Effect of different linoleic acid intakes on prostaglandin biosynthesis and kidney function in man\textsuperscript{1,3}

Olaf Adam, MD and Günther Wolfram, MD

ABSTRACT  Prostaglandin (PG) biosynthesis and kidney function was investigated in 24 adults (23 to 32 yr) during isocaloric formula diet periods, for 2 wk each, providing a linoleic acid supply of 0, 3, 3.5, 4, 6, 8, 13, 17, 18, or 20% of total energy intake. Total protein intake (15 energy %) was constant, as well as 5 g NaCl, 3 g KCl, and 0.6 g cholesterol per 2200 kcal formula diet. The amount of PG metabolites, PG-E, sodium, and creatinine in 24-h urine increased with augmented linoleic acid intake. Comparing a linoleic acid intake of 0 and 20 energy %, an increase of sodium (8%) and creatinine (16%) in 24-h urine was found on the 5th day of high linoleic acid supply. Coincidently a stimulated PG biosynthesis could be measured. Potassium, water, and PG-F excretion showed no relation to linoleic acid intake. It is concluded that linoleic acid in the diet stimulates PG-E biosynthesis in man, leading to effects in systems which control renal function, and may have clinical relevance for the sodium and potassium balance in man.  \textit{Am J Clin Nutr} 1984;40:763–770.

KEY WORDS  Prostaglandin biosynthesis in man, linoleic acid intake, formula diet, renal function

Introduction

Prostaglandins (PG) are biological active lipids, which are considered to act on renal tubular electrolyte transport and to cooperate with renal and extrarenal hormones in the control of sodium, potassium and water excretion (1–6). Results in man mostly refer to changes observed with inhibitors of PG synthesis that are known to exert effects on platelet aggregation and on metabolic processes, eg, the release of antidiuretic hormone (ADH) (7) or of phospholipase \textit{A}\textsubscript{2} (8). Experiments in which PG synthesis has been stimulated by administration of precursor substances were conducted only for hours instead of weeks.

We investigated the effect of linoleic acid intake, a known stimulus of PG formation (9, 10), on renal function in healthy subjects, receiving different amounts of linoleic acid with formula diets (FD) for 2 wk each. To evaluate the effect of dietary linoleic acid on PG biosynthesis a group of healthy females was included, making it possible to rule out the unpredictable amount of PG in urine derived from the reproductive system in males.

Methods

\textit{Experimental subjects}

Twenty-four students between 23 and 32 yr of age were selected on the basis of the following criteria: their body weight was within 10% of the ideal weight of a standard weight table (11), they were free of known metabolic abnormalities, and routine laboratory findings and clinical examination were unremarkable. They continued their normal life and normal physical activities throughout the experiment, but came to the metabolic ward every morning, where patient reports (body weight, determination of energy and water intake, frequency and consistency of stools etc.) were recorded.

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FD

The composition of FD, which were prepared in our metabolic kitchen, are shown in Table 1. Fat intake was 0.25, 0.30, 0.33, or 0.36 of total energy intake. With a fat intake of 25% (FD25), 33% (FD33), and 36% (FD36) three variations of the fat were given (polyunsaturated (P)/saturated (S) fatty acids—ratios 0.4, 0.4, and 1.8). The FD with the same P/S ratio 0.4 had different amounts of monounsaturated fatty acids. In the experiment with females (FD30), one FD was without fat and the other two FD provided a fat supply of 30 energy %. With these FD the linoleic acid intake was 0.3%, 3%, 3.5%, 4%, 6%, 7%, 8%, 13%, 17%, 18%, and 20% of total energy intake. Protein intake (15 energy %) was constant with all FD and oligopolymers of glucose supplied the rest of the energy intake. Water was given ad libitum, 3 g KCl, 5 g NaCl, and 0.6 g cholesterol were substituted per 2200 kcal FD, Protovita (Roche, Grenzach, FRG), one capsule per day, served as a source of vitamins and 100 mg of Fe-II-chloride was given every second day.

