Chronic intake of potato chips in humans increases the production of reactive oxygen radicals by leukocytes and increases plasma C-reactive protein: a pilot study1-3

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ABSTRACT
Background: Relatively high concentrations of acrylamide in commonly ingested food products, such as French fries, potato chips, or cereals, may constitute a potential risk to human health.

Objective: The objective of this pilot study was to investigate the possible connection between chronic ingestion of acrylamide-containing potato chips and oxidative stress or inflammation.

Design: Fourteen healthy volunteers (mean age: 35 y; 8 women and 6 smokers of >20 cigarettes/d) were given 160 g of potato chips containing 157 mg acrylamide daily for 4 wk.

Results: An increase in acrylamide-hemoglobin adducts in blood was found in all the study subjects, with a mean of 43.1 pmol·L−1·g−1 hemoglobin (range: 27–76; P < 0.01) in nonsmokers and 59.0 pmol·L−1·g−1 hemoglobin (range: 43–132; P < 0.05) in smokers. Concurrently, a significant increase (P < 0.01) in the oxidized LDL, high-sensitivity interleukin-6, high-sensitivity C-reactive protein, and γ-glutamyltransferase concentrations was observed in both smokers and nonsmokers. A significant increase in reactive oxygen radical production by monocytes, lymphocytes, and granulocytes and an increase in CD14 expression in macrophages (P < 0.001) were found after intake of potato chips. Twenty-eight days from the discontinuation of the experiment, the variables under study decreased to some extent. It has been shown also that acrylamide increases the production of reactive oxygen species in isolated human monocyte-macrophages in vitro and decreases the cellular glutathione concentration.


INTRODUCTION
The published data pertaining to the effects of acrylamide on human health have focused on the influence of this compound on the function of the central and peripheral nervous systems (1). Most investigations of acrylamide toxicity have concerned themselves with the pure chemical form of the substance. Occupational exposure to acrylamide in chemical industry employees, as well as acrylamide intake in potable water or in cigarette smoke, have been studied (2). Swedish researchers reported that acrylamide could be formed in various heat-treated, carbohydrate-rich foods (3, 4). A particularly high concentration of acrylamide was found in potato chips, breakfast cereals, and crisp bread (5). It has been shown that acrylamide content in food results from heat-induced reactions between the amino group of the free amino acid asparagine and the carbonyl group of a reducing sugar (6). In humans, acrylamide is absorbed mainly via ingestion or inhalation and forms adducts with hemoglobin, which appear to be useful biomarkers of acrylamide exposure (7). Conjugation with glutathione (GSH) catalyzed by glutathione-S-transferase is the main pathway of acrylamide metabolism and detoxification (8).

Environmental factors, such as diet and nutritional habits, have a significant impact on the course of cardiovascular disorders with underlying atherosclerosis. In recent years, nutritional habits have changed significantly and acrylamide-containing products, such as potato chips, have become widely popular (9).

Because the typical consumption of acrylamide-rich foods has increased, it is more likely that the compound will exert its effects within blood vessels. Therefore, studies assessing acrylamide influence on cardiovascular risk factors may be necessary, especially because many in vitro studies indicate that exposure of various cells to acrylamide leads to a significant reduction in their GSH concentration (10). This in turn may contribute to the creation of oxidative stress conditions that aggravate atherosclerosis (11). Therefore, the purpose of our study was to assess the effect of the chronic intake of acrylamide contained in commonly ingested potato chips on the free radical production reported that acrylamide could be formed in various heat-treated, carbohydrate-rich foods (3, 4). A particularly high concentration of acrylamide was found in potato chips, breakfast cereals, and crisp bread (5). It has been shown that acrylamide content in food results from heat-induced reactions between the amino group of the free amino acid asparagine and the carbonyl group of a reducing sugar (6). In humans, acrylamide is absorbed mainly via ingestion or inhalation and forms adducts with hemoglobin, which appear to be useful biomarkers of acrylamide exposure (7). Conjugation with glutathione (GSH) catalyzed by glutathione-S-transferase is the main pathway of acrylamide metabolism and detoxification (8).

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in leukocytes and on inflammation assessed through measurements of C-reactive protein (CRP) concentrations.

