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Physiological and behavioral responses to intermittent starvation in C57BL/6J mice

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ABSTRACT

The dual intervention point model states that body mass is controlled by upper and lower intervention points, above and below which animals (and humans) intervene physiologically to bring their body mass back into the acceptable range. It has been further suggested that the lower intervention point may be defined by the risk of starvation, while the upper intervention point may be defined by the risk of predation. The objective of the present study was to test whether the risk of starvation determines the lower intervention point and to examine the physiological and behavioral mechanisms that underpin the regulation of body mass, when the risk of starvation is increased. Sixty-four mice were exposed to random days of complete fasting or 50% food restriction and their body mass and fat mass responses were measured. Food intake, physical activity and body temperature were measured throughout the experiment. In addition, plasma leptin and insulin, triglyceride and non-esterified fatty acids, along with hypothalamic neuropeptides gene expression in the arcuate nucleus were assessed after 13 and 42 days of treatment. We found that C57BL/6J mice increased body mass and fatness in response to a short-term (13 days) intermittent fasting, which was restored to baseline as the treatment was prolonged. In contrast, intermittently 50% food restricted mice showed no significant changes in body mass or fatness. Over the first 13 days of treatment the data were consistent with the dual intervention point model as the mice showed both increased body mass and adiposity over this period. Over the more protracted period of 42 days the effect waned and was therefore inconsistent with the model. The body mass and fat mass gains in intermittently fasted mice were mainly accounted for by increased food intake. Elevated NPY gene expression after 13 days (three 24 h fasting events) may have driven the increase in food intake. However, no changes were observed in such neuropeptides as POMC, CART, AgRP, Ob-Rb and SOCS 3 or circulating levels of leptin, insulin, NEFA and TG. Hypothermia during fasting days may have also contributed to the increase in body mass. Over 42 days of treatment (nine 24 h fasting events) cumulative food intake was not affected by intermittent starvation. However physical activity, mainly activity during the light phase was lowered suggesting an adaptation to unpredictable starvation. Overall, mice exhibited different behavioral and physiological responses to intermittent starvation depending on the duration of treatment.

1. Introduction

1.1. Body mass regulation

The global epidemic of obesity has raised the need for a better understanding of the mechanisms that regulate body and fat mass. A balance between energy intake and energy expenditure is necessary to maintain a stable body mass. It is well established that the central nervous system (CNS) regulates food intake and energy expenditure in response to neuronal, hormonal and nutrient signals [1–3]. The hypothalamus is the most studied area in CNS with respect to the regulation of energy homeostasis. The arcuate nucleus in particular is known to play an important role in energy homeostasis because it contains two distinct populations of neurons: pro-opiomelanocortin (POMC) neurons that express the endogenous anorectic melanocortin receptor agonist (α-melanocyte stimulating hormone, α-MSH) along with cocaine-and-amphetamine-regulated transcript (CART), and neurons that express neuropeptide Y (NPY) and also the endogenous orexigenic melanocortin receptor antagonist, agouti-related protein (AgRP) [4–6]. These neuronal populations respond to peripheral signals, such as leptin, insulin and gastrointestinal hormones by modifying the production of the above neuropeptides that modulate energy balance [7]. Moreover, leptin signaling within neurons is dependent on the presence of the long form of the leptin receptor (Ob-Rb) [8], which signals principally via the Janus kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3) pathway [9]. The suppressor of cytokine signaling 3 (SOCS3) is a negative regulator of the leptin receptor which is stimulated by the JAK/STAT2

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pathway [10–13]. Gene expression levels of Ob-Rb and SOCS3 are markers of leptin sensitivity [14].

There have been several theoretical models that have attempted to conceptualize the mechanisms involved in the regulation of body mass and fatness. Kennedy (1953) proposed the lipostatic set point model which suggested that the size of body fat depots is sensed by a 'lipostat', which adjusts food intake and energy metabolism to maintain the body and fat masses at a set-point [15]. Although leptin has been often interpreted as the molecular manifestation of the lipostatic fat signal [16], this model is in conflict with the evidence in patterns of changes in animal and human body mass [17,18]. An alternative interpretation emerged suggesting that body mass is not regulated by a set-point, but rather is controlled by upper and lower intervention points, above and below which animals (and humans) intervene physiologically to bring their body mass back into the acceptable range [19,20]. In humans the upper intervention point may be located at different positions in different individuals explaining why some individuals become obese when exposed to environments with readily available food supplies, but others are able to regulate their body weights at normal levels. Based on data from small mammals and birds, Speakman (2007) has further suggested that the lower intervention point may be defined by the risk of starvation while the upper intervention point may be defined by the risk of predation [20]. In this scenario, one would expect that an increased risk of starvation would increase the lower intervention level and animals would gain fat and body mass.

1.2. Intermittent starvation

Periods of negative energy balance arising from restricted feeding or total starvation are common events [21]. Throughout their lives, small mammals must face periodic food shortages interspersed by periods of food abundance.

