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Metabolizable energy intake during long-term calorie restriction in rhesus monkeys ☆,☆☆

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Abstract

Calorie restriction (CR) is a dietary intervention shown to increase maximum life-span. The aim of this study was to compare the metabolizable energy of the pelleted semi-purified diet with estimated energy intake from food weight. Energy density of diet, urine and feces were measured by bomb calorimetry in rhesus monkeys (23–29 years old) on CR (CR, $n = 11$) and control (C, $n = 9$). Food moisture was measured to be 2-fold higher ($9 \pm 1\%$) than indicated on the label (~5%). The measured gross energy of diet was 4.4 kcal/g dry weight of CR and 4.5 kcal/g dry weight of C diets. In a two-day trial, food intake (mean ± SD) was 112 ± 20 g and 136 ± 26 g of dry mass/d in the CR and C monkeys, respectively ($p = 0.003$). The fraction of the diet absorbed (CR = 0.91; C = 0.95) was different ($p < 0.001$) between CR and C monkeys. Using these coefficients, the metabolizable energy intake averaged over 6 months was 450 ± 53 and 534 ± 97 kcal/d in CR and C monkeys, respectively (Diff = 16%; $p = 0.03$). These values were compared with energy expenditure (EE), as measured annually by indirect calorimetry (490 ± 61 kcal/d in CR and 532 ± 62 kcal/d in C monkeys). Adjusted for changes in body composition (2 ± 10 kcal/d in CR and -7 ± 12 kcal/d in C), energy balance was not different from zero in CR (-42 ± 42 kcal/d) and C (9 ± 61 kcal/d) monkeys. Use of diet weight is a reasonable estimate of the level of CR when food waste is assessed.

Keywords: Energy balance; Absorption efficiency; Food waste; Monkey chow composition; Energy expenditure; Calorimetry; Doubly labeled water; Fecal energy loss

1. Introduction

Calorie restriction (CR) without malnutrition has been shown to delay aging and attenuate age-related diseases in various organisms (Weindruch and Walford, 1988; Hunt et al., 2006). Reduced energy intake rather than a reduction in any specific macronutrient has been shown to be the primary cause of the life span extension (Iwasaki et al., 1988; Masoro, 1988). CR's ability to retard aging has been attributed to many reasons including a decrease in the metabolic rate (Harman, 1981) and associated reduction in oxidative damage (Bevilacqua et al., 2004; Masoro et al., 1991; Sohal and Weindruch, 1996). Although it is well established that an acute decrease in energy intake decreases lean mass-adjusted metabolic rate (McCarter, 1991; McCarter et al., 1985; McCarter and McGee, 1989), it is still debated whether this reduction is maintained during long-term CR or if the long-term reduction is explained by a decrease in body and organ mass (Gallagher et al., 1998).

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While the effects of reduced energy intake have been studied extensively, many studies have estimated the degree of restriction based on the degree of reduction in the mass of diet that disappears from the food holder. This, however, may not accurately reflect the true degree of restriction. For example, it is known that the energy released when food is completely combusted in a bomb calorimeter does not exactly equal the metabolic energy provided to the body due to digestibility and incomplete utilization – i.e. energy losses in urine and feces. This reduction in energy available to the body has given rise to the term 'metabolizable energy' (ME), which is defined as the energy available to the body after correcting for losses in urine and feces (Moe, 1994). The systematic approach to measuring metabolizable energy was refined by Atwater and coworkers as extensively discussed in USDA Handbook #74 (Merrill and Watt, 1973). This body of work resulted in the determination of the general Atwater factors, which are the well-known values of 4, 9, and 4 kcal/g of metabolizable energy for average dietary carbohydrate, fat and protein, respectively. In addition, specific Atwater factors for the macronutrients in specific foods have been developed for an increasingly wide range of food items.

Calorie restriction in itself may also influence the metabolizable energy of a diet. Long-term CR has been shown to cause a variable decrease in the organ masses (Weindruch and Sohal, 1997) including intestinal mass which decreases to a greater extent than the degree of restriction (Weindruch and Sohal, 1997; Greenberg, 1999; Greenberg and Boozer, 2000). This decrease in intestinal mass could lead to a decrease in absorption of nutrients (Karasov et al., 2004). Notwithstanding, the feeding behavior in CR monkeys is such that all the food given to them is eaten in a relatively short amount of time followed by long post-absorptive periods. This may lengthen the transit time in the gut leading to better absorption of nutrients. Published data, however, is lacking with regard to either of these hypothesized effects of long-term CR on the absorption of nutrients.

The main aim of this study was to accurately determine the difference in metabolizable energy intake between CR and C monkeys. We assessed the effects of reduced food intake on the digestibility and metabolizability of food by measuring the waste energy in two consecutive 24-h urinary and fecal collections in 23- to 29-year-old male monkeys. We also tested the accuracy of the estimates metabolizable energy by comparing the results against measured energy expenditure and changes in body composition.

2. Methods

2.1. Animals

Twenty ($n = 11$, CR; $n = 9$, C) late middle aged (23–29 years old) rhesus monkeys (*Macaca mulata*) that are part of an ongoing, long-term (14 y) caloric restriction study (Ramsey et al., 1997) were studied. When the study was initiated, the CR group was restricted by 30% based on individually measured food intake prior to the imposition of CR. During the subsequent 14 years, the control group has reduced its voluntary energy intake and the weight of diet provided to the CR group was reduced accordingly on two occasions. There was an 18% restriction based on weight of diet of CR compared to C at the time of this study. Animals are fed a semipurified diet (Teklad, Madison, WI) containing 15% lactalbumin, 10% corn oil, and ~65% carbohydrate. Additional details of this study were previously published (Ramsey et al., 1997; Kemnitz et al., 1993). The protocol was reviewed and approved by the Institutional Animal Care and Use Committee of the Graduate School at the University of Wisconsin.

2.2. Food intake measurement and gross energy intake

Throughout the duration of the main study, food intake was measured daily and these values were averaged by week and then by year. Because the food intakes were stable and composition was constant, we utilized the measured intake on the same two consecutive days for which we performed urinary and fecal collections without the aid of fecal markers. As part of the standard protocol, a measured amount of food was put into individual diet hoppers in the morning. At the end of the day any remaining food in the hoppers was collected and measured. Standard procedure for the main CR study and hence for the 12-month intakes reported herein were that any whole or large partial pellets that fall through the cage floor were counted and converted to the nearest gram. Food intake was calculated for each individual monkey as the difference between the weights of food given to the monkeys minus the food left in the hoppers including the pellets and partial pellets from the pans under the cages. During this two-day period of metabolizable energy study, we altered this procedure by collecting and weighing all of the small pieces of uneaten food (pellets plus partial pellets or crumbs) that fell to the pan. These crumbs of food were collected, dried in an oven at ~50 °C to remove the urinary moisture contamination and weighed.

In the current study, the moisture content of the food pellets was measured as the weight lost when feed was dried in an oven at 50 °C. Food pellets were sampled from various food-barrels within a lot. Each barrel of CR and C food was sampled three times before the barrel was empty and from three portions of the surface, per sampling. We sampled various barrels from three different lots to get a good estimate of the moisture content of food. These collected food pellets were dried in an oven at ~50 °C until two consecutive weights were the same (indicating complete drying). These dried food samples were combusted in an adiabatic bomb calorimeter to obtain the gross energy (GE) of CR and C food pellets.

2.3. Fecal and urinary collections

While the monkeys remained in their usual cages (home cage), two consecutive 24-h urine and fecal samples were col-