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Short-term calorie and protein restriction provide partial protection from chemotoxicity but do not delay glioma progression

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ABSTRACT

Short-term starvation (STS) protects normal cells while simultaneously sensitizing malignant cells to high-dose chemotherapeutic drugs in mice and possibly patients. The fasting-dependent protection of normal cells and sensitization of malignant cells depends, in part, on reduced levels of insulin-like growth factor-1 (IGF-1) and glucose. Calorie restricted diets with defined macronutrient (carbohydrate, protein, fat) ratios were evaluated for the effects on stress sensitization markers and protection in mice treated with high-dose chemotherapy. We show that short-term CR significantly reduced both glucose and IGF-1 levels, but when specific macronutrient deficiencies were tested, only the complete lack of proteins reduced IGF-1 levels. Short-term 50% CR combined with either severe protein-deficiency or ketogenic diets improved chemotoxicity resistance similarly to the standard 50% CR, but did not result in the high protection caused by STS. Notably, a high protein diet reversed the beneficial effects of short-term CR. In a subcutaneous mouse model of glioma, feeding a low protein (4% calories from protein) diet for more than 20 days did not delay tumor progression once the tumor became palpable. Also, cycles of short-term (3 days) 50% CR did not augment the chemotherapy efficacy of cisplatin in a murine breast cancer model. These results indicate that the protection from chemotoxicity and retardation of the progression of certain tumors achieved with fasting is not obtained with short-term calorie and/or macronutrient restriction.

1. Introduction

Calorie restriction (CR), defined as a reduction in calorie intake without malnutrition, is the most potent and reproducible intervention known to simultaneously protect against age-related diseases (Omodei and Fontana, 2011), including cancer (Longo and Fontana, 2010), and increase the median and maximum lifespan in mammals (Fontana et al., 2010). From an evolutionary point of view, the effects of CR may be explained by the ability of organisms to maximize survival during periods of food shortage (Kirkwood et al., 2000). CR decreases serum levels of IGF-1 and insulin, and reduces the activity of the TOR (target of rapamycin) pathway, an intracellular signal-transduction cascade conserved from yeast and plants to mammals (mTOR), where it integrates nutrient availability, as well as hormone-, growth factor-, and stress-signaling. Similarly to CR, the inhibition of TOR by genetic or pharmacological means extends lifespan (Blagosklonny, 2010; Fabrizio et al., 2001; Sharp and Bartke, 2005). CR reduces oxidative stress (Sohal and Weindruch, 1996; Youngman et al., 1992) and cell proliferation while enhancing autophagy (Cuervo et al., 2005; Wohlgemuth et al., 2007) and certain DNA repair processes (Weraarchakul et al., 1989).

Macronutrient defined diets, with altered proportions of fat, carbohydrates and protein, do not generally have major effects on the lifespan and healthspan of rodents unless calorie restriction is applied to these modified diets (Iwasaki et al., 1988; Masoro, 1990; Ross and Bras, 1973). Of note, a severe restriction of dietary protein (or specific amino acids) can extend the lifespan of rodents by up to 20% independently of the caloric intake (Pamplona and Barja, 2006), may reduce tumorigenesis (Youngman, 1993) and protects against renal and hepatic ischemic injury, resulting in reduced inflammation and preserved organ function (Peng et al., 2012). Reduced levels of serum IGF-1 in rats and mice fed with protein-restricted diets might explain the beneficial effects on longevity (Sonntag et al., 1999).

Based on the ability of complete starvation to have more potent and rapid effects on cellular protection, resistance to ischemia reperfusion injury and longevity of certain organisms compared to CR (Longo et al., 1997; Mitchell et al., 2010; Wei et al., 2008), we have described the beneficial role of cycles of several days of a complete lack of calories (short-term starvation, STS), followed by a period of *ad lib* feeding that allows rodents and humans to rapidly regain normal weight, in cancer treatment (Lee and Longo, 2011). STS selectively protects normal cells,

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mice, and possibly patients from chemotoxicity without interfering with the therapeutic outcome on cancer cells, an effect we termed Differential Stress Resistance (DSR) (Lee et al., 2010; Raffaghello et al., 2008; Safdie et al., 2009). Furthermore, fasting sensitized 15 out of 17 malignant cell lines tested to chemotherapeutic treatment *in vitro* and augmented the efficacy of chemotherapeutic agents in mouse models of tumor progression, including breast cancer, melanoma, neuroblastoma and glioblastoma multiforme (Differential Stress Sensitization or DSS) (Lee et al., 2012; Safdie et al., 2012). The fasting-induced DSR may be attributed to the redistribution of finite energy and resources from reproduction/growth to cellular protection/maintenance in normal, but not cancer cells, when nutrients are scarce or absent (Kirkwood, 2005), driven in part by differential regulation of the nutrient-sensing TOR network (Blagosklonny, 2010). The enhanced stress resistance of normal cells and the sensitization of tumor cells are in part modulated by reduced glucose availability and dampening of IGF-1 levels (Lee and Longo, 2011; Lee et al., 2010, 2012; Raffaghello et al., 2008).

