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Ketogenic diet prevents impaired prepulse inhibition of startle in an acute NMDA receptor hypofunction model of schizophrenia

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ABSTRACT

Recent transcriptomic, proteomic and metabolomics studies have highlighted an abnormal cerebral glucose and energy metabolism as one of the potential pathophysiological mechanisms of schizophrenia. This raises the possibility that a metabolically-based intervention might have therapeutic value in the management of schizophrenia, a notion supported by our recent results that a low carbohydrate/high-fat therapeutic ketogenic diet (KD) prevented a variety of behavioural abnormalities induced by pharmacological inhibition of NMDA glutamate receptors. Here we asked if the beneficial effects of KD can be generalised to impaired prepulse inhibition of startle (PPI), a translationally validated endophenotype of schizophrenia, in a pharmacological model in mice. Furthermore, we addressed the issue of whether the effect of KD is linked to the calorie-restricted state typical of the initial phase of KD. We fed male C57BL/6 mice a KD for 7 weeks and tested PPI at 3 and 7 weeks, in the presence and absence of a significant digestible energy deficit, respectively. We used an NMDA receptor hypo-function model of schizophrenia induced by acute injection of dizocilpine (MK-801). We found that KD effectively prevented MK-801-induced PPI impairments at both 3 and 7 weeks, irrespective of the presence or absence of digestible energy deficit. Furthermore, there was a lack of correlation between PPI and body weight changes. These results support the efficacy of the therapeutic KD in a translational model of schizophrenia and furthermore provide evidence against the role of calorie restriction in its mechanism of action.

1. Introduction

Schizophrenia is a complex neuropsychiatric disorder with diverse aetiology and mechanisms that result in abnormal function of synaptic communication (Forsyth and Lewis, 2017; Pocklington et al., 2014; Sarkar et al., 2017; van Os and Kapur, 2009). Normal synaptic communication requires appropriate formation of dendritic spines and the synthesis, release and recycling of neurotransmitters. Constantly forming and remodelling spines and maintaining neurotransmission is energetically expensive (Harris et al., 2012). Glucose is the main energy substrate for the brain (Magistretti and Allaman, 2015). From glucose, the high-energy molecule adenosine triphosphate (ATP) is produced through glycolysis in the cytoplasm and the tricarboxylic acid (TCA) cycle and oxidative phosphorylation in the mitochondria (Magistretti and Allaman, 2015). Reversing the ion movements that generate postsynaptic responses consumes the majority of the energy used from ATP (Harris et al., 2012). Glucose is not only the major substrate for ATP but glucose metabolism also results in production of glutamate and subsequently GABA. Therefore, deficits in glucose and synaptic energy supply lead to impaired synaptic communication and can ultimately result in abnormal brain function and behaviour (Kann, 2016).

Accumulating evidence suggests that bioenergetic function is impaired in schizophrenia (Ben-Shachar and Ene, 2017; Rajasekaran et al., 2015; Sullivan et al., 2018b). Systemic glucose metabolism abnormalities are manifested as hyperglycaemia, impaired glucose tolerance, and/or insulin resistance in first-onset, antipsychotic-naive patients with schizophrenia (Chouinard et al., 2018; Pillinger et al., 2017; Steiner et al., 2017). Numerous transcriptomic, proteomic and metabolomic studies have consistently identified the glycolysis pathway as being disrupted both in brain and CSF (Holmes et al., 2006; Prabakaran et al., 2004a; Zuccoli et al., 2017). In vivo evidence for brain bioenergetic abnormalities in patients with SZ and their unaffected siblings have been recently provided by using ^{31}P magnetization transfer spectroscopy (Chouinard et al., 2017; Du et al., 2014). A number of enzymes involved in glycolysis have been found to be dysregulated in schizophrenia (Prabakaran et al., 2004a; Sullivan et al., 2018a) and its

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translational animal models, such as pharmacological and genetic glutamate/NMDA receptor hypofunction models (Ernst et al., 2012; Funk et al., 2018; Martins-De-Souza et al., 2012; Wesseling et al., 2013). Collectively, these results suggest that a metabolically-based treatment that circumvents the affected glycolytic processes and impaired mitochondrial function, may have therapeutic benefit.

