The Sex-Dependent Impact of Chronic Clozapine and Haloperidol Treatment on Characteristics of the Metabolic Syndrome in a Rat Model

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Key words
- clozapine
- haloperidol
- metabolic syndrome
- sex-dependence

Abstract

Introduction: An increased risk for metabolic syndrome has been described for patients with psychotic disorders. Antipsychotic drugs possibly contribute to metabolic changes.

Methods: Haloperidol or clozapine was orally fed to male and female Sprague Dawley rats for 12 weeks, and body weight gain, food and water intake were measured. The serum levels of fasting glucose, HbA1c, triglycerides, cholesterol, HDL and LDL, insulin, leptin, adiponectin and ghrelin were determined. Gonadal and perirenal fat pads were removed and weighed.

Results: We found increased body weight in the male clozapine group, but decreased ones in the male haloperidol group. Clozapine-treated male and female animals had higher fasting glucose, adiponectin, leptin, ghrelin, cholesterol, HDL and LDL levels, whereas haloperidol caused increased levels of insulin and decreased values of HbA1c, cholesterol, HDL and LDL.

Conclusion: Both antipsychotic drugs cause sex-dependent metabolic changes, which are risk factors for the metabolic syndrome, be it hyperinsulinemia under haloperidol treatment or hyperglycemia, hyperleptinemia and hyperlipidemia under clozapine.

Introduction

The metabolic syndrome is a cluster of metabolic dysfunctions containing risk factors such as visceral adipositas, dysregulation of glucose metabolism including insulin resistance, atherogenic dyslipidemia and hypertension. It increases the susceptibility for diabetes mellitus type II and cardiovascular diseases associated with a totally increased morbidity and mortality [1, 2].

Diverse findings point to a correlation between several psychiatric disorders and the prevalence of metabolic syndrome [3–5]. The mortality of schizophrenia patients due to adipositas, diabetes mellitus and cardiovascular diseases is 2.5-fold higher than in the healthy population [6]. But since most patients have a history of long-term antipsychotic treatment, the differentiation between dysfunctions resulting from the disease and drug-induced metabolic dysfunctions is difficult. Thakore et al. [7] reported that – independently of drug medication – schizophrenia patients possess more intra-abdominal fat than control subjects. Drug-naïve schizophrenic patients have a higher prevalence of impaired fasting glucose and a higher level of insulin resistance [8]. Other studies again found no differences between healthy controls and drug-naïve patients in this regard [9, 10], for which reason there seems to be lacking agreement whether metabolic changes are drug-induced or disease-related. Another approach [11] reports glucose-insulin homeostasis and lipid profiles in clozapine-induced schizophrenic obesity to differ from those in non-psychiatric obesity with similar anthropometric parameters, body weight and BMI, also observed with typical agents [12].

According to diverse studies, atypical antipsychotic drugs increase the risk of developing a metabolic syndrome and thus forward the risk for diabetes mellitus and insulin resistance [13]. Correspondingly, De Hert et al. [14] indicated a prevalence of metabolic syndrome of 36% within schizophrenia patients with antipsychotic medication. Additionally, Lamberti et al. [15] found a respective prevalence in 53.8% clozapine-treated patients compared to 20.7% in the general population. Some antipsychotic drugs induce higher food consumption and/or a reduced activity with serious impact on the energy balance. Unlike haloperidol, in schizophrenia patients clozapine increases body weight gain [16] and can cause

received 07.02.2012
revised 28.06.2012
accepted 11.07.2012

Bibliography
DOI http://dx.doi.org/10.1055/s-0032-1321907
Published online ahead of print: 22 August 2012
Pharmacopsychiatry 2013; 46: 1–9

ISSN 0176-3679

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metabolic abnormalities like hyperinsulinemia, hyperlipidemia, hyperleptinemia [17] and dysfunction of the glucose metabolism [18].

Evidence suggests effects of antipsychotic drugs on weight gain [19], metabolic homeostasis and lipid metabolism to be gender-dependent [20]: men present a higher level of waist-hip ratio, triglycerides and insulin resistance index than women. This is reflected by animal models, showing that estrogen deficiency results in increased prevalence of cardiovascular diseases in female rats suggesting estrogens to ameliorate the metabolic syndrome as well as insulin resistance [21]. Transient prenatal androgen exposure produces features of the metabolic syndrome in adult female rats [22].

Studies in humans are frequently contradictory and cannot clearly distinguish between disease and treatment-related effects. Experimental research on rodents might support an understanding of the mechanisms underlying antipsychotic-induced metabolic changes which contribute to the development of the metabolic syndrome. Accordant studies were carried out on different rat strains like Wistar [23], including Han-Wistar [24], Sprague Dawley (SD) [25] and Long-Evans [26]. In general, in contrast to female Wistar rats, males did not show body weight gain or increased food intake under antipsychotic treatment [27–29]. SD rats differed by exhibiting diet-induced obesity [30] and increased plasma glucose levels [31,32] plus increased body fat deposition [33,34]. Research frequently has focused on metabolic effects in male or female animals. There nevertheless exist some studies directly comparing the effects of antipsychotic drug impact between male and female rats [35]. The aim of our investigation consisted in clarifying the impact of chronic treatment with the typical neuroleptic haloperidol and the atypical antipsychotic clozapine on male and female SD rats. We accordingly investigated body weight gain, food and water intake, fat distribution, and serum levels of parameters correlated with metabolic activity, which is based on the hypothesis that clozapine treatment differs from haloperidol in changing metabolic parameters in male and female SD rats.

