Impaired Catecholamine Inactivation
A Prohypertensive Stimulus after Dietary Linoleate Deficiency in Salt-Loaded Rats?

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SUMMARY Experiments were carried out on salt-loaded rats (1.5% NaCl as drinking fluid) to further explore the mechanisms by which blood pressure increases after a linoleic acid-deficient (LAd) diet. In 4-week-old LAd rats (0.5 cal% LA, hydrogenated palm kernel fat) compared to linoleic acid-rich rats (LAr, 13.3 cal% LA, sunflower oil), we observed, from the base of a reduced content of ω-6-polyunsaturated fatty acids in the tissues, an increase in blood pressure by 12 mm Hg (p < 0.001), a diminished formation of prostaglandin E (PGE), and an unchanged formation of PGF in the aorta as well as a reduction in the in vitro uptake of 14C-norepinephrine into cardiac, aortic, and renal tissues, and a reduced degradation rate of 14C-norepinephrine in cardiac tissue. These differences in LAr vs LAd rats were not exaggerated. With respect to aortic PGE formation, 14C-norepinephrine uptake into aortic and renal tissues and 14C-norepinephrine degradation even lessened when the diet was begun prenatally, although the reduction of ω-6-polyunsaturated fatty acids in the tissues was aggravated. Our conclusion is that a fault in catecholamine inactivation may be involved in the pathogenesis of increased sympathetic activity and blood pressure elevation in LAd-fed, salt-loaded rats, possibly via alterations of endogenous prostanoid formation. (Hypertension 5: 968-975, 1983)

KEY WORDS • diet • linoleate • salt-loaded rats • arterial hypertension • prostaglandins • sympathetic nervous system

The first experiments into the significance of dietary linoleic acid (LA) in the regulation of blood pressure in salt-loaded rats showed a blood pressure increase after an LA-deficient (LAd) diet, compared with an LA-rich (LAr) diet of 15-20 mm Hg in studies lasting 4 to 6 weeks.1-3 Further experiments revealed that the blood pressure increase in the LAd rats was accompanied by an increased vessel reactivity4 and a reduced excretory capacity of the kidney.1,5,6 Moreover, an elevated plasma catecholamine level was found in these rats, and it was demonstrated that an enhanced sympathetic activity was essential to the blood pressure increase after LAd diet in salt-loaded rats.7 For an explanation of the increased plasma catecholamine levels, alterations in prostaglandin (PG) biosynthesis found in the LAd rats were examined.5,6 It was hypothesized that the diminished aortic and renal PGE formation, after LAd diet, could suppress the negative feedback of PGE on the adrenergic transmitter release.5,7 This hypothesis was confirmed by the finding that the PG biosynthesis inhibitor, indomethacin, increased the low plasma catecholamine levels in the LAr rats but did not affect the high plasma catecholamine levels in the LAd animals.5

In the evaluation of increased catecholamine spillover to plasma in LAd rats, it seemed to be important to differentiate between a true increase in catecholamine release and an increased spillover from defective local inactivation of the transmitter. One aim of our present paper was, therefore, to compare the inactivation rate (uptake and degradation) of catecholamines in LAr and LAd rats. Furthermore, we wanted to examine whether the differences between LAr and LAd rats with respect to blood pressure, PG formation, and possibly catecholamine inactivation could be increased by a prolongation of the feeding period. Therefore, we extended the feeding period to the prenatal period in an additional experiment.
Material and Methods

Animals and Feeding Procedure

Pregnant Wistar rats were either fed ad libitum an LAr (13.3 cal% LA) or an LAd diet (0.5 cal%), during the last week of pregnancy. The exact composition of the diets is summarized in table 1. The diets were continued during suckling, and the offspring received the same diets. The male offspring aged 3 months were then salt-loaded by means of a 1.5% sodium chloride solution drinking fluid, ad libitum, for 4 weeks. At the same time, commercially available food was given to another group of pregnant rats and the offspring. The male offspring aged 3 months received either an LAr or an LAd diet, and they, too, were salt-loaded for 4 weeks. Thus, four different age-matched groups were obtained: LAr diet postnatal (LAr), LAd diet postnatal (LAd), LAr diet prenatal (LArp), LAd diet prenatal (LAdp). Altogether, we fed 140 rats, 35 in each group.

