Augmentation of prostaglandin production by linoleic acid in man

MURRAY EPSTEIN*, MEYER LIFSCHITZ† AND KENNETH RAPPAPORT*
Departments of Medicine, Veterans Administration Medical Centers, Miami, Florida* and San Antonio, Texas and University of Miami School of Medicine, Miami, Florida and University of Texas Health Sciences Center, San Antonio, Texas†, U.S.A.

(Received 29 January/7 June 1982; accepted 21 July 1982)

Summary

1. Since several lines of evidence have suggested that a diminution of endogenous prostaglandins (PG) may participate in the pathogenesis of a variety of disease states, it would be advantageous to develop therapeutic approaches that augment endogenous prostaglandin synthesis. Since linoleic acid is readily converted to arachidonic acid, we assessed the effects of linoleic acid administration on immunoassayable PGE and 6-oxo-PGF₁α excretion as indices of PGE₂ and PGI₂ production.

2. Six normal subjects were studied twice: during a seated control study and during an 8 h infusion of a 10% emulsion of safflower oil (Liposyn) containing approx. 77% linoleic acid, in a dose of 1.5 g/kg body weight.

3. Liposyn induced a profound increase in immunoassayable 6-oxo-PGF₁α excretion.

4. Simultaneously, immunoassayable PGE excretion increased modestly.

5. The current study raises the possibility that linoleic acid administration might constitute a new therapeutic approach in managing disorders characterized by an impairment of the ability to augment prostaglandin synthesis.

Key words: fatty acid emulsion, linoleic acid, 6-oxoprostaglandin F₁α, prostaglandin E.

Abbreviation: PG, prostaglandin.

Introduction

Several lines of evidence suggest that an impairment of the ability to augment the synthesis of endogenous prostaglandins underlies the renal dysfunction in a number of clinical disorders. Thus, the administration of prostaglandin synthetase inhibitors to patients with decompensated cirrhosis, congestive heart failure, and nephrotic syndrome sometimes results in significant decrements in renal blood flow, glomerular filtration rate, and sodium excretion [1–3]. Implicit in these reports is the possibility that the augmentation of endogenous prostaglandins might improve renal function.

Theoretical considerations suggest that the provision of essential fatty acids would augment endogenous prostaglandins. Under normal circumstances the body converts linoleic acid to arachidonic acid. Since arachidonic acid is the precursor of prostaglandins of the two series including E₂ and I₂, it appeared reasonable to provide this precursor to assess the augmentation of endogenous prostaglandins. In the present study, we administered Liposyn, a fat emulsion derived from safflower oil, to a group of normal subjects.

Methods

A total of ten studies (five control and five fatty acid infusion) were carried out in six normal male subjects (four of the subjects underwent both studies). In the four subjects who underwent both studies, the order of studies was varied so that two underwent a control study followed by fatty
acid infusion, whereas the remaining two under-
went a fatty acid infusion first. The subjects
ranged in age from 22 to 35 years [mean 29 ± 2
(±SE)]. The mean body weight of the group was
71.4 ± 2.7 kg (range 65–80 kg). All subjects
were normotensive at the time of study, and all
had negative histories for hypertension, cardio-
vascular disease and diabetes. Clinically apparent
renal disease was excluded by documenting a
normal urine sediment, creatinine clearance, and
negative urine cultures. The subjects did not
ingest diuretics, nonsteroidal anti-inflammatory
drugs, or other agents that might have influenced
prostaglandin synthesis. An interval of at least
5–6 days separated the control and fatty acid
infusion studies in the four subjects who under-
went both studies. The subjects ingested un-
restricted diets. Mean urinary sodium excretion
on the day before study was 153 ± 27 mmol/day.

The experimental protocols on the two study
days were similar and were carried out as follows.
After 11 h of overnight fluid restriction, the
subject voided at 08.00 hours and then sat quietly
for 1 h. At 08.15 hours, an oral water load of
300 ml was administered. At 09.00 hours, after
voiding and completely emptying his bladder, the
subject once again assumed the seated position
for the remaining 8 h. Before and after the study,
blood samples were obtained for sodium,
potassium, and creatinine determinations.

