Effect of short-term fasting on urinary excretion of primary lipid peroxidation products and on markers of oxidative DNA damage in healthy women

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The goal of this study was to determine whether short-term fasting changes in urinary biomarkers related to oxidative stress: malondialdehyde (MDA), 8-isoprostaglandin F₂α (8-isoPGF), 8-hydroxydeoxyguanosine (8-OHdG) and 1, N²-ethenodeoxyadenosine (εdA) among female volunteers participating in the short-term fasting program in South Korea. The study participants were 52 healthy women (mean age 28, range 15–48 years old) who provided urine samples both before and after the fasting program (average 7.2, range: 3–11 days). Urinary MDA was measured by HPLC-UV and εdA levels were measured by immunofluorescence detection. Urinary 8-OHdG and 8-isoPGF concentrations were determined by ELISA. Plasma leptin levels were also measured by radioimmunoassay. Information on demographic characteristics, personal habits (smoking and alcohol consumption) and previous medical history were collected by a self-administered questionnaire. Percent loss of body weight (average 6.3%, 4.28 ± 0.25 kg) was significantly correlated with fasting duration (r = 0.70, n = 52, P < 0.01). The plasma leptin levels after fasting (5.89 ± 1.10 ng/ml) were significantly lower than before fasting (6.91 ± 1.13 ng/ml) (n = 27, P = 0.05).

Urinary MDA levels after fasting (0.18 ± 1.10 mg/g creatinine) were significantly lower than before fasting (0.37 ± 1.11) (n = 51, P < 0.01). Urinary 8-isoPGF also were significantly reduced after fasting (n = 47, P < 0.01). However, there was no significant difference in 8-OHdG or εdA. There was a statistically significant correlation between % change of urinary MDA level with % change of 8-isoPGF level (partial correlation coefficient r = 0.57, n = 46, P = 0.01). The correlations between % change of 8-OHdG and plasma leptin was also significant (partial correlation coefficient r = 0.51, n = 27, P = 0.02). Our results demonstrate that the short-term fasting reduces lipid peroxidation products but does not affect oxidative stress-induced DNA damage.

Introduction

Obesity is related to increased morbidity and mortality of cancer and cardiovascular disease which are two major causes of deaths in the USA (1,2). Dietary restriction has been shown to have several health benefits including increased insulin sensitivity, stress resistance, reduced morbidity and increased life span (3,4).

Fasting has been suggested to have beneficial effects on glucose regulation and neuronal resistance to injury (4). Short-term fasting has been shown to prolong the life span of autoimmune-prone mice (5) and to reduce plasma leptin concentration in dairy cattle, non-obese healthy humans and sympathectomized men (6–9). A moderate degree of short-term weight reduction significantly decreases leptin levels in otherwise healthy overweight adults (10).

Although the underlying mechanism involved in the beneficial effects of dietary restriction are not known, oxidative stress has been proposed as an important intermediate event. Caloric restriction can decrease oxidative DNA damage (11). Caloric restricted C57BL/6 mice show a lower concentration of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-OHdG), the most widely studied markers of oxidative DNA damage (12,13). Caloric restriction also reduces malondialdehyde (MDA), a product of lipid peroxidation (LPO), in skeletal muscles of Rhesus monkeys (14) but not in streptozotocin-induced diabetic rats (15,16). 8-Isoprostaglandin-F₂α (8-isoPGF) is the prostaglandin-like compound produced by non-enzymatic mechanism of peroxidation of lipoproteins (17). It is chemically stable and can be measured in human urine (18–19). Diet and exercise intervention can reduce 8-isoPGF levels and short-term weight loss is also associated with significant reduction of 8-isoPGF (17,20).

Abbreviations: 8-isoPGF, 8-isoprostaglandin-F₂α; 8-OHdG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; ELISA, enzyme-linked immunosorbent assay; LPO, lipid peroxidation; MDA, malondialdehyde; TBA, 2-thiobarbituric acid; εdA, 1,N²-ethenodeoxyadenosine.
1. A$^\text{2}$-Ethenodeoxyadenosine (e$^\text{da}$) is formed as stable DNA base adducts from reactive aldehydes such as $\text{trans}$-$4$-hydroxy-2-nonenal during oxidative stress or LPO (21). Urinary e$^\text{da}$ as a whole body marker for LPO has been reported to be associated with salt-induced inflammation and $\alpha$-$6$-polysaturated fatty acid intake in postmenopausal Japanese women (22).