Materials and Methods

Animals and housing conditions

All experiments were carried out in accordance with the laws of the local authorities for animal experiments approved by the Landesamt für Natur, Umwelt- und Verbraucherschutz NRW, Recklinghausen (Reference number 9.93.2.10.34.07.227). On postnatal day 21 (PD 21), 30 male and 30 female pups of SD rats (Taconic, Denmark), were separated from their mothers, and housed in groups of 5 same-gender animals with free access to water and ground food pellets. The latter was composed of 89.0% dry matter, 19.0% crude protein, 4.0% crude fat, 6.0% crude fibre, 7.5% crude ash with additionally 15% fat in the dry matter (Altromin Spezialfutter GmbH, Germany). The animals were maintained on a 12:12 h light/dark cycle (lights off at 20.00 h) at a temperature of 21 °C and 60% humidity. To limit possible individual weight gain effects, the rats of each treatment group emanated from different litters with identical litter size, so each group was equally composed of rats with different parentage.

Treatment with antipsychotics

The effects of clozapine and haloperidol in adolescent and adult rats differ by substantial pruning and re-organization of the dopamine system occurring from adolescence to adulthood [36]. To avoid these puberty effects, the test started at week 13 (PD 85). The healthy animals were individually housed and weighed twice per week (weighing at Monday and Thursday, averaged for a week). Water consumption was calculated for each week and food intake measured each day, averaged for a week, including the loss of ground pellets in the litter. Food conversion ratio FCR (food intake/weight gain), food conversion efficiency FCE [%] (weight gain/food intake × 100) and growth rate [%/day] (end weight–starting weight) × 100/starting weight/test time) were calculated [37]. Based on the study of Kapur et al. [38], who criticized the current chronic dosing of haloperidol in animal studies to be inappropriately low, we used oral doses commensurate to those of the studies of Minet-Ringuet et al. [33,34] who applied dosages correspondend to 50% of the maximal physiological human dose. Starting at week 13 (PD 85) to week 25 (PD 169), 10 males and 10 females received 1 mg/kg body weight (BW) haloperidol (Haloneurol®, Hexal, Germany) per day in a measured quantity of ground pellets, corresponding to an effective average dose rate of 0.8 ± 0.03 mg/kg BW for males and 0.6 ± 0.08 mg/kg BW for females. 10 males and 10 females daily received 20 mg/kg BW clozapine (Leponex®, Novartis, Germany), corresponding to 18.5 ± 0.26 mg/kg BW for males and 17.7 ± 0.38 mg/kg BW for females. In a previous study we were able to depict that weekly relative food and water consumption (in relation to 1 kg body weight) are changing parameters under antipsychotic treatment [29]. The control groups with 10 males and females were merely fed with pure ground pellets. Via dosage and oral application we considered the reported 4–6 times shorter half-life of antipsychotic drugs in rodents compared to humans and intended to avoid stress reactions caused by daily drug injections. Perez-Costas et al. [39] asserted that in rodents appropriate doses of haloperidol in drinking water equalled receptor occupancies comparable to clinical levels, which again does not seem to apply to clozapine. To address these shortcomings, an oral application in ground pellets seemed suitable for investigating chronic treatment periods. Administering olanzapine by minipumps seems to be limited to 2 weeks and is not viable for long-term drug administration [40].

Locomotor activity

Locomotor activity under antipsychotic drug medication was measured by voluntary wheel running in a rat activity wheel (Tecniplast, Germany) compared to untreated controls. The animals were individually housed in a home cage equipped with a running wheel and free access to food and water for 24 h. A magnetic switch counted complete rotations of the activity wheel.

Determination of metabolic parameters

Food was removed 12 h prior anesthesia for determining fasting glucose. On PD 169 (week 25) the animals were anesthetized by pentobarbital (Narcoren, Merial, Germany), blood was collected by aorta puncture, perirenal and gonadal (epididymal and periovarian) fat tissue was removed and immediately weighed. Serum levels of clozapine, *N*-desmethylclozapine and haloperidol were quantified by HPLC in the biochemical laboratory of the LVR Klinikum Düsseldorf. Briefly, HPLC analyses of clozapine, *N*-desmethylclozapine and haloperidol were performed using equipment from Dionex with the 580 pump and the GINA50 autosampler. For detection, the LC spectrometer Lambda Max 481 (Waters) was used. Drugs were extracted from the blood

samples with ethyl acetate by rigorous vortexing for 30 min at room temperature. Prior to extraction, step LY170222 (Lilly) and chlorinated haloperidol analogue (Sigma-Aldrich) were added as internal standards for the quantification of clozapine as well as *N*-desmethylclozapine and for haloperidol, respectively. After ethyl acetate extraction, samples were centrifuged at 2 500 × g for 15 min, the supernatants were collected and evaporated to dryness with a speed vac (SC110A). Residues were dissolved in mobile phase (ammonium acetate (pH 4.5) with 30 % acetonitrile). Separation was performed with a Hypersil CPS (MZ, Germany) combined with Phenomenex security guard cartridges at 36 °C. The flow rate was adjusted to 1 mL/min and the adsorbance was measured at 254 nm. Drug quantification was performed with the Chromeleon software (Dionex).

The serum levels of leptin, adiponectin, ghrelin and insulin were determined by a rat leptin and adiponectin ELISA kit (Mediagnost, Germany), by a rat ghrelin Elisa kit (USBiological, USA) and by a rat high range insulin ELISA kit (DRG, Germany) in ng/ml or µg/ml following manufacturer's recommendations. Blood parameters as HbA1c, fasting glucose, total cholesterol, HDL and LDL cholesterol and triglycerides were determined in the clinical laboratory of the LVR Klinikum Düsseldorf.

Statistical analysis

Statistical analyses were performed with SPSS version 17.0 for Windows. All data are presented as mean ± SEM and all tests were 2-tailed. Distributions for all dependent variables were examined in both groups using the Kolmogorov-Smirnov test on normality. Our results suggest a normal distribution of the data concluding analysis by parametric tests.

