

Energy Restriction and Oxidative DNA Damage in Humans¹

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Abstract

The cancer-preventive effect of energy restriction in rodents has been related to a decrease in oxidative damage to DNA. We have investigated the effect of energy restriction on the rate of oxidative DNA modification estimated from the urinary excretion of the repair product, 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG), in healthy, normal weight men. Before and after 10 weeks on a diet containing 80 ($n = 16$) or 100% ($n = 8$) of the estimated weight-maintaining energy, resting metabolic rate (RMR) was measured and 24-h urine was collected for 8-oxodG determination by HPLC.

During the study, the weight loss was 10 and 2.5% of the initial weight, mostly in terms of fat, and the RMR decreased by 13 and 8% in the energy-restricted and control groups, respectively. With the use of *t* tests there was no significant difference within or between groups with respect to 8-oxodG excretion. However, if RMR was included as a covariate in multifactorial ANOVA, an average relative 17% (2-31%; 95% confidence interval) increase in 8-oxodG excretion in the energy-restricted group was significantly different from the corresponding value of the control group ($P < 0.02$). In the energy-restricted group the change in 8-oxodG excretion was correlated closely with the decrease in RMR ($r = 0.63$; $P = 0.013$).

In the present study, 20% energy restriction for 10 weeks did not reduce oxidative DNA damage; we question a beneficial effect on cancer risk in normal weight humans. Moreover, the data suggest that unless the metabolic rate decreases correspondingly, energy restriction may even increase the rate of oxidative DNA modification in humans. This apparently complex effect of energy restriction on oxidative DNA damage warrants additional investigation.

Introduction

Energy restriction consistently increases life span and reduces the incidence of spontaneous as well as carcinogen-induced tumors in rodents (1, 2). In humans, however, the consequences of energy restriction on cancer risk are still unknown. Although

some epidemiological studies show positive associations between energy and fat intake and the risk of colon cancer, causal relationships have not been established (1, 3, 4). Prospective studies of energy restriction and cancer incidence in humans require considerable and prolonged lifestyle changes from a large number of participants. Thus, controlled studies involving mechanistic risk factors as end points may provide important data. Energy restriction for 16 weeks in obese humans reduced rectal epithelial cell proliferation, a biomarker related to colon carcinogenesis (5). Similar results with parallel reduction in tumor incidence have been obtained in rats (6). The initiating event for most cancers, however, is considered to be damage to DNA, leading to a mutation. Thus, biomarkers of DNA damage may be useful with respect to beneficial effects of energy restriction in humans.

Oxidative modifications of mammalian DNA are abundant, *e.g.*, one 8-oxodG³ per 10^4 - 10^5 dG is found in DNA from normal tissue, and increased numbers are seen with advanced age, in tumors or after treatment with ionizing radiation or chemical oxidants (7-10). The responsible ROS may be generated during mitochondrial respiration as 1-5% of the processed oxygen undergo single electron transfer generating the superoxide anion radical, which in turn may be reduced to hydrogen peroxide and subsequently to the hydroxyl radical (11-13). Thus, the urinary excretion of repair or turnover products (*e.g.*, 8-oxodG) of oxidative DNA damage correlates across species with the metabolic rate and cumulated cancer risk (12, 14, 15). Even among humans the excretion of 8-oxodG correlates with oxygen consumption (16). In rodents, energy restriction reduces oxidative modification of tissue DNA and proteins, which may explain the reduced cancer risk and increased longevity (17-19). In humans, BMI (body weight divided by the square of the height) has shown an inverse relationship with the risk of lung cancer (20, 21), whereas hyperthyroid women have been reported to have a sustained, increased risk of respiratory and pancreatic cancer (22). In normal weight humans, energy restriction has been shown to reduce the RMR more than predicted from the FFM (23). Thus, a potential beneficial effect of energy restriction on cancer risk in humans may be reflected in the rate of oxidative DNA modification.

