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Effect of Every-Other-Day Fasting on Spontaneous Chromosomal Damage in Rat’s Bone-Marrow Cells

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Dietary restriction in experimental rodents, either by calorie restriction (CR) or by every-other-day fasting (EODF), was shown to protect against cancer and increase lifespan. One of the suggested hypotheses to explain the beneficial effects of dietary restriction is that the diet stabilizes the integrity of the genetic information. The effects of EODF on the spontaneous frequency of sister chromatid exchanges (SCE) and chromosomal aberrations (CA) were examined in bone-marrow cells of 3-mo-old Wistar male rats. After 12 wk of EODF diet, significant reduction in the frequency of SCE and total number of CA was observed. Data indicate a protective effect of EODF diet against spontaneous mutations in rats.

Reducing the amount of food consumed in experimental rodents either by calorie restriction (CR) or by every-other-day fasting (EODF) has been shown to (1) protect against cancer, (2) reverse most physiological changes that are associated with aging, and (3) increase lifespan (Goodrick et al., 1982; Ingram et al., 1987; Means et al., 1993; Weindruch, 1996; Kritchensky, 2001; Heilbronn & Ravussin, 2003; Ahmet et al., 2005; Johnson et al., 2006). One of the suggested hypotheses to explain the beneficial effect of caloric restriction is the enhancing effect of such a paradigm on the stability and integrity of the genetic information and its expression. Most investigations that examined this hypothesis were conducted using a diet restriction (DR) paradigm. Data showed a protective effect of CR against mutagen-induced DNA damage following a reduction in diet (Shima et al., 2000; Cooney et al., 2004). In addition, the fidelity of certain forms of DNA polymerase and DNA repair systems was found to be enhanced by CR (Haley-Zitlin & Richardson, 1993; Cabelof et al., 2003). Moreover, CR was found to prevent the aberrant expression of a number of oncogenes and tumor suppressor genes (Nakamura et al., 1989). Finally, different laboratories showed that DR reduces endogenous damage to both nuclear DNA and mitochondrial DNA (Chung et al., 1992; Dempsey et al., 1993; Kaneko et al., 1997; Zhou et al., 2000; Cassano et al., 2004).

Despite the similarities in the beneficial effects of CR and EODF, the effect of EODF on the integrity of the genome remains unknown. In this study, the effects of EODF were examined on the spontaneous rate of sister chromatid exchanges (SCE) and chromosomal aberrations (CA) in rat bone marrow cells. SCE are developed from reciprocal DNA exchange in homologous loci of sister chromatids during DNA replication, while CA derive from various endogenous and exogenous mutagenic agents (Wilson & Thompson, 2007). SCE and CA occur spontaneously at certain frequencies in all cells, and the frequency may be enhanced by certain treatments or associated with diseases (Ray et al., 2001; Karaman & Aliagaoglu, 2006; Norppa et al., 2006). The aim of this study was to determine SCE and CA in rats subjected to EODF.

Methodology
The animals used in the study were 6-mo-old naïve Wistar male rats. For the first 3 mo, the animals were reared in the animals care facility, at Jordan University of Science and Technology (JUST), Irbid, Jordan, at 24 ± 1°C with a 12:12-h light/dark cycle (light onset at 07.00 h). The study procedure was approved by the Animal Care and Use Committee at JUST. Rats were caged individually in polycarbonate cages with wire lids and ad libitum access to rodent chow (Sahil-Huran Animal Food Company, Ramtha, Jordan) and tap water.
water. The composition of the diet was as follows: protein 20% (minimum), lipid and oil 5% (minimum), fiber 5% (maximum), ash 6% (maximum), carbohydrates 50% (maximum), metabolic energy 2400 kcal/kg (minimum), water 14% (maximum), plus various amino acids, minerals, and vitamins (data obtained from food analysis at Clinical Pharmacology Department/JUST). For the experiment, two groups (every-other-day fasting [EODF] and control [AL], n = 14–16) were assigned and were kept at the animal care facility under the same conditions. In the EODF group, animals were fed on every other day. AL rats were given more food than they consumed daily, such that food was available to animals at all times. Similarly, EODF rats were given more food than they consumed in the eating day. This feeding paradigm was carried on for 12 wk. The increase in the body weights of animals was monitored by weighing each animal every week. Food consumption was measured daily at light onset.