SUBJECTS AND METHODS

Fourteen healthy volunteers (8 women and 6 men) with a mean age of 35 y (range: 22–56 y) were recruited. In this population, 4 women and 2 men smoked >20 cigarettes/d. Subjects were excluded if they were known to have a history of diabetes, hypertension, or hyperlipidemia or were on any medications, vitamins, or dietary supplements. Pregnant women and those with elevated CRP concentrations >5 mg/L at baseline and a body mass index (BMI; in kg/m²) >25 were also excluded.

The study period was divided into 3 phases. In the first 2 wk, all study subjects were required to ingest 400 g of boiled potatoes daily with amounts of fat [a mixture of liquid and hardened vegetable fats heated at 180°C for ≈20–30 min; peroxide value (POV) < 1.0 mEq/kg; carbonyl value (COV) = 7.5] and salt corresponding to those contained in commercially produced potato chips. Subsequently, for the next 28 d, the study subjects ingested 160 g of potato chips daily from one manufactured batch purchased from a shop. The potato chips contained 878 kcal, of which 4.3% was energy from protein, 65.6% was energy from fat with a polyunsaturated/saturated fatty acid ratio of 0.51, and 30.1% was energy from carbohydrates, together with 1374 mg of sodium and 5 mg of vitamin E. The acrylamide content was 980 μg/kg (156.8 μg per daily ingested 160-g portion), and the concentration of trans fatty acids was <1% (12). The POV value was <1.2 mEq/kg and the COV was 10.1. For the following 28 d, the study subjects were strictly forbidden to eat potato chips, which were replaced once again by boiled potatoes. Throughout the experiment, the study subjects maintained their baseline ingestion level of products that can potentially influence acrylamide concentrations, ie, French fries, coffee, and crisp bread. For the entire duration of the study, the participants remained under medical supervision.

The study was conducted according to the Declaration of Helsinki guidelines and was approved by the Institutional Ethics Board of the National Institute of Food and Nutrition. The study commenced in 2005 and was completed in 2007.

After routine physical examination, blood samples were drawn after an overnight fast and stored at −70°C. Plasma lipids and lipoproteins, glucose, uric acid, and γ-glutamyltransferase (GGT) were measured by standard techniques. Plasma total homocysteine concentrations were measured by using HPLC with fluorescence detection and test kits from Bio-Rad (Munich, Germany). Serum concentrations of CRP were assessed using a high-sensitivity (hs) method (immunonephelometry; Dade Behring GmbH, Marburg, Germany). Oxidized LDL (ox-LDL) was quantified by using a noncompetitive enzyme-linked immunosorbent assay (Mercodia, Uppsala, Sweden). The CV was <5.5%. Serum concentrations of interleukin-6 (hs IL-6) were measured by quantitative sandwich enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN). The limit of sensitivity for the assays was 0.02 mg/dL and the CV was 3.5–4.1%.

The N-alkyl Edman method for analysis of adducts to N-terminal valines in hemoglobin was used with modifications for analysis of acrylamide adducts (13, 14). Globin was isolated by precipitation from thawed samples of erythrocytes. The globin samples were derivatized with pentafluorophenyl iso-thiocyanate, leading to detachment of the acrylamide adduct as N-(2-carboxamoyethyl) valine-PFPTH (AA_Val-PFPTH), which was then extracted from the rest of the protein. The deuterated internal standard, AA-d5-Val-PFPTH, was added to the derivative samples before extraction. The analysis was performed by gas chromatography–tandem mass spectrometry (Finnigan TSQ 700; ThermoScientific, Waltham, MA) in the negative ion/chemical ionization mode.

Production of reactive oxygen radicals by monocytes, granulocytes, and lymphocytes was analyzed by burst test (Orpegen Pharma, Heidelberg, Germany) using flow cytometry (FACS Scan; Becton Dickinson, San Jose, CA). Cells were stimulated with the use of phorbol myristate acetate (PMA) and monocytes, granulocytes, and lymphocytes were gated (10⁴ cells) on the basis of forward-angle light scatter and single-angle light scatter.

Expression of CD14 antigen on monocytes was determined using fluorescein-5-isothiocyanate–labeled monoclonal antibodies (Becton Dickinson).