The purpose of this study was to determine whether short-term fasting changes urinary biomarkers related to oxidative stress. The markers included in the study were urinary MDA, 8-isoPGF, 8-OHdG and urinary e$^\text{da}$. The subjects recruited for this study were healthy female volunteers participating in a short-term fasting program.

**Materials and methods**

**Materials**

Methanol and acetonitrile were purchased from HAYMAN (Witham, Essex, UK) with a purity of 99.85%, 2-Thiobarbituric acid (TBA) and MDA standard were purchased from the Sigma–Aldrich Korea (Yongin, Kyunggi, Korea). The enzyme-linked immunosorbent assay (ELISA) kit for 8-isoPGF was obtained from OxisResearch (Portland, OR). The commercial ELISA kit for 8-OHdG was from the Japan Institute for the Control of Aging (Fukuroi, Shizuoka, Japan) designed for quantitative measurement of the oxidative DNA adduct 8-OHdG in urine. All other chemicals were obtained in the greatest purity available from commercial suppliers.

**Study subjects**

The study subjects consisted of 58 volunteers who participated in a fasting program (7.2 days, range: 3–11 days) and provided first morning void urine samples both before and after the fasting program in South Korea. Four males were excluded because of a small proportion (7%) and gender-specific differences in the formation of certain biomarkers for oxidative stress (e.g. 8-isoPGF, e$^\text{da}$) (23). Two females who did not complete the questionnaires were also excluded. Finally, 52 women remained in the study (mean age 28.3, range 15–48 years old). Of these, only 30 people provided pooled blood samples. All participants did not have food during the fasting program; however, water was permitted. Participants were allowed to do light exercise during the fasting program including jogging and yoga but strenuous physical activities (e.g. running, cycling, heavy weight lifting, tennis, soccer, etc.) were avoided. Every volunteer had a baseline physical examination. All participants were screened for diabetes, hypertension and other major systemic illnesses such as gastrointestinal disturbance, liver function and nephropathy. At the end of the fasting period, the volunteers started on liquids first and gradually changed to a regular diet. The study was approved by the institutional review board at the Seoul National University College of Medicine and each subject gave written informed consent. Information on demographic characteristics such as smoking, alcohol consumption, diet, medical history and medicine use was collected using a self-administered questionnaire.

**Sample collection**

Urine and blood samples were collected before and after the fasting. First morning urine samples were collected before and after the fasting program started and after the program ended. Urine samples were collected in 50 ml polypropylene tubes and stored at $-20\,^\circ\text{C}$ until analysis. Blood samples (5 ml) were collected in EDTA vacutainer tubes and kept at $-80\,^\circ\text{C}$ until analysis.

**Analysis of urinary MDA**

MDA was measured using a method previously described (24) with minor modifications. The most common method of measuring MDA is based on the reaction with TBA. A 10 mmol/l stock standard of MDA was prepared by dissolving 123.5 $\mu$mol of 1,1,3,3-tetraethoxypropane in 50 ml of ethanol (40% ethanol by volume). TBA–MDA adducts were prepared in glass tubes with a polystyrene stopper. In each tube, 300 $\mu$l of phosphoric acid (0.5 M) was mixed with 50 $\mu$l of urine and 150 $\mu$l of TBA reagent. The mixtures were incubated at 95°C for 1 h and methanol (500 $\mu$l) was added in each tube. Following a 5 min centrifugation (5000 g), the samples were analyzed using HPLC on a 4 x 150 mm Xtra C18 column with UV (wavelengths, 532 nm). The mobile phase was potassium phosphate (0.05 mol/l; pH 6.8) and methanol (58:42, v/v). The flow rate was 0.8 ml/min.

**Analysis of urinary 8-isoPGF**

The urine samples were analyzed for 8-isoPGF levels by a competitive enzyme-linked immunoassay (ELISA) (25). This ELISA kit can be used for the quantitation of free 8-isoPGF in urine samples without the need for prior purification or extraction. In brief, the samples were mixed with an enhancing reagent that essentially eliminates interference due to non-specific binding. Following substrate addition, the intensity of the color was noted to be inversely proportional to the amount of unconjugated 8-isoPGF in the sample or standard.