During the study, each subject stood briefly to
void spontaneously at 2 h intervals. To maintain
an adequate urine flow, 200 ml of water was
administered orally every 2 h during the study.
Sodium, potassium and creatinine were measured
in samples of the 2 h urine collections, and
samples of each urine specimen were frozen
promptly for determination of immunoassayable
PGE and immunoassayable 6-oxo-PGF1α.

During the fatty acid infusion study, each
subject received an infusion of long-chain
essential fatty acids as a 10% emulsion of safflower
oil (Liposyn) in a dose of 1.5 g/kg body
weight. The fatty acid components of safflower
oil are: linoleic, 77%; oleic, 13%; palmitic, 7%;
stearic, 2.5%. The fat emulsion was administered
into a peripheral vein. At 09.00 hours, an infusion
of the fat emulsion was started at a rate of 0.1
ml/min. If after 15 min no adverse effects were
observed, the rate was increased to 100–110
ml/h. Vital signs, including pulse rate and blood
pressure, were noted prior to and at the termin-
ination of study. No special side effects were noted
aside from a feeling of discomfort at the infusion
site in two instances when the infusion transiently
exceeded 110 ml/h.

Urine PGE was assayed by a radioreceptor
assay utilizing extraction and assay procedures
detailed in a previous communication from this
laboratory [4]. In brief, a 2 ml sample of urine
was adjusted to pH 6.8 and extracted with
benzene/butyl chloride (1:1, v/v). The pH was
then adjusted to 3.5 with formic acid and the
fatty acids (including PGE) were extracted with
20 ml of chloroform. The chloroform was then
flash evaporated and the residue was applied to a
Sephadex LH 20 column. The appropriate
fractions were collected and after concentration
were entered into the assay procedure. The assay
was carried out utilizing rat liver membranes
which contain a class of receptors binding
specifically to PGE. Previous studies in this
laboratory have confirmed the specificity of this
class of receptors. PGE1 cannot be differentiated
from PGE2 as there is almost identical binding to
these two compounds, but prostaglandins of the
B and F series, including 6-oxo-PGF1α, demonstrate no measurable binding, and PGA1, binds with approx. 1/16 the affinity of PGE1. Recovery of unlabelled PGE1 was determined over a range of 200 to 10,000 pg, and in nine separate samples
determined on different days, the measured
values averaged 99 ± 9% of the predicted values.
All samples from a given patient were analysed at
the same time. All samples were measured in
duplicate and at two different dilutions. Since
these receptors are relatively indifferent to PGE1
and PGE2, the present results are expressed as
PGE.

Urinary 6-oxo-PGF1α was determined by
radioimmunoassay as follows. A 1 or 2 ml
sample of urine was labelled with approx. 1000
c.p.m. of 6-oxo-[3H]PGF1α. The pH of the
samples was adjusted to 3.0 with 0.1 mol/l HCl.
The samples were twice extracted with 20 ml of
chloroform. The chloroform was then flash-
evaporated and the 6-oxo-PGF1α extract was resolubilized in a phosphate/saline buffer, pH
3.0. This phosphate/saline buffer, containing
6-oxo-PGF1α, was passed through a Sep-Pak
The present study was designed to separate the 6-oxo-PGF1α from other prostaglandin products. The appropriate
fractions eluted from the high pressure liquid
chromatography. Recovery of the radio-active 6-oxo-PGF1α with this procedure averaged
72 ± 7%. High pressure liquid chromatography
with a reverse phase column and acetonitrile/water (7:18, v/v) as the solvent system was then
performed to separate the 6-oxo-PGF1α from
other prostaglandin products. The appropriate
fractions eluted from the high pressure liquid...
chromatography column were entered into a traditional radioimmunoassay along with standard chemical 6-oxo-PGF\(_{1\alpha}\) (obtained from the Upjohn Company) and trace amounts of tritiated 6-oxo-[\(^3\)H]PGF\(_{1\alpha}\) (New England Nuclear). Antibody directed towards 6-oxo-PGF\(_{1\alpha}\) was used in a final dilution of 1:20 000. All tubes were incubated in a covered rack at 37°C in a shaking water bath for 1 h and subsequently placed in an ice bath. Separation of bound from free 6-oxo-PGF\(_{1\alpha}\) was effected with dextran-coated charcoal. All samples were run in duplicate and at either two or three dilutions. The antibody is quite specific for 6-oxo-PGF\(_{1\alpha}\), although when PGI\(_2\) was added to the assay tube it reacted as well as 6-oxo-PGF\(_{1\alpha}\), perhaps due to conversion to 6-oxo-PGF\(_{1\alpha}\) in the assay tube itself. Because of the degree of sensitivity of the standard curve, calculations of samples were corrected for the chemical amount of 6-oxo-PGF\(_{1\alpha}\) included in the radioactive trace. In eight different samples run on four different days, recovery of added known amounts of 6-oxo-PGF\(_{1\alpha}\) averaged 111 ± 15%.

