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Original Article

# Chronic effects of clozapine administration on insulin resistance in rats: Evidence for adverse metabolic effects

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ABSTRACT

Chronic treatment with the atypical antipsychotics clozapine has been associated with an increased risk for deterioration of glucose homeostasis, leading to hyperglycemia and insulin resistance diabetes. The present study mainly aimed to investigate possible mechanisms underlying clozapine-induced hyperglycemia. Male Wistar albino rats were randomly divided into two groups (each consists of 12 rats). The first group received clozapine orally at a dose of 10 mg/kg body weight daily for 6 weeks, while the other group received the drug vehicle only and served as the control group. At the end of the six weeks, hyperglycemia, hyperinsulinemia and insulin resistance, as indicated by Homeostatic model assessment of insulin resistance (HOMA-IR), were observed in the clozapine group as compared with the control group. This disturbance in glucose regulation was associated with non-significant changes in body weight, serum cortisol level, and hepatic glycogen content. The Clozapine group showed a significant increase in hepatic phosphorylase activity and in the gene expression level of hepatic glucose-6-phosphatse (G6Pase) enzymes compared to the control group. It can be concluded that clozapine-induced hyperglycemia and insulin resistance occur in a manner mostly independent of weight gain, and may be attributed to an increase in hepatic phosphorylase activity and increased expression level of G6Pase.



## Introduction

Antipsychotic drugs (APDs) are widely used for the treatment of schizophrenia [1,2]; however, they have a number of serious adverse effects that may limit their clinical utility. The older conventional sector of these drugs, represented by fluphenazine and haloperidol, produces extrapyramidal symptoms in many patients like Tardive dyskinesia [3]. The newer sector, referred to as the atypical form, represented by clozapine, may alter metabolic function and induce diabetes and weight gain during treatment [4,5].

Clozapine was the first atypical antipsychotic drug to be introduced in clinical use in several European countries in the late 1960s [6]; since then, the drug has become available almost worldwide. For many years, clozapine has virtually been the only psychopharmacological choice for patients with schizophrenia who did not respond to typical neuroleptics [7].

As reported previously, the causes and possible mechanisms for hyperglycemia are due to impairment of insulin action and insulin resistance [8], or may be due to certain defects of metabolic pathways causing either glycogenesis decrease or accelerated glycogenolysis. It has been indicated that the most common type of insulin resistance is associated with overweight, reaching 4–12 kg in 13–85% of patients, and obesity in a condition known as metabolic syndrome [9]. Although adiposity can be considered as a reasonable factor for glucoregulatory disturbances [10], several studies have demonstrated that antiphsycotic treatment could cause impaired glucose regulation independent of adiposity, as non-obese patients on clozapine and olanazapine still display significant insulin resistance [11,12].

Clozapine was found to accumulate in liver tissue, reaching certain concentrations up to 10 umol or higher, which are several folds higher than plasma levels [13]. A recent study indicated that clozapine and olanzapine can disturb the metabolism by direction of body mechanism to extract energy from fat instead of carbohydrate. This, in turn, may lead to a high level of carbohydrates and the development of insulin resistance [14]. Therefore, the association between antiphsychotic medications and the new onset or worsening of existing diabetes mellitus is difficult to interpret,

*Abbreviations:* APDs, antipsychotic drugs; HOMA-IR, homeostatic model assessment of insulin resistance; 5-HT, 5-hydroxytryptaine; G6Pase, glucose-6-phosphatase.

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and evidence for the causative association is non-conclusive [15].

Endogenous glucose production usually results from glycogenolysis and gluconeogenesis. Glycogenolysis represents glycogen breakdown to G-6-phosphate (G-6-P) and glucose via phosphorolysis by the enzyme glycogen phosphorylase [16], which takes place in muscle cells and liver tissues in response to hormonal and neural signals. In myocytes, glycogen degradation serves to provide an immediate source of G-6-P for glycolysis to provide energy for muscle contraction [16]. In hepatocytes, the main purpose of glycogen breakdown is the release of glucose into the blood stream when the blood glucose level drops, as it does between meals for uptake by other tissues. The phosphate group of G-6-P is removed by G-6-phosphatase (G6Pase), which is not present in myocytes [16].

Hormones such as epinephrine, insulin and glucagon regulate glycogen phosphorylase using a secondary messenger amplification system that is linked to G proteins. Gluconeogenesis is induced in both the liver and intestine by increased cAMP levels [17]. G6Pase is the key enzyme for gluconeogenesis in the liver and intestine. Accordingly, both enzymes, namely glycogen phosphorylase and G6Pase, have a major impact on glucose production.

Therefore, the present study generally focuses on certain factors affecting their functions, which may provide an explanation for how clozapine can induce hyperglycemia and insulin resistance.

