High Fat Diet Induced Oxidative DNA Damage Estimated by 8-Oxo-7,8-dihydro-2'-Deoxyguanosine Excretion in Rats

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The role of dietary fats and energy in carcinogenesis has been partly related to oxidative damage to DNA. We have investigated the effect of dietary fat content and saturation on the urinary excretion of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) in male and female rats. Groups of Fischer F344 rats ($n = 6$–10) were fed control chow (3.4\% fat) or diets containing 21.8\% corn oil or 19.8\% coconut oil + 2\% corn oil for 12–15 weeks. At the end of the diet intervention period 24 h urine was collected for determination of 8-oxodG by HPLC.

In the male groups fed control, corn oil and coconut oil diet the excretion of 8-oxodG was $403 \pm 150$, $932 \pm 198$ and $954 \pm 367$ pmol/kg 24 h, respectively ($p < 0.05$). In the female groups fed control and corn oil diet the excretion of 8-oxodG was $752 \pm 80$ and $2206 \pm 282$ pmol/kg 24 h, respectively ($p < 0.05$). Calculated per whole animal the excretion was $137 \pm 51$, $324 \pm 70$ and $328 \pm 128$ pmol/24 h in the control, corn and coconut oil male groups and $156 \pm 21$ and $464 \pm 56$ pmol/24 h in the control and corn oil female groups, respectively ($p < 0.05$). Thus, per animal or per consumed energy there was much less difference in 8-oxodG excretion between the corresponding male and female groups and only significant difference between the high fat groups. There was a close correlation ($r = 0.7; p < 0.05$) between 8-oxodG excretion and the energy intake.

The present study suggests that a high fat diet increases oxidative DNA modification substantially irrespective of the saturation level of the fat. Energy intake appears to be the major determinant of the rate of modification.

Keywords: 8-OxodG, dietary fat, rats, metabolism, oxidative DNA damage

INTRODUCTION

Fat and energy intake have been shown to be important dietary factors modulating tumour incidence in experimental animals. Thus, energy restriction consistently increases life span and reduces the incidence of spontaneous as well as carcinogen induced tumours in rodents.\textsuperscript{[1,2]} Similarly, in rats and mice mammary tumour

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incidence correlates with dietary fat,[3] which may act as a promoter as first described by Tannenbaum.[4] However, dietary fat is an energy source and effects of a high fat diet may partly be related to a high energy content. In support, energy restriction effectively reduces the tumour incidence even with high fat content in the diet (reviewed by Kritchovsky[5]).

A common mechanism of energy and fat in carcinogenesis may involve the generation of reactive oxygen species (ROS) and resulting oxidative damage in particular to DNA.[6] Indeed, oxidative modifications of mammalian DNA are abundant, e.g. one 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) per 10⁴–10⁵ dG is found in DNA from normal tissue, and increased amounts are seen with advanced age, in tumours or after treatment with ionising radiation or chemical oxidants.[7–10] In rodents energy restriction decreases oxidative modification of both tissue DNA and proteins which may explain the reduced cancer risk and increased longevity.[11–13] However, such effects have not been reproduced in humans subjected to approximately 20% energy restriction.[14]

So far, the effect of fat diets on oxidative DNA damage has been studied in few tissues, mammary gland, liver, lung and peripheral leukocytes, and with apparently conflicting results.[12,15–19] The fat composition may be of importance as shown by an apparently linear relationship between 8-oxodG in mammary gland DNA related to the extent of unsaturation of high fat diets fed to rats[16] although there was no difference between the levels after a diet rich in lard or corn oil.[19] Indeed, lipid peroxidation products generate 8-oxodG in DNA in vitro.[20]

Changes in the levels of oxidative adducts in tissue DNA may be tissue specific and may be caused by changes in either the rate of damage or the rate of repair. In contrast, the urinary excretion of repair products of oxidative DNA modifications, such as 8-oxodG, reflects the rate of damage as an integrated average of all the body.[21–24] Accordingly, the use of such biomarkers may provide important information on the effect of dietary fats on oxidative DNA damage. In the present study we investigated the effect of dietary fat content and saturation on the urinary excretion of 8-oxodG in male and female rats.