Diets low in carbohydrates and high in fat such as the KD, mimic the effects of fasting (Koppel and Swerdlow, 2017). The consequential reduction in circulating insulin and insulin signalling promotes a metabolic shift toward fatty acids utilisation (Kennedy et al., 2007; Paoli et al., 2013). β-hydroxybutyrate (BHB), the main circulating ketone body, is fully oxidised as an energy substrate in the brain. Furthermore, it inhibits histone deacetylases and upregulates genes involved in metabolic regulation, protecting against oxidative stress and inflammation (Achanta and Rae, 2017). The beneficial effects of the KD to normalise brain energy metabolism is achieved by circumventing glycolysis, providing alternative energy substrates in the form of ketone bodies, and resetting the processes underlying glucose and energy metabolism (Bough, 2008; Branco et al., 2016; Koppel and Swerdlow, 2017). In addition, the KD reduces glutamate toxicity, promotes GABA inhibitory tone and reduces the formation of reactive oxygen species, all of which can enhance neuronal function (Rogawski et al., 2016). Based on these results we have recently demonstrated that the hyperactivity, stereotypy, impaired sociability, and impaired working memory induced by acute treatment with the NMDA antagonist MK-801, behaviours that parallel positive, negative and cognitive symptoms of SZ, are prevented by three weeks of KD in mice (Kraeuter et al., 2015). KD was also effective in normalising dysfunctional hippocampal inhibitory circuits involved in auditory sensory gating in DBA/2 mice, a model relevant to schizophrenia (Tregellas et al., 2015).

In the present study we aimed to further investigate the effects of KD in a translatable mouse model that taps into the pathophysiological role of glutamate hypofunction in schizophrenia (Paz et al., 2008) by using an evolutionary-conserved schizophrenia-like behavioural endophenotype, the impairment of sensorimotor gating measured by prepulse inhibition of startle (PPI). Furthermore, being aware of the energy deficit during the initial phase of the KD and resultant weight loss together with the overall beneficial health effects of starvation/calorie restriction (Mattson et al., 2018), we studied the possible contribution of decreased caloric intake in the mediation of the therapeutic effect of KD in this schizophrenia model.

2. Methods

2.1. Animal and procedures

All experiments were approved by the Animal Ethics Committee of James Cook University (A2036) and were conducted according to the NHMRC/AVCC Statement and Guidelines on Research Practice (1997). Young male C57BL/6 mice ($n = 36$) (7 weeks old at the beginning of the study) maintained at 22 ± 1 °C with a 12 h dark/light cycle (lights on at 7:00 AM) and ad libitum food and water were used. Male mice were chosen due to more robust effects of MK-801 in acute pharmacological models of schizophrenia compared to female mice (van den Buuse et al., 2017). It has been shown that the dose of MK-801 used in this study, 0.25 mg/kg, failed to induce prepulse inhibition of startle (PPI) deficits in intact female C57Bl/6 mice (van den Buuse et al., 2017).

Mice were randomly assigned to either KD ($n = 18$) (SF14-063; Specialty Feeds, Western Australia) or Standard Diet (SD) (n = 18) (Goldmix Stockfeeds, Norco, Lismore, NSW, Australia) (Table 1). Throughout the study, food consumption was recorded daily and body weight was measured weekly. Mice on both KD and SD were randomly allocated to receive either saline vehicle (SD-Saline and KD-Saline) or MK-801 (SD-MK-801 and KD-MK-801) and tested at Week 3 on the diet for sensorimotor gating. Throughout and after testing the animals remained on their allocated diets for another 4 weeks. At Week 7 the same animals were tested again for PPI in a cross-over design. After completion of the second round of PPI testing, animals were humanely killed and blood was collected for BHB analysis.

2.2. Drugs

MK-801 (dizocilpine; 0.25 mg/kg; Sigma-Aldrich, St. Louis, USA) dissolved in 0.9% NaCl (saline vehicle), or saline vehicle as control solution was injected intraperitoneally (i.p.) 30 min prior to behavioural testing. The MK-801 dose was selected on the basis of published work using the same mouse strain, C57Bl/6, to achieve impaired PPI (Dean et al., 2010; van den Buuse et al., 2017).

2.3. Sensorimotor gating

Animals were acclimatised to the behavioural testing room for 12 h. Sensorimotor gating was measured by PPI during the light phase using automated startle chambers (SR-Lab; San Diego Instruments, San Diego, CA, USA), which produced both background noise and acoustic startle stimuli. Responses were recorded on a computer with the SR-Lab software (San Diego Instruments). The testing schedule included 3 min of acclimatization, which was then followed by eight startle stimuli (40 ms burst of 115 dB white noise). Afterwards 88 pseudo-randomized trials with 16 startle stimuli, four groups of eight prepulse-pulse trials at 2, 4, 8, and 16 dB over baseline with an inter-stimulus interval of 30 ms and 100 ms, as well as eight NOSTIM trials (no stimulus) were applied. The 104 trials were concluded with a further eight startle stimuli (Chavez et al., 2009; Dean et al., 2010). Because prepulse-pulse trials of 2 dB over baseline did not alter startle, the responses to these trials were not included in the analysis.

