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The influence of different calorie restriction protocols on serum pro-inflammatory cytokines, adipokines and IGF-I levels in female C57BL6 mice: Short term and long term diet effects

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ABSTRACT

Calorie restriction (CR) is an effective intervention to prevent chronic diseases including cancer. Although many factors, i.e., sex hormones, IGF-I and mTOR have been studied in response to CR, the molecular mechanisms of CR remain to be identified. Our objective was to determine the short and long-term effects of different CR protocols on pro-inflammatory cytokines. Our hypothesis was that Intermittent CR (ICR) would result in greater inhibition of pro-inflammatory serum cytokines compared to Chronic CR (CCR) as we previously found ICR to be more protective in the prevention of mammary tumor development. From ten weeks of age female C57BL6 mice were maintained on either ad libitum (AL) fed, ICR or CCR protocols (overall CR of ~75% of AL) for up to 74 weeks of age. Blood samples were collected for measurements of serum interleukin-6 (IL-6), tumor necrosis factor–alpha (TNF-α), adiponectin, leptin, IGF-I and insulin at specified ages. For ICR mice samples were collected following 3 weeks of restriction (ICR-R) and after one week of refeeding (ICR-RF). In general, both modes of CR significantly reduced serum IL-6, TNF-α, IGF-I and leptin levels compared to AL with IL-6 levels 24 and 3.5 fold and TNF-α levels t 11 and 1.5 fold lower in ICR and CCR groups, respectively at study termination. There was a trend for adiponectin and insulin to be highest in ICR-RF mice. Body weights were positively correlated with IL-6, TNF-α, insulin and leptin but negatively correlated with adiponectin-to-leptin ratio. Moreover, there was a positive correlation between IL-6 and TNF-α. Beneficial effects of ICR may function through pro-inflammatory cytokine pathways.

1. Introduction

Calorie restriction (CR) is one of the most effective experimental interventions to prevent chronic health problems such as cardiovascular disease, type-2 diabetes, dyslipidemia, asthma and cancer (Dogan et al., 2010; Gonzalez et al., 2012; Harvie and Howell, 2012; Imayama et al., 2012; Johnson et al., 2007). For example, researchers have reported that CR prevents both spontaneous and carcinogen induced mammary tumor (MT) incidence in rodents (Dogan et al., 2010; Cleary et al., 2007; Gillette et al., 1997; Hursting et al., 2003) with up to 95% decrease when caloric intake is limited to 60–80% of their ad libitum (AL) fed consumption (Dogan et al., 2010; Cleary et al., 2007; Gillette et al., 1997). In general, two types of CR, i.e., chronic calorie restriction (CCR) and intermittent calorie restriction (ICR), a regimen for alternate periods of "on" and "off" caloric restriction, have been used (Dogan et al., 2010; Hursting et al., 2003; Cleary et al., 2002; Jiang et al., 2009). Although the effects of CCR on cancer prevention have been studied extensively, ICR investigations have been more limited. Interestingly, it has recently been reported that when compared directly in the same study, ICR was more effective than CCR in MT prevention (Dogan et al., 2010; Cleary et al., 2007; Rogozina et al., 2011). For example, MMTV-TGF-α mice fed AL had MT incidence in the range of 80%, while the MT incidence rate was 27–44% in CCR mice but was only between 3 and 15% in ICR mice restricted to a similar overall degree but in an intermittent fashion (Dogan et al., 2010; Cleary et al., 2007; Rogozina et al., 2009).