In the present study we investigated the effect of restricting dietary energy intake on the rate of oxidative DNA modification estimated from the urinary excretion of 8-oxodG in 16 healthy men as compared to 8 men continuing on a weight-maintaining diet. Moreover, the effect on 8-oxodG excretion was related to the reduction in RMR caused by energy restriction.

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³ The abbreviations used are: 8-oxodG, 8-oxo-7,8-dihydro-2'-deoxyguanosine; dG, deoxyguanosine; ROS, reactive oxygen species; BMI, body mass index; RMR, resting metabolic rate; FFM, fat-free mass; T₄, thyroxine; T₃, triiodothyronine; rT₃, reverse T₃.

Materials and Methods

The study protocol has been described in detail elsewhere (24). Briefly, 24 healthy, nonsmoking men (ages 35–50 years) participated in the study after giving their informed consent. The subjects were of normal weight and had a BMI from 20.6 to 27.2 kg/m² and energy intake from 9.3 to 13.2 megajoules.

The total study lasted 12 weeks. During the first 2 weeks (run-in period) all subjects received a weight-maintaining diet based on a 7-day dietary record. Subjects who lost more than 1 kg body weight/week during the run-in had their estimated weight-maintaining dietary energy increased by 770 kilojoules. After these 2 weeks the subjects were assigned randomly to an energy-restricted group ($n = 16$) and a control group ($n = 8$) matched for age and BMI. During the following 10 weeks, the 16 subjects received a nutritionally adequate test diet containing 80% of the energy of their weight-maintaining diet, whereas the 8 control subjects continued their weight-maintaining diet. The 80% energy level was considered safe and was achieved by substitution of low fat and artificially sweetened products for ordinary products. The 20% energy restriction was achieved such that the relative contribution of carbohydrates, fat, and protein to total energy was 51, 34, and 15% versus 47, 36, and 17% in the control and energy restricted group, respectively. The content of micronutrients was kept constant and in excess of recommended dietary allowance. All foods and drinks (except water) to be consumed during these 12 weeks were supplied to the subjects. The subjects were asked to keep their level of activity constant.

Before and at the end of the dietary intervention the body weight and composition, RMR, and respiratory quotient were measured, and 24-h urine was collected for determination of 8-oxodG excretion. Urine was not collected from one subject from the energy-restricted group. The absence of significant changes in 8-oxodG in lymphocyte DNA and measures of lipid oxidation and antioxidant enzymes in plasma after the energy restriction will be reported elsewhere.⁴

RMR was measured in the morning after the subjects had stayed and fasted overnight in a metabolic ward. While lying for 30 min in supine position, the subjects were breathing through a low-resistance breathing valve (No. 2700; Hans Rudolf, Kansas City, MO). Oxygen consumption (paramagnetic analyzer, Beryl 102; Cosma, Igny, France) and CO₂ production (AR-400 IR analyzer; Anarad, Santa Barbara, CA) were measured under thermoneutral temperature conditions. RMR was determined as the mean of two measurements with the use of Weir's formula for calculation (25).

Body composition (fat mass and FFM) was computed by means of the three-compartment model of Siri (26) and measurements of body density was determined with the use of the hydrostatic technique of Brozek *et al.* (27). Weight and underwater weight were determined to the nearest 0.1 kg by a balance (ED-60-T and SK; Berkel, Ridderkerk, the Netherlands), whereas the residual lung volume was determined simultaneously by helium dilution and a spirometer (Volutes; Mijnhardt, Bunnik, the Netherlands). Total body water was determined by deuterium dilution. The final estimation of FFM was not performed in two energy-restricted subjects. For relation to RMR and 8-oxodG excretion in these two subjects, FFM was estimated from the initial value and the average decrease (1.1 kg) in the remaining energy-restricted subjects.