2.4. Blood analysis

Analysis for the ketone body, β-hydroxybutyrate (BHB), was performed using a colorimetric assay kit and performed as per manufacturer's instructions (Cayman Chemical Company, MI).

2.5. Statistical analysis

Data were analysed using SPSS Version 24 software package (IBM SPSS Statistics). BHB levels were analysed using two-tailed *t*-test. Body weight, energy and habituation were analysed using a repeated measures two-way ANOVA followed by post-hoc comparisons. Energy was calculated as follows as digestible energy of the diet x (average weekly food consumption/1000).

All PPI data were analysed using two-way ANOVA followed by post-hoc comparisons. PPI was generally slightly higher at the 30 msec ISI than at the 100 ms ISI (main effect of ISI at 3-weeks: $F_{(1,30)} = 48.4$, $p < 0.001$; at 7 weeks: $F_{(1,31)} = 42.4$, $p < 0.001$), but because there were no interactions of ISI with diet, PPI data at 30 ms and 100 ms were pooled. $p < 0.05$ was considered to be statistically significant. Data were expressed as the mean ± standard error of the mean (SEM).

Table 1
Comparison of the nutritional parameter of Standard diet and Ketogenic diet.

Nutritional parameters	Standard diet	Ketogenic diet
Protein, %	17.65	9.50
Fat, %	6.09	77.60
Carbohydrates, %	67.61	9.40
Digestible energy MJ/kg	12.87	30.80

3. Results

3.1. Metabolic parameters

An independent sample *t*-test showed that at the end of the study (Week 7) BHB plasma levels were significantly higher in mice that received KD, indicating a strong metabolic ketosis ($p < 0.001$) (Fig. 1A).

