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Schizophrenia Research

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## Letter to the Editor

### Ketogenic diet reverses behavioral abnormalities in an acute NMDA receptor hypofunction model of schizophrenia

Dear Editors,

Recent transcriptomic, proteomic and metabolomics studies suggest that abnormal glucose and energy metabolism may underlie the pathophysiology of schizophrenia (Harris et al., 2013). We hypothesized that interventions that influence energy metabolism might be therapeutically beneficial. One such intervention is the high-fat/low-carbohydrate ketogenic diet (KD) that has been effectively used in drug-resistant epilepsies (Paoli et al., 2013). To test our hypothesis we fed mice with KD for 3 weeks and induced acute NMDA receptor hypofunction by MK-801 (dizocilpine) administration to model the hypo-glutamatergic state that has been hypothesized to contribute to schizophrenia (Amann et al., 2010). We measured psychomotor hyperactivity and stereotyped behavior, social withdrawal and working memory deficits, reflecting the positive, negative and cognitive symptoms of schizophrenia, respectively (Jones et al., 2011).

Male C57BL/6 mice (~7 weeks old at the beginning of the experiment) were used. Ketogenic diet (SF14-063, Specialty Feeds, WA, Australia) or standard mouse diet (SD; Goldmix Stockfeeds, Lismore, NSW, Australia) were fed to mice for 3 weeks (Supplementary materials 1). Body weights were measured before the initiation of the diet and weekly afterwards. MK-801 (dizocilpine, Sigma-Aldrich, St. Louis, USA) or 0.9% NaCl was administered intraperitoneally (i.p.) 30 min before behavioral testing. Psychomotor activity, stereotyped behavior and ataxia were measured in an open field arena (420 × 420 × 420 mm). Social interaction was measured in the open field arena following the open field test. A target mouse, matched by age, size/weight and gender, and derived from a cage different from that of the test animal, was placed in the center zone and both animals were free to interact for five minutes. Anogenital sniffing, social avoidance and percentage time spent in social contact were measured. Video-recorded locomotor activity was analyzed using TopScan Light® (Clever Sys Inc., Reston, Virginia, USA) and stereotyped behavior (grooming), ataxia and social interaction were scored by two trained investigators blind to the treatment condition (Supplementary materials 2). Spatial working memory was measured by using the continuous spontaneous alternation task in a Y-maze (210 × 70 × 155 mm). An alternation was defined as entering all three arms consecutively. The percent alternation was calculated by the following equation: % Alternation = (Number of alternations / Total number of arm entries – 2) × 10. Plasma was obtained after centrifugation of trunk blood to analyze glucose (AU 480 Chemistry System as per manual instructions, Beckman Coulter) and the ketone body, β-hydroxybutyrate (BHB; colorimetric assay kit per manufacturer's instructions, Cayman Chemical Company, MI, USA). Statistical analysis was performed using the SPSS version 22 software package (IBM SPSS Statistics) using a two-way ANOVA followed by a Tukey's test for post-hoc comparison. Glucose and BHB levels were analyzed using Student's t-test. A  $p < 0.05$  was

considered significant. All data are displayed as Mean ± Standard error of mean (SEM).

The body weight of the KD animals was at all times lower compared to SD animals (Fig. 1A). KD mice had significantly increased BHB and decreased glucose (Fig. 1B) levels compared to the SD. KD attenuated the low-dose MK-801-induced hyperactivity, stereotyped behavior and ataxia (Table 1 in Supplementary materials 3; Fig. 1D, F, G). The social interaction and spatial working memory impairment induced by MK-801 were normalized by KD (Table 1 in Supplementary materials 3; Fig. 1H–L). The lack of correlation between MK-801-induced hyperactivity (combined grooming and ataxia or hyperactivity in the Y-maze) and social interaction or working memory (SD, social interaction:  $r = -0.252$ ;  $p = 0.37$ ; working memory:  $r = 0.196$ ;  $p = 0.52$  and KD, social interaction:  $r = -0.378$ ;  $p = 0.15$ ; working memory:  $r = -0.417$ ;  $p = 0.16$ ) argues against the suggestion that KD primarily interferes with the MK-801-induced excessive stereotypy, which may result in an indirect, non-specific effect on social interaction and working memory deficits.