Isolation of peripheral blood mononuclear cells and cultivation of monocyte-macrophages, as well as measurement of reactive oxygen species (ROS) production with 2′,7′-dichlorofluorescein, were performed as previously reported (15). Cellular GSH concentrations were determined according to Griffith (16) (Figure 1). The results are presented as means ± SDs. Arithmetic means were compared using Student’s t test and 1- or 2-factor analysis of variance followed by Tukey’s test. All calculations were made with the StatSoft statistical package (version 5.1 PC; StatSoft Poland, Warsaw, Poland).

The results obtained for hs CRP were compared using the nonparametric Wilcoxon’s test for paired values. Two-sided P values <0.05 were considered to be significant.
RESULTS

In all study subjects, significant increases in acrylamide-hemoglobin (AAHb) adducts in blood were found after potato chip intake, which confirms the volunteers’ compliance with the experimental conditions. Because the baseline values of AAHb and hs CRP in smokers were significantly higher compared with nonsmokers, the results for the groups are presented separately. As shown in Table 1, no changes were detected in BMI, blood pressure (data not shown), or in the plasma concentrations of lipids, lipoproteins, homocysteine, uric acid, or glucose throughout all the experimental periods. There was a significant increase in the concentration of AAHb in nonsmokers (P < 0.01) and in smokers (P < 0.05) after 28 d of potato chip intake.

In absolute values, the increase in AAHb was significantly larger in smokers than in nonsmokers (mean: 59.0 and 43.1 pmol·L⁻¹·g⁻¹ hemoglobin, respectively; P < 0.05). A potential interaction of potato chip intake with smoking was also analyzed with 2-factor analysis of variance; however, no significant relations were found. After the 28-d period with no potato chip intake, the concentrations of AAHb were partially reduced yet still remained significantly higher (P < 0.05) than the baseline values.

During the potato chip intake period, there was a significant increase in the following concentrations for nonsmokers and smokers, respectively: hs IL-6 concentrations of 20.6% (P < 0.01) and 15.1% (P < 0.01); ox-LDL concentrations of 21.0% (P < 0.01) and 29.1% (P < 0.01); hs CRP concentrations of 56.0% (P < 0.01) and 43.0% (P < 0.05), and GGT concentrations of 21.7% (P < 0.006) and 29.6% (P < 0.01).

For the evaluation of the oxidative burst activity of leukocytes in heparinized whole blood, flow cytometry and a test kit were used. After the potato chip intake period, a significant increase in reactive oxygen radical production by monocytes, lymphocytes, and granulocytes after stimulation with PMA was observed in those cells.

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DISCUSSION

In this study, we have shown for the first time that chronic ingestion of dietary acrylamide might induce oxidative stress in humans through leukocyte activation and increased production of reactive oxygen radicals. As a consequence, a significant increase in the ox-LDL, hs IL-6, and hs CRP concentrations occurred, with all these factors recognized as capable of enhancing atherosclerosis progression. This could be related to the lowering of the reserves of an important cellular antioxidant, GSH, by acrylamide contained in potato chips (17).

Indeed, GSH is used in the process of detoxification and acrylamide removal from the body in the form of mercapturic acid (2). In our study, we actually demonstrated that high acrylamide ingestion is followed by an increase in GGT, which serves as a rescue enzyme for cellular GSH synthesis (18). In the subsequent direct in vitro experiment, we confirmed the actual involvement of acrylamide in the production of ROS by macrophages as well as in the lowering of GSH concentrations in

### TABLE 1

<table>
<thead>
<tr>
<th>Variables</th>
<th>Nonsmokers (n = 8)</th>
<th>Smokers (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before consumption</td>
<td>28 d after consumption</td>
</tr>
<tr>
<td>AAHb (pmol/g hemoglobin)</td>
<td>43.88 ± 31.30</td>
<td>87.00 ± 47.05</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>5.11 ± 0.92</td>
<td>5.39 ± 1.00</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.12 ± 0.80</td>
<td>1.10 ± 0.51</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.54 ± 0.28</td>
<td>1.64 ± 0.34</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>3.05 ± 1.05</td>
<td>3.29 ± 0.98</td>
</tr>
<tr>
<td>ox-LDL (U/L)</td>
<td>68.35 ± 15.92</td>
<td>82.7 ± 12.85^2</td>
</tr>
<tr>
<td>hs IL-6 (ng/mL)</td>
<td>3.25 ± 0.22</td>
<td>3.92 ± 0.38^6</td>
</tr>
<tr>
<td>hs CRP (mg/L)</td>
<td>1.53 ± 0.75</td>
<td>2.39 ± 0.78^2</td>
</tr>
<tr>
<td>GGT (mg/L)</td>
<td>25.56 ± 3.27</td>
<td>33.86 ± 3.95^2</td>
</tr>
<tr>
<td>Uric acid (µmol/L)</td>
<td>291.44 ± 67.35</td>
<td>297.40 ± 61.39</td>
</tr>
<tr>
<td>Homocysteine (µmol/L)</td>
<td>9.86 ± 2.69</td>
<td>10.17 ± 3.45</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.93 ± 0.35</td>
<td>5.02 ± 0.24</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.06 ± 1.88</td>
<td>23.15 ± 2.01</td>
</tr>
</tbody>
</table>

