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Highlights

- Administration of a ketogenic diet altered the copulatory sequence in male rats
- The expected pattern of increased likelihood of ejaculation with experience was delayed
- These males may require more sexual stimulation in order to successfully copulate

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Manuscript Title: Alterations to the copulatory sequence in young adult male Sprague-Dawley rats administered a ketogenic diet

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Abstract

Ketogenic diets (KDs) have shown therapeutic potential for a range of neuropsychiatric disorders; however, there is insufficient data regarding the behavioral impacts of KDs in healthy populations. Here, we examined the impact of a KD on sexual behavior in young adult male Sprague-Dawley rats maintained on either a KD or standard chow diet (SD). We found that KD males exhibited higher mount rates, higher intromission rates (third and fourth tests only), and lower ejaculation likelihood (second test only) compared to SD males. Consequently, it may be that experience-dependent changes in the processing of sexual stimuli are not occurring as efficiently in KD males, thereby yielding the observed copulatory sequence alterations.

Keywords: Copulation; Ketogenic; Metabolic therapies; Sexual behavior

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1. Introduction

Metabolic therapies including high-fat, low-carbohydrate ketogenic diets (KDs) have been increasingly examined for therapeutic potential across a wide range of clinical disorders [1]. Indeed, KDs have an extensive record of clinical efficacy for epilepsy [2] and are now being examined for efficacy in animal models of neuropsychiatric conditions ranging from autism [3–7] to drug addiction [8–12]. KDs have also achieved more widespread use as a means to lose weight and increase athletic performance [13,14]. With increasing application of these diets to both clinical and non-clinical populations, there is a pressing need to assess the impacts of KDs on a wider range of functions, including sexual behavior.

To date, little is known about the impact of KDs on sexual functioning. One study wherein a KD was administered to obese adults examined various self-reported measures of sexual health [15]. There was no change in sexual functioning in men over the four month diet period; however, in women, several indices showed improvement in participants on a KD vs. those on a standard diet (SD), including orgasm, excitation, and lubrication. Given that these female participants were not only obese but were also rated as having overall sexual dysfunction at baseline (prior to KD administration), it is difficult to disentangle the impact of reducing BMI/weight vs. any more direct impacts that a KD may exert on sexual functioning. It is, however, important to note that an inconsistent correlative relationship was observed between changes in BMI/weight across time and sexual functioning in women for at least some sexual health measures (e.g., lubrication), suggesting that changes in BMI/weight alone likely do not fully explain the observed KD effects. A single study examining effects of a KD on sexual functioning in male mice utilized a genetic model of epilepsy (EL mice) [16]. This study found that a subset of males exhibit spontaneous death due to a urogenital obstruction occurring when

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seminal fluid is not fully ejaculated and instead coagulates in the urethra; this obstruction does not occur in EL males maintained on a KD. Although this study demonstrates a valuable benefit of the KD in a clinical model featuring obstructive uropathy, the broader impact of a KD on male sexual functioning in rodents remains unclear.

Studies examining the effects of KDs on social (non-sexual) interactions in healthy rodents indicate that KD males show increased sociability towards same-sex conspecifics [17,18]; this KD-enhancement of sociability is further supported by evidence of amelioration of the social deficits that would otherwise be observed in rodent models of autism [3–7] following administration of a KD. Given that (1) factors that negatively impact sociability in male rats (e.g., repeated social defeat) [19] also negatively impact male rat copulatory performance [20,21], and (2) the positive impact of a KD on sociability as highlighted above, we predicted that copulatory responses would be enhanced in males administered a KD. To determine whether this is indeed the case, we administered a KD or SD to juvenile male rats and then tested these males as young adults for copulatory responses across repeated sexual behavior tests. We found that there were specific alterations to the copulatory responses exhibited by KD males, including increased rate of intromissions (Sex Tests 3 and 4) and decreased likelihood of ejaculation (Sex Test 2).

2. Materials and methods

2.1 Animals

Male and female Sprague-Dawley rats were first generation offspring of breeders originally obtained from Charles River Laboratories (Kingston, NY). Following weaning, offspring were group-housed (two same-sex animals per cage) in polycarbonate cages with wire lids and woodchip bedding. Animals were maintained on a 12:12 light:dark cycle (lights off at 12

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pm), with all testing occurred during the dark phase of the cycle. All animals were provided with *ad libitum* access to food and water. Animal procedures were carried out in accordance with the *Guide for the Care and Use of Laboratory Animals: Eighth Edition* [22] and were approved by the Trinity College Animal Care and Use Committee.

2.2 Diet

At five weeks of age, rats were randomly assigned to either remain on a SD (LabDiet 5001, W. F. Fisher & Son, Somerville, NJ) or switched to a KD (Bio-serv F3666, Flemington, NJ). A detailed comparison of the dietary constituents of the specific SD and KD used here are provided in Table 1. Animals remained on their assigned diets for the duration of their involvement in the study. Although intake of the assigned diet was not regulated or monitored in the present study, it has been previously reported that male rats maintained on this KD have increased caloric intake compared to SD males [18].

Table 1

Composition of Experimental and Control Diets

	BioServ F3666		LabDiet 5001	
	% Composition	kcal/g	% Composition	kcal/g
Fat	75.1	6.76	6.4	0.56
Protein	8.6	0.34	24.1	1.18
Carbohydrate	3.2	0.13	48.1	2.34
Total	**86.9***	**7.23**	**78.6***	**4.08**

Note. *Diets also contain fiber, ash, and moisture.

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2.3 Ovariectomy surgeries

Sex stimulus female rats were ovariectomized at 5-6 weeks of age via the bilateral, dorsal approach described previously [23], with a few notable alterations to the prior published procedure. Specifically, females were anesthetized with isoflurane (Zoetis, Kalamazoo, MI) in oxygen gas (5% induction; 1.5-2.5% maintenance), the isolated ovaries were removed via cauterization, the muscle incision sites were closed with absorbable sutures (Ethicon, Somerville, NJ, USA), and the skin incision sites were closed with wound clips (Stoelting, Wood Dale, IL). Females received s.c. injections of meloxicam (2.5 mg/kg; Patterson Veterinary, Devens, MA) for analgesia, once just prior to surgery and again within 24 hours of surgery. Wound clips were removed one week following surgery. At the conclusion of the three day post-surgical monitoring period, females were placed on either a KD or continued on a SD; females remained on these assigned diets through the conclusion of their use in behavioral testing.