Even though the protective effect of CR in many pathological conditions including cancer prevention has been well established, the mechanisms of this intervention, particularly how ICR interacts in signaling pathways have not been identified. In this context, the roles of many factors such as sex hormones, IGF-I and its binding proteins (IGFBPs), insulin, adipokines, nuclear factor kappa B (NFκB), reactive oxygen species (ROS), prostaglandins, special microRNAs and mTOR have been studied (Dogan et al., 2010; Hursting et al., 2003; Dogan et al., 2007; Dogan et al., 2011; Lukanova et al., 2004; Olivo-Marston et al., 2014; Lashinger et al., 2014; Simpson and Brown, 2013a; Hursting et al., 2013; Schetter et al., 2010; Hursting, 2014; Harvey et al., 2014; Cao,

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2014; Shinmura, 2013; Harvey et al., 2013), but still many questions remain unanswered. Recent findings from in-vitro and in-vivo studies demonstrated that leptin and adiponectin, which are primarily secreted from adipose tissue (adipokines), play important roles in mammary tumor development and cell proliferation through different intracellular signaling pathways (Dogan et al., 2010; Olivo-Marston et al., 2014; Hursting, 2014; Cao, 2014; Harvey et al., 2013; Dieudonne et al., 2006; Gonzalez et al., 2006; Zhou et al., 2011; Zheng et al., 2013) and their secretion is modulated by calorie restriction (Dogan et al., 2010; Rogozina et al., 2011; Harvie et al., 2011; Vona-Davis and Rose, 2007).

In the current paper, we measured the effects of two different calorie restriction protocols in female C57BL6 mice on serum concentrations of pro-inflammatory cytokines as this is an understudied area of interest with respect to the preventive impact of CR especially for ICR. Wild-type mice were used so that disease conditions did not interfere with the measured factors. Systemic chronic inflammation caused by higher body mass index (BMI) is considered to be a major pathological event for many health disorders including cancer (Imayama et al., 2012; Olivo-Marston et al., 2014; Harvey et al., 2014; Cao, 2014; Shinmura, 2013; Zheng et al., 2013; Harvie et al., 2011; Hotamisligil et al., 1993; Morris et al., 2011; Vozarova et al., 2001; Simpson and Brown, 2013b). Conversely, both animal and human studies have suggested that CR results in favorable changes of many biochemical factors including cytokines (Gonzalez et al., 2012; Imayama et al., 2012; Hursting et al., 2003; Olivo-Marston et al., 2014; Hursting et al., 2013; Hursting, 2014; Harvey et al., 2014; Shinmura, 2013; Harvey et al., 2013; Harvie et al., 2011; Vona-Davis and Rose, 2007; Morris et al., 2011; Harvie and Howell, 2006; Higami et al., 2006; Csiszar et al., 2014; Harvie et al., 2013; Csiszar et al., 2013). Reduction in fat mass correlates with decrease in pro-inflammatory cytokines levels in serum implying that approaches designed to promote fat loss should be useful in attenuating the pro-inflammatory setting associated with higher body weight (Imayama et al., 2012; Harvie et al., 2011; Cottam et al., 2002). To our knowledge no previous reports have been published on the long-term effects of CR specifically ICR on pro-inflammatory cytokines, adiponectin (an anti-inflammatory adipokine), IGF-I and insulin in non-transgenic mice model that mimics healthy human population with normal body mass. Therefore, the main objective of the current study was to determine the short- and long-term effects of different CR protocols on pro-inflammatory cytokines/adipokines, IGF-I and insulin levels in wild-type mice.

