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Research report

Alterations in male sexual behaviour, attractiveness and testosterone levels induced by an adult-onset calorie restriction regimen

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ABSTRACT

Despite an abundance of research on calorie restriction (CR) altering gonadal and appetite regulating hormones, the sexual behavioural consequences of CR remain to be examined systematically. This study compared the sexual behaviour, partner preference, serum testosterone and leptin levels of male adult Hooded Wistar rats administered a CR (continuous 25%, 50% CR or a temporary restriction) with ad libitum fed controls. The temporary restriction (Previous CR) failed to alter sexual behaviour, partner preference and levels of testosterone and leptin. The moderately 25% CR males did not demonstrate an impairment in sexual behaviour but did demonstrate a reduced level of attractiveness to females in one measure of partner preference. Sexual performance was affected by a substantial CR, as the CR50% group exhibited a longer latency to the first intromission, indicating alteration in sexual arousal. Females also consistently demonstrated a clear preference for the control group compared to the CR50% group. These findings indicate a possible reduction in the overall reproductive potential of the substantially CR animals. Testosterone levels were equally suppressed by both the 25% and 50% CR, while leptin levels were only reduced in the CR50% group. Leptin, rather than testosterone, may have influenced the impairment in sexual behaviour only demonstrated by the substantially CR animals. Testosterone, may, however, play a role in modulating the preference of control over CR males, as attractiveness was totally reduced by a substantial CR, and partially reduced by a moderate restricted regimen.

1. Introduction

Calorie restriction (CR) both initiated in adulthood and during the perinatal period, has consistently been shown to alter a variety of physiological measures. The documentation of the behavioural alteration in adult CR animals, however, remains fragmented and relatively unexplored. This is especially so in relation to sexual behaviour. Even though a comprehensive characterization of sexual behaviour and performance in an adult-onset CR group is lacking, it is increasingly clear that CR has inhibitory effects on certain physiological attributes and processes related to sexual functioning, such as fertility and sexual maturation [30,46]. There is reason to suggest that sexual behaviour may also be affected in animals undergoing CR, as physiological responses related to sexual behaviour are altered by CR. For instance, not only does an adult-onset 50% CR reduce total androgens and consequently retard the growth of androgen sensitive tissue in musk shrews, but it also prevents the increase of total androgens and growth of androgen sensitive tissue that normally occur in males in the presence of estrous females [47]. This inhibition may consequently impact on the ability of CR animals to engage in sexual activity.

CR may also influence the potential of males to be chosen as mates. Since a large amount of energy is invested during pregnancy, lactation and rearing pups; females are typically the selective sex [44]. Females typically choose males that possess quality genes and resources, which increase the potential for offspring survival [2]. There is indication that CR influences the success of males at being chosen as mates, as females overwhelmingly prefer the odours of control house mice over mice that were born to mothers administered a CR during gestation [28]. Females may evaluate the olfactory signature of CR animals as less attractive, and therefore judge them as less appropriate mates. Testosterone has been suggested to play a significant role in mediating female partner preference, as females can distinguish between odours of males with different gonadal or endocrine profiles [51], and prefer males with high levels of

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testosterone [50]. This preference gradually increases with incremental increases of exogenous testosterone [15], and indicate that testosterone may modulate the lack of preference females demonstrate for the testosterone suppressed CR animals.

Alterations in the synthesis and release of hormones as a consequence of CR are relatively consistent between studies. Significant alterations are documented in several neuroendocrine axes, including the stimulation of the hypothalamic–pituitary–adrenal (HPA) axis [4,11,20] and a suppression of the hypothalamic–pituitary–gonadal (HPG) axis [14,22,27]. Luteinizing hormone (LH) and testosterone, in particular, have consistently been demonstrated to be reduced as a consequence of an adult-onset CR regimen [26,36]. Appetite regulating hormones are also altered by CR. Decreases in anorexogenic hormones, especially the adipocyte hormone leptin, are typical of food deprivation [39] and CR regimens [18,24,48], as it acts as a signal for changes in energy balance, energy stores and satiety [19,21].

An overall suppression of HPG function by CR has obvious implications for the expression of sexual behaviour. Leptin also impacts reproductive functioning. For instance, leptin administration can reverse the disruption of ovulation in food-restricted females [33,38] and also restore fertility and sexual behaviour to the infertile and sexually immature ob/ob mice [12,29]. As of this date, however, the concurrent investigation of adult-onset CR, sexual behaviour, and underlying neuroendocrine changes to testosterone and leptin has not been conducted.

The aim of the current study was to obtain a comprehensive estimation of the sexual behaviour of males administered an adult-onset CR. Therefore, two tests which encompass major features of sexual behaviour were assembled, these included measures of sexual arousal, performance and attractiveness. An additional aim of the study was to document the changes to two hormones, testosterone and leptin, that are implicated in both the expression of sexual behaviour and altered as a consequence of CR. As a clear characterization of the sexual behaviour of adult-onset CR is lacking, two levels of restriction, moderate (25%) and substantial (50%) were employed to establish a possible dose-dependant effect on behaviour. Temporary restrictions to diet have been demonstrated to alter a variety of physiological systems and ultimately result in long-lasting alterations in behaviour [32] and anxiety [23]. Therefore, a previously restricted condition was also included to examine any persistent effects of a short bout of CR on subsequent adult male sexual behaviour and hormone levels.

2. Materials and methods

2.1. Animals

Adult male (286 ± 4 g) Hooded Wistar rats procured from Melbourne University, Victoria, were used as the experimental subjects for the sexual behavioural tests and partner preference (n = 45). Experimental rats were housed individually in plastic cages and ranged from 7 to 9 weeks old at the beginning of experimentation. They were maintained on a 12-h light:12-h dark cycle (lights on 6:00 h) in a temperature controlled room set at 22 ± 1 °C. Water was available ad libitum; however, CR regimens were administered as described below.