To evaluate whether Liposyn itself might interfere with the assay procedures, we performed extractions on three samples of urine to which Liposyn was added. Following the addition of 0-1 ml of Liposyn emulsion to either 1 or 2 ml samples of urine, these samples were carried through the extraction procedure and assayed by radioreceptor assay (PGE) or radioimmunoassay (6-oxo-PGF\(_{1\alpha}\)), and the results were compared with the same samples run without additional Liposyn. There was no increase in the amounts of either immunoassayable PGE or 6-oxo-PGF\(_{1\alpha}\) in these urine samples as compared with the sample of urine without addition of Liposyn. Thus, there are apparently no substances in Liposyn that react in the extraction or assay procedures as either immunoassayable PGE or immunoassayable 6-oxo-PGF\(_{1\alpha}\). Analytical methods for sodium, potassium, and creatinine have been reported previously [4].

In the presentation of the data, mean values are followed by the standard error of the mean as an index of dispersion. Data were evaluated statistically by paired or unpaired t-tests or by two-factor analyses of variance for experiments having repeated measurements on the same subject [5]. If a significant effect was detected, the Newman–Keuls test was used to determine which treatment means were significantly different [5]. Differences with \(P < 0.05\) were considered significant.

Permission for the study was obtained from each subject after a detailed description of the procedure and potential complications. The protocol was approved by the Human Experimentation Committees of the University of Miami School of Medicine and the Miami Veterans Administration Hospital and was in compliance with the principles set forth in the Declaration of Helsinki. No complications occurred.

### Results

**Fluid and electrolyte excretion**

The effects of a control study and the infusion of fatty acids on urinary fluid and electrolyte excretion are shown in Table 1. Urinary flow rate (\(V\)) tended to increase during the control study, although mean \(V\) exceeded the pre-study value only during hour 3. The infusion of fatty acids tended to result in increases in \(V\) during all four periods (\(P < 0.05\) compared with preinfusion), but the mean values were not significantly different from the corresponding control values.

The rate of sodium excretion \(U_{Na}V\) was relatively constant throughout the control study, ranging from 120 to 134 \(\mu\)mol/min. Mean \(U_{Na}V\) during the infusion of fatty acids was unaltered.

