Dietary Phosphate Modulates Atherogenesis and Insulin Resistance in Apolipoprotein E Knockout Mice—Brief Report

Timothy Ellam, Martin Wilkie, Janet Chamberlain, David Crossman, Richard Eastell, Sheila Francis, Timothy J.A. Chico

Objective—Epidemiological studies link higher serum phosphate and the phosphatonin fibroblast growth factor 23 with cardiovascular events and atheroma, and they link lower serum phosphate with insulin resistance and the metabolic syndrome. We investigated whether manipulating dietary phosphate influences atherogenesis or insulin sensitivity in mice.

Methods and Results—Apolipoprotein E knockout mice were fed an atherogenic diet with low (0.2%), standard (0.6%), or high (1.6%) phosphate content. Serum phosphate and fibroblast growth factor 23 significantly increased with increasing dietary phosphate intake, but lipid profile and blood pressure were unaffected. After 20 weeks, mice on the higher phosphate diet had significantly more atheroma at the aortic sinus (42±1.9% versus 30±1.5% for high versus low phosphate, P<0.01). Compared with standard and high-phosphate diet groups, mice on a low-phosphate diet had more adipose tissue and a 4-fold increase in insulin resistance measured by homeostatic model assessment (43.7±9.3 versus 8.9±0.7 for low versus high phosphate, P<0.005).

Conclusion—A high-phosphate diet accelerates atherogenesis in apolipoprotein E−/− mice, whereas low phosphate intake induces insulin resistance. These data indicate for the first time that controlling dietary phosphate intake may influence development of both atherosclerosis and the metabolic syndrome. (Arterioscler Thromb Vasc Biol. 2011;31:1988-1990.)

Key Words: atherosclerosis ■ insulin resistance ■ prevention ■ fibroblast growth factor 23 ■ phosphate

In human observational studies, higher serum phosphate levels within the normal range have been associated with a marked increase in cardiovascular events.1,2 Adverse outcomes linked to such elevations in serum phosphate include myocardial infarction,2 coronary artery disease,3 coronary calcification,4 and cardiovascular mortality.2 These associations are observed despite adjusting for recognized risk factors. The phosphate-regulatory hormone fibroblast growth factor 23, which increases in response to increased dietary phosphate intake, has similarly been linked to cardiovascular disease.5 Conversely, lower serum phosphate has been associated with insulin resistance and the metabolic syndrome,6 which are themselves risk factors for atherosclerosis.

See accompanying article on page 1951

Methods

An expanded Methods section is given in the supplemental material, available online at http://atvb.ahajournals.org.

Animals and Diets

Eight-week-old male apolipoprotein E knockout mice were randomly assigned to atherogenic diets (21% fat, 0.2% cholesterol, 0.03% cholate) with low (0.2%), standard (0.6%), or high (1.6%) phosphate content. Groups were fed the test diets for 12 weeks (group 1, n=14) and 20 weeks (group 2, n=19, 5 to 8 per diet) before euthanization under pentobarbital anesthesia for cardiac puncture and tissue collection.

Atheroma Analysis

Atheroma burden was quantified using morphometric analysis in cross-sections through the aortic sinus at the level of the aortic valves and stained with Alcian Blue/elastic van Gieson. Calcification was assessed by von Kossa stain, and smooth muscle cell and macrophage content was assessed by immunostaining.
Statistical Methods
Data are presented as mean±SEM. Groups were compared by 1-way ANOVA with Tukey post hoc analysis.

Results
Increased Dietary Phosphate Intake Accelerates Atherogenesis in Apolipoprotein E−/− Mice
Increased dietary phosphate intake caused a significant increase in serum phosphate, parathyroid hormone, and fibroblast growth factor 23 and a small but significant reduction in serum calcium (Figure 1 and Table). The low-phosphate diet increased weight gain, but there were no significant differences in blood pressures, lipid profile, urea, or total chow consumption (see supplemental material).

Increased dietary phosphate intake had no effect on aortic sinus atheroma at 12 weeks but was associated with significantly more atheroma at 20 weeks (Figure 1A and 1B): 30±2%, 33±2%, and 42±2% for low-, standard-, and high-phosphate groups, respectively (*P<0.01 for low versus high), corresponding to a 40% increase in atheroma burden for the high versus low dietary phosphate groups. Calcification was absent by von Kossa staining, and lesions did not differ in vascular smooth muscle cell or macrophage content (data not shown).