Experimental design

Experimental subjects recorded their normal diet for a period of 8 days. They were then put on isocaloric FD, two persons starting with one of the three fat compositions. Each of the three fat compositions (or the FD without fat) was given for 2 wk to these groups in a different order. So every person was on FD for 6 consecutive wk. Sodium and potassium intake, energy uptake, body weight and 24-h urine volume were recorded during a period 3 days before and after, and throughout the experiments. Fluid intake was calculated from metabolic water in FD (1 g carbohydrates 0.6 ml, 1 g protein 0.4 ml, 1 g fat 1.1 ml) and from the intake of free water. Samples were taken from every 24-h urine and were deep frozen at −20°C until the analyses of sodium, potassium, creatinine, and uric acid. In the experiment FD30, in addition, PG-E, PG-F and tetranorprostanedioic acid (TNPDA) were determined.

Informed consent, approved by the institutional research committee responsible for monitoring human experimentation, was obtained from each subject.

Measurements

Determinations of uric acid and creatinine were done on a Technicon Autoanalyzer II system (Technicon Instruments Corp, Tarrytown, NY) by routine methods (Boehringer, Mannheim, Federal Republic of Germany, Test-set no 124 761 and no 441 716). Sodium and potassium were determined by flame photometry (Eppendorf FMC/AMF 5051 Hamburg, Federal Republic of Germany).

Analysis of TNPDA in urine was done by gas-liquid chromatography on two packed columns with liquid phases of different polarity as previously described (13). Native PG in urine were measured by radioimmunoassay with commercially available antibodies [Institute Pasteur, Paris, France, Code 79585 (PG-E) and Code 79570 (PG-F)]. Tritium labeled PG were purchased from Amersham (Braunschweig, Federal Republic of Germany). For calculation of recovery, T(H [5, 6, 8, 11, 12, 14, 15 (n)] PG-E (1000 cpm) or T(H [5, 6, 8, 9, 11, 12, 14, 15 (n)] PG-F was added to each ml of urine sample. Each sample was extracted with 10 ml of ethylacetate:isopropanol:0.1 N HCl (3:1:1;v/v/v). After extraction 10 ml of 0.9 % NaCl-solution and 6 ml of ethylacetate were added. After phase-separation the organic phase was dried at 40°C with nitrogen. PG fractions were separated with open silic acid chromatography in columns. Recovery of labeled PG-E (or PG-F) was 40 to 60%. All experimental values were corrected for recovery.

When starting with FD, and at the end of the 2-wk lasting periods, fasting blood samples were drawn for the determination of fatty acids in serum cholesterol esters by gas-liquid chromatography (14).

Statistical evaluation

Statistical analyses were performed using the non-parametric two-way analysis of variance (Friedman test), statistical differences were determined with the Wilcoxon signed rank test (12). The resulting two-by-two frequency table was evaluated descriptively by the χ2 test [exact Fisher test (12)].

In order to avoid influences from the previous period comparisons of creatinine, sodium, potassium, and water excretion were done with the values between day 4 and 14 of every FD period. All values given represent mean ± SD

Results

Compliance with FD was good, as assessed by the reduction of uric acid in 24-h
urine to basal levels during the purine-free FD. Mean uric acid excretion was 243 ± 13 mg/day and showed no change with the different FD. Resorption of FD was checked by the determination of linoleic acid in cholesterol esters in the serum. At the end of the 2-wk periods the interindividual differences in fatty acid composition of cholesterol esters were small and the volunteers showed the expected increase in cholesteryl-linoleate with higher linoleic acid intake.

The low amount of sodium in the FD caused an average loss of body water of 1 kg during the first days of FD. After that time only minor changes in body weight were noted.