### Table 1. Urinary excretory patterns during control and fatty acid infusion studies

<table>
<thead>
<tr>
<th>Time periods</th>
<th>Pre-study</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<td><strong>Group</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.3 ± 0.3</td>
<td>1.9 ± 0.4</td>
<td>2.0 ± 0.3</td>
<td>2.7 ± 0.3</td>
<td>1.9 ± 0.2</td>
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<tr>
<td>Fatty acids</td>
<td>0.7 ± 0.1</td>
<td>2.5 ± 0.5</td>
<td>3.3 ± 0.7</td>
<td>3.4 ± 0.5</td>
<td>2.2 ± 0.4</td>
</tr>
<tr>
<td>Control</td>
<td>124 ± 33</td>
<td>134 ± 26</td>
<td>132 ± 39</td>
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<td>121 ± 30</td>
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<td>Fatty acids</td>
<td>88 ± 20</td>
<td>96 ± 16</td>
<td>82 ± 20</td>
<td>68 ± 24</td>
<td>35 ± 12*</td>
</tr>
<tr>
<td>Control</td>
<td>61 ± 21</td>
<td>53 ± 14</td>
<td>68 ± 12</td>
<td>64 ± 6</td>
<td>60 ± 9</td>
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<tr>
<td>Fatty acids</td>
<td>54 ± 21</td>
<td>52 ± 18</td>
<td>53 ± 13</td>
<td>36 ± 4*</td>
<td>26 ± 4*</td>
</tr>
<tr>
<td>Control</td>
<td>162 ± 19</td>
<td>129 ± 9</td>
<td>122 ± 5</td>
<td>122 ± 6</td>
<td>123 ± 7</td>
</tr>
<tr>
<td>Fatty acids</td>
<td>132 ± 9</td>
<td>130 ± 6</td>
<td>120 ± 6</td>
<td>115 ± 7</td>
<td>105 ± 12</td>
</tr>
</tbody>
</table>
compared with the control, aside from a significant decrease during period 4. The rate of potassium excretion ($U_k V$) was relatively constant throughout the control study. The infusion of fatty acids was associated with a decrement in $U_k V$ during periods 3 and 4 compared with the corresponding control study. Creatinine clearance ($C_{cr}$) was unaltered throughout the control study. Fatty acid infusion did not alter $C_{cr}$ as compared with either the corresponding control study or the pre-infusion hour.

**Urinary immunoassayable prostaglandin excretion**

**Urinary immunoassayable 6-oxo-PGF$_{1a}$**. The effects of control and fatty acid infusion on urinary immunoassayable 6-oxo-PGF$_{1a}$ excretion are shown in Fig. 1. Immunoassayable 6-oxo-PGF$_{1a}$ excretion was relatively constant during the control study, ranging from 1.3 to 1.9 ng/min. Fatty acid infusion was associated with a profound increase in immunoassayable 6-oxo-PGF$_{1a}$ excretion, which increased from a pre-infusion mean of 1.8 ± 0.2 to peaks of 5.6 ± 0.9 and 5.5 ± 1.1 ng/min during periods 2 and 3 of infusion ($P < 0.01$ and <0.05, respectively). Furthermore, a comparison of control and fatty acid infusion disclosed that the latter values exceeded control during all four periods of study.

The cumulative quantities of immunoassayable 6-oxo-PGF$_{1a}$ excreted over the 8 h period of control and fatty acid infusion were also compared. Mean immunoassayable 6-oxo-PGF$_{1a}$ excretion during the infusion of fatty acids was 2504 ± 187 ng/8 h, markedly greater than the 790 ± 160 ng/8 h excreted during the control period ($P < 0.001$).

**Urinary PGE**. The effects of control and fatty acid infusion on urinary immunoassayable PGE excretion are shown in Fig. 2. During the 9 h of control, mean $U_{PGE} V$ was relatively constant, ranging from 1.3 to 2.9 ng/min. In contrast, fatty acid infusion resulted in an increase in immunoassayable PGE excretion during periods 2, 3 and 4 of infusion as compared with the pre-infusion hour ($P < 0.05$). A comparison of control and

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**Fig. 1.** Effect of an 8 h infusion of fatty acid emulsion on immunoassayable 6-oxo-PGF$_{1a}$ excretion in five normal subjects. Immunoassayable 6-oxo-PGF$_{1a}$ excretion was relatively constant during the control study. In contrast, fatty acid infusion resulted in a marked increase in immunoassayable 6-oxo-PGF$_{1a}$ excretion which was sustained during the last three periods of infusion. (Comparisons with the control study are indicated by the appropriate significance levels.) ---, Infusion; --, control.

**Fig. 2.** Effect of an 8 h infusion of fatty acid emulsion on immunoassayable PGE excretion in five normal subjects. Immunoassayable PGE excretion was relatively constant during the control study. Fatty acid infusion resulted in an increase in immunoassayable PGE excretion during periods 2, 3 and 4 of infusion as compared with the pre-infusion hour ($P < 0.05$). A comparison of control and infusion values, however, disclosed a significant difference only during the final period. ---, Infusion; --, control.
infusion values, however, disclosed a significant difference only during the final 2 h period.