Low Dietary Phosphate Intake Increases Insulin Resistance, Adiposity, and Hepatic Steatosis
Epididymal fat pad mass was significantly greater in the low versus high dietary phosphate group (84±23 mg versus 19±8 mg, *P<0.05, Figure 2A). Insulin resistance measured by homeostatic model assessment was increased 4-fold on a low-phosphate diet in comparison with other dietary groups (43.7±9.3 versus 11.1±0.8 and 8.9±0.7, *P<0.005, Figure 2B and 2D). This was not accounted for by adiponectin, which did not differ between low and standard dietary phosphate groups (Figure 2E). Hepatic steatosis was induced in the low-phosphate group and was accompanied by greater liver weight and alanine transaminase (Figure 2F to 2I).

Discussion
This is the first interventional study demonstrating an atherogenic effect of dietary phosphate supplementation. In keeping with human observational data, atheroma burden was least in the low-phosphate group despite the induction of insulin resistance. Modulation of components of the phosphate homeostatic axis (fibroblast growth factor 23, parathyroid hormone, calcitriol) could be responsible for this accelerated atherogenesis. Phosphate itself has been implicated as an endothelial toxin, and postprandial peaks may lead to accumulation of endothelial damage that over time predisposes to atherosclerosis.8

Low dietary phosphate intake has not previously been demonstrated to induce insulin resistance, adiposity, or steatosis. Xie et al reported increased plasma glucose after phosphate deprivation in rats, attributed to increased hepatic glucose export.9 We observed induction of insulin resistance measured by homeostatic model assessment, in keeping with the observational population data.6 This may be a consequence of increased adiposity, impaired phosphometabolite synthesis, or another mechanism.

In summary, we have demonstrated causal relationships between increased dietary phosphate and atherosclerosis, without significant alteration of lipid levels, and between low dietary phosphate and insulin resistance. Given the substantial increases in cardiovascular disease associated observationally with higher phosphate and the massive global burden

Table. Plasma Biochemistry Results and Weight by Dietary Phosphate Group at 20 Wk

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Dietary Phosphate Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.2%</td>
</tr>
<tr>
<td>Phosphate, mmol/L</td>
<td>2.02 (0.28)</td>
</tr>
<tr>
<td>Calcium, mmol/L</td>
<td>2.27 (0.04)</td>
</tr>
<tr>
<td>PTH, ng/L</td>
<td>26 (5)</td>
</tr>
<tr>
<td>FGF23, ng/L</td>
<td>58 (18)</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>33.5 (1.8)</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>6.47 (0.20)</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>23.6 (1.5)</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>7.60 (0.98)</td>
</tr>
<tr>
<td>Final weight, g</td>
<td>34.7 (1.5)</td>
</tr>
</tbody>
</table>

*PTH indicates parathyroid hormone; FGF, fibroblast growth factor; NS, not significant; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
of cardiovascular disease, these findings are potentially of major significance, particularly because interventions to lower serum phosphate (dietary restriction and oral phosphate binders) are already available.

Further discussion is available in the supplemental material.

Sources of Funding
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Disclosures
None.

References
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Supplemental Material

Methods

Animals and Diets

All experiments were approved by the University of Sheffield Project Review Committee and conformed to UK Home Office ethical guidelines (project licence number 40/3307). ApoE\(^{-/-}\) mice on a C57BL/6 background were purchased previously from JAX labs (JAX 2052) and a breeding colony maintained in our unit. Male ApoE\(^{-/-}\) mice obtained from this colony were housed in a controlled 22°C environment with 12-hour light/dark cycle and free access to food and water. From weaning until 8 weeks, all mice were fed standard chow (4.3% fat, 0.02% cholesterol, Special Diet Services, UK). Animals were subsequently fed the phosphate-modified atherogenic diets (custom made, Harlan Teklad, USA) with fixed content of lipid (21% milkfat, 0.2% cholesterol and 0.03% cholate), calcium (1%) and vitamin D (1IU/kg). The phosphate content was modified by varying the relative contributions of calcium monophosphate, calcium diphosphate and calcium carbonate, allowing uniform total calcium content. Sodium content of the diets was matched, and the diets were isocaloric (energy density differences <2%). Phosphate content of the test diets was confirmed by independent laboratory assessment (N.P Analytical Laboratories, USA).