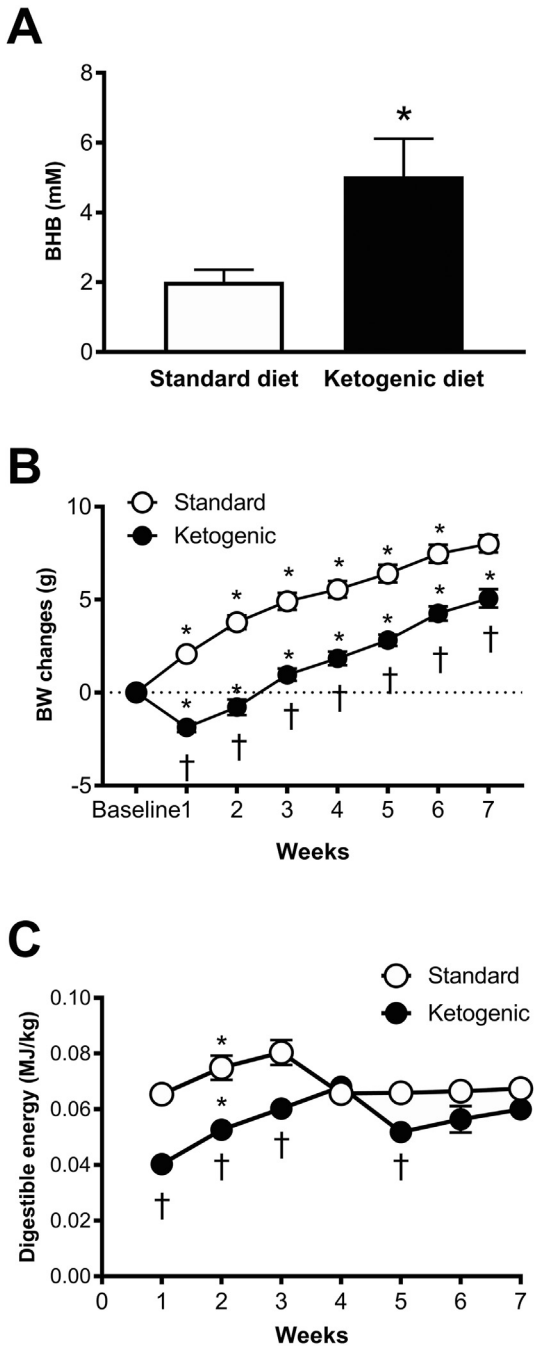

Fig. 1. (A) Mice on the ketogenic diet (KD) for 7 weeks showed significantly higher BHB concentration in plasma compared to standard diet (SD) controls. *$p < 0.05$ (B) SD mice significantly gained weight throughout the experiment, with KD mice having significantly lower body weights than SD controls; *$p < 0.05$ compared to the previous week, †$p < 0.05$ comparison between the diets. (C) Energy consumption at week 1, 2, 3 and 5 was significantly lower in mice of the KD but no significant differences in energy consumption were found at 4,6 and 7 weeks; *$p < 0.05$ compared to the previous week, †$p < 0.05$ comparison between the diets. All data are presented as group means ± SEM. SD: $n = 18$, KD: $n = 18$.

Repeated-measures ANOVA of body weight revealed a significant main effect of Diet ($F_{(1,34)} = 52.9$, $p < 0.001$) and Time ($F_{(7,238)} = 252.7$, $p < 0.001$), as well as a significant Diet x Time interaction ($F_{(7,238)} = 20.0$, $p < 0.001$) (Fig. 1B). Post-hoc tests showed that SD and KD animals progressively gained weight during the course of the experiment but that KD animals weighed less at all time-points compared to SD animals (all weeks: $p < 0.001$) (Fig. 1B).

Repeated-measures ANOVA of digestible energy consumption showed a significant main effect of Diet ($F_{(1,6)} = 19.7$, $p = 0.004$) and Time ($F_{(6,36)} = 14.6$, $p < 0.001$) as well as a Diet x Time interaction ($F_{(6,36)} = 10.7$, $p < 0.001$). Energy consumption significantly increased between Week 1 and Week 2 in SD ($p = 0.009$) and KD mice ($p = 0.002$), whereas it decreased between Week 4 to Week 5 ($p = 0.022$) in KD animals (Fig. 1C). Significant differences in digestible energy consumption were found between animals on SD and KD at Week 1 ($p < 0.001$), Week 2 ($p = 0.003$), Week 3 ($p = 0.006$) and Week 5 ($p = 0.002$) but not toward the end of the experiment at Week 6 and 7 (Fig. 1C).

3.2. PPI after 3 weeks of KD

As expected, the level of PPI was significantly dependent on the prepulse intensity ($F_{(2,60)} = 555.8$, $p < 0.001$) and MK-801 treatment resulted in significantly lower PPI values ($F_{(1,30)} = 11.5$, $p = 0.002$), an effect which was also dependent on the prepulse intensity ($F_{(2,60)} = 4.52$, $p = 0.015$). A significant diet x MK-801 treatment interaction ($F_{(1,30)} = 5.6$, $p = 0.024$) reflected that MK-801-treated SD mice showed lower PPI than saline-treated SD mice and MK-801-treated KD mice independent of prepulse intensity (Fig. 2A & B). Post hoc tests confirmed that PPI was significantly lower by 15% after MK-801 treatment compared to saline in the SD group ($F_{(1,15)} = 21.3$, $p < 0.001$) but there was no such effect in the KD group (Fig. 2A & B). Moreover, while PPI was not different between the SD and KD groups after acute saline injection, after MK-801 treatment PPI was significantly lower by 13% in SD mice than in KD mice ($F_{(1,15)} = 10.5$, $p = 0.005$).

Analysis of startle amplitudes showed significant startle habituation over the course of the PPI session ($F_{(2.4, 71.7)} = 31.6$, $p < 0.001$) but there were no effects of diet or MK-801 treatment on startle or its habituation (See Fig. 3A for average startle amplitudes).

