	139.1	0.015	437.8	39.7	247.8	57	0.1	632.1	228.6	602.7	225.1	0.817
20a-Dihydrocortisol	64.7	9.7	42.3	8.3	0.042	74.8	17.4	37.3	20.4	0.099	57.4	4.5	45.9	4.4	0.304
20b-Dihydrocortisol	142.2	29.7	89.3	15.9	0.02	120.7	18.1	57.4	16.9	0.04	157.5	21.6	112.1	21.3	0.211
6b-OH-cortisol	116	22.7	84.4	10	0.146	169.9	40.8	78	13.5	0.092	77.5	15.2	89	15	0.274
18-OH-cortisol	238.9	48.2	152.9	20.4	0.098	352.9	88.1	144.4	29.9	0.095	157.5	30.2	159	29.8	0.945
Sum of all	24412.7	4158.2	17243.9	2527.9	0.018	25523	6112.4	13926.4	2200.2	0.095	19537.9	5291.8	17999.8	5308.4	0.12

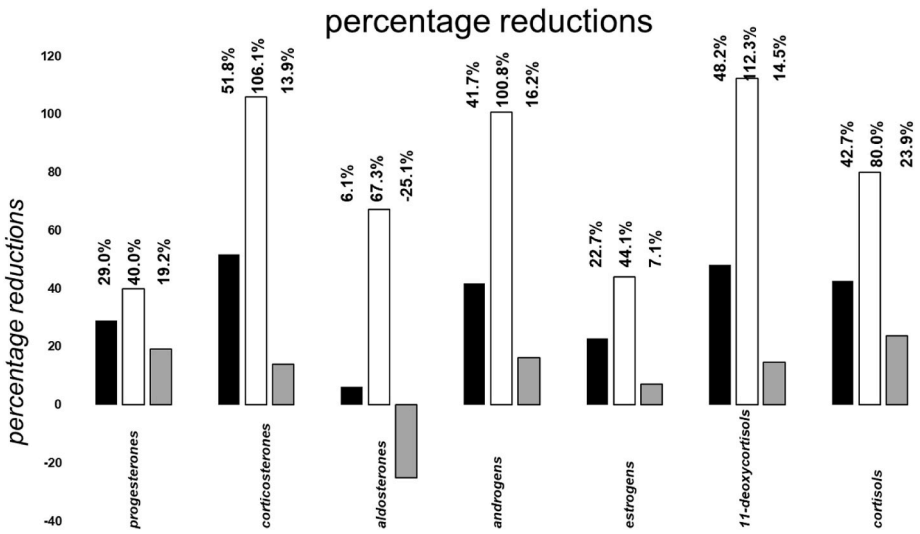

Fig. 2. Percentage change of the main classes prior to and post metformin treatment (progesterone, corticosteron, mineralcorticoid, androgens, oestrogen, 11-deoxycortisol, cortisol metabolites); black = total sample, white = female sample, grey = male sample).

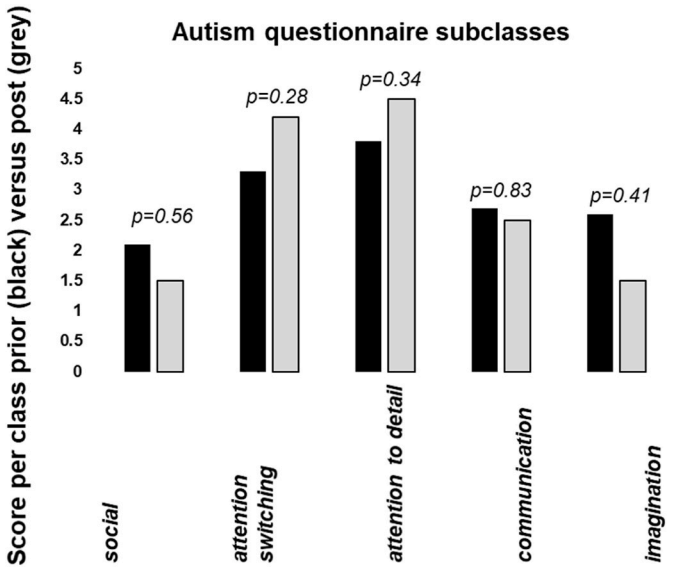

Fig. 3. Changes in scores of the main classes prior (black rectangles) to post (grey rectangles) metformin treatment (social skills score, attention switching skills, attention to detail skills, communication skills, imagination items). The Autism Quotient was developed because of a lack of a quick and quantitative self-report instrument for assessing how many autistic traits any adult has. The minimum score on the Autism quotient is 0 and the maximum 50. If an adult has equal to or more than 32 out of 50 such traits, this is highly predictive of Autism [20–22]. Regarding the social skills, the scores were prior 2.1 ± 2.4 and 1.5 ± 1.6 post-treatment ($p = 0.56$); attention switching skills 3.3 ± 1.2 prior- and 4.2 ± 1.7 post-treatment ($p = 0.28$); attention to detail skills 3.8 ± 1.2 prior-and 4.5 ± 1.4 post -treatment ($p = 0.34$); communication skills 2.7 ± 2.3 prior- and 2.5 ± 1.8 post-treatment ($p = 0.83$); imagination items 2.2 ± 1.6 prior and 1.5 ± 1.5 post-treatment ($p = 0.41$). Furthermore, when analyzing differences of the autism quotient in male versus female, no significant differences were found.

reduced in epithelial and stromal cells in comparison to their levels before treatment [46]. Interestingly, corticosterones, 11-deoxycortisol and cortisol (metabolites with glucocorticoid action) seemed to show a prominent reduction. Glucocorticoids are regulated via the adrenal gland and underlie the control of the hypothalamus-pituitary adrenal axis, especially over the CRH-ACTH system over feedback and feedforward mechanism [7,10]. Therefore, a direct relationship of metformin on the mediating role of central nervous system is implied based on these findings.