2.4 Hormones and injections

Estradiol benzoate (EB; E8515 Sigma-Aldrich, St. Louis, MO) and progesterone (P; P0130 Sigma-Aldrich) were dissolved in cottonseed oil to a concentration of 0.05 mg/ml (EB) or 5 mg/ml (P), and injected s.c. at a volume of 0.1 ml. Sex stimulus female rats received these injections of EB 48 hours, and P 4-6 hours, prior to use in behavioral tests [24]. Individual females received this pattern of injections repeatedly, with each injection of EB spaced by at least 72-96 hours.

2.5 Behavioral testing

Starting at eight weeks of age, male rats received a series of four sex tests across the duration of the experiment. Each test was separated by 96 hours. This spacing between tests was chosen due to unpublished data from our own lab and published work of others [25,26]

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indicating that male rats are more likely to show high levels of intromissions and are more likely to ejaculate when sex tests are spaced by at least four days. All tests occurred in glass aquariums (76 x 31 x 48 (l x d x h) cm) with woodchip bedding. Males were habituated to the testing apparatus for 30 min [27] prior to the start of each sex test. Hormone-primed and diet-matched sex stimulus females were then introduced into the apparatus and the animals were allowed to freely interact until either an ejaculation occurred or 30 min elapsed, whichever occurred first. Each male received a novel sex stimulus female across the four sex tests. Tests were video recorded; the number of mounts, intromissions, and whether an ejaculation occurred during testing were scored live, whereas the latency to first mount, intromission, and ejaculation scored from the recordings.

2.6 Blood collection and analyses

Rats were euthanized 24-48 hours following the last sex test. Trunk blood samples were collected into tubes and centrifuged at a rate of 12,500 RPM for 5 min at 24°C. Serum ketone (i.e., β-hydroxybutyrate) and glucose levels were quantified using Precision Xtra meters (Abbott Laboratories, Bedford, MA), in order to confirm successful induction of ketosis in KD rats.

2.7 Data analyses

All data were analyzed using JASP (v 0.16) software. Data were first examined for skewness, kurtosis, and normality (Shapiro-Wilk test); when identified, outliers (scores outside the interquartile range [IQR] by more than 1.5 +/- IQR) were removed. This resulted in measure-specific test-day data from one KD outlier (mount rate), one SD outlier (mount latency), and one SD outlier (intromission rate) being removed, yielding analyzed sample sizes of 9-10 individuals per treatment group. Repeated factors with more than three levels were examined for evidence of sphericity; when identified, the Huynh-Feldt correction was applied. The number of

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mounts and intromissions were transformed into rates (#/min) to account for differences in total test time across tests/animals. Latency data for these two measures were log-transformed in order to normalize distributions. In all cases, *p* values of less than 0.05 were considered *a priori* to be statistically significant. Effect sizes are reported as partial η^2 (ANOVA analyses).

Mount and intromission (rate/latency) as well as weight data were examined for the effects of diet (SD vs. KD) and time (mount/intromission: Sex Tests 1-4; weight: Baseline, Sex Test 1, and Sex Test 4) using mixed-design ANOVAs. Significant interactions were further examined for the simple effect of diet at each time point (ANOVAs calculated using pooled error terms, as discussed in Howell [28]). The effect of diet on likelihood of ejaculation within each test was examined using the exact binomial test with Benjamani-Hochberg correction for multiple comparisons [29], whereas the effect of test on likelihood of ejaculation within each diet group was examined using Fisher's exact test. The effects of diet on blood glucose and ketone levels as well as average ejaculation latency were examined using one-way ANOVAs.

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3. Results

The effects of diet (KD vs. SD) and test (Sex Tests 1-4) on the rate (# per minute) of mounts and intromissions, as well as the log-transformed latency to exhibit each of these behaviors, were examined using repeated measures ANOVAs (Figure 1). There were significant main effects of diet on mount rate, $F(1,17) = 16.16$, $p < 0.01$, $\eta_p^2 = 0.49$ (Figure 1A), and intromission rate, $F(1,17) = 7.24$, $p = 0.015$, $\eta_p^2 = 0.30$ (Figure 1C), with KD males exhibiting significantly higher mount/intromission rates than SD males. In addition, there were significant main effects of test on mount rate, $F(3,51) = 12.67$, $p < 0.01$, $\eta_p^2 = 0.43$, and intromission rate, $F(3,51) = 26.62$, $p < 0.01$, $\eta_p^2 = 0.61$, with the rate of intromissions observed in a given test