3.3. PPI after 7 weeks of KD

The level of PPI was significantly dependent on the prepulse intensity ($F_{(1.6,51.4)} = 284.1$, $p < 0.001$) and MK-801 treatment resulted in significantly lower PPI values ($F_{(1,31)} = 14.1$, $p = 0.001$). The diet x MK-801 treatment interaction did not reach significance ($F_{(1,31)} = 3.8$, $p = 0.059$) but there was a significant diet x MK-801 x prepulse intensity interaction ($F_{(1.7,51.4)} = 3.6$, $p = 0.042$). Post hoc tests showed that, in the SD group, PPI was significantly lower by 14% after MK-801 treatment compared to saline ($F_{(1,16)} = 21.0$, $p < 0.001$; MK-801 x prepulse intensity interaction was significant ($F_{(2,32)} = 6.2$, $p = 0.005$). The effect of MK-801 was significant at PP8 (11% decrease) and PP16 (24% decrease), but not at PP4. In contrast, in the KD group there was no significant effect of MK-801 at any prepulse intensity (Fig. 2C & D). Post hoc tests by acute treatment showed that PPI was not different between the SD and KD groups after acute saline injection at any of the prepulse intensities. However, after MK-801 treatment PPI was significantly lower by 10% in SD mice than in KD mice at PP8 ($F_{(1,15)} = 5.6$, $P = 0.032$) and at PP16 ($F_{(1,15)} = 13.6$, $P = 0.002$) but not at PP4.

Analysis of startle amplitudes showed the expected startle habituation ($F_{(3,93)} = 20.0$, $p < 0.001$) but this was not influenced by diet or MK-801 treatment (Fig. 3). A diet x MK-801 treatment interaction was found ($F_{(1,31)} = 4.4$, $P = 0.045$) but post hoc analysis by diet group showed no effect of MK-801 in either SD mice or KD mice (Fig. 3B).