Blood Pressure Measurement

Systolic blood pressure of prewarmed (infrared light) conscious rats was measured in the morning by a tail-cuff plethysmographic method. Blood pressure of each rat was measured at least three times before the start of the experiment in order to acclimatize the rats to the blood pressure measurement procedure.

Biochemical Examination

After the salt-loading period of 4 weeks, the rats were decapitated under slight ether narcosis. The organs necessary for the estimation of fatty acid pattern and PG formation (heart, kidney, aorta) were quickly removed, cleaned of fat, immediately deep frozen, and stored at −20°C for a maximum of 1 month until assayed. Investigations of 14 C-norepinephrine uptake and degradation were performed immediately in fresh organs.

Fatty Acid Composition of Cardiac and Renal Lipids

The whole frozen hearts and kidneys were cut into small pieces. The pieces were immediately placed into a chloroform-methanol solution (2:1 vol/vol) and homogenized. Lipids were extracted according to Folch et al. and separated into several neutral lipid classes (total phospholipids, triglycerides, cholesterol esters, free fatty acids) by thin layer chromatography. Gas chromatography was used to gauge the fatty acid composition by the formation of methyl esters using our recently described method.

Estimation of Aortic Prostaglandin Formation

The aorta (arcus aortae to aorta abdominalis) was incubated in 10 ml Tyrode solution at a temperature of 37°C. After 60 minutes, the PGs in the incubation medium were extracted using ethyl acetate and separated by column chromatography (Silicar CC-4 Byk, Malinckrodt GMBM, 4230 Wesel, Federal Republic of Germany). The eluting solvent was benzene-ethanol (60:40:2 vol/vol for PGE and 60:40:20 vol/vol for PGF). PGE and PGF were determined by radioimmunoassay using commercial kits (Clinical Assays, Cambridge, Massachusetts). We measured the cross-reactivity of the Clinical Assay Kit for PGE (measured as PGB2) in our laboratory: the antibody cross-reacted less than 1% with PGF1α, PGF2α, PGD2, 13,14-dihydro-15-keto-PGF2α, 6-keto-PGF1α, and 1.45% with PGB2. The intraassay variation of the radioimmunoassay was 10%; the interassay variation was 13%. The recovery after extraction was 75%.

Uptake and Degradation of 14C-Norepinephrine

Uptake and degradation in cardiac, aortic, and renal tissues were estimated as previously described. In brief, thoracic aorta, heart, and kidney were removed and kept in ice-cold Tyrode solution. Atria and kidney cortex were cut into small pieces and 100 mg of these tissues as well as thoracic aorta were preincubated in Krebs-Henseleit solution bubbled with 5% CO2 in O2 at 37°C. After the preincubation period (30 minutes), 14C-DL-norepinephrine (60 mCi/mMol) was added to give a final concentration of 10−6M, and the tissues were incubated for a further 60 minutes. Ascorbic acid (0.2%) was added to protect 14C-norepinephrine from oxidation. The subsequent steps for tissue preparation were done using the method of Whitby et al. Uptake of 14C-norepinephrine is expressed as a percentage ratio of the radioactivity found in the tissue to the total radioactivity added initially to the incubation medium. 14C-norepinephrine and its metabolic products, 14C-normetanephrine, 14C-dihydroxy-mandelic acid, and 14C-3-methoxy-4-hydroxy-mandelic acid, were separated using the method of Kopin et al. The distribu-

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**Table 1. Composition of Linoleic Acid-Rich (LAr) and Linoleic Acid-Deficient (LAd) Diets**

<table>
<thead>
<tr>
<th>Ingredients (weight%)</th>
<th>LAr diet (13.3 cal% LA)</th>
<th>LAd diet (0.5 cal% LA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Starch</td>
<td>58</td>
<td>58</td>
</tr>
<tr>
<td>Cellulose</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Salt mixture</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Vitamin mixture</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 sunflower oil</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>14 hydrogenated palm kernel oil</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Chart**

