In Vivo Doses of Acrylamide and Glycidamide in Humans after Intake of Acrylamide-Rich Food

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For assessment of cancer risk from acrylamide (AA) exposure through food, the relation between intake from food in humans and the in vivo doses (area under the concentration-time curve, AUC) of AA (AUC-AA) and of its genotoxic metabolite glycidamide (GA) (AUC-GA) is used as a basis for extrapolation between exposure levels and between species. In this study, AA-rich foods were given to nonsmokers: a high intake of 11 µg AA/kg body weight (bw) and day for 4 days or an extra (medium) intake of 2.5 µg AA/kg bw and day for a month. Hemoglobin (Hb)-adduct levels from AA and GA, measured in blood samples donated before and after exposures, were used for calculation of AUC-AA and AUC-GA using reaction rate constants for the adduct formation measured in vitro. Both AA- and GA-adduct levels increased about twofold after the periods with enhanced intake. AUC for the high and medium groups, respectively, were for AA 212 and 120 and for GA 49 and 21. The AA intake in the high group was better controlled and used for comparisons with other data. The AUCs per exposure dose obtained in the present human study (high group) are in agreement with those previously obtained at 10^7 times higher exposure levels in humans. Furthermore, the values of AUC-AA and AUC-GA are five and two times higher, respectively, than the corresponding values for F344 rats exposed to AA at levels as in published cancer bioassays.

Key Words: AUC; interspecies extrapolation; acrylamide; glycidamide; human exposure.

The finding that acrylamide (AA) is formed in foods during cooking/processing at high temperatures (Tareke et al., 2000, 2002) led to concerns about cancer risks associated with the dietary intake of AA (Dybing et al., 2005; WHO, 2002). AA is present in many common foodstuffs (IRMM; Rosén and Hällénäs, 2002), and the mean exposure levels have been estimated to between 0.5 and 1.0 µg/kg body weight (bw) and day (reviewed in Dybing et al., 2005; JECFA, 2010).

AA is carcinogenic in cancer tests with rodents and is classified as “probably carcinogenic to humans” (IARC, 1994). The epidemiological studies concerning cancer risk associated with AA in food have with a few exceptions shown no associations between AA intake and cancer (Hogervorst et al., 2010). However, it has been argued that the possibility to detect an increased cancer risk from dietary intake of AA by epidemiology is very small as there is exposure to everyone, assumed to contribute to small increments in the individual risks, without large variations in the population (Hagmar and Törnvist, 2003; Törnvist et al., 2008). In addition, it is difficult to estimate the individual intakes of AA because AA is present in many foods with large variations in the concentration, even in foods of the same type (Wirfalt et al., 2008).

The estimates of AA intakes in epidemiological studies are generally based on information from food frequency questionnaires (FFQs) and data on AA contents in food products. An alternative to estimate exposure is to use biomarkers like hemoglobin (Hb) adducts, which reflect exposure to the adduct-forming reactive compound during the last 4 months. Hb-adduct levels could be used to infer the “area under the concentration-time curve” (AUC) in blood and thus also reflect absorption and metabolic rates (Ehrenberg et al., 1983; Törnvist et al., 2002). In efforts to characterize the exposure to AA from food, individual Hb-adduct levels of AA have been compared with intake estimates from FFQs (Kütting et al., 2008; Wilson et al., 2009a,b; Wirfalt et al., 2008). These studies have shown uncertainties associated with the use of FFQs for AA-intake assessments (Hagmar et al., 2005; Kütting et al., 2008).
In the absence of strong epidemiological data, which could prove or disprove a cancer risk from AA at low doses, risk estimation has to be based on toxicological data. AA is metabolized to the epoxide glycidamide (GA) in rats, mice, and humans (Bergmark et al., 1993; Calleman et al., 1990; Settels et al., 2008; Sumner et al., 1992). DNA adducts from GA, but not from AA, are formed in rodents exposed to AA (Gambo da Costa et al., 2003; Segerbäck et al., 1995). GA is genotoxic in several test systems (reviewed by Rice, 2005) and is shown to be the predominant genotoxic agent in AA exposure (Ghanayem et al., 2005a,b,c; Mei et al., 2010; Paulsson et al., 2003b). It is widely assumed that GA is the major cancer-initiating principle in the carcinogenicity tests with AA (Rice, 2005), and therefore, the response in the bioassays should be related to the in vivo dose (AUC) of GA (AUC-GA).