Blood Pressure Measurement

Systolic and diastolic blood pressure measurements were performed twice weekly on 4 animals per dietary group (total N=12) using tail cuff measurements (Visitech 2000, Visitech Systems, NJ, USA). Mean arterial pressure was calculated from each pair of systolic/diastolic measurements. Animals were acclimatized to the technique by one week of daily training (readings taken but data discarded). Measurements were performed at the same time of day and an initial set of 10
acclimatization measurements taken on each occasion (data discarded). Results were based on the subsequent 20 attempted measurements (cuff inflations), with a mean number of successful measurements of 12 per mouse per session.

**Biochemical Analyses**

Fasting glucose was measured on whole blood (Medisense, Optium Xceed). Commercial sandwich ELISA assays were used to quantify plasma insulin (Crystalchem, USA), adiponectin (R&D systems, UK), parathyroid hormone (Immutopics, USA) and FGF23 (KAINOS laboratories, Japan), according to the manufacturers’ instructions. All other plasma biochemistry measurements were performed by autoanalyzer (Beckman Coulter DxC). LDL cholesterol was calculated from other lipid fractions according to the Friedewald formula\(^1\). Insulin resistance was measured by standard homeostatic model assessment\(^2\) (HOMA-IR) = fasting glucose (mmol/l) × fasting insulin (mU/l)/22.5.

**Tissue collection**

Mice were sacrificed by intraperitoneal injection of an overdose of pentobarbitone (4mg) and blood aspirated by cardiac puncture. The vasculature was then flushed with phosphate-buffered saline and perfusion-fixed by ventricular injection of 10% formalin. Thoracic aortae were dissected free of connecting tissue from the heart to the level of the diaphragm and fixed in 4% paraformaldehyde. Following fixation in 10% formalin, hearts and livers were dehydrated and embedded in paraffin wax. Epididymal fat pads, kidneys, lungs, livers and spleens were dissected free of connective tissue before weighing.

**Atheroma Analysis**
Cross sectional quantification of atheroma burden was performed on 7 micron sections taken through the aortic sinus. The section with 3 valves most clearly visible was selected for analysis and stained with Alcian blue/elastic van Gieson. Atheroma burden was measured using morphometric analysis software (NIS-elements Br 3.0, Nikon Instruments, USA) and expressed as a percentage of the total aortic sinus lumen area. Lumen area was calculated from perimeter measurements to correct for fixation and sectioning errors. Analysis was performed on 2 occasions by an assessor blinded to the dietary group and the average result taken (within specimen coefficient of variation 6%). Calcification was assessed by von Kossa’s staining with 2% silver nitrate, counterstained with nuclear fast red. Smooth muscle cell content of atherosclerotic lesions was assessed by alpha smooth muscle actin immunostaining (DAKO, Denmark) and macrophage content by F4/80 immunostaining (Abcam, UK).

**Hepatic Steatosis Assessment**

Sections were taken through the liver in transverse plane and stained with haematoxylin and eosin. The presence of hepatic steatosis was assessed under low power magnification (10x) by an assessor blinded to the dietary phosphate group.

**Results**

**Effects of Dietary Phosphate Group on Urea, Weight and Blood Pressure**

Dietary phosphate intake had no significant effect on urea (7.3±0.5mmol/L, 8.0±0.5mmol/L and 7.0±0.5mmol/L for low, standard and high dietary phosphate groups respectively). Lower dietary phosphate intake was associated with a trend towards greater chow consumption and BMI, with a significantly greater final weight and body length (Table I and Figure I). With the exception of
liver and epididymal fat pads, other organ weights (heart, lung, kidneys, and spleen) did not differ between groups. Blood pressure also did not differ between groups (Figure II).

Discussion

Despite extensive clinical observational data linking higher-normal phosphate with adverse cardiovascular outcomes, no interventional data has hitherto been available to indicate whether phosphate exposure plays a causative role in atherosclerosis. Reported associations between higher-normal phosphate and cardiovascular events persist despite adjusting for all recognized risk factors. In particular, adjusting for calcium, excretory kidney function and albuminuria does not abolish the associations, which are apparent in healthy populations without renal impairment.