Circulating IGF-1, acting synergistically with other hormones and growth factors, regulates energy metabolism, cell proliferation and differentiation, body size and lifespan in response to calorie and protein availability (Flototto et al., 2001; Giovannucci et al., 2003; Prisco et al., 1999; Yu et al., 2003). In addition, IGF-1 exerts a potent tumorigenic effect on a variety of cancer cells by promoting proliferation and inhibiting apoptosis (Prisco et al., 1999; Ramsey et al., 2002). The reduction in IGF-1 plays a key role in protecting against cancer and slowing aging in mammals (Colbert et al., 2009; Hursting et al., 1999; Sonntag et al., 1999). However, in humans, long-term CR causes a modest reduction in fasting glucose and has no significant effect on IGF-1 if not combined with protein restriction (Fontana et al., 2008). Further, CR requires months to years to be effective in humans and is not a practical preventive or treatment strategy for cancer patients since it may exacerbate weight loss in patients prone to it and cause weight loss in patients who may otherwise not lose and even gain weight (Lee and Longo, 2011). In contrast, fasting for an average of 60 h prior to and 24 h post chemotherapy, which has been shown to lower IGF-1 by 40% or more and cause a major reduction in glucose levels, was well tolerated by patients receiving a variety of chemotherapy drugs. These patients reported a reduction in common side effects caused by chemotoxicity (Safdie et al., 2009).

Here we have begun to address the question of whether different types of macronutrient restriction or CR can partially mimic the effects of fasting on serum levels of IGF-1 and glucose, protection of mice and sensitization of cancer cells in response to chemotherapy treatment.

2. Material and methods

2.1. Mice

All animal protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Southern California. 12–15 week old female CD-1, BalB/C or C57BL/6N mice (Charles River) were maintained in a pathogen-free environment throughout the experiments.

2.2. Macronutrient defined diets

AIN93G standard chow (Harlan) was used as the reference diet and supplied to all mice if not indicated otherwise. Diets modified in the macronutrient composition (fat, protein and carbohydrates) were all based on AIN93G (Fig. 1 and Table S1). Diets 20% P-1 (soybean oil as fat source) and 20% P-2 (coconut oil as fat source) had calories from protein sources reduced to 20% compared to the AIN93G formulation; the 0% P diet contained no protein; all these diets were isocaloric

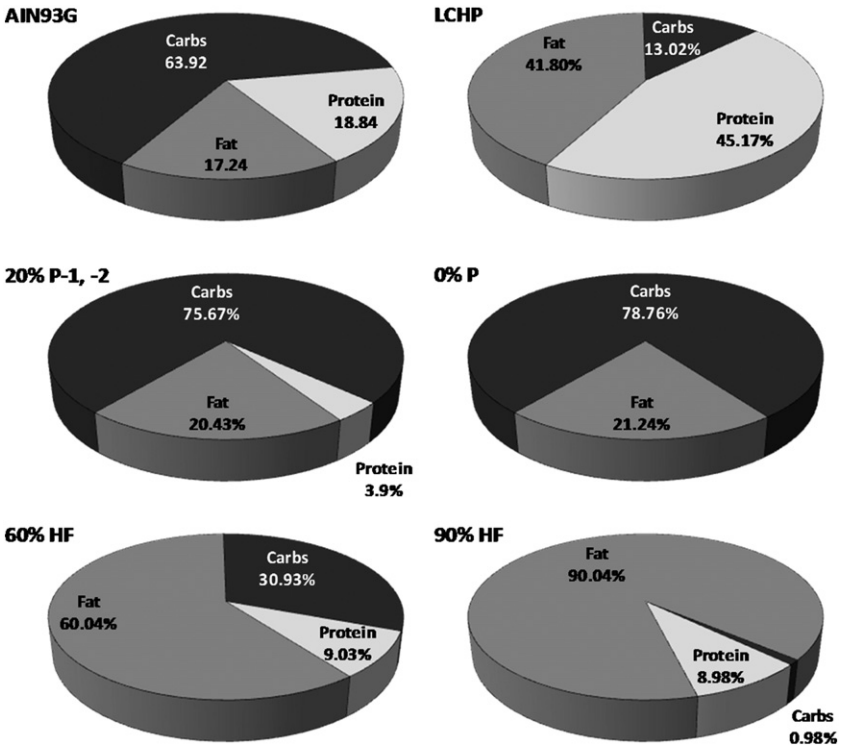

Fig. 1. Calories supplied by macronutrients of the experimental diets in %. AIN93G standard chow was the reference diet supplied to mice. The experimental diets modified in the macronutrient composition (fat, protein and carbohydrates) were all based on this diet. The low-carbohydrate LCHP diet had calories from carbohydrates reduced to 20% compared to the AIN93G formulation (13% *vs.* 63.9%) but contained more protein (45.2%) and fat (41.8%). Diets 20% P-1 (soybean oil as fat source) and 20% P-2 (coconut oil as fat source) had calories from protein sources reduced to 20% compared to the AIN93G formulation; the 0% P diet contained no protein; all these diets were isocaloric to the AIN93G standard chow. The ketogenic high fat diet 60% HF was designed to supply 60% of the consumed calories from fat sources, the calories coming from protein and carbohydrates were reduced proportionally. The 90% HF diet was a ketogenic diet which contains 90% of fat while supplying only minimal carbohydrates (less than 1%) and half of the protein content (9%). Detailed diet composition and calorie content are summarized in Table S2.