- Fatty acid pattern (%)
  - C12:0: 1
  - C14:0: 5
  - C16:0: 7
  - C16:1: 1
  - C18:0: 7.5
  - C18:1: 29
  - C18:2: 46
  - C18:3: 3.5

- Ingredients (weight%)
  - Casein: 20
  - Starch: 58
  - Cellulose: 5
  - Salt mixture: 3
  - Vitamin mixture: +
  - Fat (%)
    - 14 sunflower oil
    - 14 hydrogenated palm kernel oil

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**Notes**

- Animals and Feeding Procedure: Pregnant Wistar rats were either fed ad libitum an LAr (13.3 cal% LA) or an LAd diet (0.5 cal%), during the last week of pregnancy. The exact composition of the diets is summarized in Table 1. The diets were continued during suckling, and the offspring received the same diets. The male offspring aged 3 months were then salt-loaded by means of a 1.5% sodium chloride solution drinking fluid, ad libitum, for 4 weeks. At the same time, commercially available food was given to another group of pregnant rats and the offspring. The male offspring aged 3 months received either an LAr or an LAd diet, and they, too, were salt-loaded for 4 weeks. Thus, four different age-matched groups were obtained: LAr diet postnatal (LAr), LAd diet postnatal (LAd), LAr diet prenatal (LArp), LAd diet prenatal (LAdp). Altogether, we fed 140 rats, 35 in each group.

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Fatty Acid Pattern and Prostaglandin Formation

In the kidneys of the LAd rats, compared with the LAr rats, there was found to be a significant reduction of LA and its metabolic product, arachidonic acid (AA), in the free fatty-acid fraction ($p < 0.01$ and $p < 0.001$, table 3). In the triglycerides, the LA content was reduced ($p < 0.001$) and the AA content was slightly but significantly enhanced ($p < 0.01$) following the LAd diet. The 4-week regimen did not affect LA and AA content in the renal phospholipids and cholesterol esters ($p > 0.05$). Following the prenatal diet, LAdp rats showed a reduction of LA and AA in all lipid classes, compared with LArp rats. In the phospholipids, the free fatty acids, the cholesterol esters, and the triglycerides of the heart, there was found to be a reduction of LA, in both LAd and LAdp diet-fed rats. The effect was slightly more pronounced after the prenatal LAd diet. But in all lipid classes, a significant reduction of AA after an LAd diet was not to be found, either after postnatal or prenatal feeding.

| TABLE 2: Body Weights (g) of Linoleic Acid-Rich and Linoleic Acid-Deficient Diet-Fed Rats with Prenatal and Postnatal Regimens Before and After the Salt-Loading Period (n = 35 in each group) |
|---|---|---|---|
| LAr | LAd | LArp | LAdp |
| Before salt loading | 257 ± 9 | 265 ± 9 | 277 ± 10 | 232 ± 5 |
| After salt loading | 315 ± 7 | 304 ± 10 | 326 ± 8 | 275 ± 7 |

LAr = linoleic-acid-rich postnatal; LAd = linoleic-acid-deficient postnatal; LArp = linoleic-acid-rich prenatal; LAdp = linoleic-acid-deficient prenatal.
Table 3. Linoleic Acid (LA) and Arachidonic Acid (AA) Content (in Percentage of Whole Fatty Acids) in Renal and Cardiac Lipids of Prenatally and Postnatally Linoleic-Acid-Rich (LAr) and Linoleic-Acid-Deficient (LAd) Diet-fed, Sult-loaded Rats

