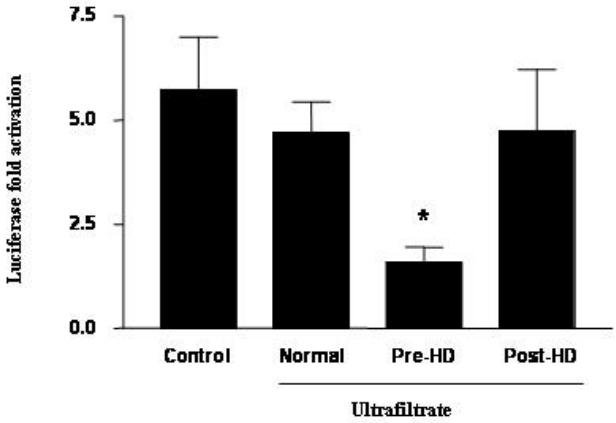

Figure 7
Uremic toxin (s) impair (s) T_3-dependent transcriptional activation in U937 cells. A reporter construct consisting of two copies of a direct repeat thyroid response element (DR-4) 5'AGGTCAcaggAGGTCA 3' cloned upstream from the minimum thymidine kinase (TK) promoter, linked to the luciferase gene was examined in U937 cells. After electroporation, cells were transferred to fresh RPMI-1640 medium without (Control) or with normal or uremic ultrafiltrate solution, collected before (Pre-HD) or after hemodialysis (Post-HD). The cells were then plated in 12-well dish and treated with T_3 10^{-7}M. After 24 h, cells were assayed for luciferase and β-galactosidase activities. * $p < 0.05$ versus control and normal ultrafiltrate.

reduced peripheral tissue sensitivity to thyroid hormone [17]. Recent data from our laboratory indicate that in order to maintain the euthyroid state showed that uremia increases T_3 influx across erythrocyte's membrane [35]. Taken together, these findings suggest that CRF affects thyroid function in multiple ways. The molecular mechanisms involved and the role of thyroid hormone receptor in this dysfunction, however, are not fully understood.

In the present study we observed that uremic plasma impaired the ability of TRβ1 and VDR heterodimers (TRβ1-RXRα and VDR-RXRα) to bind to DNA (DR-4 and DR-3 respectively), whereas that the ability of PPARγ-RXRα to bind to DNA (DR-1) was not altered. Interestingly, there was no correlation between the inhibitory activity and the plasma levels of urea, creatinine, parathyroid and thyroid hormone of the patients enrolled in this study. However, the small number of patients precludes any definitive conclusions.

To investigate whether these findings were secondary to the presence of uremic dialyzable toxins, we compared the effect of uremic plasma collected before and after hemodialysis, on TRβ1-RXRα or VDR-RXRα binding to DNA. Our results showed that the inhibitory effect of uremic plasma was significantly reduced by hemodialysis, suggesting that dialyzable toxins were in fact involved. We did not identify which toxin is responsible for this effect, but our results suggest the presence of thermo-resistant molecule(s). Further analyses of these dialyzable toxins are currently being conducted to identify and characterize the molecules responsible for this inhibitory effect.

The mechanisms responsible for our findings are not clear. In uremic syndrome the reduced clearance of many toxins plays a key role in this pathogenesis. Although VDR degradation has been suggested in renal failure [36], the uremic inhibition of TRβ1-RXRα binding to DNA could not be explained by proteolytic activity of uremic plasma since our SDS-PAGE did not show any uremic plasma-dependent degradation of TRβ1.

Our results are in agreement with other studies, which have shown that uremic toxins are involved in VD_3 resistance observed in patients with chronic renal failure [37]. Uremic ultrafiltrates derived from hemo or peritoneal dialyzed patients have been shown to inhibit the interaction of VDR with DNA [27,28]. Further studies showed that the VDR complex formation on different types of VDREs can be reduced by uremic solutions collected from patients after hemo or peritoneal dialysis [28]. Our results allow us to speculate on the possibility of a common inhibitory mechanism involving the same uremic toxin(s) inhibiting both TR-RXR-DR-4 and VDR-RXR-DR-3 complex formation.

To evaluate whether uremic toxins also affect other members of the nuclear receptor family, we studied the effect of uremic plasma on PPARγ-RXRα binding to DR-1. Contrary to what we observed with TRβ1 and VDR, pre-incubation of PPARγ with uremic plasma did not influence PPARγ-RXRα binding to DNA. This result suggests that the inhibition of protein-DNA complex caused by uremic plasma occurs only with some nuclear receptors. Taken together, our results indicate that uremic toxins exert their inhibitory effect by acting specifically on TRβ1 and VDR heterodimers.

The molecular mechanism involved in this phenomenon is not clear. The fact that PPARγ-RXRα heterodimer was not affected by uremic plasma suggests that these toxins do not interact directly with RXRα. Another possible model to explain the effects of the toxins from uremic plasma on the binding of TR to DNA would be a direct action on DNA that would block its interaction with TRβ1 and VDR heterodimers. However, even though we used the DR-1 in PPARγ assay, in contrast to DR-4 (TRE) and