**MATERIAL AND METHODS**

Male and female Fischer F344 were kept in a 12 h light cycle under constant humidity and with free access to drinking water. The rats were fed ad lib with chow containing 3.4% fat (Bomholtgard). At the age of 4–5 weeks the rats were put on the experimental diets fed ad lib for 16–20 weeks (Table I). One group of 10 male rats and one group of 6 female rats continued with the chow containing 3.4% fat and based on bruised soy, barley, wheat and fishmeal. One group of 6 female rats and one group of 10 male rats were fed a high fat

<table>
<thead>
<tr>
<th>TABLE I</th>
<th>Compositions of experimental diets</th>
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<tbody>
<tr>
<td></td>
<td>Low fat</td>
</tr>
<tr>
<td>Total fat (% of diet)</td>
<td>3.4</td>
</tr>
<tr>
<td>Saturated fatty acids* (% of fat)</td>
<td>21</td>
</tr>
<tr>
<td>Monounsaturated fatty acids* (% of fat)</td>
<td>19</td>
</tr>
<tr>
<td>Polyunsaturated fatty acids* (% of fat)</td>
<td>58</td>
</tr>
<tr>
<td>Energy from fat (% of total energy)</td>
<td>10</td>
</tr>
<tr>
<td>Total protein (% of diet)</td>
<td>24</td>
</tr>
<tr>
<td>Total carbohydrate (% of diet)</td>
<td>40</td>
</tr>
<tr>
<td>Energy (kJ/g)</td>
<td>12.0</td>
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</tbody>
</table>

*Gaschromatographic analysis.
diet containing mainly unsaturated fat in terms of corn oil (21.8%), whereas one group of 10 male rats was fed mainly saturated fat in terms of coconut oil (19.8%) and corn oil (2%). Both high fat diets were based on bruised soy wheat, skim milk powder and bone glue meal and butylated hydroxytoluene (150 ppm) was added to the oils. The composition of individual fatty acids of each diet was determined by gas chromatography.\textsuperscript{23}

The energy content of each diet was calculated using standard Atwater value estimates for physiological values.\textsuperscript{26} After 12–16 weeks on the diet the rats were placed in metabolic cages allowing determination of food consumption and complete collection of urine in refrigerated containers (4°C) for 24 h. The urine was analysed for 8-oxodG by a triplecolumn HPLC technique with electrochemical detection as previously described.\textsuperscript{27} The intra- and interassay coefficients of variation of the method were 8% and 10%, respectively. The rats continued on the diets for other studies and it was thus not possible to obtain tissues for determination of 8-oxodG in DNA.

The diet groups were compared by means of ANOVA with Duncans multiple range test for post hoc comparison. The effect of the diet and sex on the 8-oxodG excretion was analysed in ANCOVA with the energy intake as covariate. Linear regression was done by the method of least squares. Probability values less than 0.05 were considered significant.

**RESULTS**

Within each sex body weight was not different between the diet groups whereas male rats were 1.7-fold heavier than female rats (Table II). Food consumption was similar in all groups on a weight basis except that the male group fed the corn oil based high fat diet consumed significantly less food than the female group fed the same. However, in spite of a lower volume the groups on the high fat diets consumed more energy than the corresponding groups on the low fat diet. Despite a considerably lower body weight the female rats consumed more energy than the corresponding males. This is consistent with the observation that the former are more physically active than the latter.\textsuperscript{25}

A high fat diet increased the excretion of 8-oxodG 2.5–3.0 times ($p < 0.05$) in both male and female rats irrespective of the saturation level (Table II). Thus, there was no significant difference between the male groups fed the corn and coconut oil based high fat diet. Calculated per whole animal there was no significant difference in 8-oxodG excretion between the corresponding male and female control groups, whereas the female corn oil fed rats excreted 1.4 times more 8-oxodG per animal than the corresponding male rats ($p < 0.05$). Calculated per g body weight the female rats excreted 1.8 and 2.3 times more 8-oxodG than the male rats in the control and corn oil fed groups, respectively.