We have used a dietary approach to manipulate serum phosphate levels, an intervention which can be applied directly to humans. A typical laboratory rodent dietary phosphate content is 0.6% and this was taken as the standard phosphate content for our study. Restricted and supplemented dietary phosphate contents of 0.2% and 1.6% were based on previous studies demonstrating modest changes in serum phosphate and regulatory hormones at these levels. Although mice have a greater dietary phosphate intake on a per-calorie basis than humans (approximately 2-fold greater comparing our standard phosphate dietary group with typical intakes for a human male), the range of dietary phosphate contents used in our study produced proportional changes in serum phosphate similar to those that have been achieved by dietary manipulation in humans; Portale et al. reported a safe 40% reduction in time-averaged serum phosphate in a cohort of healthy subjects given a controlled phosphate diet and oral phosphate binders. In the absence of overt hypophosphatemia, there is no evidence that lower dietary phosphate intake has adverse effects on bone health. Rather, concerns have previously been raised regarding effects on bone of the high
phosphate content of Western diets. There is therefore likely to be scope for safely reducing the dietary phosphate exposure of Western populations.

Observational studies linking higher-normal phosphate with adverse cardiovascular outcomes have not concomitantly measured levels of phosphate-regulating hormones. It is consequently unknown whether differences in the levels of these hormones account for the observed association between phosphate and cardiovascular events. Greater dietary phosphate intake in humans increases the levels of parathyroid hormone (PTH)\(^{10}\) and FGF23\(^{11}\) and suppresses calcitriol\(^{12}\); these changes are themselves all linked with cardiovascular disease by observational data: Higher PTH levels even within the normal range are associated with greater cardiovascular mortality in community populations\(^{13}\) and although associations with atheroma burden have been inconsistent\(^{14}\) PTH promotes a pro-atherogenic phenotype in cultured endothelial cells\(^{15}\). Lower levels of calcitriol predict cardiovascular mortality\(^{16}\) and administration of calcitriol has recently been reported to inhibit atherogenesis in ApoE\(^{-/-}\) mice\(^{17}\). Greater fibroblast growth factor 23 levels are associated with cardiovascular events\(^{18}\), atheroma burden\(^{19}\) and endothelial dysfunction\(^{20}\).

Direct toxic effects of phosphate on endothelial cells have been demonstrated in vitro at hyperphosphatemic levels seen in kidney failure\(^{21, 22}\), but whether this is relevant to the clinical associations with high-normal phosphate is unknown. Further work will be required to determine whether phosphate levels per se or accompanying changes in regulatory hormones are responsible for the acceleration of atherogenesis by dietary phosphate supplementation in our model.

The induction of insulin resistance by low dietary phosphate intake may reflect reduced glycolytic phosphometabolite synthesis due to intracellular phosphate depletion. Inhibition of glycolysis under conditions of phosphate depletion has been documented previously\(^{23}\) and a phosphate infusion increases the glucose disposal rate (insulin sensitivity) in healthy subjects during euglycemic insulin infusion\(^{24}\). The increased adiposity of mice fed a low phosphate diet may also have contributed to the insulin resistance in our model and is a new finding that parallels observed associations between lower phosphate and obesity in humans. Metabolic effects of phosphate
depletion are not confined to carbohydrate metabolism; hypophosphatemia also impairs lipid metabolism in rat cardiomyocytes\textsuperscript{25}, though effects on adipocytes have not yet been investigated. Mitochondrial oxidative phosphorylation has been shown to be modulated by phosphate concentration\textsuperscript{26} so may plausibly link lower phosphate with reduced basal energy consumption and greater weight gain.

The induction of hepatic steatosis in the low dietary phosphate group is consistent with a metabolic syndrome phenotype. Non-alcoholic fatty liver disease is associated clinically with the presence of obesity and insulin resistance; our findings may reflect either a pathologic consequence of insulin resistance or another effect of lower phosphate on hepatocyte lipid metabolism. Further work is needed to determine how dietary phosphate restriction promotes fatty liver disease and whether increasing phosphate exposure is of clinical benefit in selected patients with this pathology.

Supplementary References


Supplementary Figure Legends

Online Supplementary Data Table I: Effects of dietary phosphate on chow consumption and body mass index in ApoE<sup>−/−</sup> mice.

Online Supplementary Data Figure I: Effects of dietary phosphate intake on weight gain in ApoE<sup>−/−</sup> mice.