One prerequisite to improve the interspecies extrapolation in the cancer risk estimation of AA from carcinogenicity tests (Friedman et al., 1995; Johnson et al., 1986) is to have data on the AUC of AA (AUC-AA) and AUC-GA in rats exposed as in the cancer bioassays and the corresponding AUCs per exposure dose of AA in humans exposed to AA through food. The values for the AUCs in AA-exposed rats have been obtained in an earlier study (Törnqvist et al., 2008).

The aim of the present study is to determine the relationship in humans between intake of AA via food and the corresponding AUC-AA and AUC-GA. This is achieved through measurements of Hb-adduct levels from AA and GA in humans with controlled dietary intake of AA and measurement of rate constants for the formation of the Hb adducts (in vitro). The obtained data are then used for the calculation of the AUC of AA and GA. We show that the AUC-AA in humans with intake of AA via food is considerably higher and that the AUC-GA is somewhat higher than the corresponding AUCs in rats exposed to AA at levels as in the carcinogenicity tests.

**MATERIALS AND METHODS**

**Overall Study Design**

In this report, levels of adducts from AA and GA to N-terminal valine in Hb were measured in blood collected before and after periods of increased AA intake in human volunteers. There were two studies, one with low (control) and high (760 μg/day) intake for 4 days (Abramssohn-Zetterberg et al., 2008) and one with medium (160 μg/day) intake for 28 days. The AUC-AA and AUC-GA in the “exposed” subjects were calculated from the adduct-level increments, obtained from measured Hb adducts, and the second-order rate constants (k_{val}) for adduct formation (see below). Reaction rate constants for AA (k_{val,AA}) and GA (k_{val,GA}) to the N-terminal valine in Hb were determined through incubation of human blood with different concentrations of AA and GA and quantification of the formed adduct levels. (Associations between biological effects and the AA exposure in these studies have been presented earlier, Abramssohn-Zetterberg et al., 2008; Naruszewicz et al., 2009.)

**Design of the AA-Intake Studies**

The design of study 1 has previously been described by Abramssohn-Zetterberg et al. (2008). Nineteen nonsmokers (56–85 kg bw; mean age 46 years, range 24–60 years) were included in the present study. The participants were randomly divided into two groups, given AA-poor or AA-rich food, respectively, and referred to as low and high-intake groups. The low group included 10 (four men and six women) and the high group nine persons (three men and six women). The meals consumed in the low group were prepared at max 100°C (boiled). The food consumed in the high group was heated at higher temperatures. Common food items for the low group were boiled potato and soft white bread, whereas common food products for the high group were French fries and crisp bread. The food intakes were monitored by the participants’ use of food diary information related to the AA concentration in the foodstuffs. Total AA intakes during the 4 days were 20 (range 10–35) μg and 3050 (range 2500–3500) μg for the low and high groups, respectively. The low group was used as a control. Participants donated blood at the start (after breakfast morning day 1) and at the end (after breakfast morning day 5) of the exposure period of 4 days. The study was approved by the Ethics Committee, Faculty of Medicine, Uppsala University (Dnr Ups 02-094).

The design of study 2 has previously been described by Naruszewicz et al. (2009). Nine nonsmoking participants (four men and five women; 55–77 kg bw; mean age 34 years, range 22–56) were included in the present study. Participants maintained their baseline diets during the study period. For 2 initial weeks, the participants ingested 400 g of boiled potatoes per day. After this period, the intake of boiled potatoes was changed to an intake of potato chips. The participants were administered potato chips from the same manufacturing batch, and two bags for each subject were analyzed for AA. The mean AA level in the potato chips was 980 μg AA/kg with a low variation and in the boiled potato below the detection level of the method (cf., Mojska et al., 2010). The intake of potato chips of 160 g/day during 28 days thus corresponded to an intake of 160 μg AA/day or totally 4.5 mg (here called medium intake) over the 28 days. After the exposure period, the potato chips were substituted by boiled potato during another 28 days (withdrawal phase). In this study, the only measured AA intake was that via the potato chips. Participants maintained their baseline intake levels of AA-containing food products, as for instance coffee and crisp bread, through the whole study period of 70 days. Baseline diets were considered to be relatively stable over the 28-day exposure period and the 28-day withdrawal phase. Participants donated blood just at the start and at the end of the exposure period of 28 days as well as after the withdrawal phase, day 56. The study was approved by the Ethics Committee, Stockholm (Dnr 35/05) and the Institutional Ethics Board of the National Institute of Food and Nutrition, Warsaw (ul. Powsińska 61/63).