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increasing with sexual experience. The main effects of diet and test day on intromission rate were moderated by a significant diet x test interaction, $F(3,51) = 4.64$, $p < 0.01$, $\eta_p^2 = 0.21$. Simple effects analyses revealed that intromission rates did not significantly differ between diet groups on Sex Test 1, $F(1,41.55) = 0.51$, $p = 0.48$, $\eta_p^2 = 0.012$, or on Sex Test 2, $F(1,41.55) = 0.33$, $p = 0.57$, $\eta_p^2 < 0.01$; however, KD males had significantly higher intromission rates on Sex Test 3, $F(1,41.55) = 10.32$, $p < 0.01$, $\eta_p^2 = 0.25$, and on Sex Test 4, $F(1,41.55) = 13.36$, $p < 0.01$, $\eta_p^2 = 0.32$. A significant main effect of test day was also observed for mount and intromission latencies (mounts: $F(3,48) = 53.25$, $p < 0.01$, $\eta_p^2 = 0.77$; intromissions: $F(2.34, 37.50) = 47.61$, $p < 0.01$, $\eta_p^2 = 0.75$), with latencies decreasing with increasing sexual experience (Figure 1B & 1D). These effects were moderated by significant diet x test interactions (mounts: $F(3,48) = 5.63$, $p < 0.01$, $\eta_p^2 = 0.26$; intromissions: $F(2.34,37.50) = 4.89$, $p < 0.01$, $\eta_p^2 = 0.23$), with simple effects analyses indicating that latencies for both measures were significantly longer for KD vs. SD males on Sex Test 2 (mount: $F(1,47.80) = 6.70$, $p = 0.013$, $\eta_p^2 = 0.14$; intromissions: $F(1,41.22) = 12.78$, $p < 0.01$, $\eta_p^2 = 0.31$). No significant differences were observed on Sex Test 1 (mounts: $F(1,47.80) = 3.58$, $p = 0.065$, $\eta_p^2 = 0.075$; intromissions: $F(1,41.22) = 0.067$, $p = 0.80$, $\eta_p^2 < 0.01$), Sex Test 3 (mounts: $F(1,47.80) = 0.32$, $p = 0.58$, $\eta_p^2 < 0.01$; intromissions: $F(1,41.22) = 0.38$, $p = 0.54$, $\eta_p^2 < 0.01$) or Sex Test 4 (mounts: $F(1,47.80) = 0.56$, $p = 0.46$, $\eta_p^2 < 0.01$; intromissions: $F(1,41.22) = 0.28$, $p = 0.60$, $\eta_p^2 < 0.01$).

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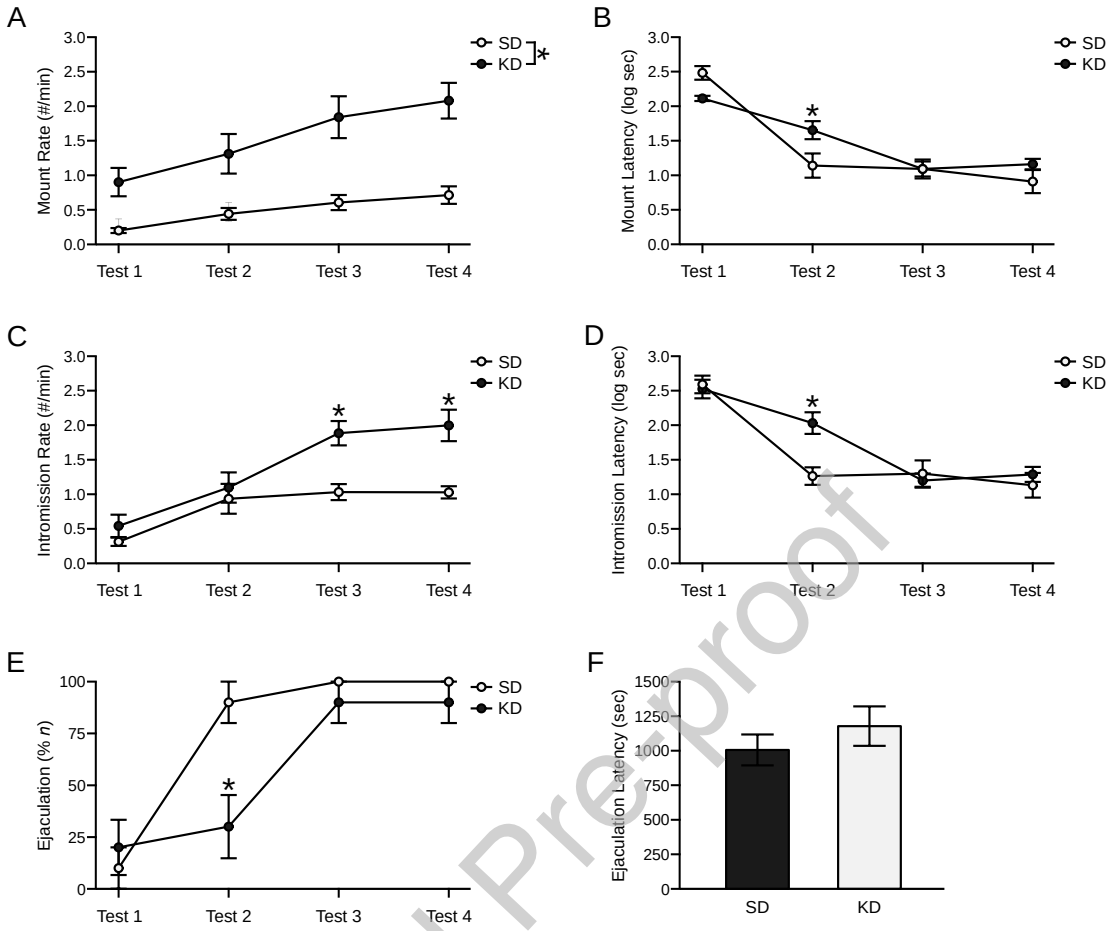

Figure 1. Copulatory behaviors observed during sexual behavior tests for male rats administered either a standard (SD) or ketogenic (KD) diet. All data are mean (± SEM), with the exception of the percent ejaculation data (E). KD animals exhibited significantly higher mount rates (collapsed across test day; A) and intromission rates (Sex Tests 3 and 4; C), and were less likely to ejaculate (Sex Test 2; E), compared to SD males. Further, KD males had significantly longer log-transformed latencies to mount (B) and intromit (D) on Sex Test 2. There were no significant differences in the latency to ejaculate (data averaged across all tests wherein an individual ejaculated; F). * $p < 0.05$, main effect of diet (A) or simple main effect of diet within a specific test day (B-E). Note that data from one KD outlier (A), one SD outlier (B), and one SD outlier (C) were removed from the respective datasets.