Online Supplementary Data Figure II: Effects of dietary phosphate intake on blood pressure in ApoE<sup>−/−</sup> mice.

Supplementary Data Table I.

<table>
<thead>
<tr>
<th></th>
<th>0.2%</th>
<th>0.6%</th>
<th>1.6%</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=6)</td>
<td>(n=8)</td>
<td>(n=5)</td>
<td>0.2% vs. 1.6%</td>
</tr>
<tr>
<td>Final BMI (kg/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>1.03(0.03)</td>
<td>0.94(0.03)</td>
<td>0.91(0.03)</td>
<td>NS</td>
</tr>
<tr>
<td>Final weight (g)</td>
<td>34.7(1.5)</td>
<td>30.3(1.03)</td>
<td>28.7(1.3)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Tail length (mm)</td>
<td>89.7(0.7)</td>
<td>90.0(1.3)</td>
<td>89.3(0.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Body length (mm)</td>
<td>183(1.9)</td>
<td>180(1.1)</td>
<td>177(1.4)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Daily chow consumption (g)</td>
<td>2.66(0.09)</td>
<td>2.51(0.07)</td>
<td>2.40(0.09)</td>
<td>NS</td>
</tr>
</tbody>
</table>
식이를 통한 인의 섭취량이 죽상경화증과 인슐린 저항성의 발생을 조절한다.

문 민 경 교수
서울대학교 보라매병원 내분비내과

Summary

목적
여러 역학 연구들에서 혈중 인과 FGF-23 (phosphatonin fibroblast growth factor 23)의 농도가 높은 수록 심혈관질환 및 죽상반의 발생이 증가함이 보고되었다. 또한 낮은 혈중 인 농도는 인슐린 저항성과 대사증후군과 연관이 있다. 연구자들은 mice 모델에서 식이 중 인의 섭취 조절이 죽상경화증의 발생 혹은 인슐린 감수성에 영향을 끼치는지 알아보고자 하였다.

방법 및 결과
아포지단백 E 결핍 mice (Apolipoprotein E knock-out mice)에 저인 식이(0.2%), 표준 식이(0.6%), 또는 고인 식이(1.6%) 세 단계로 구분한 죽상경화증 유발 식이를 시행하였다. 혈청 인과 FGF-23 농도는 식이 중 인 섭취가 증가함에 따라 유의하게 증가하였다. 그러나, 혈중 지질 농도와 혈압에 유의한 영향은 없었다. 20주 후 고인 식이를 시행한 mice에서 aortic sinus의 죽상반의 발생이 유의하게 증가하였다 (42±1.9% vs 30±1.5% for high vs low phosphate, П<0.01). 표준 식이나 고인 식이와 비교했을 때 저인 식이를 시행한 mice에서 지방조직이 더 많이 증가하였고, homeostatic model assessment에 의해 측정한 인슐린 저항성이 4배 증가하였다 (43.7±9.3 vs 8.9±0.7 for low vs high phosphate, П<0.005).

결론
고인 식이는 아포지단백 E 결핍 mice에서 죽상경화증의 발생을 증가시킨 반면 저인 식이는 인슐린 저항성을 유발하였다. 본 연구의 이러한 결과들은 식이 중 인 섭취의 조절이 죽상경화증과 대사증후군의 발생에 영향을 줄 수 있음을 보여 준다.
높은 혈중 인 농도가 만성 신질환 환자에서는 물론이고 신기능이 정상인 사람들에서도 심혈관질환 발생 증가와 관련되어 있다는 많은 연구 결과들이 있다. 생체에서 인은 수많은 세포 기능에 필수적인 요소로 어류에서 인간에게 이르기까지 모든 척추동물에서 체내 인을 보존하기 위한 시스템이 존재한다. 혈중 인의 농도를 조절하는 주된 호르몬은 부갑상선호르몬(PTH)과 골세포 및 조골세포에서 분비되는 FGF-23이다. 정상적으로 혈중 인의 농도가 상승하면 PTH와 FGF-23의 분비가 증가하고 이는 근위 세뇨관에서 sodium-dependent phosphate cotransporter NPT2a와 NPT2c의 발현을 감소시킴으로써 요장 인 배설량을 증가시켜 혈중 인 농도를 정상으로 유지한다. 이러한 FGF-23의 작용은 초기 만성 신질환에서 혈중 인 농도의 정상화에 기여하지만, 1,25-dihydroxyvitamin D의 생성을 감소시켜 PTH 분비를 증가시켜 결국 PTH 분비를 촉진함으로써 혈중 인 농도의 증가를 가져온다. 이러한 작용은 혈중 인 농도의 증가를 예측할 수 있다.