The concentration of 8-oxodG in 24-h urine was measured by an automated three-dimensional HPLC method with isocratic separation and electrochemical detection as reported previously (28). The intra- and interday coefficients of variation for the analysis were 8 and 10%, respectively. All samples were analyzed repeatedly on at least 2 different days, and the average value was used for calculation. The concentration of 8-oxodG was constant in urine samples stored at -20°C for at least 3 years. Plasma T₄, T₃, and serum rT₃ levels were analyzed by means of commercial RIA kits (EURO/DPC; Llanberis, United Kingdom, and Techland, Liège, Belgium).

Changes within groups were tested by means of the paired Student's *t* test. Differences in changes between the two groups were tested by means of the unpaired Student's *t* test. The change in 8-oxodG excretion was also compared between the two groups by means of multifactorial ANOVA with the change in RMR as covariate. The relationship between the change in 8-oxodG excretion and the other variables subject to change over time was investigated by backward stepwise multiple linear regression analysis on the whole group, as well as within each treatment group. Spearman correlation coefficients between the changes in 8-oxodG excretion and RMR were calculated for each group. Probability values <0.05 were considered significant.

Results

During the 10-week energy restriction period the 16 subjects lost 10% of their initial weight, almost exclusively in terms of fat (Table 1). Thus, a statistically significant loss of 1.5% of FFM represented only 12% of the total weight loss. Moreover, at the end of the intervention period the subjects were still losing weight, and there was no tendency for stabilization. Despite the efforts to supply a weight-maintaining diet, the control subjects also lost some weight, *i.e.*, 2.5% of their initial weight, exclusively in terms of fat ($P < 0.05$; Table 1). At the second measurement RMR had decreased significantly in both groups, although by 13% (10–16%; 95% confidence interval) in the energy-restricted group as opposed to 8% (5–11%) in the control group ($P = 0.06$ between groups). This decrease corresponded a similar decrease in plasma T₃ in both groups and a significant increase in the reverse T₃ test in the energy-restricted group (Table 1). Plasma T₄ and the respiratory quotient were not changed significantly in any group during the study (Table 1).

In a stepwise multiple linear regression analysis none of the recorded parameters in Table 1 was a significant predictor of the initial excretion of 8-oxodG (data not shown). With the use of *t* tests there was no statistically significant change over time or difference between the energy-restricted and control groups with respect to the excretion of 8-oxodG (Table 1). However, with inclusion of the change in RMR as a covariate ($P = 0.04$) in multifactorial ANOVA the change in 8-oxodG excretion was significantly different between the two groups ($P = 0.02$; Table 1). Thus, in the energy-restricted group there was an average relative increase in 8-oxodG excretion expressed per FFM of 17% (2–31%; $P = 0.02$).

In a multiple regression analysis involving all 23 subjects, the significant predictors of the treatment-related change in 8-oxodG excretion were the decrease in RMR and the relative energy supply (Table 2), whereas the weight loss and changes in the respiratory quotient or in measures of thyroid function had no predictive value (*F* values < 1.04). In the energy-restricted subjects, the change in the excretion of 8-oxodG was closely correlated with the decrease in resting metabolic rate ($r = 0.63$; $P = 0.013$; Fig. 1; Table 2). Virtually identical correlations were obtained if 8-oxodG excretion and/or RMR

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Table 1 Anthropometric data, RMR, thyroid function parameters, and excretion of 8-oxodG (mean \pm SD) in 23 subjects before and the change measured after a 10-week period with 80 or 100% of the energy supply estimated to be weight maintaining