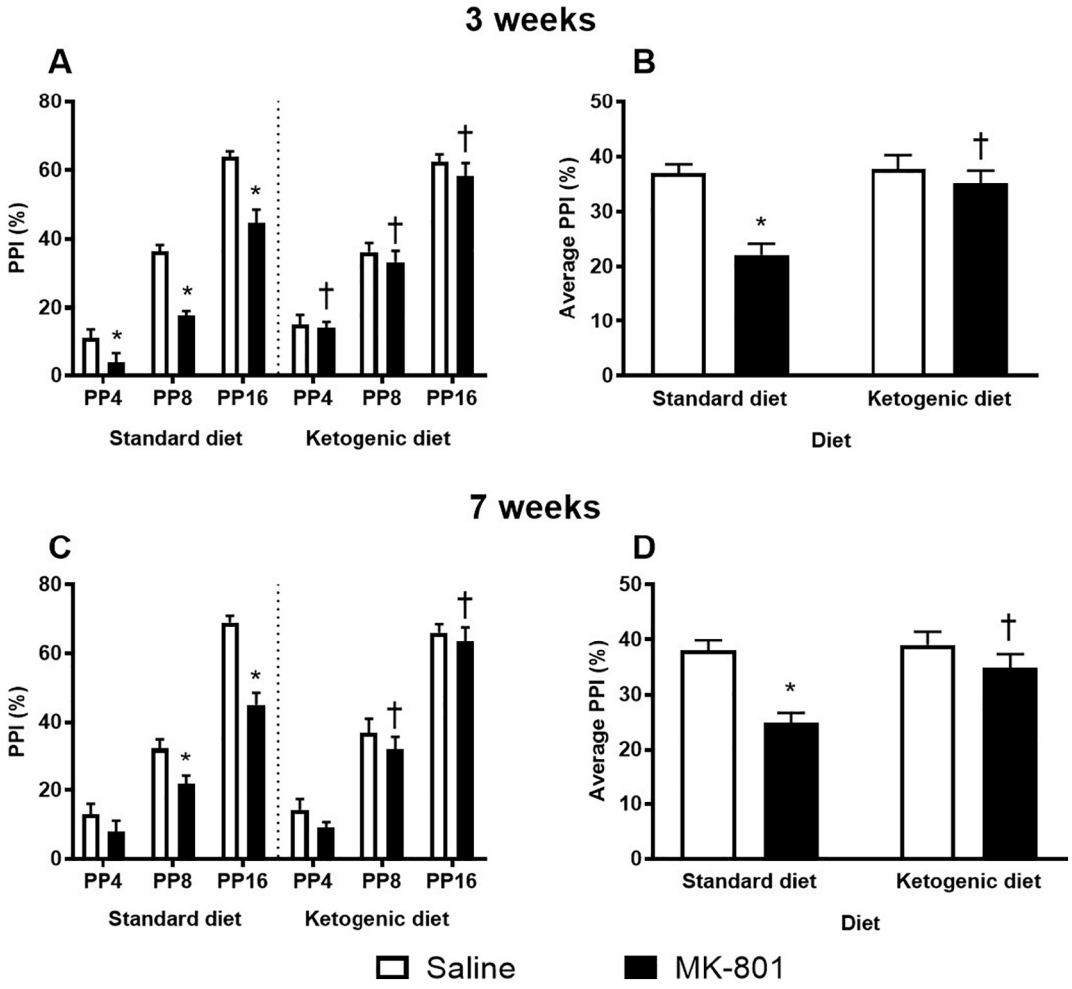

Fig. 2. Prepulse inhibition at different prepulse intensities (A, C) and expressed as average PPI (B, D). Acute treatment with MK-801 significantly impaired PPI at different prepulse intensities in mice on the standard diet (SD), but not the ketogenic diet (KD). *$p < 0.05$ compared to acute saline vehicle treatment, †$p < 0.05$ comparison between the diets. All data are presented as group means ± SEM. SD-Saline: $n = 16$, SD-MK-801: n = 18, KD-Saline: n = 18, KD-MK-801 $n = 16$.

4. Discussion

Here we demonstrated, for the first time, that ketogenic diet rescues a sensorimotor gating deficit in a translationally valid animal model. Importantly, we showed that the effect of KD does not depend on the previously described beneficial effects of calorie restriction (Duan et al., 2003). We found effective KD-induced rescue both at Week 3 on KD, when the energy intake was significantly below that of the SD-fed mice, and at Week 7 on KD when such difference was no longer present. These results, together with our earlier findings that KD normalises a variety of behavioural impairments induced by acute MK-801 administration that reflect positive, negative and cognitive symptoms of schizophrenia (Kraeuter et al., 2015), support the potential of this intervention in the management of schizophrenia.

In keeping with the published literature (Dean et al., 2010; Geyer et al., 2001;van den Buuse et al., 2017), we found that acute MK-801 administration resulted in a PPI deficit. In our study feeding mice with KD resulted in an antipsychotic-like effect by preventing the MK-801 induced PPI impairment. One plausible explanation for the observed beneficial effects of KD might be that this dietary intervention results in a fasting-like metabolic state (Veech et al., 2017). In fact, we observed a significant decrease in digestible energy consumption at Week 3 on KD, when the rescue of the MK-801 induced PPI deficit was observed. Fasting, and therefore decreased energy consumption in itself, has been shown to exert a variety of benefits on neuroplasticity, brain health and behaviour (Mattson et al., 2018). To investigate this possibility we continued the KD for another 4 weeks and then retested its effects on the PPI deficit induced by acute MK-801 administration. We found that although at this time point mice on KD consumed the same amount of digestible energy as SD-fed animals, the effect of the diet on PPI was still present. This strongly suggests that fasting and calorie restriction are unlikely to explain the effect of KD on the MK-801 induced PPI deficit. Another, alternative potential mediator of the effect of KD is the elevated BHB concentration in the blood, as was found in this study, in keeping with the fact that BHB is the main circulating ketone body during the KD. An elevated level of BHB is accompanied by cellular and molecular adaptations of neural networks in the brain that enhance their functionality and bolster their resistance to stress, injury and disease (Newman and Verdin, 2017; Sleiman et al., 2016). Specifically, BHB is an alternative fuel source, an epigenetic modulator, an immunomodulator and signalling molecule (Achanta and Rae, 2017).

The microbiome-gut-brain axis has been of great interest in psychiatry in general and in its involvement in schizophrenia in particular (Cryan and O'Mahony, 2011; Dinan et al., 2014). The effects of KD on the gut microbiome have been associated with anti-seizure effects in epilepsy (Olson et al., 2018; Zhang et al., 2018). Furthermore, a recent study showed that the gut microbiome is altered in individuals with first episode psychosis (Schwarz et al., 2018). Moreover, emerging literature suggests that the antipsychotic drug action may involve the gut microbiome (Cussotto et al., 2018; Maier et al., 2018). Therefore, it is