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The likelihood of ejaculation on a given test was first compared across test days, separately for each diet condition, using the exact binomial test with Benjamani-Hochberg correction for multiple comparisons [29]. Although only 10% of SD males ejaculated during Sex Test 1, the likelihood of ejaculating increased significantly by Sex Test 2 (90% ejaculating) and remained significantly higher when comparing Sex Test 3 (100% ejaculating) and 4 (100% ejaculating) vs. Sex Test 1, all $p < 0.05$ (Figure 1E). This pattern was altered in KD animals; specifically, the likelihood of ejaculating for these males did not increase significantly from Sex Test 1(20% ejaculating) to Sex Test 2 (30% ejaculating), $p > 0.05$, although there was an increased probability of ejaculation when comparing the first sex test against Sex Test 3 (90% ejaculating) and 4 (90% ejaculating), all $p < 0.05$. Fisher's exact test was then used to compare the likelihood of ejaculation during a test across diet conditions. This analysis revealed that SD males were more likely to ejaculate during Sex Test 2 compared to KD males, $p = 0.02$; no significant differences were observed on the other test days, all $p > 0.05$. Given the number of males that did not ejaculate in either of the first two tests (e.g., 8 or 9 (out of 10) per group in Sex Test 1), the average latency to ejaculate for each male across any tests in which that male ejaculated was calculated and then averaged within each diet condition. There were no significant differences in averaged ejaculation latency across treatment groups, $F(1,18) = 0.77$, $p = 0.39$, $\eta_p^2 < 0.041$ (Figure 1F).

Twenty-four hours following Sex Test 4, male rats were euthanized and trunk blood was collected and analyzed for glucose and ketone levels (Figure 2). There was no significant effect of diet condition on blood glucose, $F(1,18) = 1.53$, $p = 0.23$, $\eta_p^2 = 0.079$ (Figure 2A). As expected, there was a significant main effect of diet on blood ketone levels, $F(1,8.14) = 33.34$, p

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< 0.01, η_p^2 = 0.69, with KD animals exhibiting elevated blood ketone levels compared to SD animals (Figure 2B).

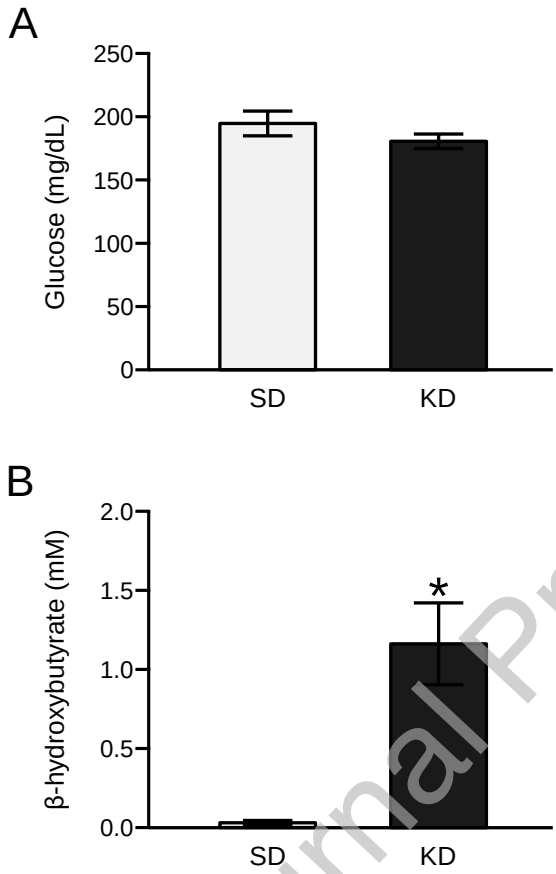

Figure 2. Mean (± SEM) blood glucose and blood ketone (β-hydroxybutyrate) levels in male rats taken at the conclusion of the experiment. Blood glucose levels were not significantly different in male rats maintained on a standard diet (SD) vs. a ketogenic diet (KD) (A). In contrast, blood β-hydroxybutyrate levels were significantly higher in KD vs. SD rats (B). * p < 0.05, SD vs. KD comparison.

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The effect of diet (KD vs. SD) on body weight was assessed at Baseline (prior to diet assignment), at Sex Test 1, and at Sex Test 4. In agreement with previous reports [10], there was a significant main effect of diet on weight, $F(1,18) = 242.81$, $p < 0.01$, $\eta_p^2 = 0.93$, with SD males weighing significantly more than KD males (Figure 3). There was also a significant main effect of time on weight, $F(1.26,22.74) = 838.25$, $p < 0.01$, $\eta_p^2 = 0.98$, with weight increasing across time, as well as a significant diet x time point interaction, $F(1.26,22.74) = 574.84$, $p < 0.01$, $\eta_p^2 = 0.97$. This interaction was further examined for the simple main effect of diet at each time point. Importantly, weight did not differ across assigned diet conditions at Baseline, $F(1,26.34) = 0.37$, $p = 0.55$, $\eta_p^2 = 0.014$. However, weight was significantly lower in KD vs. SD animals at Sex Test 1, $F(1,26.34) = 298.49$, $p < 0.01$, $\eta_p^2 = 0.92$, and at Sex Test 4, $F(1,26.34) = 394.20$, $p < 0.01$, $\eta_p^2 = 0.94$.

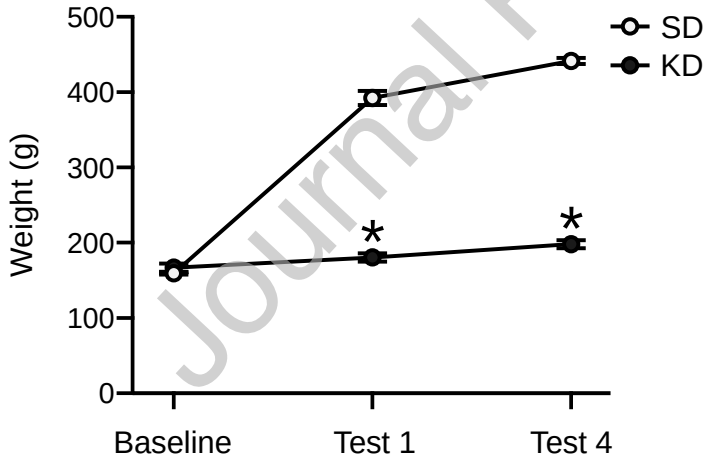

Figure 3. Mean (± SEM) body weight of male rats maintained on a standard (SD) or ketogenic (KD) diet. Weight was assessed at Baseline, at Sex Test 1, and at Sex Test 4. There were no significant differences in weight at Baseline; however, SD rats weighed significantly more than

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KD rats at Sex Test 1 and Sex Test 4. * $p < 0.05$, simple main effect of diet within a specific test day.

