



Article

# Clozapine Worsens Glucose Intolerance, Nonalcoholic Fatty Liver Disease, Kidney Damage, and Retinal Injury and Increases Renal Reactive Oxygen Species Production and Chromium Loss in Obese Mice

Geng-Ruei Chang <sup>1,†</sup>, Hsien-Yueh Liu <sup>2,†</sup>, Wei-Cheng Yang <sup>3</sup>, Chao-Min Wang <sup>1</sup>, Ching-Fen Wu <sup>1</sup>, Jen-Wei Lin <sup>2</sup>, Wei-Li Lin <sup>2,4</sup>, Yu-Chen Wang <sup>5-8</sup>, Tzu-Chun Lin <sup>1</sup>, Huei-Jyuan Liao <sup>1</sup>, Po-Hsun Hou <sup>9,10,\*</sup>, Chee-Hong Chan <sup>11,\*</sup>, and Chuen-Fu Lin <sup>12,\*</sup>

**Citation:** Chang, G.-R.; Liu, H.-Y.; Yang, W.-C.; Wang, C.-M.; Wu, C.-F.; Lin, J.-W.; Lin, W.-L.; Wang, Y.-C.; Lin, T.-C.; Liao, H.-J.; et al. Clozapine Worsens Glucose Intolerance, Nonalcoholic Fatty Liver Disease, Kidney Damage and Retinal Injury and Increases Renal Reactive Oxygen Species Production and Chromium Loss in Obese Mice. *Int. J. Mol. Sci.* **2021**, *22*, 6680. <https://doi.org/10.3390/ijms22136680>

Academic Editor: Abdelkrim Hmadcha

Received: 28 April 2021

Accepted: 18 June 2021

Published: 22 June 2021

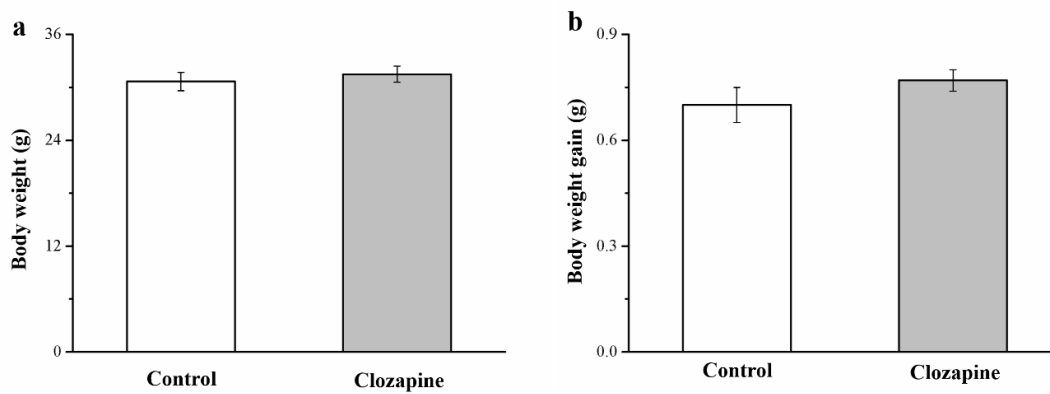
**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



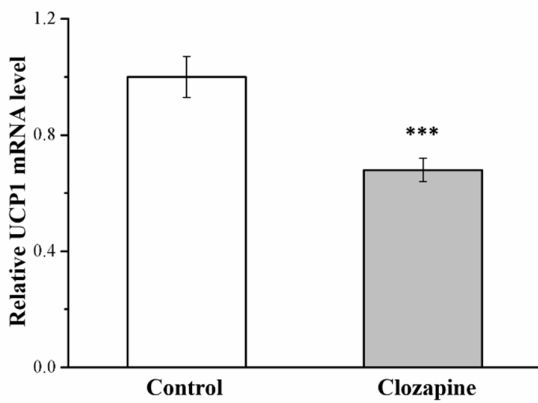
**Copyright:** © 2021 by the author. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).