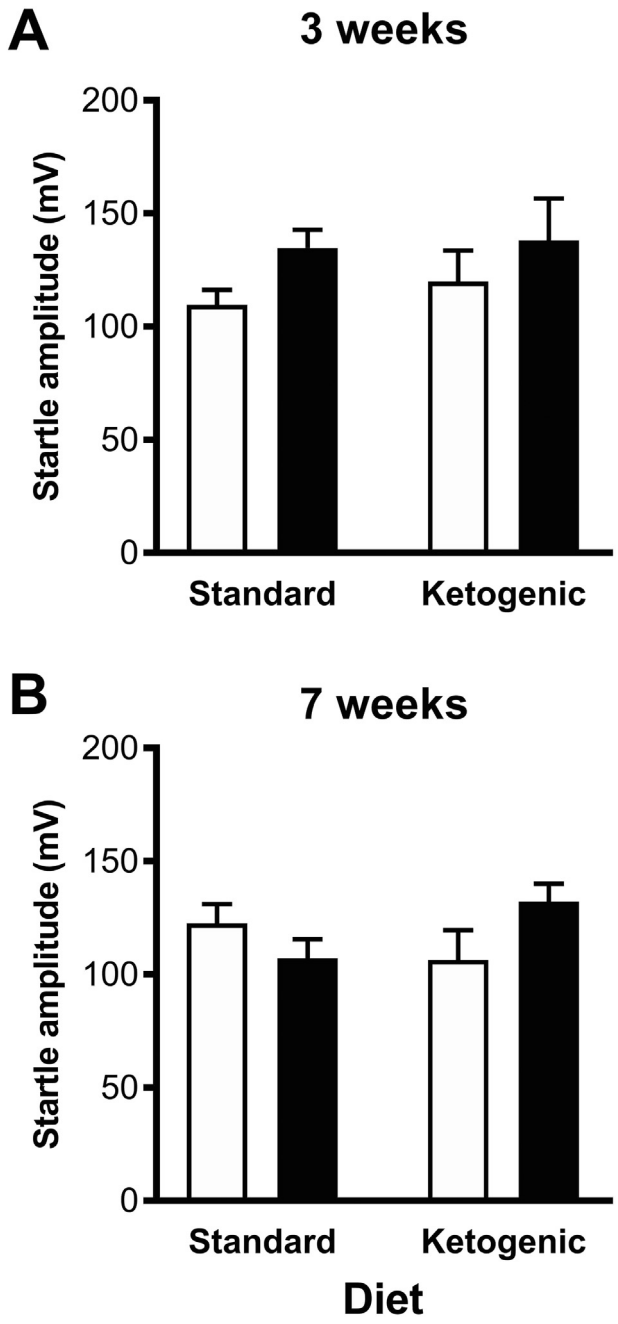

Fig. 3. Acute treatment with MK-801 had no significant effect on average startle at 3 weeks and 7 weeks of standard diet or ketogenic diet. All data are presented as group means ± SEM. SD-Saline: n = 16, SD-MK-801: n = 18, KD-Saline: n = 18, KD-MK-801 n = 16.

possible that beneficial behavioural effects observed in this study might be, at least in part, due to alterations in gut microbiome induced by the KD.

We found that neither MK-801 nor KD altered startle responses. Similarly to our present results, it was previously shown that neither MK-801 (Dean et al., 2010) nor antipsychotics (Olivier et al., 2001), which have an effect similar to KD in attenuating this PPI deficit, affect startle responses. These results also suggest that the effect of KD on PPI cannot be explained by a parallel effect on startle amplitudes.

Our current experimental design did not allow us to directly investigate the molecular mechanisms of KD in the context of the hypoglutamatergic model of schizophrenia. However, emerging evidence highlights brain bioenergetics processes to underlie some aspects of schizophrenia pathophysiology which can be specifically influenced by KD and ketogenic metabolites (Sullivan et al., 2018b). In their pioneering study, Prabakaran et al. (2004b) found the glycolytic pathway to be downregulated both at the transcript and protein levels, with four glycolytic genes and seven out of the 10 key glycolytic enzymes decreased in post-mortem dorsolateral prefrontal cortical (DLPFC) samples from individuals with schizophrenia. To further substantiate the notion of abnormal bioenergetics function in schizophrenia, it has been recently reported that key glycolytic enzymes hexokinase-1 and phosphofructokinase-1, as well as glucose transporter-1 and -3 mRNA expression, are downregulated in a neuron-specific manner in the pyramidal cell of the DLPFC in post-mortem schizophrenia samples (Sullivan et al., 2018a). A wealth of proteomics studies have provided strong evidence that enzymes involved in glycolysis are down-regulated in the DLPFC in schizophrenia (Nascimento and Martins-de-Souza, 2015; Zuccoli et al., 2017). Beyond establishing the molecular signature of impaired bioenergetics functions such as glycolysis in post-mortem human brains, recent in vivo results lend further support to the pathophysiological role of impaired energy metabolism. For example, brain bioenergetic abnormalities pointing toward impaired glucose metabolism have been recently found by using ^{31}P magnetization transfer spectroscopy in patients with schizophrenia and their unaffected siblings (Chouinard et al., 2017; Du et al., 2014). Importantly, similar abnormalities implicating impaired glucose metabolism have been identified in translational, hypo-glutamatergic animal models of schizophrenia (Funk et al., 2018; Iasevoli et al., 2012; Martins-De-Souza et al., 2012). Proteins specifically related to glucose metabolism and utilisation were altered in the brain of mice with genetically-reduced expression of the NMDA glutamate receptor, consistent with a reduction in glucose and lactate transport into neurons (Funk et al., 2018). Collectively, these converging human and preclinical findings support the notion that an abnormal glucose and energy metabolism, perhaps through impaired glycolysis and resulting bioenergetics deficits, may play an important role in the pathophysiology of schizophrenia (Sullivan et al., 2018b). The low-carbohydrate/high-fat KD provides an alternative energy substrate in the form of BHB, the main circulating ketone body, by circumventing glycolysis to produce acetyl-coenzyme A (acetyl-CoA), which links glycolysis with the tricarboxylic acid (TCA) cycle and ultimately to the energy producing oxidative phosphorylation in the mitochondria (Bough, 2008; Rho and Stafstrom, 2012). Through these metabolic effects, KD also influences brain excitability (Lutas and Yellen, 2013) and the excitation/inhibition balance that has been shown to underlie circuit abnormalities in the prefrontal cortex in schizophrenia (Hoftman et al., 2017; Lisman, 2012; Rosen et al., 2015).