본 연구에서는 아포지단백 E 결핍 mice에 인 함량이 각각 0.2%, 0.6%, 1.6%인 저인 식이, 표준 식이, 고인 식이를 2주간 한 뒤 각 군에서 발생한 죽상반의 크기를 비교하였다. 고인 식이군에서 저인 식이군에 비하여 죽상반의 크기가 40% 증가하였다. 식이 중인 함량의 증가에 따라 혈중 인 농도가 유의하게 증가하였을 뿐 아니라 PTH의 증가, FGF-23의 증가 및 칼슘의 감소가 유의하였다. 이 연구에서는 식이 중인섭취량의 증가가 죽상경화증의 발생 원인임을 보여 주었으나, 죽상경화증의 유발에는 기전에 대한 연구는 이루어지지 않았다. 또한, 이 연구는 소수의 특정 동물 모델을 이용한 실험이 한계가 있다는 점을 고려해야 할 것이다. 통상적으로 mice의 식이는 사람에 비해 두 배 가량의 인 함량 차이가 나고, 실제 이 실험에서 저인 식이를 시행한 mice의 혈중 인 농도가 2.02mmol/L(6.25mg/dL)로 사람의 정상 혈중 인수치보다 훨씬 높았다. 혈중 인 농도의 증가는 다양한 기전으로 죽상경화증의 발생과 연관될 수 있다. 혈중 인 농도의 증가는 1,25-dihydroxyvitamin D의 합성 감소를 가져오며 혈중 인 농도의 증가는 심근 수축력 감소시키고 관상동맥의 석회화를 증가시킨다. 또한, 혈중 인 농도의 증가는 직접 혈관 손상을 유발한다는 보고도 있다. 소의 동맥 내피세포에 고인식이를 처리하면 NAD(P)H oxidase가 활성화되어 산화 스트레스의 발생이 증가하고, nitric oxide의 생성이 감소한다. 사람에서 고인 식이를 하게 되면 혈관내피세포 기능이 감소한다는 연구가 있다. 혈중 인 농도의 증가는 PTH의 증가를 유발하고 이는 IL-6 등의 생성을 증가시키고 염증을 유발할 수 있다. 또한, 심혈관질환 발생 기전으로 FGF-23의 역할을 생각할 수 있다. FGF-23 역시 만성 신질환 환자에서 심혈관질환 발생 및 사망의 독립적인 위험인자로 알려져 있다. FGF-23이 혈중 인 농도의 증가에 연관되어 있다는 보고도 있지만, FGF-23의 역할은 아직도 연구가 미루어 볼 때 심혈관질환의 발생, 증가, 치료에 대한 몇 가지 기전이 제시되고 있다.
가능성이 높다. 노화와 함께 신기능의 감소가 동반되므로 혈중 인과 FGF-23의 농도는 나이가 증가함에 따라 증가 될 것이고 이런 변화가 노화와 함께 증가하는 심혈관질환 발생에 기여할 수 있을 것으로 생각된다. 저인 식이 혹은 Phosphate binder에 의한 혈중 인의 감소가 심혈관질환의 발생 혹은 사망을 감소시키는데 기여할 수 있을지에 대한 연구가 기대된다.

한편 본 연구에서 또 하나 흥미로운 점은 인 섭취가 적을 경우 지방조직의 증가와 함께 인슐린 저항성이 나타났다는 것이다. 비슷한 결과로 정상혈당 클램프를 하는 동안 인산을 주입하면 인슐린 감수성이 증가한다는 보고도 있다. 제2형 당뇨병처럼 인슐린 저항성과 심혈관질환의 발생 위험이 공존하는 사람에게서 가장 적절한 혈중 인 농도를 결정하는 연구도 필요할 것으로 생각된다.