<table>
<thead>
<tr>
<th></th>
<th>Postnatal diet</th>
<th>Prenatal diet</th>
<th>p</th>
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<tbody>
<tr>
<td></td>
<td>LAr (n = 10)</td>
<td>LAd (n = 10)</td>
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</tr>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LA</td>
<td>10.5 ± 0.5</td>
<td>9.6 ± 0.7</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>AA</td>
<td>34.9 ± 1.3</td>
<td>32.8 ± 1.3</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>FFA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LA</td>
<td>19.1 ± 0.3</td>
<td>14.7 ± 2.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>AA</td>
<td>27.7 ± 2.3</td>
<td>16.4 ± 3.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CE</td>
<td></td>
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<tr>
<td>LA</td>
<td>22.2 ± 1.3</td>
<td>23.5 ± 4.4</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>AA</td>
<td>9.5 ± 2.2</td>
<td>8.5 ± 1.7</td>
<td>&gt;0.05</td>
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<tr>
<td>TG</td>
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<tr>
<td>LA</td>
<td>28.2 ± 1.2</td>
<td>15.2 ± 1.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AA</td>
<td>1.2 ± 0.2</td>
<td>2.8 ± 0.3</td>
<td>&lt;0.01</td>
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<tr>
<td>Heart</td>
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<td>PL</td>
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<tr>
<td>FFA</td>
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<tr>
<td>LA</td>
<td>26.0 ± 1.9</td>
<td>9.5 ± 0.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AA</td>
<td>12.5 ± 2.1</td>
<td>12.4 ± 1.1</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>CE</td>
<td></td>
<td></td>
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<tr>
<td>LA</td>
<td>21.0 ± 3.1</td>
<td>11.2 ± 1.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AA</td>
<td>9.8 ± 3.4</td>
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<tr>
<td>TG</td>
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<tr>
<td>LA</td>
<td>34.6 ± 1.1</td>
<td>6.5 ± 0.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AA</td>
<td>1.8 ± 0.2</td>
<td>1.6 ± 0.4</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

PL = phospholipids; FFA = free fatty acids; CE = cholesterol esters; TG = triglycerides; LA = linoleic acid; AA = arachidonic acid. For other abbreviations see Table 1.

Figure 2 illustrates that PGE formation in the aorta of LAd rats was significantly reduced compared with the LAr rats (p < 0.02), whereas reduction of PGF formation was statistically insignificant (p > 0.05). The differences in aortic PG formation between LAr and LAd rats were not increased after prenatal feeding (p > 0.05). This effect resulted from a diminished PG formation in the LArp rats.

Uptake and Degradation of 14C-norepinephrine

After postnatal feeding, in all tissues investigated uptake of 14C-norepinephrine was markedly reduced in LAd rats as compared with LAr rats (p < 0.05) (fig. 3). After prenatal feeding, the 14C-uptake was significantly reduced only in the heart of LAdp rats (p < 0.01) (fig. 3). The distribution of 14C-norepinephrine and its metabolites in cardiac, aortic, and renal tissues of the four dietary groups after a 60-minute incubation period is shown in table 4. In the hearts of LAd rats, the content of 14C-norepinephrine was significantly increased and the content of its methylation and deamination products was significantly reduced, as compared with LAr rats. In the aorta and kidney there were no significant differences in the distribution of 14C-norepinephrine and its metabolic products in LAr vs LAd rats (p > 0.05), with one exception: the 14C-normetanephrine content was increased in the aortas of LAd rats (p < 0.01).

Following prenatal feeding we observed no differences in the distribution of 14C-norepinephrine and its metabolites in the different tissues between the two dietary groups (p > 0.05).
Discussion

In accordance with previous dietary studies with salt-loaded rats lasting 4–6 weeks, our results show an increase in blood pressure after a dietary linoleate deficiency compared with an LAr diet. Moreover, our results confirm that salt loading is a condition sine qua non for blood pressure increase with an LAd diet, as indicated by the absence of increased blood pressure in the prenatally LAd diet-fed rats before salt loading. In addition to this, the present experiments show that the slight but significant and reproducible blood pressure increase after LAd diet could not be augmented by a prolongation of the diet period in the pregnant mothers. So we have, as far as dietary LA is concerned, an all-or-nothing phenomenon: the prohypertensive effect of the LAd diet is fully developed after a 2–3 weeks...
regimen and can neither be increased by the prolongation of the diet period, as shown by the present experiments, nor by further restriction of dietary LA, as described earlier.5