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4. Discussion

We show here that administration of a KD alters the copulatory sequence across repeated sexual experiences in young adult male Sprague-Dawley rats. Specifically, male KD rats exhibited a higher mount rate, higher intromission rate (Sex Tests 3 & 4 only), increased latencies to mount and intromit (Sex Test 2 only), and reduced likelihood of reaching ejaculation (Sex Test 2 only), compared to SD males. Further, administration of a KD significantly reduced weight gain and increased blood ketone levels in these males, in agreement with our prior work. Taken together, these findings indicate that although both groups of males increased their rates of sexual contact and likelihood of ejaculation with repeated sexual experience, KD males may do so with less efficiency compared to SD males.

The observed improvements in the copulatory efficiency of SD and KD males following repeated sexual experience is in excellent agreement with past literature. Specifically, the overall reductions in mount and intromission latencies closely parallels what has been shown previously in similarly within-subjects [e.g., 25] as well as in between-subjects [e.g., 30] design studies. Although sexually-naïve males will form a conditioned place preference to either intromissions alone or to intromissions plus ejaculations, indicating that both types of stimulation are rewarding to these males, experienced males will only form a CPP for ejaculation [31]. Sensory information relevant for the reinforcing aspects of copulation travel from the penis to the spinal cord via the pudental and dorsal penile nerves; this information is then relayed from the spinal cord to limbic/hypothalamic areas such as the medial preoptic area (MPOA) [32]. Indeed, copulatory stimuli induce expression of immediate early genes such as *c-fos* [33] and *Arc* [27] in MPOA of male rats. Interestingly, males with repeated sexual experience show enhanced copulation-induced *c-fos* expression in this brain area compared to inexperienced males, despite

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receiving fewer intromissions [34]. Considered together, these data indicate that experience may lead to differential processing of sensory stimuli to enhance copulatory efficiency. Our findings that KD males show reduced likelihood of ejaculation (Sex Test 2) and increased rate of intromissions (Sex Tests 3 & 4) compared to SD males suggest that this experience-dependent shift may not be occurring as efficiently in KD males. In possible agreement with this point, it may be that the increased mount/intromission latencies observed in Sex Test 2 in KD males are indicative of a persisting focus on anogenital investigation and related precopulatory responses vs. mounting/intromitting; however, this is strictly speculative given that precopulatory behaviors were not analyzed in the present study. Additional research will be required to determine the extent to which sexual experience impacts sexual reinforcement (and the processing of sexual reinforcement-relevant stimuli) in KD animals.

One mechanism whereby a KD could impact processing of sexually-relevant stimuli to influence copulation is via alterations to gonadal steroid hormone signaling. Past work in castrated male rats has demonstrated a negative relationship between testosterone (T) dose administered and intromission latency, as well as a positive correlation between T dose and likelihood of ejaculation within 30 min [35]. Furthermore, those authors report that copulatory performance is most broadly disrupted when circulating T is substantially below levels normally observed in intact males. However, administration of a KD does not impact serum T levels in adult male rats maintained on this diet [36]. This is in contrast to data in humans showing that a KD increases serum T in both obese [37–39] and healthy [40] men. As such, it appears unlikely that circulating T levels were decreased in KD male rats utilized in the present study. Androgens such as T exert their effects on male copulatory responses via actions at androgen receptors (ARs). Gonadectomized, T-implanted male rats administered the AR antagonist flutamide show

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reduced mount, intromission, and ejaculation frequencies compared to T-implanted males administered vehicle [41]. These effects are likely mediated by ARs in MPOA, given that implantation of hydroxyflutamide into MPOA decreases copulatory responses in T-implanted males [42]. It therefore may be the case that AR expression/function is altered in male rats maintained on a KD, leading to the altered sexual functioning observed in these males.

An additional, non-mutually exclusive mechanism driving behavioral effects observed in the present study may be diet-induced alterations to adenosinergic/dopaminergic signaling. Ketones such as β-hydroxybutyrate are efficient energy sources; indeed, mice maintained on a KD have higher brain levels of ATP [43] as well as the ATP metabolite adenosine [44], compared to SD mice. Caffeine, a non-specific adenosine receptor antagonist, increases the number of mounts and intromissions in adult male rats [45]. Although not directly examined, it could be that enhanced brain adenosine signaling (via KD administration) would also decrease these responses. Both adenosine A_1 and A_{2A} receptors are found within brain reward areas such as the nucleus accumbens (NAc) [46], forming antagonistic heterodimers with dopamine receptors (dopamine D_1 and D_2 receptors, respectively) [47]. Adenosine signaling could therefore act to counter the effects of dopamine within NAc; this is in fact a major, hypothesized mechanism of action for this diet's effects on responses to drugs of abuse [48]. Infusion of the D_2-like receptor antagonist haloperidol failed to affect copulatory responses in male rats [49]; however, infusion of the nonselective dopamine receptor antagonist cis-flupenthixol into MPOA reduces the number of mounts, intromissions, and ejaculations in these males [50]. Interestingly, dopamine levels increase in MPOA of copulating male rats, but decrease in those that fail to copulate [51]. Considered together, these data suggest MPOA, rather than NAc, may be more critical for the effects of dopamine on copulatory responses. Whether adenosine acts to modulate

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dopamine signaling within MPOA, and further, whether this modulation underlies the impact of a KD on male rat copulatory responses, are important unanswered questions to be addressed moving forward.