Since 21 dependent variables were examined, the level of significance for the main analyses was adjusted to $\alpha = 0.05/21 = 0.0024$.

Repeated measures ANOVA with TREATMENT and SEX as between-subject factors and TIME as the repeated measures factor was carried out to elucidate the effects of body weight gain, food and water intake. The results of the activity counts, blood analyses and the Elisa-tests were examined by 2-way ANOVA with the between-subject factors TREATMENT and SEX. For all dependent variables, in case of significant group effects, post hoc tests between the 3 groups with Bonferroni adjusted level of significance for the number of subgroup comparisons ($\alpha = 0.05/6 = 0.0083$) were performed separately for male and female rats. In the case of significant sex-related effects, post-hoc tests between male and female rats were computed for all 3 groups (level of significance Bonferroni adjusted: $\alpha = 0.05/3 = 0.0167$). (For body weight, food and water intake subgroup analyses were calculated for the mean data over all measurement points.)

A bivariate correlation procedure with Spearman's rho coefficient was carried out to test the relationship between fat tissue (perirenal and gonadal) and weight, as well as fat tissue and the serum levels of leptin, adiponectin, ghrelin and insulin, glucose and insulin with $p \leq 0.05$ as significant.

Results

Serum levels of clozapine, *N*-desmethylclozapine and haloperidol

We found 29.4 ± 8.4 ng/mL haloperidol in the serum of male and 31.0 ± 5.6 ng/mL in female rats after 12 weeks of oral medication with haloperidol (**Fig. 1**); 80.4 ± 5.6 ng/mL clozapine and 69.8 ± 5.5 ng/mL *N*-desmethylclozapine were detected in male rats and 31.2 ± 2.4 ng/mL clozapine and 83.8 ± 7.3 ng/mL of the metabolite in females under clozapine treatment. Compared to males, the level of clozapine in females was significantly decreased ($p < 0.000001$), whereas the metabolite was increased.

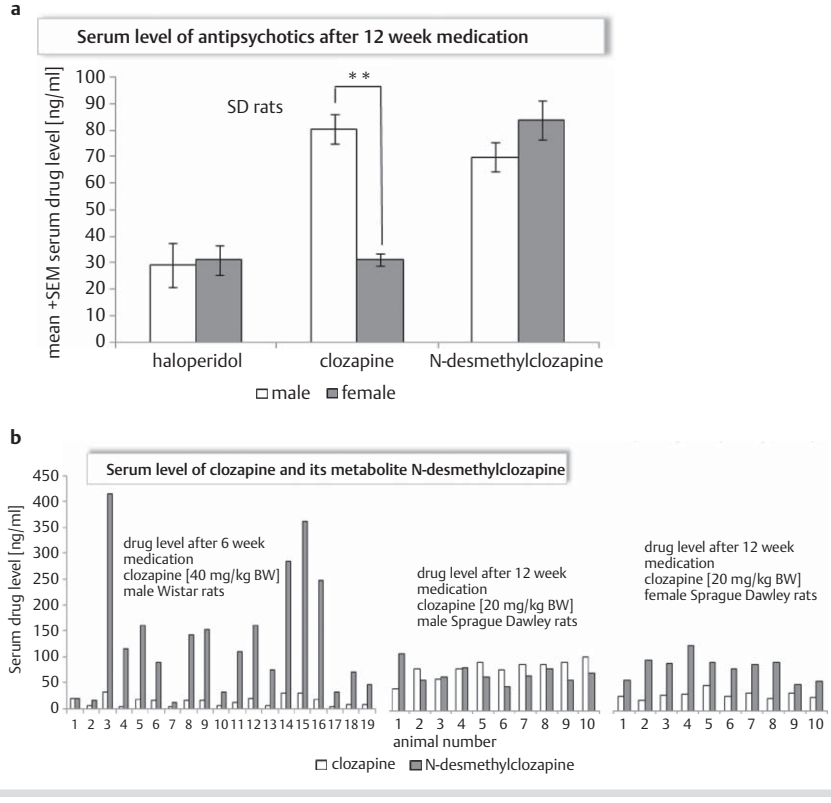

Fig. 1 **a** Serum level of clozapine, N-desmethylclozapine and haloperidol after oral drug medication (n = 10 for all groups). No differences were found between male and female rats treated with haloperidol. Females showed a significant decrease in clozapine level compared to males (** $p < 0.000001$). Males exhibited a lower level of N-desmethylclozapine, a metabolite of clozapine. **b** Serum level of clozapine and its metabolite N-desmethylclozapine in single male Wistar and male and female Sprague-Dawley rats by HPLC. Male Wistar and female Sprague-Dawley had a higher serum level of the metabolite, whereas male Sprague-Dawley rats show a higher or equal level of clozapine except animals 1 and 3.

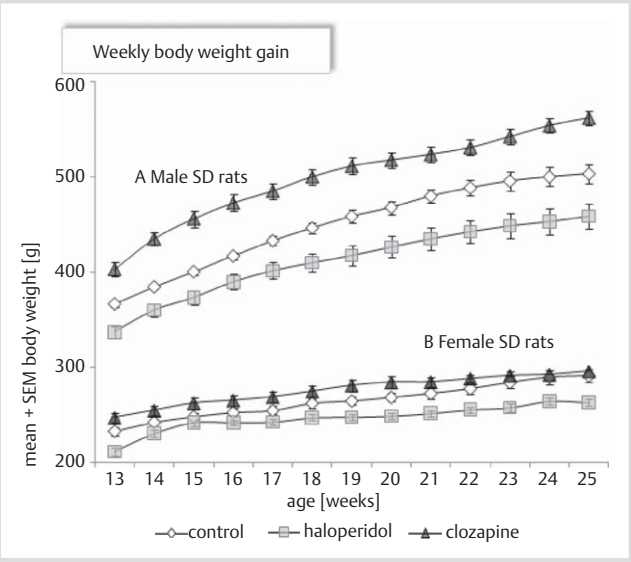

Fig. 2 Weight gain of male and female Sprague-Dawley rats: controls, haloperidol- and clozapine-treated animals (n = 10 for all groups). All animals were weighed twice per week. Depicted is the weight after application of the antipsychotic drugs at week 13 to week 25. The male and female haloperidol group gained less weight than the respective control groups (not significant), whereas the male clozapine group gained significantly more weight than the controls, $p \leq 0.0083$ as significant.