**Analysis of urinary 8-OHdG**

The level of urinary 8-OHdG was determined by a competitive enzyme-linked immunosorbent assay (ELISA) kit (JAICA, Fukuroi, Japan). In brief, 50 $\mu$l of primary monoclonal antibody and a 50 $\mu$l of sample or standard were added to microtiter plates, which were pre-coated with 8-OHdG, incubated at 37°C for 1 h and washed with 250 $\mu$l of phosphate-buffered saline (PBS). One hundred microliters of HRP-conjugated secondary antibody was then added to each well, incubated at 37°C for 1 h and washed with 250 $\mu$l of PBS. One hundred microliters of enzyme substrate was then added to each well, incubated at 37°C for 1 h and the reaction was terminated with 100 $\mu$l of 1 N phosphoric acid. Absorbance of each well was read at 450 nm by a microplate reader (ELx808, Bio-Tek, Winooski, VT).

**Analysis of urinary e$^\text{da}$**

Urine e$^\text{da}$ was analyzed according to a method published earlier (22). Two milliliters urine samples were spiked with an internal standard (1,1$^\text{2}$-ethenoadenosine-[2,8-3H]) and purified on preparative HPLC. Two fractions (internal standard and e$^\text{da}$) were collected; the earlier eluted L.$^\text{s}$ fraction was counted for recovery by liquid scintillation counter and the e$^\text{da}$ fraction was concentrated (overnight) in a speed-vac and then used for immuno-precipitation.

The immunoprecipitation of e$^\text{da}$ was performed in Tris–HCl buffer (10 mM Tris, pH 7.5, 140 mM NaCl, 3 mM Na2SO4) containing 1% BSA and 0.1% rabbit IgG (Sigma–Aldrich, Schnelldorf, Germany) and a monoclonal antibody EM-A-1 provided by Dr P. Lorenz and M. Rajewsky (Institute for Cell Biology, University of Essen, Germany). The antigen–antibody complex was precipitated with saturated ammonium sulfate. The precipitate was washed and e$^\text{da}$ eluted using 50% methanol/water and then concentrated in a speed-vac. The pellets were analyzed by an HPLC-fluorescence detector (Hewlett Packard, Waldbronn, Germany). Using a linear gradient (NLH)–IP0A, 17 mM, pH 5, buffer/methanol 9:1 to 8:2 in 30 min at a flow rate of 1 ml/min. The e$^\text{da}$ peak was detected at $\lambda$ excitation 230/ emission 410 nm and quantified by a standard curve using e$^\text{da}$ standard (Sigma–Aldrich). The detection limit of e$^\text{da}$ in this HPLC system was $\sim 5$ fmol per injection.

**Plasma leptin assay**

Plasma leptin levels were measured by a radioimmunoassay using a commercially available kit for human leptin (catalogue no. HL-81K; Linco Research, St Louis, MO), as described previously (26).

**Statistical methods**

The paired t-test was used to compare group means (before and after fasting) as smoking, alcohol consumption, diet, medical history and medicine use was collected using a self-administered questionnaire.

Results

Average body weight loss was $4.28 \pm 0.25$ kg of their initial body weight (62.18 ± 1.51 kg) (Table I). The percent loss of body weight (mean 6.3%) was significantly correlated with fasting duration (days) (Pearson’s correlation $r = 0.70$, $n = 52$, $P < 0.01$) (Figure 1).

The plasma leptin levels after fasting (5.89 ± 1.10 ng/ml) were significantly lower than before fasting (6.91 ± 1.13 ng/ml) (Table I; $n = 27$, $P = 0.05$ by paired t-test) (Table I and Figure 2).

Urinary MDA levels after fasting (0.18 ± 1.10 mg/g creatinine) were significantly lower than before fasting (6.91 ± 1.13 ng/ml) (Table I; $n = 27$, $P = 0.05$ by paired t-test) (Table I). Urinary 8-isoPGF also was significantly reduced after fasting ($n = 47$, $P < 0.01$). However, there was no significant difference in 8-OHdG or e$^\text{da}$ (Table I). Although creatinine levels increased (16%) after fasting, the results of biomarker levels before and after creatinine adjustment remained unchanged (Table II).
There was a statistically significant correlation between percent change of urinary MDA levels and percent change of 8-isoPGF levels (partial correlation coefficient $r = 0.57$, $n = 46$, $P < 0.01$) after adjusting for BMI and smoking. There was also a statistically significant correlation between percent change of urinary 8-OHdG with percent change of plasma leptin level (partial correlation coefficient $r = 0.51$, $n = 27$, $P < 0.05$, adjusted for percent change BMI and smoking status).