There are several limitations of the present work that warrant discussion. First, general locomotor activity was not assessed in this study. As such, it may be that the increased mounts and intromissions observed in KD males is reflective of a more generalized locomotor stimulating effect. Although caloric intake was not tracked in the present study, the KD utilized here does indeed have more kcal/g compared to the utilized SD and there is evidence from a prior study that male rats on a KD have higher caloric intake than those on an SD [18]. It is not unreasonable, then, to speculate that increased energy availability could yield increased locomotion in KD rats. However, in our previous work we found no differences in general ambulatory activity between KD and SD rats that were tested at similar ages, and with a similar age of assigned diet initiation/length of time on assigned diet, as in the present study [10]. Furthermore, if a non-specific locomotor-enhancing effect was driving the results observed here, it would be expected that the latency to ejaculate on Sex Tests 3 & 4 (i.e., when KD rats show significantly increased intromissions vs. SD males) would be significantly reduced. Yet no differences in ejaculation latency were observed on these test days. An additional limitation is the exclusive use of diet-matched male-female pairings in copulatory behavior tests (i.e., KD males were only paired with KD females, and SD males with SD females). As such, it is not possible to dissociate the impact of a KD on the stimulus quality of the female from the diet's impact more directly on male sexual behavior. The decision to use only diet-matched pairings was dictated by initial pilot data findings, wherein KD males (due to their smaller size) struggled to gain intromittive access to larger SD females. Furthermore, we also observed that KD animals

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of both sexes would spend substantial time engaging in coprophagia (specifically, consuming the feces of the partner) when paired with a SD partner. This was not observed in diet-matched pairs. Together, these issues precluded the use of dissimilar diet pairings, as they both substantially interfered with the ability of KD animals to perform copulatory responses.

5. Conclusions

Our findings address a significant gap in understanding how very high fat, low carbohydrate ketogenic diets can impact aspects of male sexual functioning. The alterations to the copulatory sequence across repeated sexual experience in KD males observed here suggest that a KD has the potential to alter experience-dependent changes in processing of copulatory stimuli. Further, our results provide the first indication that KDs may impact sexual functioning in a non-clinical population, an important consideration given the increased popularity of KDs in both clinical and non-clinical settings.

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Data statement

Recorded data (e.g., video files, behavioral scoring, body weight records, and serum assays) and other research materials are available by request from the corresponding author.

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References

[1] M. Mentzelou, A. Dakanalis, G.K. Vasios, M. Gialeli, S.K. Papadopoulou, C. Giaginis, The Relationship of Ketogenic Diet with Neurodegenerative and Psychiatric Diseases: A Scoping Review from Basic Research to Clinical Practice, Nutrients 15 (2023) 2270. https://doi.org/10.3390/nu15102270.

[2] M. Ułamek-Kozioł, S.J. Czuczwar, S. Januszewski, R. Pluta, Ketogenic diet and epilepsy, Nutrients 11 (2019) 2510. https://doi.org/10.3390/nu11102510.

[3] K. Castro, D. Baronio, I.S. Perry, R. dos S. Riesgo, C. Gottfried, The effect of ketogenic diet in an animal model of autism induced by prenatal exposure to valproic acid, Nutr. Neurosci. 20 (2017) 343–350. https://doi.org/10.1080/1028415X.2015.1133029.

[4] L. Qin, K. Ma, Z. Yan, Rescue of histone hypoacetylation and social deficits by ketogenic diet in a Shank3 mouse model of autism, Neuropsychopharmacology 47 (2022) 1271–1279. https://doi.org/10.1038/s41386-021-01212-1.

[5] D.N. Ruskin, J. Svedova, J.L. Cote, U. Sandau, J.M. Rho, M.K. Jr, D. Boison, S.A. Masino, Ketogenic diet improves core symptoms of autism in BTBR mice, PLOS ONE 8 (2013) e65021. https://doi.org/10.1371/journal.pone.0065021.

[6] D.N. Ruskin, J.A. Fortin, S.N. Bisnauth, S.A. Masino, Ketogenic diets improve behaviors associated with autism spectrum disorder in a sex-specific manner in the EL mouse, Physiol. Behav. 168 (2017) 138–145. https://doi.org/10.1016/j.physbeh.2016.10.023.

[7] D.N. Ruskin, M.I. Murphy, S.L. Slade, S.A. Masino, Ketogenic diet improves behaviors in a maternal immune activation model of autism spectrum disorder, PLOS ONE 12 (2017) e0171643. https://doi.org/10.1371/journal.pone.0171643.

Journal Pre-proof

Journal Pre-proof

[8] M. del C. Blanco-Gandía, F. Ródenas-González, M. Pascual, M.D. Reguilón, C. Guerri, J. Miñarro, M. Rodríguez-Arias, Ketogenic Diet Decreases Alcohol Intake in Adult Male Mice, Nutrients 13 (2021) 2167. https://doi.org/10.3390/nu13072167.

[9] A.B. Bornebusch, G.F. Mason, S. Tonetto, J. Damsgaard, A. Gjedde, A. Fink-Jensen, M. Thomsen, Effects of ketogenic diet and ketone monoester supplement on acute alcohol withdrawal symptoms in male mice, Psychopharmacology (Berl.) (2021). https://doi.org/10.1007/s00213-020-05735-1.

[10] L.A. Martinez, M.E. Lees, D.N. Ruskin, S.A. Masino, A ketogenic diet diminishes behavioral responses to cocaine in young adult male and female rats, Neuropharmacology 149 (2019) 27–34. https://doi.org/10.1016/j.neuropharm.2019.02.001.

[11] F. Ródenas-González, M.C. Blanco-Gandía, J. Miñarro, M. Rodríguez-Arias, Effects of ketosis on cocaine-induced reinstatement in male mice, Neurosci. Lett. 778 (2022) 136619. https://doi.org/10.1016/j.neulet.2022.136619.

[12] R. Trinko, D.M. Diaz, E. Foscue, S.L. Thompson, J.R. Taylor, R.J. DiLeone, Ketogenic diet enhances the effects of oxycodone in mice, Sci. Rep. 13 (2023) 7507. https://doi.org/10.1038/s41598-023-33458-8.

