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# ROLE OF CHOLESTEROL IN THE REGULATION OF RENAL PHOSPHATE TRANSPORT

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## 1. ABSTRACT

The kidney plays a critical role in the regulation of inorganic phosphate (Pi) homeostasis through changes in the proximal tubular apical membrane Na-dependent Pi (Na/Pi) transport activity. In response to alterations in dietary Pi intake and during the aging process, changes in renal Na/Pi transport activity are inversely correlated with apical membrane cholesterol content. Cholesterol regulates Na/Pi transport activity by fluidity-dependent and fluidity-independent mechanisms, including regulation of Na/Pi protein transcription, synthesis, and trafficking to and from the plasma membrane.

## 2. INTRODUCTION

Lipids play an essential structural role as a barrier between intra and extracellular medium and as a matrix providing a suitable hydrophobic environment for membrane proteins. The functional role of lipids as a selective barrier to fully or moderately water soluble compounds, and as modulators of membrane transport proteins, enzymes, and channels activity have been well recognized (1-4).

In this report we will discuss the evidence that has been obtained during the past decade which indicates an important role for alterations in renal proximal tubular cell membrane cholesterol content in the regulation of renal phosphate transport activity.

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The kidney plays a critical role in the regulation of inorganic phosphate (Pi) homeostasis. Pi is freely filtered across the glomerules, and most of the Pi is reabsorbed along the proximal tubule via a sodium gradient-dependent process (Na/Pi co-transport). The Na/Pi cotransporter is located on the apical brush border membrane (BBM) of the proximal tubule. The evidence, to date, indicates that regulation of the overall renal tubular Pi transport by dietary, hormonal, or metabolic factors occurs at the level of the proximal tubular BBM Na/Pi cotransport system (5-8).

## 3. ROLE OF CHOLESTEROL IN THE REGULATION OF RENAL PHOSPHATE TRANSPORT

The first evidence that alterations in BBM cholesterol content may modulate Na/Pi cotransport activity was provided by Molitoris *et al* (9) who showed that in rats chronically fed a low Pi diet the adaptive increase in the renal tubular reabsorption of phosphate was associated with a decrease in BBM cholesterol content. In fact, the authors demonstrated an inverse linear relationship between the tubular reabsorption of Pi and BBM cholesterol content. Since a decrease in BBM cholesterol also resulted in an increase in BBM fluidity, this study suggested that either a decrease in BBM cholesterol content or an increase in BBM fluidity could play a role in the regulation of Na/Pi transport. Subsequent studies with isolated BBM and/or renal tubular cells grown in culture demonstrated that a direct increase in membrane fluidity indeed results in an increase in Na/Pi cotransport activity (10-12). Further studies in our laboratory confirmed the findings of Molitoris *et al*, and also showed that in aged rats the decrease in the renal tubular reabsorption of Pi was associated with an increase in BBM cholesterol content (Figure 1) and a decrease in BBM fluidity (13).