Table 1 Food conversion ratio (FCR), food conversion efficiency (FCE) and growth rate over 12 week medication with clozapine or haloperidol of male and female Sprague-Dawley rats (n = 10 per group).

	FCR	FCE	Growth rate
male control	12.8 ± 0.78	8.10 ± 0.49	0.39 ± 0.025
female control	22.4 ± 2.03	4.80 ± 0.45	0.25 ± 0.025
male haloperidol	12.9 ± 0.48	7.8 ± 0.31	0.34 ± 0.019
female haloperidol	24.5 ± 1.97	4.30 ± 0.32	0.20 ± 0.017
male clozapine	12.30 ± 0.29	8.18 ± 0.20	0.27 ± 0.011
female clozapine	26.9 ± 1.82	3.89 ± 0.28	0.20 ± 0.017

Single male Wistar [29] and female SD rats had a higher serum level of the metabolite *N*-desmethylclozapine, whereas single male SD rats had a higher or equal level of clozapine, except animals 1 and 3 (see **Fig. 1b**).

Weight, food and water intake

We found a continuous weight gain (**Fig. 2**) in the control and drug-treated groups during 12 weeks of drug application TIME [$F(12, 648) = 804.2$, $p < 0.0024$]. Weight of the animals differed significantly between the groups TREATMENT [$F(2, 54) = 41.1$, $p < 0.0024$] and there were sex-dependent effects SEX [$F(1, 54) = 1289.7$, $p < 0.0024$]. Male and female rats orally treated with haloperidol had a lower mean body weight than controls and clozapine-treated animals, whereas only male rats under clozapine medication showed significantly higher body weight than controls and haloperidol treated animals (all $p < 0.0083$). For more details see **Fig. 2**.

We found no differences for food conversion ratio [$F(2, 54) = 1.00$, $p = 0.38$], for food conversion efficiency [$F(2, 54) = 0.86$, $p = 0.42$] or growth rate [$F(2, 54) = 3.1$, $p = 0.055$], but females differed significantly from males (all $p < 0.0167$) (**Table 1**).

Weekly food [$F(10,540) = 11.85$, $p < 0.0024$] differed over time. Food intake was significantly different for TREATMENT [$F(2, 54) = 51.2$, $p < 0.0024$] and SEX [$F(1, 54) = 410,4$, $p < 0.0024$], whereas water intake differed significantly for TREATMENT [$F(2, 53) = 28.4$, $p < 0.0024$]. Food intake was lower both in male and female haloperidol medicated animals compared to controls and clozapine medicated rats, whereas in male and female medicated animals water intake was decreased compared to controls (all $p < 0.0083$). Food intake in females was decreased compared to males (all $p < 0.0167$), whereas water intake in females under haloperidol was not significantly reduced.

The relative weekly food [$F(10, 540) = 61.6$, $p < 0.0024$] and water [$F(10, 530) = 29.2$, $p < 0.0024$] intake related to 1 kg body weight decreased significantly over time and was significantly different for TREATMENT [food: $F(2, 54) = 23.8$, $p < 0.0024$, water: [$F(2, 53) = 26.2$, $p < 0.0024$] and SEX [food: $F(1, 54) = 186.2$, $p < 0.0024$, water: [$F(1, 53) = 80.7$, $p < 0.0024$]. In males, relative mean food and water intake was significantly lower in the haloperidol than in the control group, whereas in female rats it was decreased in haloperidol-treated compared to clozapine-treated rats and controls (all $p < 0.0083$). In all 3 groups, relative mean food and water intake was significantly lower in male than in female rats (all $p < 0.0167$). (For more details see **Fig. 3a, b, 4a, b**).

Locomotor activity by voluntary wheel running

We found differences for TREATMENT [$F(2, 54) = 7.4$, $p < 0.0024$] and SEX [$F(1, 54) = 48.7$, $p < 0.0024$] for locomotor activity tested over 24 h. Control animals showed the highest activity (2 697 ± 296 counts for males and 8 155 ± 1 257 for females) followed by clozapine (2 516 ± 290 for males and 6 142 ± 594 for females) and haloperidol-treated animals (1 560 ± 278 for males and 4 091 ± 767 for females) confirming the higher sedative effect of haloperidol. However, only the female haloperidol group differed significantly from the controls ($p < 0.0083$). As expected [41], females generally had a higher activity level than males (all $p < 0.0167$).

Fat tissue and related hormones (Table 2)

Male clozapine-fed rats showed a trend to higher percental fat tissue than controls ($p > 0.0083$), and males had a non-significant higher percental fat level than females ($p > 0.0167$). Significantly positive correlations between endpoint weight and fat tissue were found for controls (males $\rho = 0.673$, $p = 0.033$; females $\rho = 0.634$, $p = 0.049$) and male haloperidol medicated rats ($\rho = 0.908$, $p = 0.0003$).

Male rats treated with clozapine showed a non-significant elevated plasma leptin level compared to controls. The leptin level was significantly decreased in females compared to males in both the clozapine and the haloperidol groups ($p = 0.001$ for both groups) and not significant in the control group ($p = 0.017$). Male clozapine-fed rats also showed a trend to increased adiponectin ($p = 0.042$) and ghrelin ($p = 0.077$) levels.