Discussion

There was a significant reduction of urinary biomarkers of oxidative stress related to LPO (e.g. MDA, 8-isoPGF) after short-term fasting (average 7 days). A plasma leptin level was also significantly reduced after fasting. Short-term fasting reduced both non-specific biomarkers of LPO (MDA) and a marker for \textit{in vivo} LPO (8-isoPGF) produced from arachidonic acid by COX-independent non-enzymatic mechanisms. However, biomarkers that may reflect longer-term oxidative DNA damage (e.g. 8-OHdG, edA) were not observed to change after short-term fasting.

Although obesity is associated with increased oxidative stress in animal models and an increase in 8-isoPGF in the obese Zucker rat, limited information in humans is available.

Table I. Means (±SE) and percent change of urinary biomarkers of oxidative stress (MDA, 8-isoPGF, 8-OHdG and edA) and plasma leptin level before and after fasting

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>N</th>
<th>Arithmetic mean</th>
<th>Geometric mean</th>
<th>% Change</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before fasting</td>
<td>After fasting</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>52</td>
<td>63.01 ± 1.51</td>
<td>58.69 ± 1.41</td>
<td>−6.8</td>
<td>&lt;0.01</td>
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<td>MDA (mg/g Cr)</td>
<td>51</td>
<td>0.50 ± 0.07</td>
<td>0.24 ± 0.03</td>
<td>−52.0</td>
<td>&lt;0.01</td>
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<tr>
<td>8-isoPGF (µg/g Cr)</td>
<td>47</td>
<td>9.93 ± 2.00</td>
<td>4.40 ± 0.80</td>
<td>−55.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>8-OHdG (µg/g Cr)</td>
<td>50</td>
<td>16.50 ± 2.26</td>
<td>15.75 ± 1.69</td>
<td>−4.5</td>
<td>0.97</td>
</tr>
<tr>
<td>edA (fmol/µmol Cr)</td>
<td>48</td>
<td>6.39 ± 0.61</td>
<td>7.22 ± 1.13</td>
<td>+13.0</td>
<td>0.59</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>27</td>
<td>8.03 ± 0.76</td>
<td>6.68 ± 0.66</td>
<td>−16.8</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>62.18 ± 1.02</td>
<td>57.90 ± 1.02</td>
<td>−6.3</td>
<td>&lt;0.01</td>
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Table II. Concentration of urinary creatinine and biomarkers (before creatinine adjustment)

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>N</th>
<th>Arithmetic mean</th>
<th>Geometric mean</th>
<th>% Change</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Before fasting</td>
<td>After fasting</td>
<td></td>
<td></td>
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<tr>
<td>Creatinine (mg/dl)</td>
<td>52</td>
<td>150.01 ± 11.00</td>
<td>174.28 ± 12.15</td>
<td>14.15</td>
<td>0.13</td>
</tr>
<tr>
<td>MDA (µmol/litre)</td>
<td>51</td>
<td>2.52 ± 0.28</td>
<td>1.47 ± 0.12</td>
<td>−52.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>8-isoPGF (ng/ml)</td>
<td>47</td>
<td>16.43 ± 3.43</td>
<td>7.73 ± 1.53</td>
<td>−55.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>8-OHdG (ng/ml)</td>
<td>50</td>
<td>26.40 ± 4.21</td>
<td>15.75 ± 1.69</td>
<td>−4.5</td>
<td>0.97</td>
</tr>
<tr>
<td>edA (fmol/mmol)</td>
<td>48</td>
<td>111.95 ± 18.68</td>
<td>88.32 ± 12.30</td>
<td>+29.0</td>
<td>0.10</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>27</td>
<td>8.03 ± 0.76</td>
<td>6.68 ± 0.66</td>
<td>−16.8</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>121.82 ± 1.11</td>
<td>151.46 ± 1.08</td>
<td>21.4</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Table III. Partial correlation coefficients among percent change of biomarkers [(before − after fasting)/before fasting × 100]

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>MDA</th>
<th>8-isoPGF</th>
<th>8-OHdG</th>
<th>edA</th>
<th>Leptin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.57a</td>
<td>−0.36</td>
<td>−0.01</td>
<td>−0.10</td>
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<tr>
<td>8-isoPGF</td>
<td>−0.34</td>
<td>−0.22</td>
<td>−0.31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8-OHdG</td>
<td>0.28</td>
<td>0.51b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>edA</td>
<td>−0.09</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

Fig. 1. Correlation of body weight change (%) and fasting duration (days) (Pearson’s correlation coefficient $r = 0.70$, $n = 52$, $P < 0.01$).