Sister-Chromatid Exchange (SCE) Assay

SCE assay on bone-marrow cells was conducted as previously described (Spronck & Kirkland, 2002). Bromodeoxyuridine (BrdU) (Sigma) was dissolved in distilled water containing activated charcoal (Sigma) (1 ml water for every 100 mg charcoal) to a final concentration of BrdU equal to 20 mg/ml. The mixture was magnetically stirred for 2 h in the dark at room temperature. Rats were injected ip with BrdU–charcoal mixture at final dose of 1mg BrdU/g body weight. Colchicine (0.3 mg/rat, Sigma) was injected ip 22 h after BrdU injection and then rats were sacrificed 2 h after colchicine administration. The femurs were excised quickly, and bone-marrow cells were flushed from the femur into Hanks balanced salt solution (HBSS, Promega, USA). The cellular suspension was centrifuged at 300 $\times$ g for 5 min, decanted, and the cellular pellet was resuspended in the residual HBSS. Cells were then incubated in 10 ml hypotonic solution (0.075 M KCl) at 37°C for 20 min. The cellular suspension was centrifuged at 300 $\times$ g for 5 min, decanted, and the cellular pellet was resuspended in the residual hypotonic solution. The cells were fixed with three changes of ice-cold methanol:acetic acid (3:1). The samples were incubated in fixative overnight at 4°C. The cellular suspension was then placed onto prechilled microscope slides to obtain metaphase spreads. The slides were allowed to air dry and subsequently stained with the fluorescent-plus-Giemsa technique as described previously (Perry & Wolff, 1974). The slides were analyzed using light microscopy at 400$\times$ magnification. The individual who scored SCE was blind to the treatment. For SCE analysis, 40s generation (M2) metaphase spreads were scored for the frequency of SCE per metaphase cell. The mitotic index (MI) was determined as shown previously (Spronck & Kirkland, 2002).

Chromosomal Aberrations (CA) Assay

Rats were injected ip with colchicine (0.3 mg/rat) and then sacrificed 2 h after colchicine administration. Bone-marrow cells were isolated and processed as described earlier except that slides were stained with 2% Giemsa solution (pH 6.8) for 15 min and then analyzed microscopically at 400$\times$ magnification. For CA analysis, 50s well-spread metaphase cells in each animal were scored. CA were divided into gaps (including both chromatid gaps and chromosome gaps), breaks (including both chromatid breaks and chromosome breaks), and exchanges. The individual who scored CA was blind to the treatment.

Statistical Analysis

Statistical analysis was performed using a commercial software package (SPSS version 10.0 for Windows, SPSS, Inc., Chicago, IL). Data are reported as mean ± SEM. The comparison of body weights and statistical analysis of the differences in EODF and AL groups was conducted with a one-tailed Student’s t-test. Significance of differences was examined at a p value of 5%.

RESULTS

Previous studies found that rats maintained on an EODF schedule consumed less food over time and maintained lower body weight compared to animals fed AL (Goodrick et al., 1983; Weindruch, 1996). In agreement with these findings, rats maintained on the EODF regimen for 12 wk showed significantly reduced (80%) body weight gain in EODF compared to the AL group (Figure 1).

SCE were observed in bone-marrow cells after treatment with EODF diet for 12 wk. Analysis of SCE was restricted...
to metaphase arrests displaying differentially stained sister chromatids (M2). As shown in Table 1, the frequency of spontaneous SCE in rats maintained in EODF was significantly lower than for AL. In addition to having a lower number of spontaneous SCE, EODF also decreased the average number of SCE per metaphase. Approximately, 1.4% of M2 cells from EODF rats had an average of >6 SCE per metaphase, compared to 29% in AL rats. About 72% of M2 cells from EODF rats had 0–2 SCE per metaphase, compared to 29% in AL rats. Similar values for mitotic index (MI) were observed in EODF and AL groups (MI: 1.9–2.5 and 2–2.4, respectively).

CA were observed in bone marrow cells after treatment with EODF. Gaps, breaks, and exchanges were included in the aberrations assessment (Table 2). A significant decrease in the frequency of chromosomal breaks and exchanges per metaphase cell was observed in the EODF compared to AL rats (Table 2). When gaps were included in the analysis, the difference was also significant (Table 2). Table 2 also shows that AL animals had approximately fourfold more total breaks than animals in the EODF group.