**Chemicals and Materials**

**Caution.** AA, GA, and pentafluorophenyl isothiocyanate are reactive compounds and should be handled with care.

GA was purchased from Larodan Fine Chemicals AB (Göteborg, Sweden), AA from KEBO Lab (Spånga, Sweden), and pentafluorophenyl isothiocyanate from Sigma-Aldrich (Stockholm, Sweden). All used chemicals were of highest grade commercially available. Standard compounds used for identification and quantification of Hb adducts were gifts from Dr E. Bergmark (Bergmark, 1997). Samples of whole blood from human donors used for the in vitro studies in the determination of rate constants were purchased at Karolinska Hospital (Stockholm, Sweden).

**Analysis of AA in Foods**

**Study 1.** Analysis of AA was performed by liquid chromatography tandem mass spectrometry (MS/MS) using electrospray ionization as described in Abramssohn-Zetterberg et al. (2008). Samples were homogenized and extracted with water and deuterium-labeled AA was added as an internal standard. Two types of solid phase extraction columns were used, which enabled reliable quantification of AA in solid matrices down to 5 μg/kg (Rosén et al., 2007).
The method precision was better than prescribed by international standards when evaluated in a multilaboratory collaborative trial validation study.

**Study 2.** Analysis of AA in potato chips was performed using bromination of AA. Samples were homogenized, extracted with water, and spiked with a deuterated AA as internal standard before further processing and bromination according to earlier description (Mojksa et al., 2010). Samples were analyzed with gas chromatography (GC/MS/MS) in positive ion chemical ionization mode. The method for AA determination in food was validated. Analytical quality control was implemented by the use of certified reference material, and the analysis with the method was shown to fall into the range of the certified value (Mojksa et al., 2010).

**Analysis of Hb Adducts**

The blood samples were prepared for GC-MS/MS analysis of Hb adducts from AA and GA according to the N-alkyl Edman method (Bergmark, 1997; Paulsson et al., 2003a; Törnqvist et al., 1986). Erythrocytes were isolated by centrifugation of the blood samples, further washed with 0.9% NaCl solution, and stored at −20°C until analysis. Globin was isolated from the samples of erythrocytes through precipitation with ethyl acetate from an acidified isopropanol solution (Mowerer et al., 1986). For the isolation of the adducts to N-terminal valine, the globin samples (50 mg) were dissolved in formamide and incubated with the reagent pentafluorophenyl isothiocyanate. The Hb adducts were then detached to form pentafluorophenylthiohydantoins (PFPTHs) of N-(2-carbamoyl-ethyl)valine (AA-Val) and N-(2-carbamoyl-2-hydroxyethyl)valine (GA-Val). After extraction, the samples were acetonized to prepare according to the method above. As globins with known adduct levels of both AA-Val and GA-Val were added to 40 mg of erythrocytes through precipitation with ethyl acetate from an acidified isopropanol solution (Mowrer et al., 1986). For the isolation of the adducts to N-terminal valine, the globin samples (50 mg) were dissolved in formamide and stored at 20°C for 2 days. The method precision was better than prescribed by international standards (Mojska et al., 2010). Samples were analyzed according to earlier description (Mojska et al., 1992, 2010). Samples were analyzed using the second-order rate constant for the adduct formation, for which the AUC (area under the curve) could be inferred from a measured Hb adduct level, that is, the accumulation of AA or GA adducts (1). The daily AUC could be calculated at exposure during longer times using the daily adduct-level increment (a) (for A in Equation 2).