Stochastically imposed intermittent periods of fasting (complete absence of food) or periodic food restriction (involving reduced levels relative to habitual intake, but not complete absence) may mimic the unpredictable food availability in the wild. However, few studies have investigated the physiological and behavioral responses to stochastic food exposure. Swiss mice showed increased food intake and decreased energy expenditure on days that intervened between 24 h fasting events over 4 weeks treatment (including 3 or 4 fasting days and 4 or 3 feeding days each week), and they decreased overall body mass [22]. However in another study the same group found that 4 weeks treatment including 3 fasting days and 4 feeding days each week had no effect on overall body mass [23]. Alternate day fasting (ADF) is a similar and widely studied regimen, however, most of the studies have been focused on the beneficial effects on aging and the effect of ADF on body weight is less often reported. Body mass has been shown to be highly variable in response to ADF in both humans and in animal models. In Fisher rats, when ADF regimens were applied in the short term, no effect on body weight was observed after 2 weeks [24], whereas gains in body mass were noted in C17BL/10 mice after 8 weeks [25]. However, in other studies, when ADF was administered for 12 weeks, body mass decreased in both C57BL/6 mice and rats. This variability in response is confusing and its relationship to the dual-intervention model is uncertain because in ADF the fasting days come at predictable intervals, and hence the uncertain risk of starvation is not altered by this treatment. Even in the studies performed by Zhao and colleagues [22,23] the occurrence of fasting days is so frequent that it may be predictable — leading to different responses from those predicted by the dual-intervention point model.

In the present study we aimed firstly to test whether starvation risk defines the lower intervention point for body mass and adiposity as suggested in the dual intervention point model, by experimentally increasing starvation risk over a period of 42 days, by exposing mice to nine random days of complete fasting or food restriction, and recording their body mass and fat mass responses. Second we investigated the physiological and behavioral mechanisms that underpin the regulation of body mass during intermittent fasting or food restriction, by measuring food intake, physical activity and body temperature throughout the experiment. In addition, NPY, AgRP, POMC, CART, Ob-Rb and SOCS3 gene expression in the arcuate nucleus of the hypothalamus were assessed after 13 days and 42 days of treatment. We also measured plasma leptin and insulin, as well as triglyceride (TG) and non-esterified fatty acids (NEFA). We hypothesized that if the dual intervention point model is correct randomly imposed intermittent fasting and food restriction would cause body mass and fat mass to increase as a result of an increase in the lower intervention point. Secondly, we hypothesized that physiological and behavioral compensation mechanisms would be employed to adjust body mass and fatness within the range and thirdly, that leptin, insulin and other potential signals would change in response to intermittent fasting and food restriction and neuropeptides sensitive to leptin would be involved in the body mass response.

2. Material and methods

2.1. Animal housing and intermittent starvation regimen

Sixty four female C57BL/6 mice aged 6–8 weeks were purchased from Charles River (Charles River UK Ltd, Kent, UK) and housed in single cages (M3 cage 48 × 15 × 13 cm, NKP Cages, Kent, UK) in a temperature controlled room (21 ± 1 °C) under a 12:12-hour light:dark photoperiod with the lights coming on at 06:00 and a "dawn/dusk" period of 20 min at either end of the light period. Wood shavings and shredded paper bedding were provided for enrichment. At around age 9–10 weeks mice were implanted with transmitters that measured their body temperatures and physical activity levels (details below) and were then fed a standard control diet (D12450B, 10% kcal/fat, Research Diet, New Brunswick, NJ, USA) ad libitum until 22 weeks of age when they were randomly assigned into three groups: a control group AL, fed ad libitum (n = 20); IF, intermittently fasted (n = 22); IR, intermittently 50% food restricted (n = 22). All mice had free access to water throughout the study. All procedures were reviewed by a local ethical committee and performed in accordance with UK home office regulations under license PPL 60/3707 held by JRS.

Mice were divided into two cohorts containing 32 mice in each AL (n = 10); IF (n = 11); IR (n = 11). Note the sample sizes in each group and cohort were unbalanced because the number of recording pads for the implanted transmitters (see below) was fixed at 64. Body mass and food intake were recorded for 3 days on a daily basis prior to the intermittent starvation regime. Animals were stochastically exposed to 24 h fasting or restriction on 9 occasions over a period of 42 days. Each starvation day was always followed by a non-starvation day. Probability of next day would be starvation was set at 0.21 and was decided using random integers. During the treatment, days 4, 7, 10, 14, 16, 20, 26, 29 and 39 were assigned as fasting/restriction days. On each treatment day, IF mice were completely deprived of food, while IR mice were given 50% of their average daily food intake measured over the baseline period at the start of the experiment. Mice were provided with ad libitum food between fasting/restriction days. Body mass and food intake were measured at 1500 h every day when food was given or removed. The first cohort of mice was killed on day 13 of the treatment, while the second cohort was killed on day 42.

2.2. Physical activity and body temperature

Prior to experimentation the mice were implanted with a telemetry transmitter to monitor body temperature (T_b) and physical