[13] N.B. Bueno, I.S.V. de Melo, S.L. de Oliveira, T. da Rocha Ataide, Very-low-carbohydrate ketogenic diet *v.* low-fat diet for long-term weight loss: a meta-analysis of randomised controlled trials, Br. J. Nutr. 110 (2013) 1178–1187. https://doi.org/10.1017/S0007114513000548.

[14] F.T. McSwiney, L. Doyle, D.J. Plews, C. Zinn, Impact of ketogenic diet on athletes: Current insights, Open Access J. Sports Med. 10 (2019) 171–183. https://doi.org/10.2147/OAJSM.S180409.

Journal Pre-proof

Journal Pre-proof

[15] A. Castro, D. Gomez-Arbelaez, A. Crujeiras, R. Granero, Z. Aguera, S. Jimenez-Murcia, I. Sajoux, P. Lopez-Jaramillo, F. Fernandez-Aranda, F. Casanueva, Effect of a very low-calorie ketogenic diet on food and alcohol cravings, physical and sexual activity, sleep disturbances, and quality of life in obese patients, Nutrients 10 (2018) 1348. https://doi.org/10.3390/nu10101348.

[16] M.T. Todorova, C.A. Dangler, M.G. Drage, B.J. Sheppard, J.G. Fox, T.N. Seyfried, Sexual dysfunction and sudden death in epileptic male EL mice: Inheritance and prevention with the ketogenic diet, Epilepsia 44 (2003) 25–31. https://doi.org/10.1046/j.1528-1157.2003.11402.x.

[17] A.M.S. Arqoub, K.G. Flynn, L.A. Martinez, Gestational exposure to a ketogenic diet increases sociability in CD-1 mice., Behav. Neurosci. (2020). https://doi.org/10.1037/bne0000368.

[18] D. Kasprowska-Liśkiewicz, A.D. Liśkiewicz, M.M. Nowacka-Chmielewska, J. Nowicka, A. Małecki, J.J. Barski, The ketogenic diet affects the social behavior of young male rats, Physiol. Behav. 179 (2017) 168–177. https://doi.org/10.1016/j.physbeh.2017.06.007.

[19] F. Eskandari, M. Salimi, M. Hedayati, H. Zardooz, Maternal separation induced resilience to depression and spatial memory deficit despite intensifying hippocampal inflammatory responses to chronic social defeat stress in young adult male rats, Behav. Brain Res. 425 (2022) 113810. https://doi.org/10.1016/j.bbr.2022.113810.

[20] T. Mizuno, S. Yotsuyanagi, Y. Nagasaka, M. Namiki, Dehydroepiandrosterone Alleviates Copulatory Disorder Induced by Social Stress in Male Rats, J. Sex. Med. 3 (2006) 612–618. https://doi.org/10.1111/j.1743-6109.2006.00272.x.

Journal Pre-proof

Journal Pre-proof

[21] S. Niikura, O. Yokoyama, K. Komatsu, S. Yotsuyanagi, T. Mizuno, M. Namiki, A Causative Factor of Copulatory Disorder in Rats Following Social Stress, J. Urol. (2002). https://doi.org/10.1016/S0022-5347(05)64754-4.

[22] Committee for the Update of the Guide for the Care and Use of Laboratory Animals; National Research Council, Guide for the care and use of laboratory animals: Eighth edition, The National Academies Press, Washington, D.C., 2011.

[23] D.K. Khajuria, R. Razdan, D.R. Mahapatra, Description of a new method of ovariectomy in female rats, Rev. Bras. Reumatol. 52 (2012) 466–470.

[24] D.F. Hardy, J.F. DeBold, The relationship between levels of exogenous hormones and the display of lordosis by the female rat, Horm. Behav. 2 (1971) 287–297. https://doi.org/10.1016/0018-506X(71)90003-1.

[25] P.E. Cruz-Casallas, L.F. Felicio, A.G. Nasello, A quantitative analysis of the role of experience in the regulation of sexual behavior in male rats, Psychobiology 28 (2000) 406–410. https://doi.org/10.3758/BF03331998.

[26] L.M. Shulman, M.D. Spritzer, Changes in the sexual behavior and testosterone levels of male rats in response to daily interactions with estrus females, Physiol. Behav. 133 (2014) 8–13. https://doi.org/10.1016/j.physbeh.2014.05.001.

[27] J.M. Turner, R.G. Will, E.A. Harvey, T. Hattori, D.J. Tobiansky, V.L. Nutsch, J.R. Martz, J.M. Dominguez, Copulation induces expression of the immediate early gene Arc in mating-relevant brain regions of the male rat, Behav. Brain Res. 372 (2019) 112006. https://doi.org/10.1016/j.bbr.2019.112006.

[28] D.C. Howell, Statistical Methods for Psychology, 008 edition, Cengage Learning, 2012.

Journal Pre-proof

Journal Pre-proof

[29] Y. Hochberg, A sharper Bonferroni procedure for multiple tests of significance, Biometrika 75 (1988) 800–802. https://doi.org/10.1093/biomet/75.4.800.

[30] D.A. Dewsbury, Copulatory behaviour of rats (Rattus norvegicus) as a function of prior copulatory experience, Anim. Behav. 17 (1969) 217–223. https://doi.org/10.1016/0003-3472(69)90004-9.

[31] C.M. Tenk, H. Wilson, Q. Zhang, K.K. Pitchers, L.M. Coolen, Sexual reward in male rats: Effects of sexual experience on conditioned place preferences associated with ejaculation and intromissions, Horm. Behav. 55 (2009) 93–97. https://doi.org/10.1016/j.yhbeh.2008.08.012.

[32] L.M. Coolen, Neural control of ejaculation, J. Comp. Neurol. 493 (2005) 39–45. https://doi.org/10.1002/cne.20784.

[33] L.M. Coolen, H.J.P.W. Peters, J.G. Veening, Fos immunoreactivity in the rat brain following consummatory elements of sexual behavior: a sex comparison, Brain Res. 738 (1996) 67–82. https://doi.org/10.1016/0006-8993(96)00763-9.