REFERENCES

Dietary Phosphate Modulates Atherogenesis and Insulin Resistance in Apolipoprotein E Knockout Mice—Brief Report

Timothy Ellam, Martin Wilkie, Janet Chamberlain, David Crossman, Richard Eastell, Sheila Francis, Timothy J.A. Chico

Objective—Epidemiological studies link higher serum phosphate and the phoshatonin fibroblast growth factor 23 with cardiovascular events and atheroma, and they link lower serum phosphate with insulin resistance and the metabolic syndrome. We investigated whether manipulating dietary phosphate influences atherogenesis or insulin sensitivity in mice.

Methods and Results—Apolipoprotein E knockout mice were fed an atherogenic diet with low (0.2%), standard (0.6%), or high (1.6%) phosphate content. Serum phosphate and fibroblast growth factor 23 significantly increased with increasing dietary phosphate intake, but lipid profile and blood pressure were unaffected. After 20 weeks, mice on the higher phosphate diet had significantly more atheroma at the aortic sinus (42±1.9% versus 30±1.5% for high versus low phosphate, P<0.01). Compared with standard and high-phosphate diet groups, mice on a low-phosphate diet had more adipose tissue and a 4-fold increase in insulin resistance measured by homeostatic model assessment (43.7±9.3 versus 8.9±0.7 for low versus high phosphate, P<0.005).

Conclusion—A high-phosphate diet accelerates atherogenesis in apolipoprotein E−/− mice, whereas low phosphate intake induces insulin resistance. These data indicate for the first time that controlling dietary phosphate intake may influence development of both atherosclerosis and the metabolic syndrome. (Arterioscler Thromb Vasc Biol. 2011;31:1988-1990.)

Key Words: atherosclerosis ■ insulin resistance ■ prevention ■ fibroblast growth factor 23 ■ phosphate

In human observational studies, higher serum phosphate levels within the normal range have been associated with a marked increase in cardiovascular events.1,2 Adverse outcomes linked to such elevations in serum phosphate include myocardial infarction,2 coronary artery disease,3 coronary calcification,4 and cardiovascular mortality.2 These associations are observed despite adjusting for recognized risk factors. The phosphate-regulatory hormone fibroblast growth factor 23, which increases in response to increased dietary phosphate intake, has similarly been linked to cardiovascular disease.6 Conversely, lower serum phosphate has been associated with insulin resistance and the metabolic syndrome,6 which are themselves risk factors for atherosclerosis.

It is unknown whether these observations reflect causal relationships between phosphate levels within the normal range and cardiovascular disease. Demonstration of a causal relationship would have profound public health implications, because Western diets are high in bioavailable phosphate as a consequence of phosphate-containing additives and high animal protein content.7

Methods

An expanded Methods section is given in the supplemental material, available online at http://atvb.ahajournals.org.

Animals and Diets

Eight-week-old male apolipoprotein E knockout mice were randomly assigned to atherogenic diets (21% fat, 0.2% cholesterol, 0.03% cholate) with low (0.2%), standard (0.6%), or high (1.6%) phosphate content. Groups were fed the test diets for 12 weeks (group 1, n=14) and 20 weeks (group 2, n=19, 5 to 8 per diet) before euthanization under pentobarbital anesthesia for cardiac puncture and tissue collection.

Atheroma Analysis

Atheroma burden was quantified using morphometric analysis in cross-sections through the aortic sinus at the level of the aortic valves and stained with Alcian blue/elastic van Gieson. Calcification was assessed by von Kossa stain, and smooth muscle cell and macrophage content was assessed by immunostaining.
comes linked to such elevations in serum phosphate include marked increases in cardiovascular events. Adverse outcomes include increased cardiovascular disease, renal disease, and increased serum parathyroid hormone. The phosphate-regulatory hormone fibroblast growth factor 23, which increases in response to increased dietary phosphate content, is available at http://atvb.ahajournals.org DOI: 10.1161/ATVBAHA.111.231001

**Objective**

We investigated whether manipulating dietary phosphate influences atherogenesis or insulin sensitivity in Apolipoprotein E Knockout Mice—Brief Report

**Methods**

Blood samples were collected 12 weeks after dietary phosphate supplementation. Serum phosphate and fibroblast growth factor 23 significantly increased with higher phosphate intake. Phosphate deprivation in rats, attributed to increased hepatic glucose output, demonstrated to induce insulin resistance, adiposity, or steatosis.