Prostaglandin biosynthesis

PG were determined in 24-h urine of the six females, who were given FD30. PG-E and PG-F were determined every second day during the FD periods in which a linoleic acid intake of 0 or 20% of total energy was provided. We found an increase of PG-E by 47% on average (Table 2), which was observed after 4 days on the linoleic acid enriched FD, as shown in Table 3. Comcomitantly, a small, statistically not significant increase of PG-F in the 24-h urine was found.

Mean values of TNPDA during normal diet were 306 ± 63 µg/day in the healthy females. Daily determinations of TNPDA showed a decrease to 123 ± 46 µg/day within the first day on the linoleic acid-deficient diet.

<table>
<thead>
<tr>
<th>TABLE 2</th>
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<tbody>
<tr>
<td>Relationship between linoleic acid intake and amount of PGE2, PGF2α, and PG-M (PG metabolites convertible to tetranorprostanedioic acid) in 24-h urine*</td>
</tr>
<tr>
<td>Linoleic acid intake (energy %)</td>
</tr>
<tr>
<td>8</td>
</tr>
<tr>
<td>20</td>
</tr>
</tbody>
</table>

* The given values represent mean ± SD of determinations daily (PG-M) or every 2nd day (PGE2 and PGF2α) in six healthy females during the indicated periods of FD 30 and during 6 days on their normal Western diet (free diet) before the experiment.

<table>
<thead>
<tr>
<th>TABLE 3</th>
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<tbody>
<tr>
<td>The given values represent daily determinations (mean ± SD) of PG-M (PG-metabolites convertible to tetranorprostanedioic acid) and the determinations of PGE2 and PGF2α every 2nd day in the 24-h urine of females (n = 6) during the indicated periods of formula diets</td>
</tr>
<tr>
<td>Linoleic acid intake</td>
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<tr>
<td>energy %</td>
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<td>1-4</td>
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<tr>
<td>5-10</td>
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<tr>
<td>11-14</td>
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<tr>
<td>124 ± 42</td>
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<tr>
<td>0</td>
</tr>
<tr>
<td>20</td>
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</tbody>
</table>

* Significant effect of linoleic acid intake; p < 0.05 (Wilcoxon signed rank test).

FD and the values remained within the range of 64 to 218 µg/day during this FD period. Switching to a linoleic acid intake of 20 energy % caused an increase in TNPDA to 355 ± 76 µg/day. After the fourth day on the linoleic acid enriched FD, this difference was statistically significant (p < 0.05) (Table 2 and 3).

Creatinine excretion

During creatinine-free formula diets creatinine excretion is almost constant and related to the muscle mass. We assumed a similar muscle mass in our experimental subjects because of their normal body weight. Therefore, we performed a comparison of creatinine excretion during different FD with different experimental subjects.

Creatinine excreted in 24-h urine, calculated per kg body weight, was related to the amount of fat and of linoleic acid in the FD, as shown in Table 4. An exception was the fat-deficient FD, during which values for creatinine excretion were 17.4 ± 2.4 mg/kg body weight and higher than those during FD25. This may be explained by a weight loss which was small, but higher than during all other FD. Thus a loss of body protein may have contributed to the creatinine excretion during this FD. A linoleic acid supply, between 13 and 20 energy % corresponded to an elevation of creatinine in 24-
h urine, as shown in Table 4. Plasma creatinine levels tended to be lower during high linoleic acid intake, mean values of experimental subjects on FD25 are shown in Table 5. Accordingly, mean values of creatinine clearance increased in all persons during FD25, FD30, FD33, and FD36 with increasing linoleic acid intake. Changes were most pronounced with FD25. In this FD fat I and II provided a daily linoleic acid supply of 6.5 and 12.4 g/day, which was lower than the usual uptake. With fat III linoleic acid intake was 37.5 g/day and an increase of creatinine clearance was noted, when compared to the values during fat I and II of FD25. Only females participated in FD30 and linoleic acid intake was 0, 4, or 20 of total energy intake. A statistically significant (p < 0.05) stimulation of creatinine excretion was only found with the highest linoleic acid intake. Increasing the amount of fat in FD lowered the difference in creatinine excretion, observed with higher linoleic acid intake. This applies for FD36, during which no statistically significant difference was found for creatinine excretion comparing the effects of fat I and III.