The percentage of fat tissue was positively correlated with leptin serum level in the control and haloperidol group (male control $\rho = 0.83$, $p = 0.003$; male haloperidol $\rho = 0.75$, p = 0.013; female control ρ = 0.83, p = 0.003; female haloperidol $\rho = 0.82$, $p = 0.007$), but not in the clozapine groups (male clozapine $\rho = 0.50$, $p = 0.14$; female haloperidol $\rho = 0.10$, $p = 0.78$). Fat tissue was negatively correlated with adiponectin serum level in the male haloperidol group ($\rho = -0.66$, $p = 0.038$).

Glucose and related parameters (Table 2)

We found a non-significant elevation of fasting glucose in female rodents treated with clozapine ($p = 0.023$). HbA1c values were

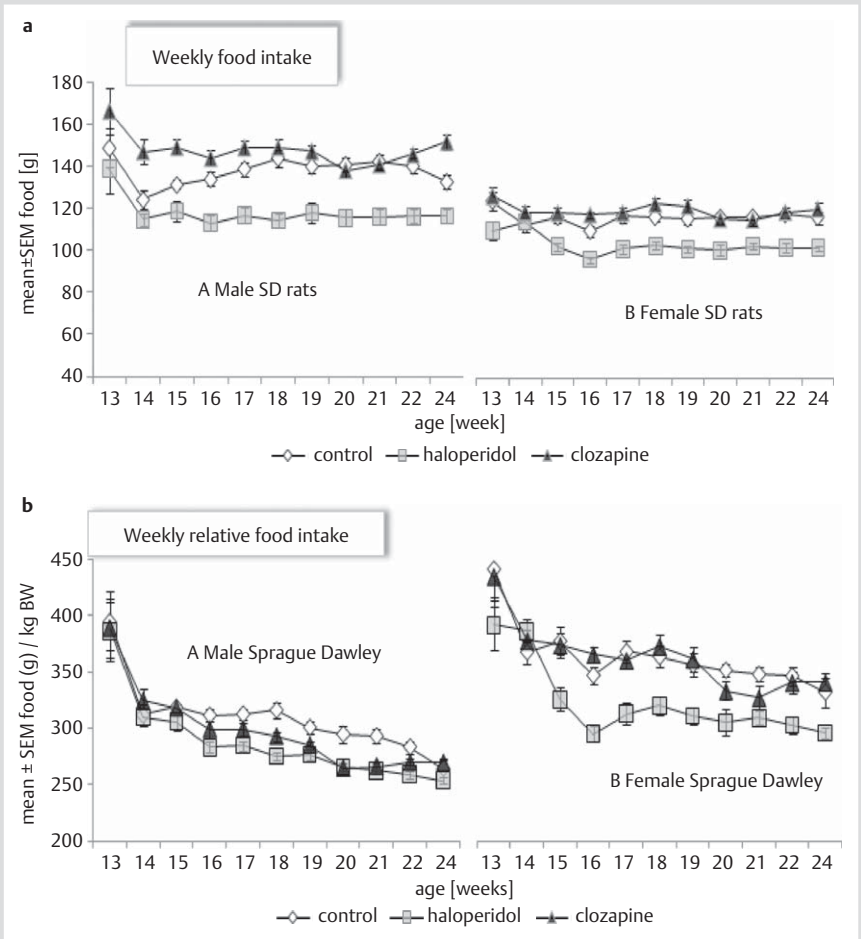

Fig. 3 Weekly food intake of male and female Sprague-Dawley rats: controls, haloperidol and clozapine treated animals (n = 10 for all groups). **a** Weekly food intake: we found no differences between controls and clozapine-medicated rats, although the male clozapine group ate more in general. Animals under haloperidol medication ate significantly less than the control and clozapine group, $p \leq 0.0083$ as significant. **b** Weekly relative food intake based on 1 kg BW. All animals showed a decreased food intake from week 13 to week 25. Animals under haloperidol medication ate significantly less than the control and clozapine group, $p \leq 0.0083$ as significant.