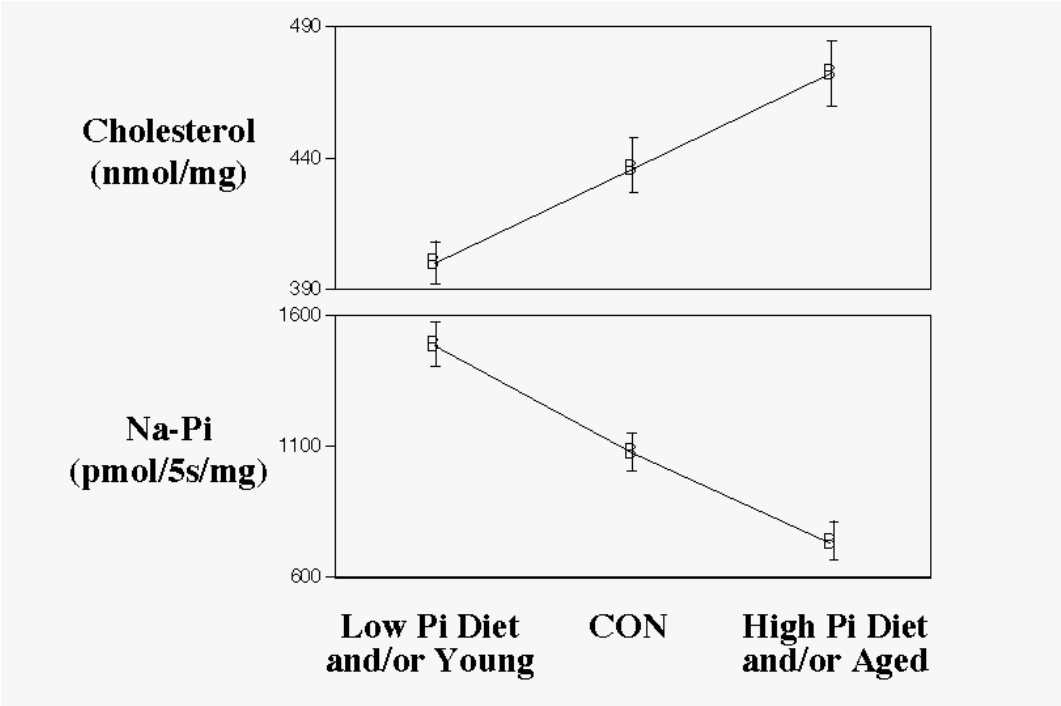


**Figure 1:** There is an inverse relationship between BBM Cholesterol content and BBM Na/Pi cotransport activity in young versus aged rats and in rats fed a low Pi versus a high Pi diet. Data adapted from References 13 and 14.

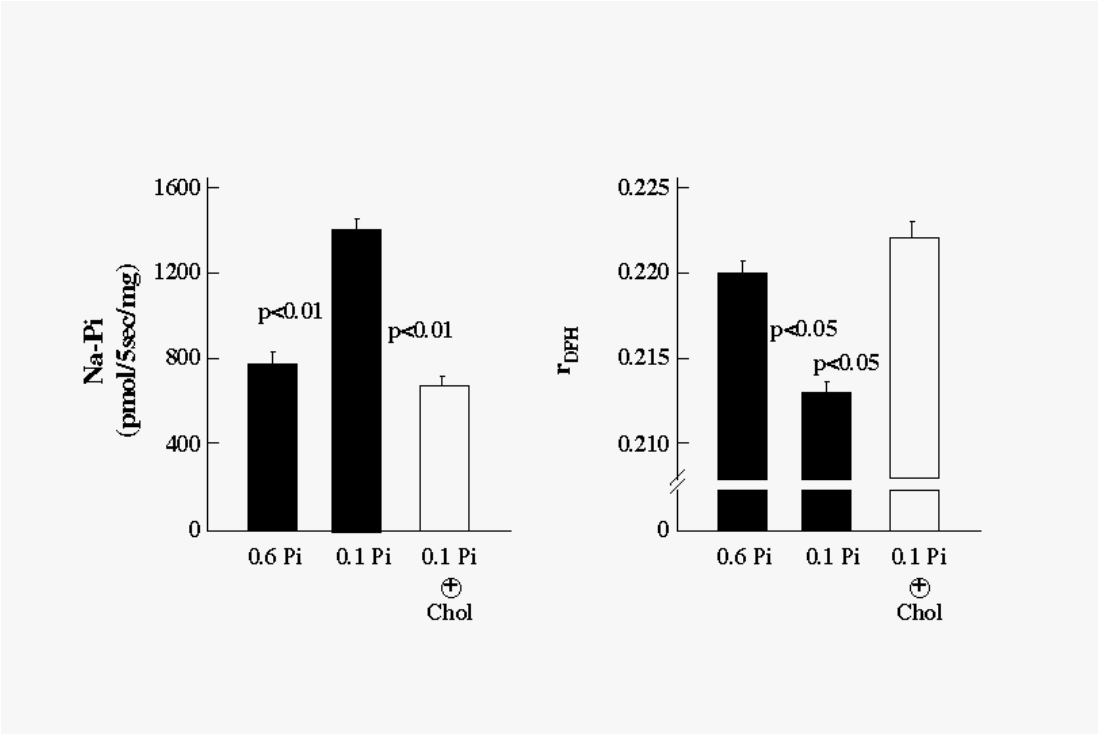


**Figure 2:** In rats which have been fed a low Pi diet, *in vitro* enrichment of the isolated BBM with cholesterol, to levels found in rats fed a normal Pi diet, reverses the adaptive increases in BBM Na/Pi cotranport activity (right panel) and BBM fluidity (inversely related to the fluorescence polarization of diphenylhexatriene, $r_{DPH}$, left panel). Adapted from Reference 14.