With regards to potential effect on autism spectrum traits, no direct effect was identified with the Autism Spectrum Quotient [20–22]. We applied our questionnaire, consisting of 50 questions, with the aim to elucidate if metformin has an effect on traits of autistic behaviour [20–22]. Neither a significant change in total score prior to post treatment, nor a significant change for the different items (social skills, attention switching, attention to detail, communicative skills, imagination items), was detected [20–22]. In principle, the Autism Quotient also reveals sex differences (males > females, not detected here) and cognitive differences (scientists > non-scientists), a pattern of results that has been closely replicated in a Japanese sample implying an independency of cultural background [20–22]. The Autism Spectrum Quotient can rapidly quantify where an adolescent is situated on the continuum from autism to normality and thus a potential shift might be detected [20–22]. Despite the advantages of the questionnaire its simplicity has to be considered as a limitation of this study as it is impossible to catch all aspects of social behaviour respectively on autism spectrum traits. Nevertheless, the sample size was relatively small and the calculated power analysis already indicates the potentially very large sample needed to detect changes. To keep in mind, the simple approach was used for pragmatic reasons to not stress subjects analyzed too much. More elaborative strategies such as an encompassing anamnesis and consequent tracking during the treatment period might have yield to the possibility to detect even smallest effects and should be used in a potential clinical trial with affected subjects with autism. Further studies in subjects with autism, which have in addition an indication for metformin would thereby help to deepen our understanding of the effects on autism spectrum traits.

Focusing on limitations of the study is the small sample size, especially in female subjects. Weakening this argument, a previously calculated power analysis implied for testosterone already for a sample size of seven a significant effect [1]. Furthermore, for ethical reasons a placebo-controlled study was impossible, as subjects with an indication based on increased blood sugar levels were included and not giving metformin as soon as possible would have been unethical. Steroid hormones exert very broad reference values and therefore, effects on absolute levels are difficult to capture [48]. Furthermore, in an optimal manner the study design would have been a crossover placebo-controlled trial. This was not possible due to the fact that participants having an indication for metformin should be administered as

soon as possible. Furthermore, characterizing the metabolite concentrations allows for descriptive development of our understanding. The complex topology of the steroid networks and receptors involved is however only partly captured with this approach (Fig. 1). For example, a glucocorticoid metabolite can bind on an androgen receptor, as well as glucocorticoid receptor. Thereby, a respective metabolite can have a similar effect to a smaller extent and/or the effect of a competitive inhibitor. The analyses performed must therefore be considered to be descriptive, without the understanding of ligand receptor activity. Finally, information about the phase of menstrual cycle were not collected and therefore this is a potential cofounding factor (see Ackermann et al. [48]. However, in a study of 10 healthy white women, (20–40 years with regular endogenous menstrual cycles between 24 and 34 days), the range of 24-h urinary excretion of androgens and glucocorticoids was in a similar range as measured in our study [48,49]. Moreover, no differences were found between menstrual, follicular and luteal phase of the menstrual cycle for the urinary excretion of androgens and of the five glucocorticoids cortisol, cortisone, TH-cortisol, allo-TH-cortisol and TH-cortisone implying some irrelevance [48,49]. In addition, a study comparing salivary cortisol after waking revealed no differences between 11 women in the luteal phase and 12 women in the follicular phase of the menstrual cycle [50].In another study, it the phase of menstrual cycle was also not recorded, because previous unpublished analyses did not reveal an impact on urinary glucocorticoid and androgen metabolites [48,51]. Nevertheless, despite this, other findings reporting changes exist [48,52,53]m, thus further studies are required to fully elucidate this.

In summary, as several lines of evidence suggested an involvement of steroid hormones in autism, and higher levels were shown in girls and boys with autism mostly pronounced androgens, Metformin was considered as potential pharmaceutical [3–5,54,60]. This was further supported as in a mice model, after dysregulation of steroid hormones Metformin improved social impairment even better than other pharmaceuticals targeting steroid hormones even upon baseline conditions [10]. These findings are now expanded with a human focus. Thereby, as steroid hormones may exert different effects on brain, respectively cognitive functions such as learning, emotional judgement or face recognition the findings might have a high value from a translational point of view [7,55]. In consequence and as it is accepted that androgens affect (neurocognitive) development during sensitive stages and we further know that metformin targets the altered cascades in subjects with autism a clinical trial might answer might answer the questions concerning the usability. We therefore conclude that based on our findings of higher levels of steroid hormones in girls and boys with autism, best effects of Metformin in a mice model of autism, a detected decrease of steroid hormone levels in humans pre versus post treatment, to think about a potential usage of Metformin in some forms of autism might be fruitful. Severe forms of autism which are for example associated with Fragile-X syndrome known for dysregulated steroid hormones should be considered first before coming to a general recommendation of an off-label use of this pharmaceutical.

Trial register

ClinicalTrials.gov (Trial registration number: NCT04930471).

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Ethics

The study was in line with the declaration of Helsinki and its later amendments and approved by the local Ethics Committee and was registered in the Clinicaltrials.gov database (Trial registration number: NCT04930471, Registered 17.06.2021).

Declaration of competing interest

The authors declare having no Conflict of interest.

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