Dose calculation from adduct level:

$$D = \frac{1}{k_{val}} \times A. \quad (2)$$

Adduct level at long-term exposure (when t < t_m):

$$A_{acc} = a \times t \left(1 - \frac{t}{t_m}\right). \quad (3)$$

In the calculation of the daily adduct increments in this study, the accumulation and elimination of adducts during the different exposure periods were considered in detail as described by Granath et al. (1992), the approach in study 2 was earlier applied by Granath et al. (1994, 1996) (calculations are approximated with Equation 3). Thus, in study 1 (4 days), the total adduct-level increment during the exposure period was divided with 3.93 days and in study 2 (28 days) with 24.7 days to obtain the daily adduct increments.

**Measurement of Second-Order Rate Constants for the Reaction with N-terminal Valine in Hb**

Second-order reaction rate constants for the formation of Hb adducts could be calculated according to Equation 2 from measured adduct level (A) in Equation 2) in Hb after treatment of blood in vitro with known concentrations of the reactive compound during short incubation times (defined concentration over time, AUC; D in Equation 2). In this study, incubations were done to measure rate constants for reactions of AA and GA with the N-terminal valine in Hb (k_{val,AA} and k_{val,GA}, respectively). Aqueous solutions (30 μl) of AA and GA were added to samples of whole blood (final volume 3 ml and the blood temperature was 37°C). Blood samples of five concentrations of AA (5–80 μM) and GA (2.5–20 μM) and blanks, all in duplicates, were incubated at 37°C for 1 h. Reactions were terminated by addition of two volumes of ice cooled saline solution, quickly followed by centrifugation and separation of the plasma. The washing of erythrocytes was repeated twice. The incubations with AA and GA were carried out with blood from two subjects. Globin was precipitated and Hb-adduct levels were measured according to the N-alkyl Edman method (see above). The concentrations of the compounds were considered constant during the short time (1 h) of incubation (as shown by Paulsson et al., 2005).

**Statistical Analysis**

Statistical analyses of the data on adduct-level changes over exposure time and of differences of AUCs were performed by a Student’s t-test (paired or unpaired). The results were considered to be statistically significant when p < 0.05.

**RESULTS**

**Rate Constants for Reaction of AA and GA with N-terminal Valine**

The in vitro studies with blood from two donors showed linear relationships between Hb-adduct levels (picomoles per gram globin) and doses (AUC in micromolar × hour) of AA and GA (r^2 ≥ 0.93) (Figs. 1 and 2). The following mean values for the second-order reaction rate constants were obtained (picomoles per gram per micromolar hour = 10^-6 liters per gram and hour):

$$k_{val,AA} = 6.4 \text{ pmol/g per } \mu \text{Mh},$$

$$k_{val,GA} = 21 \text{ pmol/g per } \mu \text{Mh}.$$
Changes of Hb-Adduct Levels in Relation to AA Intake

In both the high group in study 1 and medium group in study 2, AA- and GA-adduct levels increased significantly, about twofold, over the respective exposure periods (Figs. 3 and 5). The variations between individuals in the adduct-level increments were small (Table 1), considering possible sources of variation in exposure, uptake, and metabolism. The low (control) group in study 1 showed no change of the mean AA-adduct level after the test period (Fig. 4). A significant, but low, increase of GA-adduct levels was observed in the low group (Table 1, Fig. 4). The analysis of adduct levels after the 28-day withdrawal phase in study 2, used as a control in this study, showed a significant decrease (Fig. 5).

Calculated In Vivo Doses (AUC) from Hb-Adduct Levels

From measured adduct levels, the daily adduct-level increments were calculated (considering time periods of exposure, cf. "Materials and Methods" section) and then adjusted for intake per kg bw (Table 2). The AA intake in the high group was 2500–3500 µg during the 4 days. In study 2, the extra intake of AA over the baseline diet during 28 days was 160 µg/day. After correction for the individual bw, an average intake of 11 µg AA/kg bw and day (high intake) was obtained in study 1 and an average additional intake of 2.5 µg AA/kg bw and day (medium intake) over the baseline diet was obtained in study 2 (Table 2). The obtained mean daily adduct increments (calculated on individual values) were 1.4 and 0.77 pmol AA-Val/g per µg AA/kg bw for the group with high and medium intake, respectively (Table 2). The corresponding means of the individual GA-adduct–level increments were determined to 1.0 and 0.44 pmol GA-Val/g per µg AA/kg bw (Table 2).