## Materials and methods

### *Drugs and chemicals*

Clozapine (clozapex®) was kindly provided by Apex Pharma, Badr city, Cairo, Egypt. All other chemicals were of pure analytical grade.

### *Experimental design*

Adult male albino rats of Wistar strain weighing 150 ± 10 g (10–12 weeks old) were used for the present study. They had free access to water and food throughout the experimental period. The local committee approved the design of the experiments. After an adaptation period of two weeks, the animals were randomly divided into two groups. Each consisted of 12 rats as follows: one group received clozapine orally at a dose of 10 mg/kg body weight daily for 6 weeks [18,19]. The drug was suspended in 0.5% gum acacia and freshly delivered daily to rats. Another group of rats received the vehicle of clozapine throughout the course of the study and served as a normal control group.

At the end of the sixth week and immediately before scarification, the body weight of each rat was determined. The animals were sacrificed by decapitation. Blood samples were collected and centrifuged to separate serum. Serum glucose level was freshly determined, while remaining samples were kept at −30°C until insulin and cortisol levels were assayed. Liver samples were excised, rinsed in ice-cold saline, and dried. The first portion was quickly frozen at −30°C for the assessment of glycogen phosphorylase activity and gene expression level of G6Pase. Another portion was processed instantly for the determination of the glycogen level, where 0.1 g of liver was homogenized with 5 ml tricholoracetic acid (TCA), centrifuged, boiled in a boiling water bath for 15 min, cooled, centrifuged and the supernatant was collected and stored at − 30°C for assay of hepatic glycogen content.

### *Determination of serum glucose, insulin and cortisol levels*

The serum level of glucose was assayed using commercially available Spinreact kits (Spain), according to the methods of Trinder [20] and Abraham et al. [21], respectively. The serum insulin level was measured by immunoradiometric assay using kits provided by Immunotech, France. The serum cortisol level was determined according to the method of De Souza and Van Loon [22] and Kant et al. [23] using commercially available cortisol rat ELISA kit (EIAb, China).

Homeostatic model assessment of insulin resistance (HOMA-IR) was determined according to the formula of Matthews et al. [24] and Shoji et al. [25] as follows:

$$\frac{\text{Fasting glucose level (mg/dl)} \times \text{fasting insulin (mU/ml)}}{405}$$

### *Determination of hepatic glycogen content and glycogen phosphorylase activity*

The hepatic glycogen content was determined according to the method of Kemp and Van Heijningen [26]. The methods depend on that heating of glycogen solution with concentrated sulphuric acid induces dehydration of the former and the development of pink color, the intensity of which is proportional to the hepatic glycogen content using 1 g% stock glycogen standard solution. Hepatic glycogen content was expressed as mg glycogen/g liver.

Liver glycogen phosphorylase activity was determined according to the method of Hers and Van hoof [27]. The method depends on the action of liver glycogen phosphorylase on glucose 1-phosphate splitting it into glucose and inorganic phosphate, which is then estimated colorimetrically. Activity of glycogen phosphorylase was expressed as mM phosphate liberated/min/g liver.

### *Quantitative reverse transcription polymerase chain reaction (RT-PCR) analysis of hepatic G6Pase*

PCR was used to quantify the expression of mRNA for hepatic G6Pase. The gene expression of beta-actin was measured as a standard housekeeping gene. Total RNA was separated from liver, based on the method of Chomczynski and Sacchi [28]. The yield of total RNA obtained was determined spectrophotometrically at 260 nm. First-strand cDNA was synthesized using 10 μl RNA using a first strand cDNA synthesis kit (Stratagene USA).

The primers used were: G6Pase (F 5′-CCACCCAGGGCACGAG-3′; R 5′-GGTGCCAGCCTCATCTATTG-3′); beta actin (F 5′-TCACCCTG-AAGTACCCCATGGAG-3′; R 5′-TTGGCCTTGGGGTTCAGGGGG-3′). Primers were designed with the Primer3-Blast software (NCBI, USA). All primers were synthesized by the Midland Certified Reagent Company Inc. (Midland, Texas, USA). The amplification was performed, and typical profile times used were denaturation at 95 °C for 1 min, followed by annealing at 63 °C for 1 min, elongation at 72 °C for 2 min for 40 cycles with additional 10-min incubation at 72 °C after completion of the last cycle.

### *Semi-quantitative determination of PCR products*

The PCR products were electrophoretically resolved on 2% agarose–TBE gel containing 0.5 mg/mL ethidium bromide and run for 1.5 h at 8 V/cm. Semi-quantitation was performed using the gel documentation system (BioDO, Analyser) supplied by Biometra. The relative expression of each studied gene (R) was calculated following the formula:

$$R = \frac{\text{Densitometrical Units of each studied gene}}{\text{Densitometrical Units of}\beta\text{-actin}}$$