<table>
<thead>
<tr>
<th>TABLE II</th>
<th>Body weight and fodder consumption in rats fed diets of different fat content and composition</th>
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<tbody>
<tr>
<td></td>
<td>Male (9–10/group)</td>
</tr>
<tr>
<td></td>
<td>Low fat</td>
</tr>
<tr>
<td>Body weight</td>
<td>342 ± 19</td>
</tr>
<tr>
<td>Food intake (g)</td>
<td>11.8 ± 2.2</td>
</tr>
<tr>
<td>Energy intake (kJ/24 h)</td>
<td>142 ± 25</td>
</tr>
<tr>
<td>(kJ/kg BW 24 h)</td>
<td>414 ± 70</td>
</tr>
<tr>
<td>8-OxodG excretion (pmol/24 h)</td>
<td>137 ± 51</td>
</tr>
<tr>
<td>(pmol/kg 24 h)</td>
<td>403 ± 150</td>
</tr>
<tr>
<td></td>
<td>Female (6/group)</td>
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<tr>
<td></td>
<td>Low fat</td>
</tr>
<tr>
<td>Body weight</td>
<td>207 ± 10</td>
</tr>
<tr>
<td>Food intake (g)</td>
<td>12.5 ± 1.6</td>
</tr>
<tr>
<td>Energy intake (kJ/24 h)</td>
<td>151 ± 20</td>
</tr>
<tr>
<td>(kJ/kg BW 24 h)</td>
<td>733 ± 122*</td>
</tr>
<tr>
<td>8-OxodG excretion (pmol/24 h)</td>
<td>156 ± 21</td>
</tr>
<tr>
<td>(pmol/kg 24 h)</td>
<td>752 ± 80*</td>
</tr>
</tbody>
</table>

* $p < 0.05$ versus low fat group of same sex; † $p < 0.05$ versus group opposite sex receiving identical diet.
In ANCOVA of the 8-oxodG excretion the energy intake was a significant covariate \( (p = 0.0004) \) but the effect of a high fat diet was still highly significant \( (p = 0.000002) \). Among the individual animals from all groups there was a close correlation between 8-oxodG excretion and the energy intake whether calculated per whole animal \( (r = 0.70) \) or per g BW \( (r = 0.77) \) (Figure 1).

**DISCUSSION**

The present study demonstrated a 3-fold increase in the rate of oxidative DNA modification in rats fed a high fat diet, irrespective of saturation level, as compared with a regular low fat chow.

The effect of fat saturation on oxidative DNA modification has until now only been reported for mammary gland tissue in rats.\(^{16,19}\) In one study high fat (24.6\%) diets based on palm oil, corn oil and menhaden were compared and a significant correlation between the extent of unsaturation and 8-oxodG levels was shown.\(^{16}\) Moreover, the slope of the linear relationship was steeper in rats fed a diet deficient in vitamin E and selenium as compared to proficient animals. However, in a later study no difference in mammary 8-oxodG level was seen in rats fed a 20% fat diet based on lard or corn oil.\(^{19}\) The latter result appears more in agreement with the present lack of difference between rats fed coconut and corn oil based diets in 8-oxodG excretion, which reflects the average rate of oxidative DNA damage in the whole body. The mammary gland has an extremely high fat content and any effect may be specific to tissues with e.g. a high fat metabolism. Indeed, lipid peroxidation products from linolenate derivatives and other unsaturated fatty acids generated 8-oxodG in isolated DNA \emph{in vitro} whereas oleic acid had no such effects.\(^{20,28}\) Nevertheless, these fatty acids added to culture medium had no effect on oxidative DNA damage in human lymphocytes exposed \emph{in vitro}.\(^{28}\)