- <sup>1</sup> Department of Veterinary Medicine, National Chiayi University, 580 Xinmin Road, Chiayi 60054, Taiwan; grchang@mail.ncyu.edu.tw (G.R.C.); leowang@mail.ncyu.edu.tw (C.M.W.)
  - <sup>2</sup> Bachelor Degree Program in Animal Healthcare, Hungkuang University, 6 Section, 1018 Taiwan Boulevard, Shalu District, Taichung 433304, Taiwan; lhy\_vet@hk.edu.tw (H.Y.L.);
  - <sup>3</sup> School of Veterinary Medicine, National Taiwan University, 4 Section, 1 Roosevelt Road, Taipei 10617, Taiwan; yangweicheng@ntu.edu.tw (W.C.Y.)
  - <sup>4</sup> General Education Center, Chaoyang University of Technology, 168 Jifeng Eastern Road, Taichung 413310, Taiwan; (W.L.L.)
  - <sup>5</sup> Division of Cardiology, Asia University Hospital, 222 Fuxin Road, Wufeng District, Taichung 41354, Taiwan; richard925068@gmail.com (Y.C.W.)
  - <sup>6</sup> Department of Medical Laboratory Science and Biotechnology, Asia University, 500 Lioufeng Road, Wufeng District, Taichung 41354, Taiwan; richard925068@gmail.com (Y.C.W.)
  - <sup>7</sup> Division of Cardiovascular Medicine, China Medical University Hospital, 2 Yude Road, North District, Taichung 404332, Taiwan; richard925068@gmail.com (Y.C.W.)
  - <sup>8</sup> College of Medicine, China Medical University, 91 Hsueh-Shih Road, North District, Taichung 404333, Taiwan; richard925068@gmail.com (Y.C.W.)
  - <sup>9</sup> Department of Psychiatry, Taichung Veterans General Hospital, 4 Section, 1650 Taiwan Boulevard, Taichung 40705, Taiwan;
  - <sup>10</sup> Faculty of Medicine, National Yang-Ming University, 2 Section, 155 Linong Street, Beitou District, Taipei 11221, Taiwan; (P.H.H.)
  - <sup>11</sup> Division of Nephrology, Chang Bing Show Chwan Memorial Hospital, 6 Lugong Road, Lukang Township, Changhua 505029, Taiwan; cheehong.chan@gmail.com (C.H.C.)
  - <sup>12</sup> Department of Veterinary Medicine, College of Veterinary Medicine, National Pingtung University of Science and Technology, 1, Shuefu Road, Neipu, Pingtung 912301, Taiwan; cflin2283@mail.npust.edu.tw (C.F.L.)
- † These authors contributed equally to this work.  
\* Correspondence: Tel.: +886-4-23592525 (P.H.H.); +886-975-617071 (C.H.C.); +886-8-7703202 (C.F.L.)

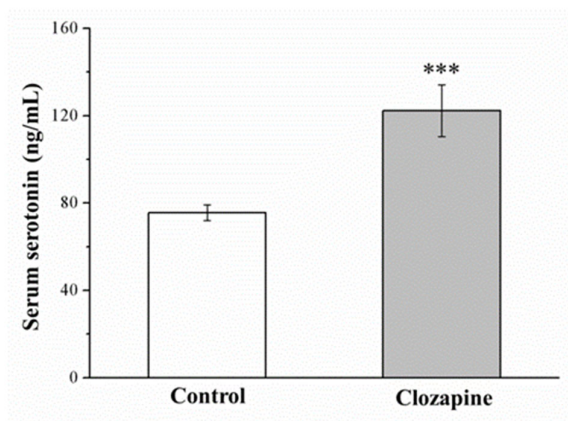
## Supplementary Figures.



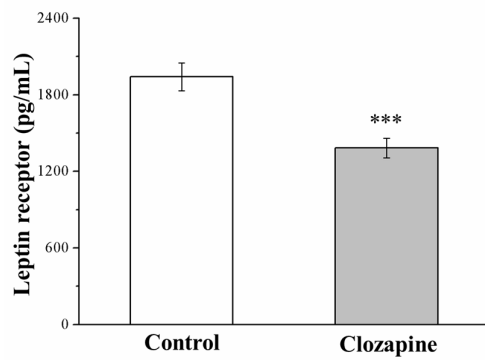
**Figure S1.** Effects of clozapine (2 mg/kg/day) on body weight and weekly weight gain measured in mice fed a standard diet and receiving control and clozapine treatment for 8 weeks.