In the next step, the in vivo doses (AUC, in nanomolar hours) per exposure dose (micrograms per kg bw) were calculated using the second-order rate constants, \( k_{\text{Val-AA}} \) and \( k_{\text{Val-GA}} \), obtained from the in vitro studies. The AUC-AA and AUC-GA per exposure dose of AA are given in Table 2. The means of the individual AUC values were significantly higher in the high group than in the medium group: 1.8 times for AUC-AA \( (p = 0.05) \) and 2.3 times for AUC-GA \( (p = 0.001) \) as calculated from the adduct-level increments. Differences in the GA metabolism were evaluated by considering the GA to AA ratio of the AUCs. The average individual GA to AA ratios of

![FIG. 1. Determination of in vitro rate constant (\( k_{\text{Val-AA}} \)) for the reaction between AA and N-terminal valine in Hb, showing measured AA-adduct levels in Hb versus the dose of AA (micromolar hours) in blood. The experiment was carried out with blood from two subjects (1 and 2).](http://toxsci.oxfordjournals.org/)

![FIG. 2. Determination of in vitro rate constant (\( k_{\text{Val-GA}} \)) for the reaction between GA and N-terminal valine in Hb, showing measured GA-adduct levels in Hb versus the dose of GA (micromolar hours) in blood. The experiment was carried out with blood from two subjects (1 and 2).](http://toxsci.oxfordjournals.org/)

![FIG. 4. Measured Hb-adduct levels in study 1, low group (control). AA (left) and GA (right)-adduct levels measured in 10 persons. From left to right in the respective groups: adduct levels measured before (day 1) and after (day 5) intake of a diet of very low AA content (intake of approximately 5 µg/day). Mean values are shown by horizontal bars.](http://toxsci.oxfordjournals.org/)

![FIG. 3. Measured Hb-adduct levels in study 1, the high exposure group. AA (left) and GA (right)-adduct levels measured in nine persons. From left to right in the respective groups: adduct levels measured before (day 1) and after 4 days (morning day 5) intake of a diet with high AA content (intake of approximately 760 µg/day). Mean values are shown by horizontal bars.](http://toxsci.oxfordjournals.org/)
the incremental AUC were about the same in the two exposed
groups, 0.23 and 0.20, respectively; with a higher variation in
the medium than in the high group (RSD 48 and 23%,
respectively).

**DISCUSSION**

The primary aim of the present study was to determine
AUC-AA and AUC-GA per exposure dose of AA in humans
after exposure via food and to compare these values with the
earlier obtained AUCs in rats exposed to AA as in the
carcinogenicity tests. The AUC reflects the net effect of all
enzymatic, chemical, and physiological factors on uptake, rates
of formation, and elimination of the measured chemical and is
the most accurate quantitative data to use in adjustment of
species differences in these respects.

**Changes of Hb-Adduct Levels in Relation to AA Intake**

Altogether, the substantial increase in the Hb-adduct levels
after intake of food with high/medium levels of AA in both
study groups is considered as a proof that the increased adduct
levels reflect the increased AA intake. It is notable that just
a few days with high intake of AA-rich food could give such
high increases (> 50 pmol/g) of the adduct levels as observed
in study 1. The low increase of GA-adduct levels after intake of the
control diet (study 1) could not be explained at present and
should be further studied. However, this has no impact on the
result from the group with high intake, as the increase is
studied at an individual level, before and after exposure at
approximately 100 times higher daily intakes than in the
control group. As expected, the adduct levels measured after
the withdrawal phase of 28 days in study 2 (day 56) somewhat
decreased; the background levels would be reached after the
erthrocyte turnover.