**Urine volume and water balance**

With a linoleic acid intake in the range of 6 to 8 energy % a decrease in 24-h urine volume was observed, compared to the values during higher (13 to 20 energy %) or lower (0 to 4 energy %) linoleic acid intake (Fig 1). This was true for FD25, FD33, and FD36. During the ingestion of the fat deficient FD (FD30, 0 energy % linoleic acid) the excretion of water was 12.7 ± 2.6 ml/kg body weight/day. This value was only surpassed by an amount of 13.1 ± 3.0 ml/kg body weight/day during the highest (FD30, 20 energy %) linoleic acid intake.

**Sodium excretion**

Sodium excreted in 24-h urine was related to the linoleic acid supply given to experimental subjects (Table 6), but not to the amount of fat in FD. In all experiments (FD25, 30, 33, and 36) the volunteers

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**Table 4**

Creatinine excreted into 24-h urine (mg/kg body wt, mean ± SD) of healthy volunteers during days 5–14 of formula diet periods, providing the indicated linoleic acid supply.

<table>
<thead>
<tr>
<th>Linoleic acid intake</th>
<th>Fat in formula diets (energy %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25</td>
</tr>
<tr>
<td>energy %</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>16.6 ± 1.8</td>
</tr>
<tr>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>16.6 ± 1.7</td>
</tr>
<tr>
<td>6</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>17.1 ± 1.6</td>
</tr>
<tr>
<td>13</td>
<td></td>
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<tr>
<td>17</td>
<td></td>
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<tr>
<td>18</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>16.8 ± 2</td>
</tr>
</tbody>
</table>

Mean of all diets†

|                     |        |        |        |        |
| 16.8 ± 2            | 18.6 ± 2 | 19.5 ± 2 | 22.6 ± 2 |

* Significant effect of linoleic acid intake; p < 0.05 (Wilcoxon signed rank test).

† The given values represent mean ± SD of creatinine in the urine of the experimental subjects during all formula diets with the indicated amount of fat.

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**Table 5**

Creatinine concentrations in the plasma (mean ± SD) and creatinine clearances of the six experimental subjects during FD25.

<table>
<thead>
<tr>
<th>Linoleic acid intake</th>
<th>Creatinine in plasma (mg/100 ml)</th>
<th>Creatinine clearance (ml/1.73 m² body surface/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>energy %</td>
<td>mg/100 ml</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.77 ± 0.1</td>
<td>109 ± 8</td>
</tr>
<tr>
<td>6</td>
<td>0.70 ± 0.1</td>
<td>111 ± 6</td>
</tr>
<tr>
<td>13</td>
<td>0.68 ± 0.1</td>
<td>121 ± 8†</td>
</tr>
</tbody>
</table>

* Determinations were done on the 21st days of each 2-wk period.

† Significant effect of linoleic acid intake; p < 0.05 (Wilcoxon signed rank test).
showed a stimulation of natriuresis with increased linoleic acid intake.

Furthermore natriuresis occurred at the same time, when a stimulated PG biosynthesis could be measured. When providing 20 energy % linoleic acid, sodium excretion in the six females did not show significant increments during the first 3 days. However, it increased significantly (p < 0.05) during the subsequent days (Fig 2).

**Potassium excretion**

In FD25, FD33, and FD36 the excretion of potassium in our volunteers paralleled the volume of 24-h urine (Fig 1). In these experiments the highest amount of potassium (547.4 ± 67 μmol/kg body weight) in the 24-h urines was measured during low linoleic acid intake of 0 to 4 energy %. The values for potassium excretion were lowest (478 ± 52 μmol/kg body weight) during a
linoleic acid supply in the range of 6 to 8% of total energy intake, and increased to 536 ± 65 μmol/kg body weight, with a linoleic acid intake of 13 to 20 energy %.