**Results**

Increased dietary phosphate intake caused a significant increase in serum phosphate, parathyroid hormone, and fibroblast growth factor 23 and a small but significant reduction in serum calcium (Figure 1A and Table). The low-phosphate diet increased weight gain, but there were no significant differences in blood pressures, lipid profile, urea, or total chow consumption (see supplemental material).

Increased dietary phosphate intake had no effect on aortic sinus atheroma at 12 weeks but was associated with significantly more atheroma at 20 weeks (Figure 1A and 1B): 30±2%, 33±2%, and 42±2% for low-, standard-, and high-phosphate groups, respectively (P<0.01 for low versus high), corresponding to a 40% increase in atheroma burden for the high versus low dietary phosphate groups. Calcification was absent by von Kossa staining, and lesions did not differ in vascular smooth muscle cell or macrophage content (data not shown).

**Discussion**

This is the first interventional study demonstrating an athero
genetic effect of dietary phosphate supplementation. In keeping with human observational data, atheroma burden was least in the low-phosphate group despite the induction of insulin resistance. Modulation of components of the phosphate homoeostatic axis (fibroblast growth factor 23, parathyroid hormone, calcitriol) could be responsible for this accelerated atherogenesis. Phosphate itself has been implicated as an endothelial toxin, and postprandial peaks may lead to accumulation of endothelial damage that over time predisposes to atherosclerosis.

Low dietary phosphate intake has not previously been demonstrated to induce insulin resistance, adiposity, or steatosis. Xie et al reported increased plasma glucose after phosphate deprivation in rats, attributed to increased hepatic glucose export. We observed induction of insulin resistance measured by homeostatic model assessment, in keeping with the observational population data. This may be a consequence of increased adiposity, impaired phosphometabolite synthesis, or another mechanism.

In summary, we have demonstrated causal relationships between increased dietary phosphate and atherosclerosis, without significant alteration of lipid levels, and between low dietary phosphate and insulin resistance. Given the substantial increases in cardiovascular disease associated observationally with higher phosphate and the massive global burden

**Table. Plasma Biochemistry Results and Weight by Dietary Phosphate Group at 20 Wk**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Dietary Phosphate Group</th>
<th></th>
<th></th>
<th>P Value, 1.6% vs 0.2%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate, mmol/L</td>
<td>0.2%</td>
<td>2.02 (0.28)</td>
<td>3.11 (0.20)</td>
<td>3.59 (0.23)</td>
</tr>
<tr>
<td>Calcium, mmol/L</td>
<td>0.2%</td>
<td>2.27 (0.04)</td>
<td>2.16 (0.05)</td>
<td>2.07 (0.01)</td>
</tr>
<tr>
<td>PTH, ng/L</td>
<td>0.2%</td>
<td>26 (5)</td>
<td>100 (17)</td>
<td>225 (58)</td>
</tr>
<tr>
<td>FGF23, ng/L</td>
<td>0.2%</td>
<td>58 (18)</td>
<td>127 (12)</td>
<td>409 (98)</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>0.2%</td>
<td>33.5 (1.8)</td>
<td>33.7 (2.2)</td>
<td>35.3 (2.1)</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>0.2%</td>
<td>6.47 (0.20)</td>
<td>6.34 (0.43)</td>
<td>6.26 (0.35)</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>0.2%</td>
<td>23.6 (1.5)</td>
<td>24.8 (1.7)</td>
<td>25.4 (1.6)</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>0.2%</td>
<td>7.60 (0.98)</td>
<td>5.68 (0.70)</td>
<td>8.16 (1.26)</td>
</tr>
<tr>
<td>Final weight, g</td>
<td>0.2%</td>
<td>34.7 (1.5)</td>
<td>30.3 (1.0)</td>
<td>28.7 (1.3)</td>
</tr>
</tbody>
</table>

PTH indicates parathyroid hormone; FGF, fibroblast growth factor; NS, not significant; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

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**Figure 1. A, Aortic sinus atheroma burden as percentage of cross-sectional area. *P<0.05, **P<0.01, n=5 to 8 per group for all comparisons. B, Representative aortic sinus lesions.**
of cardiovascular disease, these findings are potentially of major significance, particularly because interventions to lower serum phosphate (dietary restriction and oral phosphate binders) are already available.

Further discussion is available in the supplemental material.

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Disclosures
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References