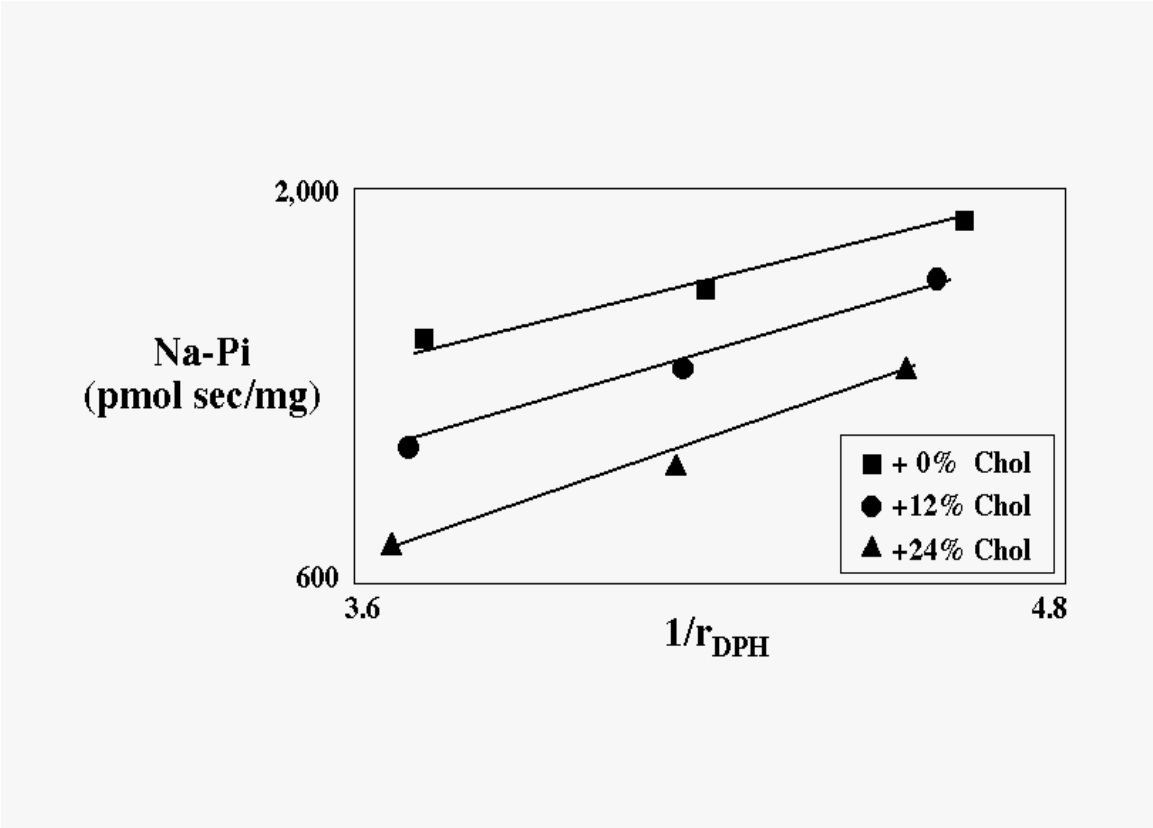


**Figure 3:** Cholesterol modulates BBM Na/Pi cotransport activity both by membrane fluidity dependent and independent mechanisms. Increasing membrane fluidity causes an increase in Na/Pi cotransport activity. However, even at an identical level of membrane fluidity, increasing cholesterol causes a decrease in Na/Pi cotransport activity. Adapted from Reference 14.

In studies designed to determine if changes in BBM cholesterol content *per se* do play a role in the modulation of BBM Na/Pi cotransport (14), we first showed that in BBM isolated from young rats chronically fed a low Pi diet *in vitro* enrichment with cholesterol, to levels present in young rats chronically fed a high Pi diet, completely reversed the adaptive increase in Na/Pi cotransport activity (Figure 2).