Here we demonstrated, for the first time, that ketogenic diet normalized pathological behaviors in an animal model of schizophrenia. Furthermore, weight loss, elevated β-hydroxybutyrate and decreased glucose levels indicate that metabolic adaptation occurred in KD-fed mice. This proof-of-concept study did not directly address potential mechanisms of action of the KD in schizophrenia. However, we know that abnormal glutamate neurotransmission (Coyle et al., 2012), GABA hypofunction (Frankle et al., 2015) and severe disturbances of glucose metabolism (Martins-de-Souza et al., 2011) may underlie the pathophysiology of schizophrenia. Recent studies revealed that KD decreases brain glutamate and increases GABA levels and provides alternative energy substrates through ketone bodies and the β-oxidation of fatty acids bypassing glycolysis (Lutas and Yellen, 2013). Therefore, we suggest that KD may exert its beneficial influence through the above multiple mechanisms to normalize underlying pathophysiological processes in schizophrenia. As KD has been safely and effectively administered to humans in different pathological conditions (Rho and Stafstrom, 2012), while our findings require confirmation in other animal models, the present results has the potential to be swiftly translated into a novel, safe and effective management of schizophrenia.

#### Conflict of interest

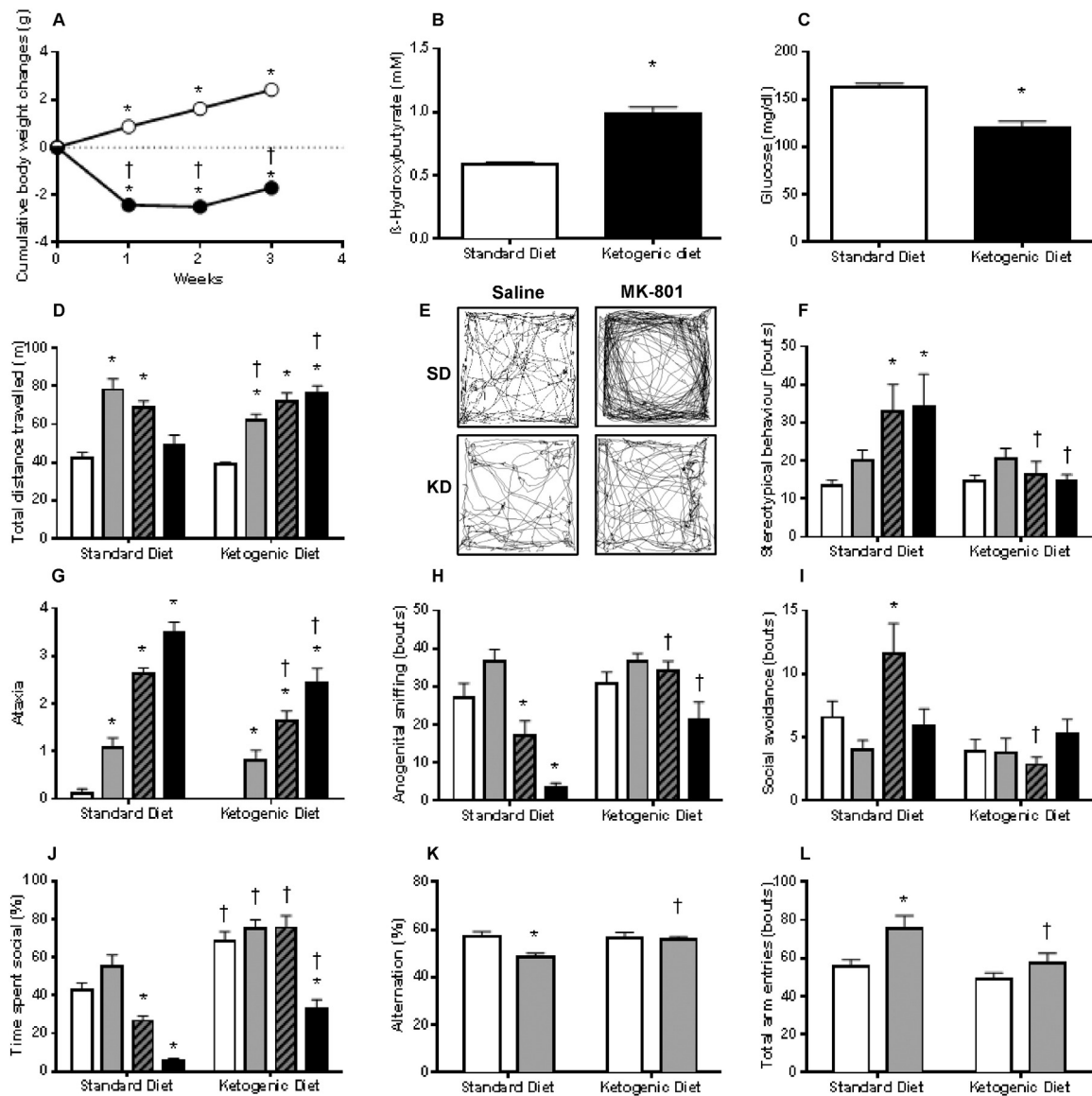
The authors declare no conflict of interest.