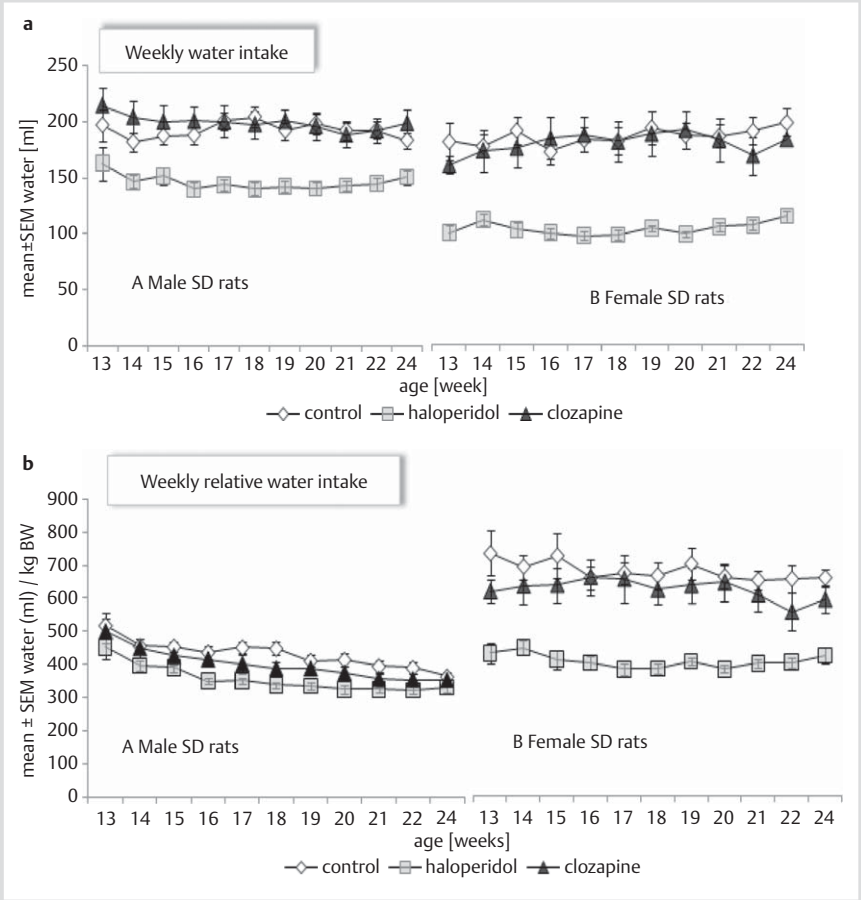

Fig. 4 Weekly water intake of male and female Sprague-Dawley rats: controls, haloperidol and clozapine treated animals (n = 10 for all groups). **a** Weekly water intake: We found no significant differences between control and clozapine-medicated animals. Animals under haloperidol medication drank less than the control and clozapine group, with $p \leq 0.0083$ as significant. **b** Weekly relative water intake based on 1 kg BW: Male animals showed a lightly decreased water intake from week 13 to week 25. Animals under haloperidol medication drank less than the control and clozapine group, with $p \leq 0.0083$ as significant.

Table 2 Weight of perirenal and gonadal fat pads, level of serum adiponectin, leptin, ghrelin, fasting glucose, insulin, HbA1c and HOMA IR after 12 week of haloperidol and clozapine medication of male and female Sprague Dawley rats (n = 10 per group).

	Fat tissue [%BW]	Adiponectin [µg/mL]	Leptin [ng/mL]	Ghrelin [ng/mL]	Fasting glucose [mg/dL]	Insulin [µg/L]	HOMA IR	HbA1c [%]
male control	5.43 ± 0.39	5.4 ± 0.4	2.7 ± 0.7	4.9 ± 1.1	200.2 ± 11.4	7.1 ± 0.1	0.87 ± 0.04	3.41 ± 0.06
female control	4.59 ± 0.39	6.0 ± 0.4	0.9 ± 0.1	6.5 ± 0.6	180.0 ± 7.8	7.5 ± 0.3	0.84 ± 0.05	3.01 ± 0.05
male haloperidol	48.9 ± 4.54	6.1 ± 0.4	2.5 ± 0.4	5.2 ± 0.8	213.1 ± 8.1	8.0 ± 0.5	1.05 ± 0.07	3.27 ± 0.08
female haloperidol	4.72 ± 0.38	6.5 ± 0.4	1.0 ± 0.1	6.7 ± 0.9	179.9 ± 8.2	8.7 ± 1.2	0.95 ± 0.1	2.80 ± 0.02*
male clozapine	6.52 ± 0.30	7.0 ± 0.5	3.7 ± 0.5	8.4 ± 1.2	216.5 ± 12.0	7.5 ± 0.3	1.00 ± 0.07	3.47 ± 0.09
female clozapine	4.86 ± 0.23	6.9 ± 0.7	1.2 ± 0.3	7.8 ± 0.6	245.6 ± 29.4	7.3 ± 0.6	1.11 ± 0.1	3.01 ± 0.09

*Significantly different to the control group ($p < 0.0083$)

Table 3 Serum levels of total cholesterol, HDL- and LDL-cholesterol, triglycerides after 12 week of haloperidol and clozapine medication of male and female Sprague-Dawley rats (n = 10 per group).

	Total cholesterol [mg/dL]	HDLcholesterol [mg/dL]	LDL-cholesterol [mg/dL]	Triglycerides [mg/dL
male control	114.0 ± 3.4	33.2 ± 0.8	60.2 ± 2.7	93.0 ± 7.4
female control	118.7 ± 2.3	37.7 ± 1.6	70.3 ± 1.7	53.8 ± 5.8
male haloperidol	104.3 ± 3.9*	31.8 ± 0.7	55.7 ± 4.4	84.4 ± 8.9
female haloperidol	101.7 ± 5.3	32.3 ± 1.1	57.3 ± 4.7	53.2 ± 6.5
male clozapine	127.8 ± 5.1	33.3 ± 1.4	75.5 ± 3.5*	94.7 ± 6.3
female clozapine	150.2 ± 6.5*	43.5 ± 1.2*	94.2 ± 5.4*	62.7 ± 5.6

*Significantly different to the control group ($p < 0.0083$

significantly decreased in haloperidol-fed rats ($p = 0.005$), and females always had significantly lower levels in all 3 groups (all $p < 0.0167$). Haloperidol-medicated rats showed a trend to increased insulin levels ($p > 0.0083$). The HOMA-IR was elevated either in clozapine or haloperidol groups (not significant, all $p > 0.0083$).

Level of total cholesterol, HDL- and LDL-cholesterol and triglycerides (Table 3)

There was a TREATMENT effect for total cholesterol [$F(2, 54) = 31.0$, $p < 0.0024$]. It was decreased in male haloperidol treated rats compared to controls and increased in female clozapine treated rats compared to the other 2 groups (all $p < 0.0083$). For HDL-cholesterol there were significant TREATMENT effects [$F(2, 52) = 14.1$, $p < 0.0024$, increased in the female clozapine group], and also sex-related effects [$F(1, 52) = 26.9$, $p < 0.0024$], since in clozapine-medicated rats females showed higher values than males ($p < 0.0167$). For LDL cholesterol, there were significant TREATMENT effects, as well [$F(2, 52) = 27.3$, $p < 0.0024$]. In male and female rats, values in the clozapine group were increased in comparison to the haloperidol and the control groups (all $p < 0.0083$).