	Energy restricted ($n = 15/16$)		Control ($n = 8$)	
	Before	Change	Before	Change
Body weight (kg)	78.6 \pm 9.3	-7.5 \pm 2.6 ^{a, b}	77.7 \pm 6.6	-2.7 \pm 2.1 ^a
Fat mass (kg)	18.1 \pm 5.2	-6.3 \pm 1.7	18.3 \pm 6.2	-1.9 \pm 1.8
FFM ^c (kg)	60.5 \pm 6.8	-1.08 \pm 1.01 ^a	59.5 \pm 5.2	-0.19 \pm 1.02
Plasma T ₃ (nM)	1.79 \pm 0.22	-0.23 \pm 0.18 ^a	1.86 \pm 0.21	-0.16 \pm 0.18 ^a
Serum rT ₃ (ng l ⁻¹)	256 \pm 82	24.4 \pm 43.5 ^a	251 \pm 60	-7.5 \pm 45.3
Plasma T ₄ (nM)	99 \pm 14	-3.2 \pm 6.9	102 \pm 17	-6.0 \pm 11.5
RMR (MJ 24 h ⁻¹)	8.02 \pm 1.26	-1.07 \pm 0.63 ^a	7.40 \pm 0.75	-0.60 \pm 0.40 ^a
RMR/FFM (J \cdot kg ⁻¹ 24 h ⁻¹)	133 \pm 16	-15.4 \pm 11.1 ^a	125 \pm 9	-9.4 \pm 6.7 ^a
Respiratory quotient	0.82 \pm 0.08	-0.03 \pm 0.08	0.76 \pm 0.10	-0.01 \pm 0.07
8-oxodG excretion (nmol 24 h ⁻¹)	37.8 \pm 13.2	3.51 \pm 11.6 ^c	39.5 \pm 12.5	-2.85 \pm 8.86
8-oxodG excretion/FFM (pmol 24 h ⁻¹ kg ⁻¹)	629 \pm 218	78 \pm 189 ^c	666 \pm 202	-43 \pm 155

^a $P < 0.05$ versus initial value.

^b $P < 0.05$ versus corresponding value of control group.

^c $P < 0.05$ versus the control group in multifactorial analysis of variance with the RMR change as covariate.

Table 2 Statistically significant results of backwards stepwise multiple regression analysis of the relationship between the decrease in RMR, change in other host factors shown in Table 1, and the change in the excretion of 8-oxodG (pmol 24 h⁻¹) in 23 subjects receiving 80% ($n = 15$) or 100% ($n = 8$) of the energy supply estimated to be weight maintaining for 10 weeks

	Coefficient (95% confidence interval)	F value	P value
All ($n = 23$)			
Constant	60 (19–101)		0.009
Energy restriction (%)	0.55 (0.12–0.98)	6.4	0.012
RMR decrease (megajoules)	12.4 (3.9–20.9)	8.1	0.0098
Energy restricted ($n = 15$)			
Constant	17.9 (7.1–28.7)		0.006
RMR decrease (megajoules)	13.6 (3.7–23.5)	8.4	0.013

was expressed per kg FFM (data not shown). There were no such significant correlations in the control group ($r = 0.26$; $P = 0.56$; $F = 0.4$ in multiple regression analysis).

Discussion

The cancer-preventive effect of energy restriction in rodents has been suggested to be related partly to a decrease in the generation of and protection from ROS and the resulting oxidative damage to DNA (2, 17, 18, 29, 30). It is therefore surprising that the present study appears to show increased oxidative DNA damage estimated by urinary 8-oxodG excretion after 20% energy restriction for 10 weeks in healthy men. However, the present change in 8-oxodG excretion was closely correlated to the extent of a variable decrease in RMR, suggesting a more complex relationship with the diet intervention. Thus, energy restriction induces catabolism, possibly increasing ROS generation, whereas the ensuing decrease in RMR may imply reduced mitochondrial respiration and formation of ROS.