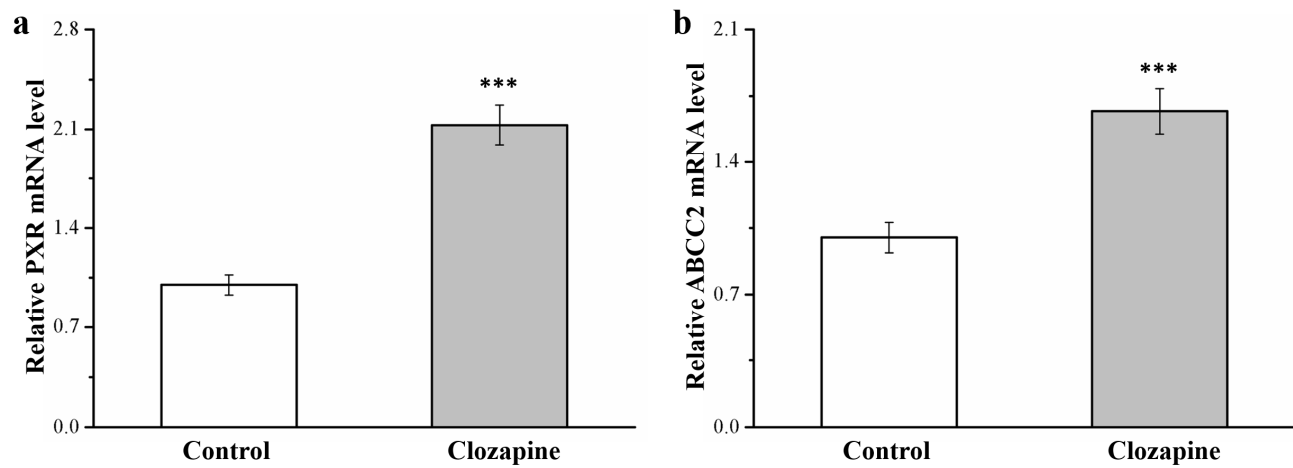
**Figure S2.** UCP1 mRNA expression measured in brown adipose tissue from the control and clozapine groups (all fed an HFD). \*\*\* $p < 0.001$ .



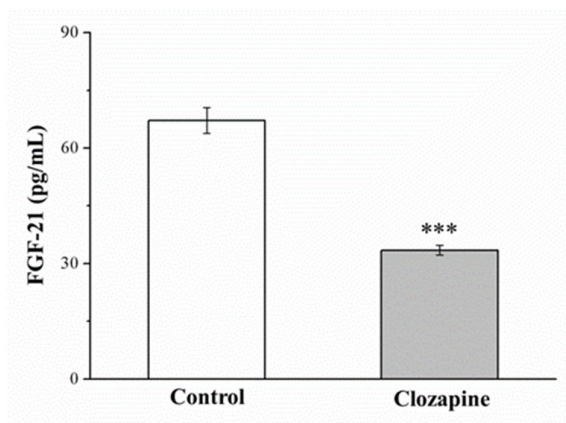
**Figure S3.** Serum levels of serotonin determined for the control and clozapine (receiving 2 mg/kg/day) groups. \*\*\* $p < 0.001$ .



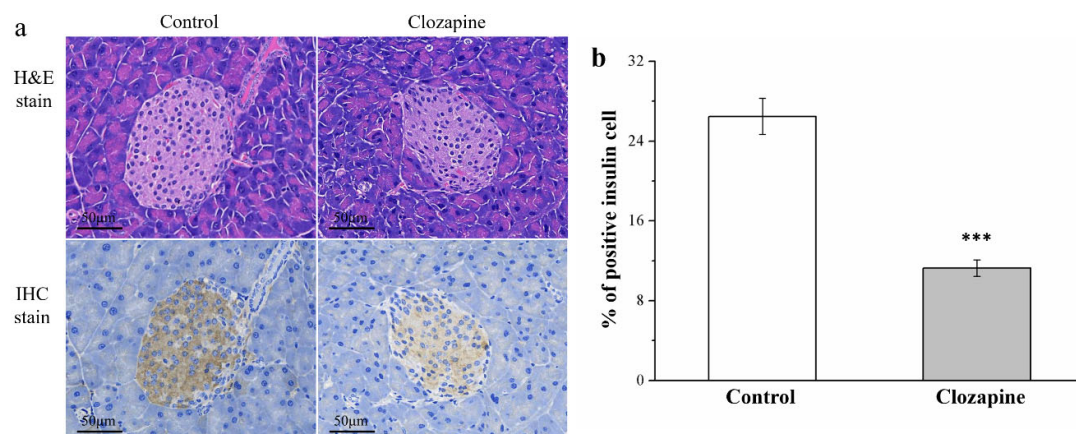
**Figure S4.** Serum levels of soluble leptin receptor determined for the control and clozapine (receiving 2 mg/kg/day) groups. \*\*\* $p < 0.001$ .