There was a significant sex effect for triglyceride levels [$F(1, 54) = 37.2$, $p < 0.0024$]. Males always had a significant higher amount of triglycerides than females ($p = 0.001$ for controls, $p = 0.011$ for haloperidol and $p = 0.001$ for clozapine medication).

Discussion

After 6 weeks, the results of SD compared to Wistar rats [26] yielded a lower food conversion ratio (FCR), higher food conversion efficiency (FCE) and higher growth rate (GR) in SD rats (unpublished data). This concurs with the findings of Kühn et al. [42], namely that higher growth rate in SD rats is completely attributed to a significant higher food conversion compared to Wistar rats, since food intake of both groups was comparable. SD rats moreover showed higher body weight, fasting glucose, fasting insulin and HOMA-IR than Wistar rats [43]. The study of Chovan et al. [44], demonstrated different pharmacokinetics and metabolic profiles. Gao et al. [45] found intrinsic metabolic differences like higher individualized metabolic variations in fasting and feeding states, and a stronger ability to recover from an altered metabolic profile with less hepatic injury in SD compared to Wistar rats. A possible explication of the metabolic differences between the different strains and sexes could be the fact that clozapine had a lower metabolic conversion rate in male SD rats: male Wistar [29] and female SD rats, both exhibiting no robust weight gain or fat deposition, showed a high level of the metabolite N-desmethyl-clozapine, whereas male SD rats had an equal or higher level of clozapine. In patients, *N*-desmethylclozapine has higher half-life elimination and possibly contributes significantly to the atypical effects of clozapine treatment by blocking the same receptors. Hence in summary we regard SD as a more appropriate rat model for metabolic changes than Wistar rats.

In our study we found significant alterations of body weight in the drug-treated group of male SD rats. Food and water intake was decreased in the haloperidol group. The reducing effect of orally applied haloperidol on body weight of male and female rats can at least partially be explained by lower food and water intake and the sedative quality of the drug. But food and water intake cannot solely be the limiting factor of body weight gain. Female haloperidol-treated animals absorbed significantly less food without a relevant impact on body weight. Male rats treated with clozapine had a slightly lower food and water intake compared to controls with increased body weight and fat weight, but no increased FCR, FCE or growth rate (see **Table 1**). We therefore assume the increased body weight of male SD rats under clozapine medication to probably result from an increased fat deposition, although our findings show only a trend toward increased fat tissue. Moreover, end weight and fat tissue are positively correlated only in controls and rats exposed to haloperidol suggesting that a high amount of fat in clozapine treated rats is not removed. The study of Jassim et al. [46] could show that clozapine is primarily associated with effects on carbohydrate

and lipid metabolism in metabolically active peripheral tissues prior to the development of drug-induced weight gain. According to other studies [47] a direct and potent effect of clozapine on adipose tissue is not an important mechanism to induce metabolic disturbances in humans. In animal models antipsychotic-induced metabolic side effects are not necessarily associated with pronounced weight gain [33]. These findings comply with a clinical study showing that antipsychotic treatment can cause impaired glucose regulation independently of adiposity [48]. Several studies found no clozapine effect on food intake and body weight in male and female rats. For example, Choi et al. [25] applied the drug by constant infusion (10 mg/kg/day) on 60-day-old SD rats for 11 days. Cooper et al. [24] found no weight gain and hyperphagia but enhanced adiposity in female Wistar rats after clozapine injections (i.p., b.i.d.) over 21 days. In our study we used a 3-month treatment period possibly useful to find alterations in weight gain. Additionally, instead of daily injections, we offered food prepared with antipsychotics in order to establish a regular supply comparable to the human one with several daily doses to ensure a durable or constant blood level of antipsychotics [34]. Such as human patients treated with antipsychotics, not all rats exhibited abnormal weight gain and/or metabolic changes. But our sample might be too small to find significantly increased fat content after treatment. Despite their controlled origin, our animals showed a widespread variability in response to drug medication. Considering the genetic background, it has been revealed that different body weight phenotypes within outbred SD populations react differently to the development of drug- or diet-induced obesity via altered anabolic features in liver and hypothalamus [49] and can be obesity-prone (OP) or obesity-resistant (OR) [50]. The underlying mechanisms are yet not known and need further investigation.

Furthermore, haloperidol medication led to reduced water intake in rats, which possibly aggravates the observed symptoms of apathy and tiredness. Mechanisms underlying drug-influenced drinking behaviour and their role in metabolism are only dissatisfyingly investigated. There exist hints [51] on a modulating interaction between leptin and nitric oxide, which seems to have a thirst- and appetite-reducing effect itself [52].

Minet-Ringuet et al. [33] showed that in male SD rats haloperidol and olanzapine did not modify caloric intake or body weight, but significantly increased adipose tissue and circulating leptin. We found a marginal effect of haloperidol on adiponectin but not on the leptin level. Clozapine-treated rats showed an increased ghrelin level, whereas the leptin and adiponectin levels were also elevated in males under clozapine medication. As expected, leptin levels were positively correlated with percent fat mass in controls and haloperidol groups, but surprisingly not in the clozapine groups, suggesting that the leptin level in clozapine-medicated rats not only depends on fat mass, but possibly also on ghrelin or adiponectin secretion. Normally, ghrelin rises during fasting phases, but clozapine seems to increase plasma ghrelin independently of the nutritional state [53, 54]. Ghrelin again enhances white adipose tissue leptin production by a direct receptor-mediated effect [55]. Obese rats generally have reduced adiponectin serum levels, but clozapine might additionally influence adiponectin regulation regardless of fat content, as verified in human patients [56]. Hyperleptinemia was independently associated with clozapine treatment in human patients [57]. According to these results we found higher leptin and adiponectin levels in male rats under clozapine medication. Clozapine did not affect fat content in female rats. Respectively medicated, they in fact suffered from hyperglycemia which led to an elevated HOMA-IR. Contrastingly, the increased HOMA-IR in the haloperidol group was not caused as a result of high glucose level but rather by high insulin level. Following changes in glucose metabolism for at least 8 weeks, HbA1c represents the blood level of glucose, which was not used in metabolism. But decreased HbA1c-level under haloperidol medication in female rats, less pronounced in males, is probably due to the anemia (decreased levels of erythrocytes, hemoglobin and hematocrit; unpublished data) in these animals. As to humans, Emsley et al. [58] found an elevated HbA1c level in schizophrenia patients treated with haloperidol. In human patients haloperidol causes only minimal changes in erythrocyte counts [59] thus exacerbating comparisons between human conditions and animal models.