Table I. Means (±SE) and percent change of urinary biomarkers of oxidative stress (MDA, 8-isoPGF, 8-OHdG and edA) and plasma leptin level before and after fasting

There was a statistically significant correlation between percent change of urinary MDA levels and percent change of 8-isoPGF levels (partial correlation coefficient $r = 0.57$, $n = 46$, $P = 0.01$) after adjusting for BMI and smoking. There was also a statistically significant correlation between percent change of urinary 8-OHdG with percent change of plasma leptin level (partial correlation coefficient $r = 0.51$, $n = 27$, $P = 0.02$) (Table III).
available (27,28). Davi et al. (18) showed that women with android obesity had higher levels of urinary 8-isoPGF than non-obese women. They also observed a statistically significant reduction of urinary 8-isoPGF in 11 women with android obesity (BMI >28 and waist-hip ratio >0.86) who successfully achieved weight loss in a short-term weight loss program (12 weeks) (18). Roberts et al. (20) reported a significant reduction of serum 8-isoPGF in 11 men on a low-fat, high-fiber diet combined with daily exercise for 45–60 min for 3 weeks. Thompson et al. (29) also reported a 35% reduction in urinary 8-isoPGF in 28 women after 14 days of consuming an array of fruits and vegetables. These findings of a lower level of urinary 8-isoPGF may result from reduced oxidative stress from the diet and exercise intervention.

However, results of the association between caloric restriction and MDA are limited and inconsistent. Although Zainal et al. (14) reported that caloric restriction lowered the concentrations of MDA in skeletal muscle in the Rhesus monkey, Ugochukwu et al. (15) failed to show the effect of dietary caloric restriction on reduction of plasma MDA in streptozotocin-induced diabetic rats. In a human intervention study, there was no significant change in urinary MDA levels in 28 women after 14 days of consuming fruits and vegetables (29). These inconsistencies might be due to insensitivity and non-specificity of urinary MDA and/or incomplete control for other factors (e.g. other diets, physical activity, etc.) which may affect MDA levels. However, the significant correlation between the change of urinary 8-isoPGF and the change of MDA during the fasting periods observed in this study suggests that short-term fasting significantly reduced oxidative stress, particularly generated by the short-term LPO process.

On the contrary to a number of previous animal studies showing significant reduction of oxidative DNA damage after short-term caloric restriction (30–32), there was no significant change in 8-OHdG or εdA levels in this study. The
finding of no significant reduction in urinary 8-OHdG levels observed in this study is consistent with the findings from 28 women in a diet intervention program (29). Although the finding of a significant correlation between the change in urinary 8-OHdG with the change of plasma leptin levels observed in this study may suggest the effect of short-term fasting on urinary 8-OHdG levels, these results could be explained by several other factors. First, short-term fasting or severe caloric restriction may not alter the steady-state level of oxidative DNA damage which is determined, in part, by the rate of hydroxyl radical generation and individual repair capacity. edA which reflects longer-term oxidative DNA damage may not be associated with short-term fasting or severe caloric restriction (23). Second, we could not control for other factors (e.g. inter-individual variation of toxicokinetics and/or repair capacity, hidden inflammatory process, etc.) possibly related to oxidative DNA damage (8-OHdG, edA) levels. The study would have been strengthened by following out for an additional time period after the restoration of the normal diet.

Although our results may not have practical implications because of the artificial nature of study design (fasting a few days), recent biochemical and microarray results summarized by Spindler (33) suggest that short-term caloric restriction acts rapidly and reversibly to extend life span in animal model. Furthermore, short-term caloric restriction (2 weeks) significantly decreased cellular oxygen consumption and reactive oxygen species production of rat muscle mitochondria (30). However, the results of this study should be cautiously interpreted and future studies of the effect of long-term fasting should be conducted.

In conclusion, although the long-term effects of fasting remain to be investigated, our results suggest that short-term fasting exerts beneficial effects and reduces urinary levels of primary LPO products (MDA, 8-isoPGF) without effecting biomarkers of oxidative DNA damage (8-OHdG, edA).

Acknowledgement

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Conflict of Interest Statement: None declared.

References


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