The investigations of the fatty acid compositions of the tissues revealed diet-induced changes in renal and cardiac lipids. After LAd diet, there was a decrease in LA (fully expressed after prenatal diet) and of the heart. But the LAd diet lowered the levels of the elongation and desaturation of AA only in the kidney lipids (fully expressed after prenatal feeding). The AA levels in cardiac lipids were not reduced, even after the prenatal LAd diet. Galli et al.17 found an even higher AA content in the four dietary groups following a 60-minute incubation period. Our results confirm the findings obtained by Gallo et al.17

Prostaglandin formation in this diet/blood pressure model has been investigated in detail in several organs.2,3,6 In accordance with previous findings, we observed in these experiments a marked decrease in aortic PGE formation without significant changes in PGF generation after 4 weeks of LAd diet compared with the LAr diet. But the differences in PGE formation in LAd vs LAr rats were not visible after prenatal feeding. Fatty acid compositions in the aortas were not investigated. Therefore, it is only speculation whether alterations in AA content or other compensatory mechanisms, e.g., cofactor availability, formation of lipid peroxides after LAr diet, were responsible for the abolition of the differences in aortic PGE formation between LAr and LAd rats after prenatal feeding.

Investigations into 14C-norepinephrine uptake revealed a strong reduction in cardiac, aortic, and renal tissues after 4 weeks of the LAd diet vs 4 weeks of the LAr diet. The effect was most markedly expressed in the cardiac tissue. It is beyond the scope of these experiments to decide whether neuronal or extraneuronal mechanisms were predominantly involved. But an important point to note is that the differences in 14C-norepinephrine uptake between LAr and LAd rats were not observed in the aorta and kidney of the prenatally fed rats, but only in the cardiac tissue.

To get some information on the catecholamine metabolism, we investigated the pattern of 14C-norepinephrine and its degradation products, namely, deamination the minor pathway for catecholamine metabolism. The investigations of the fatty acid compositions of the tissues revealed diet-induced changes in renal and cardiac lipids. After LAd diet, there was a decrease in LA (fully expressed after prenatal diet) and of the heart. But the LAd diet lowered the levels of the elongation and desaturation of product AA only in the kidney lipids (fully expressed after prenatal feeding). The AA levels in cardiac lipids were not reduced, even after the prenatal LAd diet. Galli et al.17 also observed that the tissue content of AA does not easily correlate in all organs with the amount of LA in the diet. Galli et al.17 found an even higher AA content in aortic phosphatidylinositol and all platelet phospholipid classes after feeding rabbits an LAd diet. Therefore, the prevailing concept that low levels of LA in the diet should generally decrease the levels of AA in tissues and vice versa does not hold true in all organs.

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To get some information on the catecholamine metabolism, we investigated the pattern of 14C-norepinephrine and its degradation products, namely, deamination and O-methylation products in the tissues of the four dietary groups following a 60-minute incubation period. Our results confirm the findings obtained by Kopin et al.,13 that O-methylation is the major pathway and deamination the minor pathway for catecholamine inactivation in the heart. After 4 weeks of the LAd diet, the metabolism of 14C-norepinephrine in the heart was reduced, as expressed by the increased content of unmetabolized 14C-norepinephrine at the ex-
pense of the metabolic products. Thus, in the heart the two most important mechanisms of catecholamine inactivation — uptake and O-methylation — were inhibited by 4 weeks of the LAd diet. The differences between the cardiac degradation rate of \( ^{14} \text{C}-\text{norepinephrine} \) in LAr and LAd rats were not seen in the prenatally fed rats. In the aorta and kidney, no inhibition of \( ^{14} \text{C}-\text{norepinephrine} \) degradation was observed following the feeding of the LAd diet, either in the prenatal or postnatal regimen.