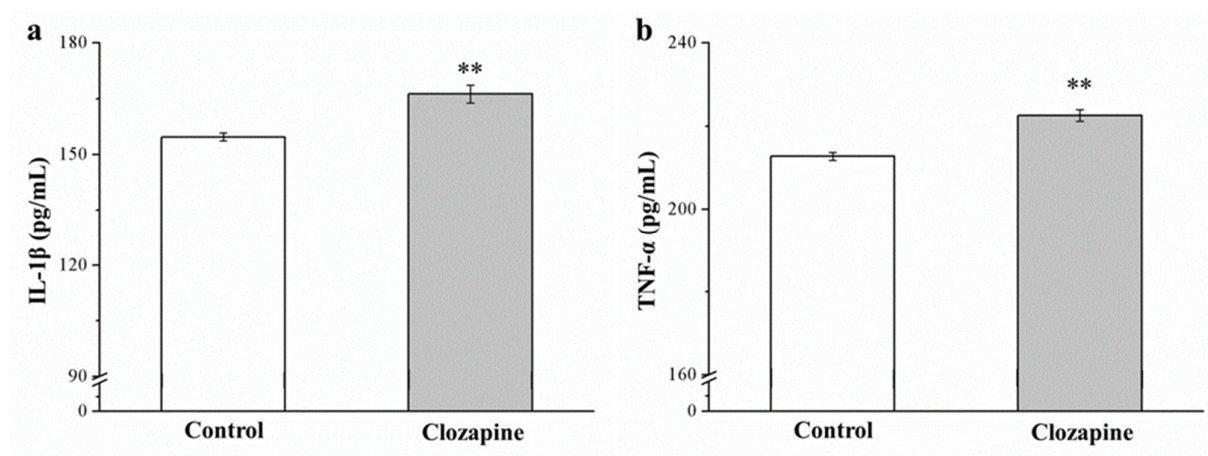
**Figure S5.** (a) PXR and (b) ABCC2 mRNA levels in the livers determined for the control and clozapine (receiving 2 mg/kg/day) groups. \*\*\* $p < 0.001$ .



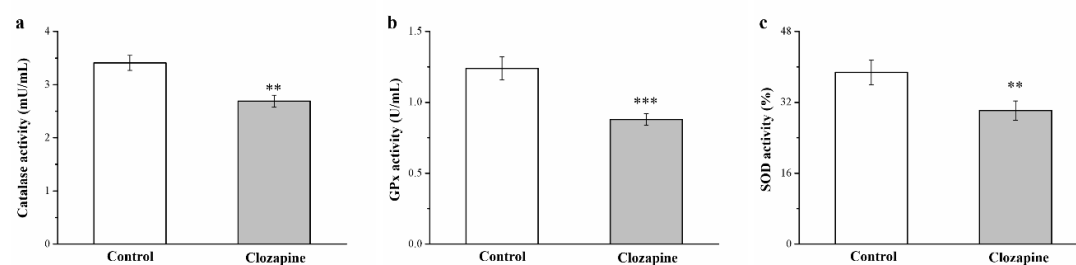
**Figure S6.** Serum levels of FGF-21 determined for the control and clozapine (receiving 2 mg/kg/day) groups. \*\*\* $p < 0.001$ .