Exact molecular mechanisms notwithstanding, it is well known that KD is safe to administer to humans over a period of time (Cai et al., 2017), it has beneficial systemic metabolic effects (Abbasi, 2018), and can potentially counteract some of the metabolic side effects of antipsychotic drugs, such as weight gain, insulin resistance and metabolic syndrome (Newcomer et al., 2002; Newcomer, 2005). Therefore, a swift clinical translation of the use of therapeutic KD in the management of schizophrenia can be proposed. In line with this, a recent case study showed reductions in auditory hallucinations and delusions, improvement in mood, and ability to concentrate, in schizoaffective disorder in response to three weeks of KD (Palmer, 2017). However, KD is difficult to adhere to and not entirely without risk (Kosinski and Jornayvaz, 2017), therefore it should be applied with caution and under medical supervision, or alternatively, the use of exogenous ketones/ketogenic substrates (Stubbs et al., 2017; Veech et al., 2017) (such as BHB) could be considered.

Limitations of the study include a possible effect of KD on MK-801 absorption, which has not yet been addressed in the literature. However, this is unlikely, as KD had no effect on the pharmacokinetics of L-DOPA in Parkinson's patients (Elbarbry et al., 2018) and did not alter serum concentrations of anti-epileptic drugs such as carbamazepine, lamotrigine, levetiracetam, topiramate and phenobarbital (Heo et al.,

2017). Valproic acid was the only anti-epileptic drug, the plasma concentrations of which were reduced in combination with KD (Heo et al., 2017) although Coppola et al. (2010) found that 1 month of KD treatment showed only a non-significant reduction in circulating valproic acid concentrations. Therefore, although direct evidence is not yet available, it is unlikely that 3 weeks of KD would significantly alter the pharmacokinetic properties of a single dose of MK-801.

Further limitations include, as discussed previously, that only male mice were used in the present study, and therefore no conclusion can be drawn on the possible effects of KD in female mice. Sex differences and other animal models of schizophrenia need to be explored to obtain more detailed knowledge of the potential therapeutic use of KD. Future research also needs to specifically investigate the role of the gut microbiota in the mediation of the beneficial effects of the KD in schizophrenia-like behaviours.

In conclusion, KD effectively prevented impaired sensorimotor gating, a translationally valid and highly conserved endophenotype related to psychosis, in an acute hypo-glutamatergic animal model of schizophrenia. These KD effects were independent of the presence or absence of digestible energy deficit. This provides initial proof of concept that schizophrenia pathophysiology might be amenable to metabolic therapeutic approaches.

Contributions

ZS and AKK conceived the idea and designed the study. AKK carried out the behavioural studies, analysed the data and wrote the first draft of the manuscript. ZS analysed some of the data and reviewed the drafts. MvdB analysed the PPI data and provided advice on the behavioural studies. All authors contributed to and have approved the final manuscript. ZS serves as the guarantor on this submission.

Conflict of interests

The authors declare no competing interests.

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Data statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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