The mechanisms by which dietary LA affects \( ^{14} \text{C}-\text{norepinephrine} \) inactivation are unknown. Alterations in the fatty acid composition of the tissues may influence membrane properties and/or carrier-mediated processes. Another possible explanation for the differences in \( ^{14} \text{C}-\text{norepinephrine} \) inactivation between the LAr and the LAd group might be related to changes in AA metabolism. Although there have been some reports that PGs do not inhibit the catecholamine reuptake mechanisms in vitro, 18 or the major catecholamine-degrading enzymes COMT and MAO, 18-20 it cannot be ruled out that changes in the endogenous synthesis of eicosanoids (PGs, prostacyclin, thromboxanes, and leukotrienes) may play a role in this connection. This idea is supported by findings obtained by Clarenbach et al., 21 who described an inhibition by indomethacin of catecholamine uptake into synaptosomes of the rat brain. More recently, Fernandez Pardal et al. 22 observed a marked augmentation in the formation of the metabolic products of norepinephrine evoked by PGE, in the isolated rat uterus, whereas PGF\(_{2\alpha}\) did not alter the metabolism of norepinephrine. In our experiments, the alterations in aortic PGE formation correlate to a certain extent to the changes in \( ^{14} \text{C}-\text{norepinephrine} \) inactivation.

In our previous experiments, 2 we observed an increased plasma catecholamine level in the LAd, compared to the LAr, salt-loaded rats after the 4-week regimen, and we could show that chemical sympathectomy abolished the increase in blood pressure as well as in the plasma catecholamines of the LAd diet-fed rats. We postulated that the increase in plasma catecholamines in the LAd rats could be due to the diminished PGE formation and thereby a suppressed negative feedback of PGE on the adrenergic transmitter release. 16 The experimental data presented here suggest that an impaired catecholamine inactivation may also contribute to the increased plasma catecholamine levels in the 4-week LAd diet-fed rats. It remains to be clarified whether catecholamine inactivation is impaired in other organs important for catecholamine inactivation (e.g., spleen, adrenal gland, lung) and also under in vivo conditions following an LAd diet. Interestingly, Brenneman and Rutledge 23 described an in vitro impairment of synaptosomal norepinephrine and dopamine uptake following an LAd diet in non-salt-loaded rats. On the other hand, salt loading diminished the uptake of norepinephrine into cardiac and aortal tissues, 24 that is to say, both salt loading and an LAd diet reduced catecholamine inactivation. This may have something to do with the finding that salt loading is a condition sine qua non regarding the blood pressure increase following a dietary linoleate deficiency.

Our results do not fit the expectation that differences between LAr and LAd rats, with respect to blood pressure, PG formation, and \( ^{14} \text{C}-\text{norepinephrine} \) inactivation, would be exaggerated following prenatal feeding. The differences between the LAr and LAd rats were, with the exception of blood pressure and \( ^{14} \text{C}-\text{norepinephrine} \) uptake into cardiac tissue, even lessened after prenatal feeding, although the reduction of \( \omega\)-polyunsaturated fatty acids in the tissue after the LAd diet was more markedly expressed in the prenatal group. We must therefore consider the possibility that regulatory mechanisms, unknown at present, work against an increase in differences following a dietary lipoleic deficiency in the prenatal group.

In genetically hypertensive rats, Salt and Iversen 25 found a decrease in the amount of \( ^{3} \text{H}-\text{norepinephrine} \) taken up by the heart. Our latest results revealed that norepinephrine uptake in tissues of genetically hypertensive rats can be increased by the feeding of an LAr diet (Hoffmann et al., manuscript in preparation). In these rats, we observed an attenuated development of hypertension. 26

In conclusion, the results presented here demonstrate that in the 4-week diet-fed, salt-loaded LAd rat, there was an increased blood pressure, a diminished aortic PGE formation, as well as a reduced uptake of \( ^{14} \text{C}-\text{norepinephrine} \) into cardiac, aortic, and renal tissues, and a reduced \( ^{14} \text{C}-\text{norepinephrine} \) inactivation rate in cardiac tissue, compared to LAr rats. These differences were not enhanced but in part were even diminished following prenatal feeding.

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