In rats restricted in energy intake by 40% for 2 weeks or up to 2 years, the level of oxidative modifications in terms of 8-oxodG and 5-hydroxyuracil was reduced by 30–40% in nuclear and mitochondrial DNA from liver and mammary glands (17, 18). This effect could be due to a decreased rate of

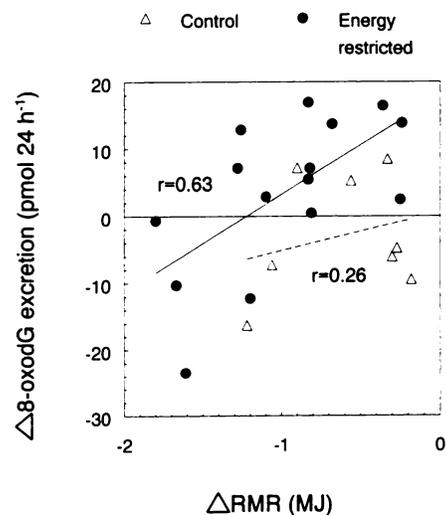


Fig. 1. Change in the excretion of 8-oxodG, a measure of oxidative DNA modification, in relation to the change in RMR during 10 weeks on a diet containing 80% ($n = 15$) or 100% ($n = 8$) of the estimated weight-maintaining energy. The lines of regression and correlation coefficients are shown for each group.

modification and/or an increased rate of DNA repair as shown in energy-restricted rats (31, 32). However, in a single human subject, with energy restriction by 40–50% for periods of 10 days, the urinary excretion of 8-oxodG and a similar biomarker (thymidine glycol) was reduced by 50–80% (15), consistent with either a decreased rate of damage and/or of repair. Moreover, in rats the activity or expression of antioxidant enzymes, including superoxide dismutase and catalase, was increased, whereas lipid peroxidation and oxidative modification of protein were reduced by similar energy restriction (2, 19, 32, 33). With the present intervention period of 10 weeks, a change in 8-oxodG excretion will reflect a changed rate of damage rather than of repair. Thus, the 20% energy restriction of the present study may have been limited to reduce the rate of oxidative

DNA modification. However, even with this moderate restriction there was no tendency for weight stabilization after 10 weeks, and more drastic or prolonged reduction in energy intake would not be safe in normal weight humans. Accordingly, the present level of energy restriction does not seem to be an efficacious way of reducing cancer risk related to oxidative DNA modification in normal weight humans.

The loss of 2.5% of the body weight in the control group indicates that the estimation of weight-maintaining energy intake was too low. Thus, the intervention group was probably restricted to approximately 70% rather than 80% of their energy requirement. Nevertheless, RMR decreased by only 13% on average, and there was no sign of weight stabilization, indicating that most of the energy-restricted subjects were catabolic at the time of measurement of 8-oxodG excretion. In contrast, the 8% reduction in RMR probably corresponded to the unintended reduction in energy intake in the control group. Catabolism was related mostly to a reduction in fat mass. Thus, the apparent increased 8-oxodG excretion in the energy-restricted group could be related to generation of ROS from oxidation of fat from body stores. Due to a decreased fat intake as part of the energy restriction, the total fat oxidation was changed minimally as supported by the unchanged respiratory quotient, although it was only measured in the morning. In women at risk of breast cancer, reduction of fat intake to a target of 15% of the energy for 3–24 months reduced the level of 5-hydroxyuracil in DNA of circulating lymphocytes by 68% in 9 as compared to 12 women who continued with a fat intake in excess of 30% of the energy (34). However, in a cross-sectional study, no relationship between the percentage of energy intake from fat and the excretion of 8-oxodG was seen (28).

The present relationship between the change in 8-oxodG excretion and the decrease in RMR is consistent with the close correlation between the excretion and 24-h oxygen consumption or total energy expenditure shown in healthy young women (16), as well as across species (12, 14, 15, 35). In the present study, with constant extent of exercise, the changes in RMR would closely reflect changes in oxygen consumption. The apparent relationship between oxidative DNA damage and oxygen consumption is thought to be due to the 1–5% fraction undergoing single electron transfers to generate ROS during mitochondrial respiration (11). Thus, hydrogen peroxide formation per mg mitochondrial protein (13) and the summed mitochondrial surface area (36) have been shown to correlate with the metabolic rate across species. Moreover, energy restriction decreases the metabolic rate calculated per animal and may improve coupling of mitochondrial respiration, thus reducing ROS formation (2, 32). In addition to this effect, energy restriction may also increase the activity of antioxidant enzymes and DNA repair in rodents as mentioned previously (2, 31, 32).

