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ATF4 is a key molecule linking food intake and skeletal development

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RSK2 is a ribosomal kinase that is inactivated in Coffin Lowry Syndrome (CLS), a mental retardation disease with skeletal manifestation that worsens over time. It has been shown that one mechanism whereby RSK2 favors skeletal development and bone formation is by phosphorylating ATF4, a transcriptional factor that itself regulates osteoblast differentiation during development and favors bone formation and osteoclast differentiation post-natally[1]. ATF4 regulates these various processes through two mechanisms, by favoring expression of some genes and by enhancing amino acid import in osteoblasts[1]. To determine the biological importance and medical relevance of this latter mode of action of ATF4 we fed pregnant *Atf4*+/- and *Rsk2*+/- mothers with either a normal diet (ND), a high fat diet (HFD) or a high protein diet (HPD). Subsequently *Atf4*- and *Rsk2*-deficient pups were kept on the same diet as their mothers received until one month of age. Remarkably HPD rescued the perinatal lethality of *Atf4*-/- mice, it also restored protein synthesis as measured by phosphorylation of GCN2 and the α subunit of eukaryotic translation initiation factor 2 in both *Atf4*-/- and *Rsk2*-/- bones as well as by determining collagen synthesis in bones. Consequently bone formation parameters were normalized and bone mass was identical between wild type, *Atf4*-/- and *Rsk2*-/- mice fed a HPD (Figure 1). Surprisingly, bone mineralization and osteoblast genes expression as analyzed by histological means and *in situ* hybridization was also corrected in *Atf4*-/- embryos. Two arguments indicate that this effect of HPD is specific of the function of the RSK2-ATF4 signaling pathway; first a HFD has no effect on bone formation parameters and bone mass, second a HPD did not affect any aspect of the phenotype in the mice lacking *Runx2*-/- and *Osterix*-/-, the crucial genes for osteoblasts' differentiation[2,3].

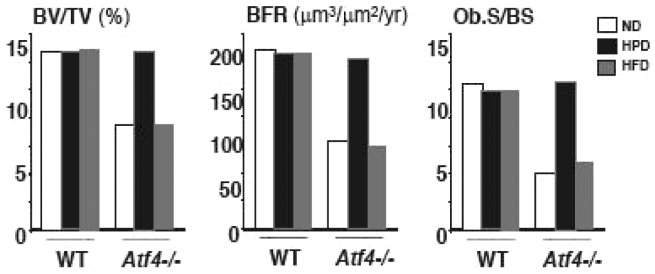

Figure 1. High protein diet (HPD) corrects bone formation in *Atf4*-deficient mice. Decreased bone volume/tissue volume (BV?TV), osteoblast surface/bone surface (Ob.S/BS), bone formation rate (BFR, $\mu m^3/\mu m^2$/year) and osteoblast surface/bone surface (Ob.S/BS) were normalized with HPD but not high fat diet (HFD) (n=6, p=0.05).

On the other hand, Neurofibromatosis type I (NF1) is a disease caused by loss of function mutations in *NF1*, a gene encoding the Ras GAP (GTPase Activating Protein) neurofibromin, characterized by nerve tumors and skeletal abnormalities. We showed that in contrast, RSK2 activity, ATF4-dependent collagen synthesis as well as osteoblast number, bone formation ratio and bone mass are increased in mice lacking neurofibromin in osteoblasts ($Nf1_{ob}$-/- mice)[4]. Furthermore, transgenic mice overexpressing *Atf4* specifically in osteoblasts (*α1(I)-Atf4*), displayed increased osteoblast numbers, bone formation ratio and bone mass, which are the same phenotypes observed in $Nf1_{ob}$-/- mice. In agreement with ATF4 function in amino acid import, a low protein diet (LPD) decreased bone protein synthesis, normalized bone

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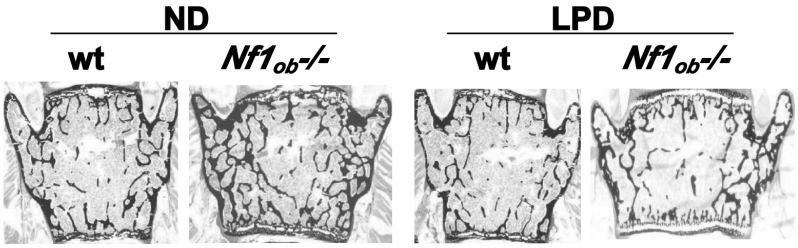

	ND wt	ND Nf1ob-/-	LPD wt	LPD Nf1ob-/-
BV/TV	15.3±1	19.6±0.4*	14.8±0.7	15.3±0.6
BF	153.3±11	313.9±7.0*	157.0±11	186.2±22
ObS/B	19.1±0.8	31.8±1.6*	19.0±0.5	19.7±1.0

Figure 2. Low protein diet (LPD) corrects bone formation of the mice lacking *Nf1* in osteoblasts ($NF1_{ob}$-/-). BV/TV. BFR and Ob.S/BS in WT and $NF1_{ob}$-/- bones under ND or LPD (n=6, $p<0.05$).

formation and bone mass in $Nf1_{ob}$-/- (Figure 2) and *α1(I)-Atf4* mice without affecting other organ weight. In addition, increased bone resorption parameters in both mice were not affected with the low protein diet, suggesting LPD corrects bone formation specifically.

In summary, these results illustrate that a simple diet manipulation could alleviate some of the symptoms in patients with genetic bone diseases. They also suggest that the knowledge of the molecular mode of action of a gene involved in a given genetic disease can have therapeutic consequences and provide evidence for a link between food intake and skeletal development.

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