[34] L.A. Lumley, E.M. Hull, Effects of a D1 antagonist and of sexual experience on copulation-induced Fos-like immunoreactivity in the medial preoptic nucleus, Brain Res. 829 (1999) 55–68. https://doi.org/10.1016/S0006-8993(99)01338-4.

[35] D.A. Damassa, E.R. Smith, B. Tennent, J.M. Davidson, The relationship between circulating testosterone levels and male sexual behavior in rats, Horm. Behav. 8 (1977) 275–286. https://doi.org/10.1016/0018-506X(77)90002-2.

[36] O.T. Kayode, A.V. Owolabi, A.A.A. Kayode, Biochemical and histomorphological changes in testosterone propionate-induced benign prostatic hyperplasia in male Wistar rats treated

Journal Pre-proof

Journal Pre-proof

with ketogenic diet, Biomed. Pharmacother. 132 (2020) 110863. https://doi.org/10.1016/j.biopha.2020.110863.

[37] A. Cignarelli, D. Santi, V.A. Genchi, E. Conte, F. Giordano, S. Di Leo, A. Natalicchio, L. Laviola, F. Giorgino, S. Perrini, Very low-calorie ketogenic diet rapidly augments testosterone levels in non-diabetic obese subjects, Andrology 11 (2023) 234–244. https://doi.org/10.1111/andr.13357.

[38] S. La Vignera, R. Cannarella, F. Galvano, A. Grillo, A. Aversa, L. Cimino, C.M. Magagnini, L.M. Mongioì, R.A. Condorelli, A.E. Calogero, The ketogenic diet corrects metabolic hypogonadism and preserves pancreatic ß-cell function in overweight/obese men: a single-arm uncontrolled study, Endocrine 72 (2021) 392–399. https://doi.org/10.1007/s12020-020-02518-8.

[39] L.M. Mongioì, L. Cimino, R.A. Condorelli, M.C. Magagnini, F. Barbagallo, R. Cannarella, S. La Vignera, A.E. Calogero, Effectiveness of a very low calorie ketogenic diet on testicular function in overweight/obese men, Nutrients 12 (2020) 2967. https://doi.org/10.3390/nu12102967.

[40] V. Vidić, V. Ilić, L. Toskić, N. Janković, D. Ugarković, Effects of calorie restricted low carbohydrate high fat ketogenic vs. non-ketogenic diet on strength, body-composition, hormonal and lipid profile in trained middle-aged men, Clin. Nutr. 40 (2021) 1495–1502. https://doi.org/10.1016/j.clnu.2021.02.028.

[41] B.A. Gladue, L.G. Clemens, Flutamide inhibits testosterone-induced masculine sexual behavior in male and female rats, Endocrinology 106 (1980) 1917–1922. https://doi.org/10.1210/endo-106-6-1917.

Journal Pre-proof

Journal Pre-proof

[42] M.Y. McGinnis, G.W. Williams, A.R. Lumia, Inhibition of male sex behavior by androgen receptor blockade in preoptic area or hypothalamus, but not amygdala or septum, Physiol. Behav. 60 (1996) 783–789. https://doi.org/10.1016/0031-9384(96)00088-1.

[43] K. Nylen, J.L.P. Velazquez, V. Sayed, K.M. Gibson, W.M. Burnham, O.C. Snead, The effects of a ketogenic diet on ATP concentrations and the number of hippocampal mitochondria in Aldh5a1−/− mice, Biochim. Biophys. Acta BBA - Gen. Subj. 1790 (2009) 208–212. https://doi.org/10.1016/j.bbagen.2008.12.005.

[44] T.A. Lusardi, K.K. Akula, S.Q. Coffman, D.N. Ruskin, S.A. Masino, D. Boison, Ketogenic diet prevents epileptogenesis and disease progression in adult mice and rats, Neuropharmacology 99 (2015) 500–509. https://doi.org/10.1016/j.neuropharm.2015.08.007.

[45] J.G. Pfaus, M.F. Wilkins, N. DiPietro, M. Benibgui, R. Toledano, A. Rowe, M.C. Couch, Inhibitory and disinhibitory effects of psychomotor stimulants and depressants on the sexual behavior of male and female rats, Horm. Behav. 58 (2010) 163–176. https://doi.org/10.1016/j.yhbeh.2009.10.004.

[46] A.K. Dixon, A.K. Gubitz, D.J.S. Sirinathsinghji, P.J. Richardson, T.C. Freeman, Tissue distribution of adenosine receptor mRNAs in the rat, Br. J. Pharmacol. 118 (1996) 1461–1468. https://doi.org/10.1111/j.1476-5381.1996.tb15561.x.

[47] S. Sheth, R. Brito, D. Mukherjea, L.P. Rybak, V. Ramkumar, Adenosine receptors: Expression, function and regulation, Int. J. Mol. Sci. 15 (2014) 2024–2052. https://doi.org/10.3390/ijms15022024.

Journal Pre-proof

Journal Pre-proof

[48] I. Ballesteros-Yáñez, C.A. Castillo, S. Merighi, S. Gessi, The role of adenosine receptors in psychostimulant addiction, Front. Pharmacol. 8 (2018). https://doi.org/10.3389/fphar.2017.00985.

[49] J.G. Pfaus, A.G. Phillips, Role of dopamine in anticipatory and consummatory aspects of sexual behavior in the male rat., Behav. Neurosci. 105 (1992) 727. https://doi.org/10.1037/0735-7044.105.5.727.

[50] E.A. Pehek, R.K. Warner, T.J. Bazzett, D. Bitran, L.C. Band, R.C. Eaton, E.M. Hull, Microinjection of cis-flupenthixol, a dopamine antagonist, into the medial preoptic area impairs sexual behavior of male rats, Brain Res. 443 (1988) 70–76. https://doi.org/10.1016/0006-8993(88)91599-5.

[51] E.M. Hull, J. Du, D.S. Lorrain, L. Matuszewich, Extracellular dopamine in the medial preoptic area: implications for sexual motivation and hormonal control of copulation, J. Neurosci. 15 (1995) 7465–7471. https://doi.org/10.1523/JNEUROSCI.15-11-07465.1995.

Journal Pre-proof