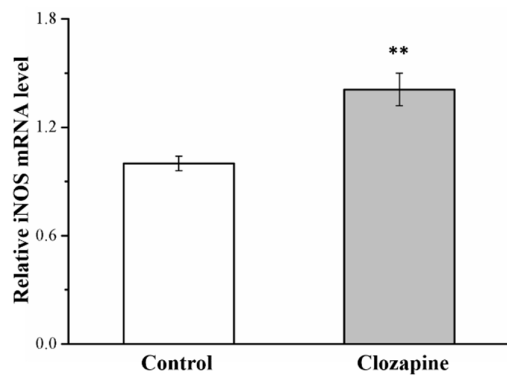
**Figure S7.** (a) H&E and immunohistochemical stains (magnification, 200×) showing islet morphology and (b)  $\beta$ -cell percentage of the control and clozapine (receiving 2 mg/kg/day) groups. \*\*\* $p$  < 0.001.



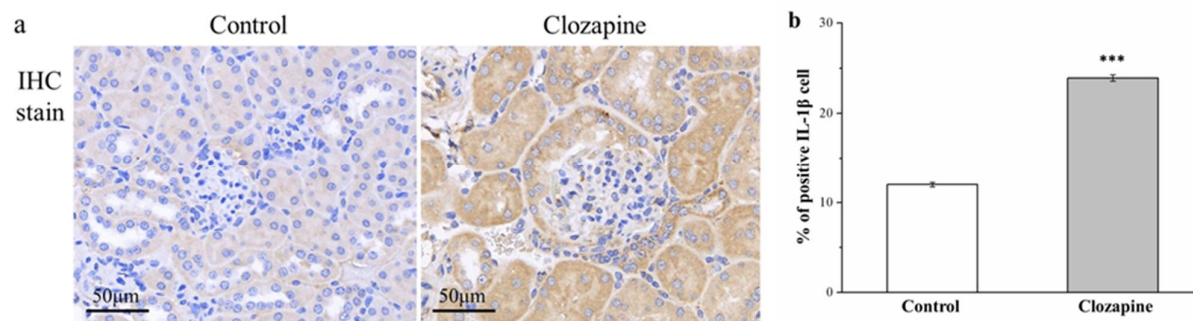
**Figure S8.** Serum levels of (a) TNF- $\alpha$  and (b) IL-1 $\beta$  determined for the control and clozapine (receiving 2 mg/kg/day) groups. \*\* $p$  < 0.01.



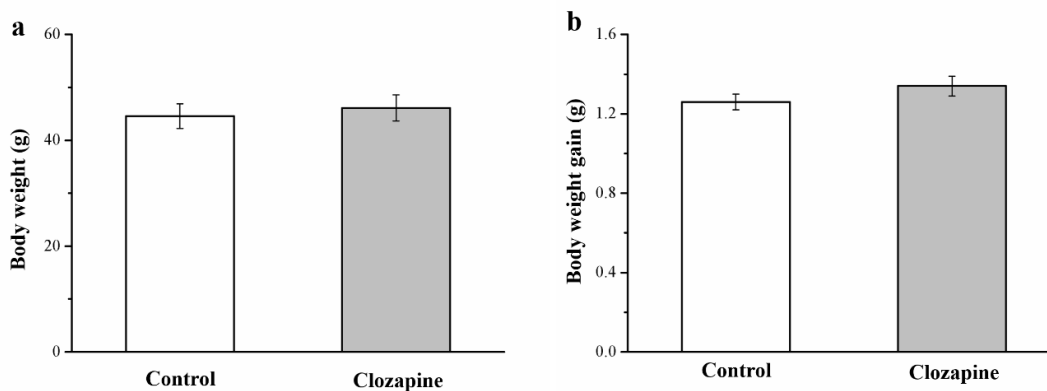
**Figure S9.** Pancreatic (a) catalase, (b) GPx, and (c) SOD activities in the control and clozapine (receiving 2 mg/kg/day) groups. \*\* $p$  < 0.01 and \*\*\* $p$  < 0.001.



**Figure S10.** *iNOS* mRNA levels in the renal determined for the control and clozapine (receiving 2 mg/kg/day) groups. \*\* $p < 0.01$ .



**Figure S11.** (a) Immunohistochemical stain (magnification, 200 $\times$ ) and (b) renal IL-1 $\beta$  levels of the control and clozapine (receiving 2 mg/kg/day) groups. \*\*\* $p < 0.001$ .



**Figure S12.** (a) Body weight and (b) weekly weight gain determined for the control and clozapine (receiving 1 mg/kg/day) groups.