### DISCUSSION

EODF usually involves an “eat day” on which food is consumed, and this day alternates with a “fast day” in which food is removed. This diet paradigm is different from CR, in which daily food consumption is reduced by 30–50%. A key difference between EODF and CR is that overall caloric intake need not be limited; instead, the frequency of food consumption is altered.

Many of the beneficial changes of EODF are shared with CR. For example, both treatments increased maximum lifespan, decreased cancer incidence, and protected against age-associated diseases and neurodegeneration (Means et al., 1993; Mattson & Wan, 2005; Johnson et al., 2006; Mager et al., 2006; Varady & Hellerstein, 2007). Both treatments produced decreased levels of plasma homocysteine, total cholesterol, and triglycerides, but increased high-density lipoprotein (HDL) and stress resistance (Anson et al., 2003; Fontana et al., 2004; Aksungar et al., 2005).

Previous studies demonstrated that CR reduces endogenous damage to both nuclear DNA and mitochondrial DNA. This effect was reported in various tissues using different assays including comet assay, CA assay, and 8-hydroxydeoxyguanosine assay (Chung et al., 1992; Kaneko et al., 1997; Zhou et al., 2000; Cassano et al., 2004). Our results demonstrate, for the first time, that EODF also reduced in vivo rates of spontaneous chromosome damage in rats assessed using bone-marrow cells for SCE and CA tests. The decrease in mutation rates was observed under normal conditions, which is more biologically relevant than changes induced by mutagens. Therefore, both EODF and CR exert a beneficial effect on the integrity of the genome.

The mechanism(s) by which EODF decreases spontaneous mutation rates is unknown. However, it might be due to the effect of EODF diet on the oxidative stress status in the animals. Several studies showed that animals on EODF display (1) a reduction in the levels of oxidative-modified proteins, lipid peroxidation products, DNA oxidation, and cellular oxygen free radical production (Loft et al., 1995; Radak et al., 2002; Descamps et al., 2005; Johnson et al., 2007; Caro et al., 2008); (2) an upregulation of the expression of antioxidant systems (Gomi & Matsuo, 1998), and (3) a decrease in the amount of respiratory complexes (Lambert & Merry, 2004; Lambert et al., 2004; Merry, 2004). A strong correlation between oxidative stress and level of mutations rat is well established.

A second mechanism by which EODF decreases spontaneous mutation rates might be due to its effects on the DNA repair systems and the fidelity of certain forms of DNA polymerase as reported with CR (Lipman et al., 1989; Haley-Zitlin & Richardson, 1993; Guo et al., 1998).

The effect of EODF on lowering the spontaneous mutation rates might play a role in the pathway that protects animals from cancer development. Previous studies showed that EODF and CR diets decrease the rates of cancer development (Descamps et al., 2005). In addition, positive correlation was established between frequency of chromosomal damage and cancer development (Norppa et al., 2006; Boffetta et al., 2007). Therefore, EODF paradigm might modify cancer development through lowering chromosomal damage in individuals. Accumulation of mutations is also associated with aging and cell senescence (Vijg, 2000; Trifunovic et al., 2005; Kukat & Trifunovic, 2008). Thus, lowering spontaneous mutations rates might also play a role in the pathway by which EODF protects the animals from age-associated diseases and neurodegeneration and subsequently a longer lifespan.

In conclusion, our results demonstrate that EODF diet lowered in vivo spontaneous chromosome damage frequency
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Animal number</th>
<th>Number of cells scored</th>
<th>Gaps</th>
<th>Breaks</th>
<th>Exchanges</th>
<th>Total aberrations (without gaps)</th>
<th>Total aberrations (with gaps)</th>
<th>Number of aberrations per cell (without gaps)</th>
<th>Number of aberrations per cell (with gaps)</th>
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<td>Control</td>
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<td>8</td>
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<td>0.16</td>
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<td>8</td>
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<td>0.06</td>
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<tr>
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<td>0.074*</td>
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Note. Asterisk indicates significant difference at $p < .05$, by Student’s t-test.
in rats and the reduction in genetic damage demonstrates an antimutagenic role for EODF paradigm.

REFERENCES