2. Materials and methods

2.1. Animals and study design

For the CCR and ICR diets nutrients were altered with the goal that except for carbohydrate all other nutrients in absolute amounts were similar among the groups. Eight-week-old female C57BL6 mice obtained from a colony maintained at the Hormel Institute were fed AIN-93M diet from 8 until 10 weeks of age and then were randomly assigned to one of three experimental groups: AL-fed, ICR, and CCR. AL mice were fed AIN-93M diet throughout the study. Mice assigned to the ICR group were provided with a 50% restriction diet (with 2-fold increases in protein, vitamin, mineral and fat content which was isocaloric compared to the AIN-93M diet) for three week restriction periods. Following restriction periods, ICR mice were provided the AIN-93M diet at 100% of age-matched AL consumption for three weeks. CCR mice were fed at 75% of AL mice using a diet formulated to be isocaloric with AIN-93M with 25% increases in protein, vitamin, mineral and fat content. The diets were purchased from Harlan Teklad (Madison, WI) and have previously been described in detail (Dogan et al., 2010; Rogozina et al., 2011). All mice had free access to water and were individually caged. Food intakes were determined daily and body weights weekly. Animals were observed for any health problems by expert veterinary technicians on a daily basis and by veterinarian when it was necessary. At designated ages: 10 (baseline), 13, 14 (Cycle 1); 37, 38 (Cycle 5); and 73, 74 (Cycle 11) weeks of age blood samples were obtained and mice were sacrificed five hours after they were given their daily allotment of food. Tissue samples were collected and sent it to pathology department for pathological analysis by an expert on blind fashion. All the samples were used in this experiments were from healthy mice which had no tumors or any other pathophysiological conditions according to their pathological reports. Weeks 13, 37 and 73 were after three weeks of restriction for ICR mice which are designated as ICR-Restricted (ICR-R) and weeks 14, 38 and 74 following one week of refeeding for ICR mice which are designated as ICR-Refed (ICR-RF). Because there were no differences in body weights and serum measurements for AL-fed and CCR mice for the one week age differences their data were combined. Number of samples ("n" values) for each group was between 4 and 16 at each time point. This study was approved by the University of Minnesota Institutional Animal Care and Use Committee and the University of Minnesota is an AAALAC accredited facility.

2.2. Measurement of pro-inflammatory cytokines, adipokines and IGF-I levels

Serum IL-6, TNF-α, insulin and leptin levels were measured using the Mouse Adipokine LINCOplex Kit 96 Well Plate Assay (LINCO Research, St. Charles, MO) and adiponectin was measured using the Mouse Single Plex Adiponectin LINCOplex Kit 96 Well Plate Assay (LINCO Research, St. Charles, MO) and read on the Luminex100 instrument. Serum IGF-I levels were measured using mouse/rat IGF-I ELISA (DSL-10-29,200, Diagnostic Systems Laboratory, Webster, TX) kit. Each serum sample was run in duplicate. Also an internal control sample was run in every plate and negative control samples were used. Inter-assay CVs in % are 7.0% for insulin, 11.9% for leptin, 10.0% for IL6 and 9.1% for TNF-a. Intra-assay CVs in % are 4.0% for insulin, 6.0% for leptin, 7.2% for IL6, 6.7% for TNF-a. The measurements were done and values were obtained by experts who have many publications in this field (Gu et al., 2009; Linkov et al., 2009). Because some of the samples we used had below the lowest standard value when the cytokine levels were calculated in pg/ml the fluorescence unit was used in the present study. For the correlation and ratio metric analysis, the values from the same individual mice were used.

2.3. Statistical analysis

Results are presented as means ± SEM and analyzed using GraphPad Prism 4 (San Diego, CA). Final body weight, fat pad weight, and serum concentrations of IL-6, TNF-α, adiponectin, leptin, adiponectin to leptin ratio (A/L), insulin and IGF-I were analyzed by one-way ANOVA followed by Newman-Keuls multiple comparison test to determine significant differences between specific groups at each age. Also within each group at each age changes were assessed. Spearman correlation test and linear regression analysis were used separately to determine whether correlation between two parameters was statistically significant. As indicated above because there were no significant difference between two consecutive time points at weeks 13 and 14, 37 and 38, and 73 and 74 for AL-fed and CCR mice data from these two time points were combined. However, for ICR mice these time points were presented separately. Statistical significance was at $p < 0.05$ or less.

3. Results

3.1. The effects of different calorie restriction protocols on body and fat pad weights (Table 1)

There were no differences in body weights among the groups when mice were assigned to the study at 10 weeks of age ($p > 0.05$). Body weights of AL mice were significantly higher than the other groups at