1 Values are means ± SDs. TC, total cholesterol; TG, triglyceride; ox-LDL, oxidized LDL; hs IL-6, high-sensitivity interleukin-6; hs CRP, high-sensitivity C-reactive protein; GGT, γ-glutamyltransferase. Interactions of chips intake with smoking: no significant relations (2-factor ANOVA and Tukey’s test).

2 P < 0.01 compared with before consumption (one-factor ANOVA and Tukey’s test).

3 P < 0.05.

4 P < 0.001 compared with before consumption (Student’s t test).

5 P < 0.05.

6 P < 0.01.
TABLE 2
Effects of potato chip consumption on reactive oxygen radicals in leukocytes after stimulation with phorbol myristate acetate and CD14 expression in monocytes

<table>
<thead>
<tr>
<th>Variables</th>
<th>Before consumption</th>
<th>28 d after consumption</th>
<th>After 28 d without consumption</th>
<th>Before consumption</th>
<th>28 d after consumption</th>
<th>After 28 d without consumption</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monocytes</td>
<td>46.38 ± 8.38</td>
<td>99.00 ± 36.93a</td>
<td>67.75 ± 20.06b</td>
<td>46.67 ± 6.74</td>
<td>87.83 ± 27.36c</td>
<td>72.17 ± 19.99c</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>17.50 ± 2.62</td>
<td>33.63 ± 11.82c</td>
<td>24.13 ± 6.53d</td>
<td>19.33 ± 2.34</td>
<td>34.00 ± 9.10b</td>
<td>24.83 ± 3.66d</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>84.13 ± 55.40</td>
<td>141.50 ± 58.22a</td>
<td>115.00 ± 55.93b</td>
<td>63.33 ± 13.37</td>
<td>115.50 ± 28.02a</td>
<td>82.00 ± 8.02a</td>
</tr>
<tr>
<td>CD14 in monocytes</td>
<td>505.00 ± 52.14</td>
<td>599.13 ± 60.91d</td>
<td>582.13 ± 59.96d</td>
<td>576.33 ± 131.13</td>
<td>679.17 ± 121.44d</td>
<td>591.33 ± 92.00d</td>
</tr>
</tbody>
</table>

1 Values are means ± SDs. Results are shown in units of fluorescence intensity for 10⁶ cells. For smokers compared with nonsmokers before consumption, there were no significant relations (Student’s t test). For interactions of potato chip intake with smoking, there were no significant relations (2-factor ANOVA and Tukey’s test).

2 P < 0.05.

3 P < 0.01.

4 P < 0.001 compared with before consumption (one-factor ANOVA and Tukey’s test).

These newly described mechanisms of the action of acrylamide and its metabolite glyciamide may also be responsible for mechanisms of molecular regulation of genes through influence on transcription factors, including nuclear factor-κB. This is confirmed by the recent studies by Clement et al (19) and Hasegawa et al (20), which were conducted with the use of genomic and proteomic analysis. The fact that acrylamide may contribute to nuclear factor-κB activation seems to explain the increased CD14 expression in the monocytes that we observed, as well as increased ROS production by leukocytes, which is associated with NADPH-oxidase stimulation. A further consequence is the induction of chronic low-intensity inflammation, shown by an elevated concentration of hs CRP, which is an independent marker of coronary heart disease events (21). Nevertheless, further studies are needed to investigate our observation that, despite a stop in potato chip intake, both the AAHb and reactive oxygen radical production in leukocytes showed a tendency to remain to some extent for the next 28 d. This could be associated with a slower rate of AAHb catabolism via the receptor-dependent endocytosis. Indeed, the latest studies by Schae et al (22) showed that some of the hemoglobin modifications could reduce its uptake via the CD163 receptor. This in turn might have effects on the maintenance of inflammation, because proper hemoglobin clearance is necessary to counteract its cytotoxicity resulting from oxidative and nitrosative processes (23).