According to the present data, energy restriction may affect oxidative DNA modification by increasing ROS formation from fat catabolism and decreasing mitochondrial ROS generation through decreased metabolic rate. In the present setting, with 20% restriction for 10 weeks and continuous weight loss the balance turned out unfavorably. However, it cannot be excluded that a beneficial balance may result, *e.g.*, after weight stabilization has been reached or with another extent of energy restriction.

Although the micronutrient content was constant it cannot be excluded that the diet change necessary for energy reduction had unwanted negative effects on the generation of or protection from ROS. However, there are no data to suggest that the artificial sweeteners used in the restricted diet could be a source of ROS. In a cross-sectional study of 83 healthy subjects,

8-oxodG excretion was not related to diet composition estimated from a 2-week weighed diet record (28).

The consequences of 8-oxodG formation in nuclear DNA have been particularly related to carcinogenesis (37). Tumor tissue DNA contains high levels of 8-oxodG, in addition to a whole series of other oxidative DNA modifications (8, 9). The present and other data regarding the urinary excretion of 8-oxodG and similar biomarkers suggest that the rate of oxidative DNA modifications corresponds to 10^4 affected bases/cell/day (14, 28, 38, 39). In replicating DNA, 8-oxodG leads to G-T transversions, as well as other mutations and codon 12 activation of *c-Ha-ras* or *K-ras* oncogenes in mammalian systems (40–43). In human tumors, G-T transversions are among the most frequent hot spot mutations in the *p53* suppressor gene (44). Reports of exponential accumulation of 8-oxodG and a correlation with deletions in mitochondrial DNA from human heart muscle indicate a role of oxidative DNA modification from the respiratory chain in the muscle weakness associated with aging (45).

In vivo, 8-oxodG in DNA is extensively repaired by nucleotide excision or the formamidopyrimidine-DNA glycosylase enzyme, resulting in two possible products, 8-oxodG or the corresponding base (8-oxoguanine), respectively (7, 10, 39, 46–48). Two involved DNA repair enzymes, one with glycosylase activity and one excising single 8-oxodG as a nucleotide, have been isolated from nuclear extracts of a human cell line (49). In excision repair-deficient human cell lines, plasmids containing 8-oxodG were replicated at a rate of only 25% of the rate of proficient cell lines and with a 3–5-fold increased frequency of G-T transversion mutations typical for 8-oxodG (50). This suggests that nucleotide excision is the most important pathway for 8-oxodG in humans. In addition, digestion of damaged DNA from cell renewal and mitochondrial turnover will liberate 8-oxodG. Oxidized nucleosides and nucleotides from the cellular pools may be incorporated into DNA and lead to mutations (40, 42) unless sanitized by an enzyme that hydrolyzes the phosphates of 8-oxodGTP with high affinity and 8-oxodG as a putative end product (51). Animal experiments have shown that injected 8-oxodG is readily excreted unchanged into the urine, whereas 8-oxodG in the diet or oxidation of dG during excretion does not contribute (39, 46, 52). Thus, although the exact relative importance of the DNA repair pathways remains to be determined, the urinary excretion of 8-oxodG reflects the general average risk of a promutagenic oxidative adduct in DNA of all tissues and organs. In the present study FFM was minimally affected by energy restriction, supporting the belief that 8-oxodG from digestion of DNA from cell turnover was constant.

In conclusion, the present study does not support the notion that energy restriction is an efficacious way of reducing the rate of oxidative DNA modification in normal weight humans. Moreover, the data suggest that unless the metabolic rate decreases, correspondingly moderate energy restriction may even increase the rate of oxidative DNA modification in humans, although a beneficial net balance cannot be excluded if weight stabilization is achieved. The apparently complex effect of energy restriction on oxidative DNA damage warrants additional investigation.

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