**Comparison with Published Data on Reaction Rate Constants**

For the calculation of AUC from the Hb-adduct level, the
second-order reaction rate constant for adduct formation has to
be carefully determined. Values for reaction rate constants of
AA or GA to the N-terminal valine in Hb have earlier been
measured (cf., Table 3). Differences in the obtained values
(Table 3) are judged primarily to be because of differences in
calibration and in experimental conditions. In the present study,
as well as in studies B and C in Table 3, we have used low
concentrations (micromolar) of AA and GA for incubations
of whole blood, which would be relevant for the calculation

### TABLE 1

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Exposure dose of AA (µg)</th>
<th>AA-adduct level (pmol/g globin) Before</th>
<th>After</th>
<th>p Value</th>
<th>GA-adduct level (pmol/g globin) Before</th>
<th>After</th>
<th>p Value</th>
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<tbody>
<tr>
<td>Study 1: 4-day test period</td>
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<tr>
<td>&quot;Low&quot; control group</td>
<td>10</td>
<td>20 ± 8.0</td>
<td>59 ± 19</td>
<td>58 ± 19</td>
<td>0.5</td>
<td>72 ± 19</td>
<td>80 ± 18</td>
<td>0.01</td>
</tr>
<tr>
<td>&quot;High&quot; exposed group</td>
<td>9</td>
<td>3050 ± 330</td>
<td>57 ± 10</td>
<td>114 ± 15</td>
<td>&lt; 0.0001</td>
<td>64 ± 27</td>
<td>106 ± 25</td>
<td>0.0001</td>
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<tr>
<td>Study 2: 28-day test period</td>
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<tr>
<td>&quot;Medium&quot; exposed group</td>
<td>9</td>
<td>4400 ± 0</td>
<td>36 ± 15</td>
<td>81 ± 30</td>
<td>0.001</td>
<td>35 ± 20</td>
<td>62 ± 23</td>
<td>0.001</td>
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<tr>
<td>Study 2: withdrawal phase,</td>
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<tr>
<td>adducts measured at day 56</td>
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<td></td>
</tr>
<tr>
<td>&quot;Medium&quot; exposed group</td>
<td>8</td>
<td>4400 ± 0</td>
<td>81 ± 30</td>
<td>63 ± 25</td>
<td>0.001</td>
<td>62 ± 23</td>
<td>51 ± 22</td>
<td>0.005</td>
</tr>
</tbody>
</table>

*Note.* In study 1, the test period was 4 days, and in study 2, the test period was 28 days. In study 2, HB adducts were measured also after the withdrawal phase (only exposure from baseline diet) at day 56. Significance of increases or decreases of adduct levels over test periods (studies 1 and 2) and withdrawal phase (study 2) was calculated with the student’s paired *t*-test.

*Significant increase.*

*Significant decrease.*

![FIG. 5. Measured Hb-adduct levels in study 2, medium exposure group.](image-url)
of AUC in blood from Hb-adduct levels. Furthermore, studies A–D have used the same analytical setup. The measured values for the reaction rate constant of AA (\( k_{\text{Val-AA}} \)) show relatively low variations between studies and between human Hb and rat Hb (Table 3). A higher reactivity of GA, \( k_{\text{Val-GA}} \), compared with AA is found in all studies; however, there are large variations between the values. In our comparison in the present study, the reactivity of GA was found to be approximately three times higher than the reactivity of AA and the reactivity of GA toward human Hb was 50% higher than toward rat Hb (A and B in Table 3).

**Comparison with Published Data on Adduct Levels and AUC**

Considering the relatively low increase of the exposure doses compared with background exposure in the medium (study 2) and in the high (study 1) groups, the AUCs per exposure dose showed surprisingly little variation within groups. The results from the two studies were supportive regardless of the different designs of the studies. However, in study 1, it was a short exposure period and AA was analyzed in the total diet, and thus, the intake assessment in study 1 was better implemented than in study 2. Therefore, we use the results from study 1 in the following comparison with other studies.

