

# Zinc Deficiency Suppresses the PI3K/Akt Survival Pathway and Enhances Apoptosis in Differentiated Human Lung Epithelial Cells

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**Introduction:** Intracellular zinc deficiency has been identified as a predisposing factor to lung epithelial damage. In this investigation we determined that zinc deficiency does not directly augment formation of reactive nitrogen species (RNS) during inflammatory stress but does down-modulate activity of the PI3K/Akt survival pathway thereby increasing the susceptibility of the lung epithelium to apoptosis.

**Hypothesis:** Zinc deficiency reduces Akt kinase activity and enhances death receptor-activation in the lung epithelium resulting in enhanced cellular apoptosis and breakdown of barrier function.

**Methods:** Cultures from multiple donors were exposed to a combination of biologically relevant factors associated with lung epithelial injury (IFN $\gamma$  250 U/ml, TNF $\alpha$  100 ng/ml, and a Fas cross-linking antibody (FasAb) 200 ng/ml) in the presence or absence of supplemental zinc. Transepithelial resistance (Rt) was measured across culture inserts with a portable ohmmeter. Apoptosis was detected immunohistochemically by cytoplasmic localization of caspase cleaved cytokeratin-18. Barrier function and paracellular transport were measured by Lucifer Yellow flux. Akt activity was measured by phospho-Akt blotting and in vitro kinase activity. RNS were measured as an azo-dye product of the Greiss reaction.

**Results:** Exposure to IFN $\gamma$ , TNF $\alpha$  and FasAb resulted in a four-fold increase in RNS formation and a moderate increase in apoptosis (10-20%). Apoptosis, but not RNS formation, was substantially increased in zinc deficient cells (50-70%) resulting in an abrupt decrease in Rt and increase in paracellular leak. Zinc deficiency also lead to a concomitant decrease in Akt function. Addition of Zinc sulphate rescued cells from apoptosis and restored Akt activity.

**Conclusions:** From our findings we conclude that subacute zinc deficiency may predispose patients to increased lung injury by down-regulating the Akt survival pathway.

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