#### Contributors

ZS conceived the idea, designed the study and analyzed some of the data. AKK carried out the behavioral studies, analyzed the data and wrote the first draft of the manuscript. HL and BCL assisted with behavioral data analysis and DR helped with the biochemical assays. All authors contributed to and have approved the final manuscript.

#### Acknowledgements

We thank Mr. Scott Blyth and Ms. Jennifer Stanford, who kindly provided support and assistance in the animal facility and members of the Laboratory of Psychiatric Neuroscience for the helpful discussions.



**Fig. 1.** Effects of KD on metabolic parameters and MK-801-induced behaviors in mice. A) Effect of SD (standard diet; open circles) and KD (ketogenic diet; closed circles) on cumulative body weight changes over three weeks. Analysis of body weight changes revealed a significant main effect of Diet ( $F_{(1,54)} = 337.06$ ;  $p < 0.001$ ), Time ( $F_{(2,108)} = 23.12$ ;  $p < 0.001$ ), and a marginally significant Diet  $\times$  Time interaction ( $F_{(2,108)} = 2.92$ ;  $p = 0.05$ ). SD animals showed significantly increased ( $p < 0.001$ ; A), while the KD significantly decreased ( $p < 0.001$ ; A) weight. B) KD (ketogenic diet; closed bar) increased ( $t_{(1,30)} = 5.51$ ;  $p < 0.001$ ) plasma  $\beta$ -hydroxybutyrate levels compared to SD (standard diet; open bar); C) KD decreased ( $t_{(1,20)} = -7.00$ ;  $p < 0.001$ ) plasma glucose levels; D) Effects of Standard Diet and Ketogenic Diet on MK-801-induced locomotor hyperactivity; E) Representative tracks in the open-field in SD (Standard Diet) and KD (Ketogenic Diet) mice in response to saline or MK-801 (0.1 mg/kg) administration, note that the MK-801-induced hyperactivity in the SD animal is nearly normalized in the KD mouse; F) Effects of Standard Diet and Ketogenic Diet on MK-801-induced stereotyped grooming behavior; G) Effects of Standard Diet and Ketogenic Diet on MK-801-induced ataxia; when considering D to G that lower MK-801 dose (0.1 mg/kg) induced hyperactivity, while further increasing the dose (0.2 and 0.4 mg/kg) resulted in stereotypy (F) and ataxia (G), that were attenuated in the KD animals compared to SD mice, shifting the high-dose MK-801 (0.4 mg/kg) effect toward hyperactivity (D); H) Effects of Standard Diet and Ketogenic Diet on MK-801-induced changes in anogenital sniffing activity; I) Effects of Standard Diet and Ketogenic Diet on MK-801-induced social avoidance; J) Effects of Standard Diet and Ketogenic Diet on MK-801-induced social interaction deficit as measured by decreased percent time spent in social interaction, note the overall prosocial effects of the Ketogenic Diet; K) Effects of Standard Diet and Ketogenic Diet on MK-801-induced changes in spatial working memory measured by spontaneous alternation percentage in the Y-maze; and L) Effects of Standard Diet and Ketogenic Diet on MK-801-induced hyperactivity during the Y-maze test expressed as total number of arm entries. From D to L: open bars, saline controls, gray bars, 0.1 mg/kg MK-801, hatched gray bars, 0.2 mg/kg MK-801 and closed bars, 0.4 mg/kg MK-801. In B and C \* $p < 0.05$  versus Standard Diet, in all other graphs \* $p < 0.05$  versus saline control and † $p < 0.05$  versus same treatment group in the Standard Diet-fed animals.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.schres.2015.10.041>.

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23 July 2015

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