In Table 4, AUC-AA and AUC-GA per exposure dose of AA obtained in the present study (A in the table) are compared with those of other studies. In study B (Törnqvist et al., 2005), F344 rats were exposed to AA in a short-term study through drinking water at three doses (0.1–2 mg/kg bw and day; 1 week), i.e., exposure levels as used in the published cancer bioassays (Friedman et al., 1995; Johnson et al., 1986). There was a linear dose-response for the AA- and GA-adduct-level increments (Törnqvist et al., 2008). In the present human study with high exposure, the figures for AUC-AA are 4.4 and 6.2 times higher than the average for the female and male rats, respectively, and the corresponding values in humans for AUC-GA are 1.4 and 2.7 times higher, respectively (A and B in Table 4). A difference between the genders was not observed in the present human study. The comparison between studies A and B in Table 4 is judged to have high accuracy as the values for the rat were determined with low SD (achieved, for instance, through repeated daily dosing for a week) and with the same analytical setup.

A comparison of our present study with a high-dose study in humans (AA administered via drinking water) (Fennell et al., 2005) is of interest for the evaluation of possible effect of exposure level on the metabolic rates (A and C in Table 4). In the study by Fennell et al. (2005), males were administered AA at about the same high AA levels (0.5–3 mg/kg bw; single oral doses via drinking water) as used in the cancer bioassays with rats (cf., Friedman et al., 1995; Johnson et al., 1986). No significant deviations from linear relationships between AA- or GA-adduct levels and exposure doses were observed in the humans exposed at these high exposure levels. The values of AUC per exposure dose in the present study are somewhat lower (0.8–0.9 times) than in the study by Fennell et al. (2005) (A and C in Table 4). The AUC-GA/AUC-AA ratio is approximately the same. Altogether, the comparison of the two human studies shows that there is no large influence on metabolic rates at \( 10^2 \)–\( 10^3 \) times higher exposure levels of AA compared with the exposure levels from intake from food.

**Table 2**

<table>
<thead>
<tr>
<th>Group</th>
<th>Exposure (( \mu g ) AA/kg bw and day)</th>
<th>Daily adduct increment per exposure dose of AA (pmol/g globin per ( \mu g ) AA/kg bw and day)</th>
<th>In vivo dose (AUC) per exposure dose of AA (nMh per ( \mu g ) AA/kg bw)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( N )</td>
<td>AA</td>
<td>GA</td>
</tr>
<tr>
<td>Study 1, “high” group</td>
<td>9</td>
<td>11 ± 1.6</td>
<td>1.4 ± 0.36</td>
</tr>
<tr>
<td>Study 2, “medium” group</td>
<td>9</td>
<td>2.5 ± 0.3</td>
<td>0.77 ± 0.26</td>
</tr>
</tbody>
</table>

Note. Figures are for the “exposed” groups in study 1 and study 2, high and medium, respectively. Mean AUCs are calculated from individual adduct-level measurements.

**Table 3**

<table>
<thead>
<tr>
<th>Reference</th>
<th>( k_{\text{Val-AA}} ) (pmol/g/( \mu M )h)</th>
<th>( k_{\text{Val-GA}} ) (pmol/g/( \mu M )h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Present study</td>
<td>6.4</td>
<td>nd</td>
</tr>
<tr>
<td>B. Törnqvist et al. (2008)</td>
<td>nd</td>
<td>4.6</td>
</tr>
<tr>
<td>C. Paulsson et al. (2005)</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>D. Bergmark (1997)</td>
<td>4.4</td>
<td>nd</td>
</tr>
<tr>
<td>E. Fennell et al. (2005)</td>
<td>4.3</td>
<td>3.8</td>
</tr>
<tr>
<td>F. Tareke et al. (2006)</td>
<td>7.4</td>
<td>2.9</td>
</tr>
</tbody>
</table>

Note. nd, not determined.
In addition, there is one human study (200 \text{lg AA/kg, oral intake}) that measured AUC through serum concentrations of AA (Kopp and Dekant, 2009), which arrives at a 70 times lower estimate of AUC-AA per intake dose than in the present study. Considering our data and the other data on AUC-AA in the rat and in human cited above (Table 4), we consider the low estimate by Kopp and Dekant (2009) of AUC-AA as an outlier.