The experimental males also served as the stimulus animals for the partner preference test. For this test, an unrelated group of adult female virgin Hooded Wistar rats served as the experimental animals (n = 10). Females were approximately 200 g at the time of arrival and were not littermates. They were maintained in the same conditions as experimental males but were pair housed and allowed free access to food and water. These also served as the stimulus animals in the sexual behaviour tests conducted 1 week after the partner preference test. All stimulus animals were used repeatedly and were distributed across all experimental conditions. Animal experimentation and care was performed in accordance with the guidelines set forth by the National Health and Medical Research Council, and were approved by La Trobe University Animal Ethics Committee, Approval Number AEC 05/02.

2.2. Calorie restriction regimen

CR was determined every week by calculating the amount of food age- and gender-matched controls ate in three consecutive days. The amount consumed by the controls was consistent across the experimental period and ranged from 24 to 28 g. The dietary composition of the food administered to controls and restricted groups are summarised in reference [25]. At the start of the experiment, rats were randomly allocated to one of four CR regimens; controls, fed ad libitum; 25% calorie restriction (CR25%), received 75% of the amount consumed by controls (~19–21 g per day); 50% calorie restriction (CR50%), received 50% of the amount consumed by controls (~12–14 g per day); a short-term CR50% (Previous CR) group that received a 50% CR on a single occasion for a 3-day period at the beginning of the experimental period and were then reverted to an ad libitum diet for the remainder of the experiment. The CR period began after a week of acclimation (approximately 8 weeks of age) and continued for 3 weeks before partner preference and sexual behaviour testing took place, on the fourth and fifth weeks of the CR period, respectively. The CR regimens extended throughout the testing period and food was delivered daily between 8:00 and 10:00 h.

2.2.1. Surgery and estrous induction

Three weeks before testing, sexually mature females rats were bilaterally ovariectomized under anaesthesia (ketamine 61 mg/kg and xylazine 9 mg/kg) and given 2 weeks to recover. Before all sessions, artificial estrous was induced by subcutaneous injections of estradiol benzoate (10 μL in 0.1 mL of olive oil) 48 and 24 h, and progesterone (1 mg in 0.2 mL of olive oil) 4 h before each test. Females were given three 30 min sessions with sexually experienced males to acquire the full range of sexual behaviours before being paired with an experimental male. Behavioural estrous was verified before each test session by exposing each female to a sexually experienced male; they were determined to be receptive if they displayed 6 or more lordosis responses to 10 mounts of a male.

2.2.2. Sexual behaviour test

A male was placed in a clear Perspex box (45 cm × 25 × 40 cm) for 5 min, before a receptive female was introduced; male sexual behaviour was then monitored and recorded for 30 min. If the male failed to display a mount within the first 15 min, the female was removed and replaced with another receptive female and the 30 min session resumed. If no mount occurred again within 15 min the trial was terminated. The arena was cleaned with ethanol after each test pair. Behavioural variables examined during the sexual behaviour tests included frequency of mounts, intromissions and ejaculations, as well as frequency of mounts and intromissions in the first series (from the start of the test until the first ejaculation). The mount and intromission latency (interval between start of test and first mount and intromission), ejaculation latency (interval between first intromission and ejaculation) and the post-ejaculatory interval (period between ejaculation and the first subsequent mount or intromission) were also recorded.

2.2.3. Partner preference test

The attractiveness of the experimental males to females was examined with the partner preference test. In this test, a female's preference for experimental males was assessed in a clear Perspex box (100 cm × 50 cm × 50 cm), separated into three compartments by wire. Each female was exposed to a total of three trials. In each trial a control rat was placed into a compartment while a rat from one of the three CR groups (either CR25%, CR50% or previously restricted) was placed in the adjoining compartment, until all three comparisons had been made. Females were never exposed to the same control animal. Furthermore, the placement of the control and CR rat in the left and right compartment alternated between all trials, in order to eliminate any effects of place preference of the female. The order of the pairings was also randomised. The male rats were placed in their respective compartments 5 min before a female was introduced. The receptive female was then placed in the neutral compartment, enabling her to explore the outside of each compartment containing one of the males. The total time spent in front of each compartment and the duration of investigative behaviours (direct sniffing) and interaction (pawing) with each partner through the wires was recorded for 15 min, whereupon all rats were returned to their home cages. The arena was cleaned with ethanol after each trial. All partner preference tests and sexual behaviour tests were conducted under red light conditions during the first half of the dark phase of the light:dark cycle. Three days after all testing, rats were decapitated by guillotine and trunk blood collected. Blood was centrifuged, serum collected and stored at −20 °C until all samples were collected for determination of serum testosterone and leptin.

2.2.4. Determination of testosterone concentration

Testosterone was determined by ELISA (IBL, Hamburg, Germany). Serum samples were thawed and plated in duplicate in antibody-coated microplate wells and incubated for 60 min at room temperature with horseradish peroxide labelled testosterone. Following the incubation period, the microplate was decanted and washed three times; 200 μL of tetramethlbenzidine (TMB) solution was added to each well and incubated at room temperature for 15 min. After incubation, colour development was stopped, and the optical density of the wells was measured at 450 nm with a Synergy HT Multi-detection Microplate Reader (Bio-Tek Instruments Inc., Winooski, Vermont, USA). Testosterone levels were calculated using KC4 v 3.4 software (Bio-Tek Instruments). Intra- and inter-assay coefficient of variation was 3.28% and 6.71%, respectively. The detection limit of the assay was 0.083 ng/mL.