Dysglycemia-like pathological fasting glucose levels or insulin resistance are risk factors for the metabolic syndrome, and impaired glucose tolerance can be found in clozapine- but not halperidol-treated patients [60]. Fortifying our results in an animal model, Tulipano et al. [32] proved clozapine to induce sex-related alterations of glucose homeostasis with the significant highest serum glucose level and reduced insulin sensitivity in female rats without effects on food intake. Impaired peripheral glucose use and/or increased glucose output from the liver might be responsible for the hyperglycemia in clozapine-treated females. It has indeed been shown that clozapine impairs glucose metabolism not by direct induction of insulin resistance but rather acts via an increase in glucagon secretion [61]. Thus clozapine possibly stimulates hepatic glucose production. A limiting factor of our study is the fact that the experimental setup is not well designed for detecting antipsychotic-induced glucose dysregulation, which would require that the system is challenged, e.g., by glucose tolerance tests and/or by hyperinsulinemic clamp experiments. However, the mechanisms underlying the resistance of male rats to clozapine-induced hyperglycemia need to be further investigated. As outlined by Best et al. [62], clozapine has no effect on basal insulin release but significantly inhibits glucose-induced secretion. In contrast, the depolarizing action on pancreatic β-cell-membrane of haloperidol might be expected to enhance insulin release. Along with our findings we suppose haloperidol and clozapine to differently influence the glucose metabolism.

Total cholesterol and LDL-cholesterol were increased in both clozapine groups, but decreased in the haloperidol groups. HDL-cholesterol was enhanced in females medicated with clozapine and reduced in the female haloperidol group. Dyslipidemia is a risk factor for the metabolic syndrome, and increased total cholesterol plus LDL-cholesterol are involved in the etiology of coronary heart diseases. Our findings of dyslipidemia match clinical studies, showing that clozapine is associated with an increase in cholesterol levels [63] in schizophrenia patients.

We did not find significant changes in the level of triglycerides. Compared to cholesterol, triglyceride concentrations show a high variability and can be nutritionally affected. Kimura et al. [64] discovered that time and intake amounts of fat influence triglyceride levels. So we assume that triglyceride levels, which presumably remain unchanged by antipsychotic drug application, are caused by the animals' overnight fasting, the time period in which rats normally have the main food intake.

However, our study entails some limitations. The depicted results appear to critically depend on the experimental procedures, especially in terms of drug application, duration of medication, rat strains used, diet and drug doses applied. Drug administration via feeding compared to gavage or injections is the least-invasive application procedure, reduces handling stress and allows the exact determination of drug, food and water intake. But oral application requires social isolation of the test animals. Social isolation, even if regarded as a minor stress factor, can change parameters of the hypothalamo-pituitary-adrenal axis (HPA axis) like corticosterone release, involved in the glucose homeostasis [65], inhibit oxidative metabolism [66], and stimulate hippocampal estradiol synthesis [67]. These effects need further investigation. In order to determine the fat content of each animal, we excised the perirenal (retroperitoneal), the epididymal and periovarian fat pads, but not the mesenteric and subcutaneous fat, which hardly can be completely removed [68]. Although this omission admittedly curtails our analysis, the procedure avoids erroneous fat removal. To determine fasting glucose, our test animals fasted 12 h (dark period) before blood withdrawal. Fasting [69] can decrease the degree of obesity induced by high fat diet, can change the effect of leptin or insulin, and can cause serious disturbance of neuroendocrine peptides. Our animals were fed with ground pellets containing 15% additional fat in the dry matter. Weight gain, fat mass, plasma glucose, cholesterol, triglyceride, leptin and insulin increase dose-dependently with increased intake of dietary fat [70]. The impact of these parameters on the results of our study needs further investigation.

Despite these limitations, we could show significant sex- and treatment-dependent differences. But the underlying mechanisms, mainly the effect of estrogens, remain unclear and should be the target of further studies. In our study we have disregarded the estrus of females, although activity, food and water intake, and body weight vary across the ovarian cycle [71].

In summary, our animal model suggests chronic antipsychotic drug medication over a 12-week test period to possibly influence body weight gain, fat tissue metabolism and the level of related hormones, to cause dyslipidemia, hyperglycemia or hyperinsulinemia with sex-related impact in SD rats. All these effects are risk factors for the metabolic syndrome. Considering the explorative design for activity counts, blood analyses and ELISA tests, our findings offer no conclusive evidence for a causal relationship resulting from multiple testing. Thus this study should be replicated in an independent larger sample to confirm our relevant positive findings. And it should be supplemented by collateral investigations in human patients to clarify effects related to the metabolic syndrome.

Acknowledgements

We thank Dr. A. Treiber, former director of the TVA, Heinrich-Heine-Universität, Düsseldorf and her assistants for excellent support regarding the animal experiments. Dr. M. Jänner, LVR Klinikum, Düsseldorf, kindly shared her expertise in statistical analysis of our results. Margit Geisler, LVR Klinikum, Düsseldorf, kindly carried out the ELISA tests and the determination of serum drug levels by HPLC.

Conflict of Interest

The authors declare that there is no conflict of interest.

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