Discussion

The most striking finding in our experiment was the dose-response relationship between dietary linoleate, total body PG and renal PG-E biosynthesis, and creatinine and sodium excretion.

The determination of TNPDA provides an indirect measure of endogenous PG synthesis (9, 13). Primary PG in the urine of females are believed to be of renal origin (15, 16). In our experiment with FD30 we found an increase in TNPDA as well as of PG-E in the urine, which occurred after 4 days of high linoleic acid intake, indicating a stimulation of renal and extrarenal PG biosynthesis. Experiments with labeled linoleic acid have shown that it takes about 3 days before orally given linoleic acid is transformed to arachidonic acid, incorporated into membrane phospholipids (17), and available for PG biosynthesis in appreciable amounts.

In animal experiments PG-E has been shown to decrease sodium transport across the collecting tubule and the thick ascending limb of Henle’s loop, and to increase renal blood flow (1-6). These effects of PG-E, stimulated in our volunteers by high linoleic acid intake, may be responsible for the observed natriuresis.

In contrast to the finding in animals (18), salt restriction from 12/g/day (with conventional diet) to 5/g/day (with FD) did not influence PG biosynthesis in our volunteers. The amount of PG-F measured in 24-h urine was not influenced by salt or linoleic acid intake. This finding confirms reports in the literature that PG-F excretion is not significantly changed with salt loading or salt depletion in man (19).

Creatinine excretion increased with a linoleic acid intake in the range of 13 to 20% of total energy intake, but was also related to the amount of fat in the diet (Table 4). Vergroesen et al (20) reported a more than twofold increase of creatinine in the urine in hypertensive subjects with a chronically high sodium intake, when linoleic acid intake was augmented by 1.7 energy %. Increasing the amount of fat caused a decrease of carbohydrates in the diets of our volunteers, and thereby an increase in plasma...
glucagon levels may be assumed. Glucagon has been shown to stimulate renal blood flow (21), thus contributing to creatinine excretion, which has been observed with higher fat intake.

When comparing the effects of a low (0 to 4 energy %), normal (6 to 8 energy %), and high (13 to 20 energy %) linoleic acid intake, similar effects on the excretion of creatinine and sodium on the one hand and of potassium and water on the other hand are found (Fig 1). During a normal (6 to 8 energy %) linoleic acid supply compared to the values during low linoleic acid intake, an increase of sodium and creatinine contrasts to a decrease of water and potassium excretion.

PG influence several factors which modify renal excretion of water, including glomerular filtration rate, ADH, and tubular function. A threshold concentration for PG is assumed, which determines the effect on renal function. Very low PG concentrations seem to act mostly on hormonal regulation (7). Gross et al (2) reported on an in vivo antagonism between ADH and PG in the dog kidney. An inhibition of ADH would promote water excretion, as observed with a low PG biosynthesis. A stimulated PG biosynthesis probably leads to hemodynamic renal effects, causing a diuresis, which was observed with high linoleic acid supply. In addition, experiments in the rat showed a dissociation of the effects of PG on renal sodium and water reabsorption by cyclooxygenase inhibitors (22).

If PG biosynthesis was inhibited by indomethacin in rats, potassium excretion was significantly, though slightly, increased (22). Similarly, kaliuresis during low linoleic acid intake may reflect low PG biosynthesis. An increased Na+ and water load, delivered to the distal tubulus, augments potassium excretion, as we could observe during a linoleic acid supply of 13 to 20 energy %.

The results of our nutritional experiment support the assumption that linoleic acid intake stimulates extrarenal PG and renal PG-E biosynthesis, leading to effects on renal function, which may have clinical relevance for the sodium and potassium balance in man.

References