There are a few other animal studies with oral exposure to AA at several levels at low doses (<100 \text{lg/kg bw and day}). Two studies have been done with repeated daily oral dosing with the lowest doses in the range of human exposure to AA in food. A linear dose-response between AA exposure and AA-adduct levels, as well as GA-adduct levels, was shown in a study with mice where AA was administered via different feed matrix at five exposure levels (3–50 \text{lg/kg bw and day; 40 days}) (Vikström et al., 2008). A study with swine, where AA was administered via drinking water or via feed at two levels (0.8 and 8 \text{lg/kg bw and day; approximately 3 months}), showed proportional adduct increments of the AA adduct at the two doses, with no significant difference depending on the route of administration (Aureli et al., 2007).

With the assumption that bioavailability and volume of distribution are about the same in rats and humans in the studies compared above, the species difference in the AUC-AA per exposure dose also reflects the species difference in the overall half-lives, as the AUC-AA in blood reflects the absorption and detoxification. The overall half-life in humans of AA is thus indicated to be about five times longer than in the rat (studies A and B in Table 4). The corresponding interspecies comparison with regard to the ratio of AUC-GA to AUC-AA reflects possible differences both in the formation and detoxification of GA. This ratio is two to three times higher for the rat compared with that for human.

### SUMMARY

The purpose of this study was to measure AUC-GA in humans after AA intake from food and compare these with the earlier obtained values for the AUCs in rats administered AA as in the carcinogenicity tests. AUC-AA and AUC-GA were determined for two groups of human volunteers with daily intakes of AA of 11 \text{lg/kg bw (high intake, study 1) or 2.5 \text{lg/kg bw (medium intake, study 2) (Table 2).}}

About half of the AUCs per intake for both AA and GA were observed in the group with medium intake compared with the group with high intake. We use the data from the measurements for the high-intake group in study 1 as a basis for the conclusions as there is a control of the total AA exposure in this study (cf., “Results” section).

In the high-intake group, the adduct-level increments in \text{Hb} per exposure dose in \text{micrograms of AA per kilogram bw} are 1.4 \text{pmol AA-Val/g} and 1.0 \text{pmol GA-Val/g}. From these data and from determined reaction rate constants for adduct formation, we calculated the corresponding AUCs of AA and GA in humans from exposure to dietary AA. The AUC-AA and AUC-GA were determined to be approximately 200 and 50 nMh per \text{lg AA/kg bw}, respectively. This means that per exposure dose of AA, the AUC-AA is about five times and the AUC-GA is nearly twofold higher in humans with intake from food compared with the average for the female and male F344 rats exposed as in the cancer bioassays. At the present state of knowledge, this is the best data for the estimation of AUC of AA and GA from exposure of AA via food in the general population, which could be used as a basis for interspecies extrapolation in cancer risk estimation.

### FUNDING


### ACKNOWLEDGMENT

Hans von Stedingk is acknowledged for technical assistance.

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### TABLE 4

Comparison of values on AUC-AA and AUC-GA, obtained from studies with AA exposure to humans and rats. Study A is the high intake group in the present investigation.

<table>
<thead>
<tr>
<th>Exposure</th>
<th>AA (nMh/\text{lg AA per kg bw})</th>
<th>GA (nMh/\text{lg AA per kg bw})</th>
<th>AUC-GA/AUC-AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Human: Repeated daily dosing during 4 days through food, the present study</td>
<td>212</td>
<td>49</td>
<td>0.23</td>
</tr>
<tr>
<td>B. Rat: Repeated daily dosing during one week through drinking water, Törnqvist et al. (2008)</td>
<td>F\textsuperscript{a}: 48; M\textsuperscript{b}: 34</td>
<td>F: 34; M: 18</td>
<td>F: 0.70; M: 0.53</td>
</tr>
<tr>
<td>C. Human: Single dose through drinking water, Fennell et al. (2005)</td>
<td>246\textsuperscript{c}</td>
<td>60\textsuperscript{c}</td>
<td>0.25</td>
</tr>
</tbody>
</table>

\textsuperscript{a}F, female.
\textsuperscript{b}M, male.
\textsuperscript{c}recalculated from figures in the cited paper.
REFERENCES