These newly described mechanisms of the action of acrylamide and its metabolite glyciamide may also be responsible for progression of cancer, irrespective of the direct effect of these substances on DNA damage (17). Despite many controversies as to the cancer-promoting action of dietary acrylamide, recent prospective studies indicate that the risk of postmenopausal endometrial and ovarian cancer increases with mean acrylamide ingestion of ≈40 μg/d (24).

Our study, which should be regarded as a pilot study, indicates that high-acrylamide ingestion may constitute a new dietary risk factor for atherosclerosis progression, both in a healthy population and in patients with coronary artery disease. This hypothesis is supported by the studies by Ashfag et al (25), in which low GSH concentration, associated oxidative stress, and increased hs CRP concentrations were shown to correlate with early atherosclerosis development in healthy people, as assessed by carotid intima-media thickness measurements. Moreover, the concentration of GGT increase after acrylamide ingestion may be a prognostic indicator of the risk of ischemic heart disease and diabetes as well as of hypertension, which was demonstrated in the CARDIA (Coronary Artery Risk Development in Young Adults) Study (26). Additionally, it has recently been demonstrated that elevated GGT may be a risk factor for restenosis in vascular stents (27).

Our study was performed under the conditions of an acute experiment in which the concentration of acrylamide ingestion was 3 times higher than the currently calculated ingestion of ≈50 μg/d in the Western diet (9). Furthermore, the study group was relatively small, which is a limitation to the statistical assessment of the results. Therefore, we recognize that the study results require corroboration by other centers. Also we cannot completely exclude the possibility that the increased uptake of partially oxidized lipids, generated in the process of the production of potato chips, could to some extent influence the results obtained in our experiments (28). However, in view of the fact that the values defining the quality of fat in the potato chips used in this study, ie, POV and COV, were within the lower limits of the acceptable range, we consider the probability of their involvement in the induction of oxidative stress to be small.

In conclusion, long-term ingestion of high-acrylamide doses with food may cause chronic inflammation and contribute to the development of early atherosclerosis as well as increase the risk of coronary artery disease.

We are grateful to I Athanassiadis for skillful assistance with mass spectrometry analysis.

The authors’ responsibilities were as follows—MN: designed the study and contributed to the drafting of the article; DZ-D: was responsible for the analysis of flow cytometry data; AK: was responsible for the flow cytometry study; GN: was responsible for the biochemistry determinations; ASV: was responsible for the acrylamide-hemoglobin adduct determinations; and MT: performed the acrylamide-hemoglobin data analysis. The authors declared no conflicts of interest.

REFERENCES
Erratum


The units used throughout the article for sulfur amino acid concentrations are incorrect. Instead of “mmol/L,” the units should be “μmol/L.”


Erratum


On page 704S, the third sentence in the second paragraph of the Results and Discussion section included values in parentheses that were erroneously transposed. The sentence should read as follows:

We also noted with interest that, in the Nurses’ Health Study, although multivitamin users in the highest plasma vitamin B-12 quartile category performed somewhat better on cognitive function tests than did subjects who did not use supplements and were in the lowest quartile category for plasma vitamin B-12 (mean difference in global cognitive score: 0.19; 95% CI: –0.01, 0.40), multivitamin users who were, nevertheless, in the lowest vitamin B-12 quartile category performed worse than the comparison subjects (mean difference in global cognitive score: –0.18; 95% CI: –0.43, 0.07) (21).

In addition, on page 706S, reference 21 should read as follows: Kang JH, Irizarry MC, Grodstein F. Prospective study of plasma folate, vitamin B-12, and cognitive function and decline. Epidemiology 2006;17:650–7.


Erratum


In the Design section of the Abstract on page 773, the units listed for acrylamide are incorrect. The sentence should read as follows: Design: Fourteen healthy volunteers (mean age: 35 y; 8 women and 6 smokers of >20 cigarettes/d) were given 160 g of potato chips containing 157 μg acrylamide daily for 4 wk.