In addition to examining in a kinetic fashion the pattern of $U_{PGE}$ during the studies, we compared the cumulative quantities of immunoassayable PGE excretion over the 8 h period of control and the infusion of fatty acids. Mean immunoassayable PGE excretion during the infusion of fatty acids was $1766 \pm 298$ ng/8 h, exceeding the $964 \pm 81$ ng excreted during the control period ($P < 0.05$).

**Discussion**

Previous studies in a number of animal models have demonstrated that the intrarenal administration of arachidonic acid can augment endogenous prostaglandin synthesis [6]. From a clinical point of view, however, this approach is not readily applicable since arachidonic acid is quite labile and exerts considerable systemic haemodynamic effects. Theoretical considerations dictate that an alternative approach may be possible, i.e. the administration of another precursor of prostaglandins, linoleic acid.

Linoleic acid, the most common dietary essential fatty acid, is readily converted to linolenic and then to arachidonic acids [7]. Subsequent conversion of arachidonic acid to prostaglandins also occurs in a number of mammalian tissues. The potential for administering linoleic acid as a means of augmenting prostaglandin production has been suggested [8, 9]. Although the observed effects in the latter studies were attributed to the actions of prostaglandins, prostaglandin levels were not measured. To our knowledge, however, the intravenous administration of linoleic acid has apparently not previously been evaluated as a means of augmenting endogenous prostaglandin production in man.

The current study demonstrates that the infusion of a fatty acid emulsion which is rich in linoleic acid resulted in a marked increase in urinary excretion of immunoassayable 6-oxo-PGF$_{1\alpha}$. In contrast, when identical studies were conducted during the same time of day but without fatty acid infusion (control studies), there was no significant increase in urine immunoassayable 6-oxo-PGF$_{1\alpha}$ excretion. Simultaneously, the infusion of fatty acids resulted in a modest increase in immunoassayable PGE excretion. Although the augmentation in PGE excretion was not as striking as that observed for 6-oxo-PGF$_{1\alpha}$, immunoassayable PGE excretion during fatty acid infusion did exceed the pre-infusion levels during three of the periods of infusion. Furthermore, cumulative immunoassayable PGE excretion during the 8 h of infusion exceeded the values documented during the corresponding control periods.

The possibility must be considered that effects related to the experimental design, other than the infusion of fatty acids, might explain the alterations in prostaglandin excretion. Although it is possible (albeit unlikely) that Liposyn itself might affect the assay procedures with a factitious increase in the levels of immunoassayable PGE and 6-oxo-PGF$_{1\alpha}$, we demonstrated that the addition of Liposyn to urine did not alter the results of the assay. Among the factors that appear to affect the rate of urinary immunoassayable PGE excretion (and perhaps 6-oxo-PGF$_{1\alpha}$ excretion) is urinary flow rate, particularly higher urine flow rates [10]. Despite such observations, it is unlikely that flow rate could have accounted for the striking differences observed during control and fatty acid infusion. Although urine flow tended to increase during the fatty acid infusion study, the values did not differ statistically from the corresponding values during the control study.

If these results, in fact, do indicate an increase in endogenous prostaglandin production following linoleic acid administration, it is of interest to consider which organs or cell type might be responsible. Although most mammalian cells are assumed to have the ability to convert linoleic acid to arachidonic acid, a recent study by Spector et al. [11] suggests that at least umbilical vein endothelial cells are lacking in this ability. Whether this generalization holds for endothelial cells of a more mature nature or endothelial cells from other organs has not been addressed at present.

It is of interest to consider the implications of an increase in 6-oxo-PGF$_{1\alpha}$ excretion. Since 6-oxo-PGF$_{1\alpha}$ is recognized as one of the major non-enzymic breakdown products of PGI$_2$, the demonstration of an increase in immunoassayable 6-oxo-PGF$_{1\alpha}$ suggests that the infusion of fatty acid augmented PGI$_2$ production. Although qualitative inferences are possible, it is not possible to assess quantitatively the magnitude of PGI$_2$ augmentation. The exact fraction of PGI$_2$ which is converted to 6-oxo-PGF$_{1\alpha}$ in mammals has been estimated to range from approx. 9% in the rat [12] to 13% in the monkey [13]. Thus, although 6-oxo-PGF$_{1\alpha}$ is a major product of PGI$_2$ catabolism, a number of other products exist as well.