In subsequent studies, we showed that in BBM isolated from young rats *in vitro* enrichment with cholesterol, to levels present in aged rats, reproduced the age-related decrease in Na/Pi cotransport activity (Figure 3). Of interest, this effect of cholesterol was specific and selective for Na/Pi cotransport, as cholesterol enrichment had no effect on BBM Na/glucose, Na/proline, and Na/sulfate cotransport, or Na/H exchange activities (14).

## 4. CELLULAR MECHANISMS AS HOW CHOLESTEROL MODULATES RENAL PHOSPHATE TRANSPORT

Since changes in BBM cholesterol content causes simultaneous changes in BBM fluidity (15,16), in the next series of studies we tried to determine if changes in BBM cholesterol content *per se*, or changes in BBM fluidity, or both modulate Na/Pi cotranport activity. In our studies, this was achieved by first modulating BBM cholesterol content *in vitro*, and then measuring BBM fluidity and BBM Na/Pi transport activity at different temperatures. Our measurements indicated that there was a direct correlation between an increase in BBM fluidity and an increase in BBM Na/Pi cotransport activity. However, at a given level of BBM fluidity, increasing BBM cholesterol content still caused a decrease in BBM Na/Pi cotransport activity (Figure 3). The data, therefore, indicates that perturbations in BBM cholesterol content and/or BBM fluidity each, independent of each other, modulate BBM Na/Pi cotransport activity.

In these studies BBM fluidity was measured by the steady-state fluorescence polarization of 1, 6-diphenyl 1,2,5,-hexatriene (DPH), which measures the time-averaged structural and dynamic properties which determine the relative order and motions of the lipid molecules in the membrane bilayer (17). In subsequent studies, when we have also performed time resolved measurements with DPH, and steady-state and time-released measurements with the phase sensitive probe 6-dodecanoyl-2-diethylamino-naphthalane (Laurdan)

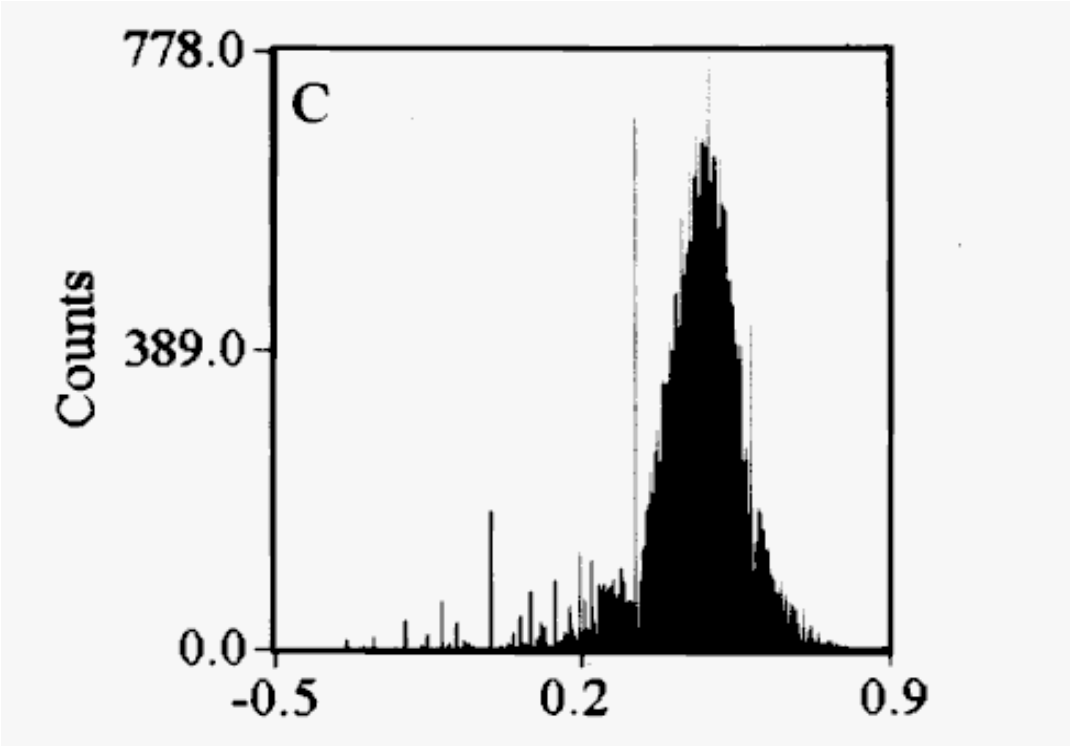


**Figure 4:** Generalized Polarization (GP) histogram of BBM labeled with Laurdan. Excitation GP is calculated according to GP = ($I_{440}$ - $I_{490}$/$I_{440}$ + $I_{490}$), where $I_{440}$ and $I_{490}$ are the fluorescence emission intensities at 440 and 490 nm. Adapted from reference 23.