In the present study the rats fed the high fat diets consumed considerably more energy than the low fat fed rats although the body weights were similar in the two groups. Indeed, there was a close correlation between energy intake and 8-oxodG excretion. Dietary fat may thus serve as an energy source allowing an energy rich diet in addition to increasing plasma thyroid hormones and consequently the rate of metabolism.\(^{29}\) In accordance, the tumour reducing effect of an energy restricted diet is present with both high or low and low fat content (reviewed by Kritchevsky.\(^{8}\)). Surprisingly, in rats fed diets either 20\% or 3\% corn oil the oxidative adduct, 5-hydroxyuracil, was significantly lower in DNA from mammary gland epithelium as compared with rats fed a control diet with 5\% corn oil.\(^{12}\) Apparently similar effects in liver DNA did not reach statistical significance in that study. However, the diet intervention lasted only two weeks and the three groups of rats consumed the same amount of energy, supporting that fat as such has no specific effect in terms of oxidative DNA modification. In mice fed a high fat diet \emph{ad lib} the 8-oxodG level in lung DNA was 7-fold...
increased\(^\text{17}\) although this was not reproduced in a later similar study.\(^\text{18}\) Both these studies showed an enhancing effect of the high fat diet on 8-oxodG formation due to intratracheal installation of diesel particles. In a cross sectional study of 83 healthy human subjects no relationship between the percentage of energy intake from fat and the urinary excretion of 8-oxodG which on the other hand was related to the total energy intake.\(^\text{27}\) In contrast, a reduction of fat intake by 42–48% for 3–24 months in women at risk of breast cancer reduced the level of 5-hydroxyuracil in DNA of circulating lymphocytes by 68%.\(^\text{11}\) In that study 9 women with a fat reduction diet were compared with 12 women who continued with a fat intake in excess of 30% of the energy whereas the total energy intake was not different between the groups. Accordingly, a high fat diet seems to increase the rate of oxidative damage to DNA but whether this is a direct effect of fat or its metabolism or an indirect effect e.g. via the energy rich diet and increased rate of metabolism has yet to be settled. Certainly, several of these mechanisms could be working in concert.

The present differences in 8-oxodG excretion per body weight between male and female rats could be related to the differences in food and energy intake per body weight. Thus, per whole animal there were minimum differences between the males and females. The higher energy intake per body weight in females thus relates to a higher level of physical activity\(^\text{28}\) and rate of metabolism. The relationship between the rate of metabolism deduced from the energy intake and 8-oxodG excretion is consistent with the close correlation between the excretion and 24-h oxygen consumption or total energy expenditure shown in healthy young women\(^\text{30}\) as well as across species.\(^\text{25,31–33}\) 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.\(^\text{34}\) Thus, hydrogen peroxide formation per mg mitochondrial protein\(^\text{35}\) and the summed mitochondrial surface area\(^\text{36}\) have been shown to correlate with the metabolic rate across species.

The high and low fat diets were somewhat different in composition in addition to the difference in the fat content. However, the protein content was largely the same and the use of fish meal in the low fat chow had minimal influence on the fatty acid composition determined by gas chromatographic analysis (data not shown). In human intervention studies antioxidant vitamins have shown no effects on the excretion of 8-oxodG.\(^\text{37,38}\) Moreover, there was close correlation between 8-oxodG excretion and total energy intake across all three diets in the present study. Accordingly, it is unlikely that differences other than the fat content in the diets were responsible for the differences in 8-oxodG excretion possibly due to differences in metabolic rate, in the present rat study.

In conclusion the present study has demonstrated that a high fat diet increases oxidative DNA modification substantially in rats irrespective of the saturation level of the fat. Energy intake appears to be the major determinant of the rate of modification.

References


\(^{[4]}\) A. Tannenbaum (1942) The genesis and growth of tumors III. Effects of a high fat diet. \emph{Cancer Research} 2, 468–475.

\(^{[5]}\) D. Kritchevsky (1993) Colorectal cancer: the role of dietary fat and caloric restriction. \emph{Mutation Research} 290, 63–70.