The disparity between the marked increase in urinary immunoassayable 6-oxo-PGF$_{1\alpha}$ excretion and the relatively modest increase in urinary immunoassayable PGE excretion merits com-
These observations are consistent with the suggestion that fatty acid administration augments primarily PG1 production and that such augmentation derives from systemic rather than renal PG1 production. Since thromboxane B2 was not measured in this study, the possible augmentation of thromboxane A2 production by Liposyn cannot be ascertained.

The absence of an increase in urine flow rate, sodium excretion, or creatinine clearance despite augmentation of PG1 production (and to a lesser extent PGE production) merits discussion. It has been previously demonstrated that both PG1 and PGE2, when administered into the renal artery, lead to increases in sodium excretion [14, 15]. Furthermore, the intrarenal administration of PGE2 markedly increases urine flow rate [15]. In the current study, the lack of a significant change in either urine flow rate or sodium excretion militates against major effects for either of these prostaglandins at the level of the kidney in the present protocol.

An alternative explanation is possible. As we have noted elsewhere [3], accumulating evidence suggests that the extent to which renal prostaglandins modulate renal function is determined in great part by the volume status of the subject. Thus, the administration of prostaglandin synthetase inhibitors to normal subjects blunted sodium excretion in the sodium-depleted state, but not in the sodium-replete state, despite comparable suppression of basal urinary immunoassayable PGE [4]. According to this formulation, renal prostaglandins are critical modulators of renal function only during conditions or disease states characterized by volume contraction. If this formulation is correct, it would be anticipated that an augmentation of renal prostaglandins might affect renal function only during the sodium-depleted state, and not in the sodium-replete state such as that which obtained in the present study.

A third explanation is possible. Although we did not estimate thromboxane A2 activity, it is possible that the increase in PG1 was attended by a concomitant increase in thromboxane A2. If such events occurred, the haemodynamic effects of PG1 might be counteracted, without a resultant change in renal haemodynamics. Additional studies will be necessary to assess this possibility.

If in fact, Liposyn administration does augment endogenous PG1 production, its potential clinical utility might be considerable. The possible role of prostaglandins in decreasing both systemic and pulmonary as well as renal vascular resistance has yet to be explored in clinical disease states for lack of a clinical means of increasing their endogenous production. It would seem appropriate to determine blood levels of 6-oxo-PGF1α during Liposyn administration to ascertain whether, in fact, these levels are increased and perhaps to monitor systemic haemodynamic consequences including cardiac output, peripheral and pulmonary vascular resistance.

In summary, the present studies demonstrate that Liposyn infusion augments the rate of excretion of immunoassayable 6-oxo-PGF1α and PGE, although levels of the former increased to a greater extent. This might be due to differences in the rate of metabolism of these two prostaglandins. These findings are consistent with the formulation that the linoleic acid found in Liposyn material is, in part, converted to arachidonic acid and subsequently to prostaglandins within the normal human subject. To our knowledge, this study constitutes the first demonstration that provision of linoleic acid can increase prostaglandin production in man. This finding may prove to have clinical utility. For example, it is conceivable that augmentation of prostaglandins might ameliorate the renal dysfunction associated with disease states such as cirrhosis of the liver in which the compensatory ability to augment prostaglandin synthesis is impaired.

Acknowledgments

We are indebted to Arthur G. DeNunzio, Steven E. Budabin, Betty Waldron, and Pearl de la Cruz for their outstanding assistance in various aspects of the study and to Susan Morantes and Tam Eggers for their expert preparation of the manuscript. These investigations were supported by funds from Abbott Laboratories, Veterans Administration Research funds and by a Program Project Grant (AM-17387).

References

Prostaglandin augmentation by linoleic acid