( 15, 18, 19, 20), we have found evidence for the presence of lipid microdomains in the BBM. In fact, in recent studies using the two-photon fluorescence microscopy of Laurdan (21-23), we have provided evidence for the presence of lipid domains within the BBM lipid bilayer of differing fluidity values (Figure 4). We have further shown that cholesterol strongly modulates the distribution of these lipid domains. It is therefore possible that cholesterol modulates BBM Na/Pi cotransport, in part, by influencing the distribution of lipid microdomains surrounding the Na/Pi cotransporter proteins, which in turn could modulate their transport function by influencing their diffusion within the BBM lipid bilayer. Alternatively, cholesterol could also modulate Na/Pi cotransport activity by direct biochemical modification of the Na/Pi cotransporters.

The studies outlined above indicate that cholesterol regulates BBM Na/Pi cotransport activity by modulating the activity of the preexisting Na/Pi cotransporters. However, it is possible that cholesterol could also modulate Na/Pi cotransport activity by regulating the *de novo* transcription, synthesis, and translocation of the Na/Pi cotransporters to the apical membrane and/or internalization from the apical membrane and eventual degradation (Table 1). In fact, we have recently shown that chronic adaptation to a low Pi diet, which is characterized by an increase in BBM Na/Pi transport, is associated with parallel increases in BBM Na/Pi transporter protein and cortical Na/Pi mRNA abundance (24). In addition, we have also shown that the age-related decrease in BBM Na/Pi transport is associated with parallel decreases in BBM Na/Pi protein and cortical Na/Pi mRNA abundance (25).

The major question is whether the decrease in renal cholesterol content during chronic dietary Pi deprivation and/or the age-related increase in renal cholesterol content can modulate Na/Pi cotranport activity through transcriptional and translational regulation. Recent studies indicate that alterations in cholesterol content control cellular functions by diverse mechanisms. In human HeLa cells and fibroblasts, a decrease in membrane cholesterol induces proteolysis of a membrane-bound transcription factor and activates transcription of the genes for the LDL receptor and HMG CoA synthase (26-27). Cholesterol also regulates endothelin gene expression in endothelial cells (28). In rats, a high cholesterol diet causes increases in the glomerular transforming growth factor β (TGF-$\beta_1$) and fibronectin mRNA levels (29). In MA 104 cells, a monkey kidney epithelial cell line, lowering the cell cholesterol content decreases the clustering of the GPI-anchored folate receptors and inhibits receptor-mediated transport of folate (30). Cholesterol also

**TABLE 1. MECHANISMS HOW CHOLESTEROL COULD MODULATE RENAL PHOSPHATE TRANSPORT**

| | |
|---|---|
| 1. | Transcriptional control and/or mRNA stability |
| 2. | Synthesis and targeting (exocytosis) of the Na/Pi transporter to the apical membrane |
| 3. | Internalization (endocytosis) of the Na/Pi transporter from the apical membrane and its degradation |
| 4. | Lateral and/or rotational diffusion of the Na/Pi transporter in the apical membrane lipid bilayer |
| 5. | Localization of the Na/Pi transporter within specific lipid domains |
| 6. | Direct chemical modification of the Na/Pi transporter or modification by lipid- modulated kinases and phosphatases |

modulates the apical membrane domain formation and internalization of alkaline phosphatase, another GPI-anchored protein (31).

## 5. SUMMARY

In summary, in addition to the chemical and physical effects on the Na/Pi cotransport proteins at the level of the plasma membrane, in intact renal tubular cells cholesterol could also regulate Na/Pi cotransport activity by modulating Na/Pi mRNA level and/or BBM Na/Pi protein abundance (Table 1).

Further studies in renal tubular cells grown in culture should elucidate the cellular and molecular mechanisms how perturbations in cholesterol metabolism and cholesterol content modulate Na/Pi cotransport activity.

## 6. ACKNOWLEDGMENTS

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