LOW EXTRACELLULAR SODIUM PROMOTES ADIPOGENIC COMMITMENT OF HUMAN MESENCHYMAL STROMAL CELLS: A NOVEL MECHANISM FOR CHRONIC HYPONATREMIA-INDUCED BONE LOSS

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BACKGROUND

Hyponatremia represents an independent risk factor for osteoporosis and fractures, affecting both bone density and quality. A direct stimulation of osteoclastogenesis and bone resorption in the presence of reduced extracellular sodium concentrations ([Na^+]) has been shown, but, to date, the effects of reduced [Na^+] on osteoblasts have not been elucidated.

This study investigated the effects of a chronic reduction of extracellular [Na^+], independently of osmotic stress, on human mesenchymal stromal cells (hMSC) from bone marrow, which represent the common progenitor for osteoblasts and adipocytes.

MATERIALS AND METHODS

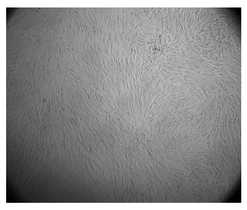

Fig 1: hMSC isolated from the iliac crest of normal donors' marrow aspirates (magnification 5x)

[Na]+	NaCl (g/L)	Mannitol (g/L)
153 mM	5.508	-
147 mM	5.16	2.18
141 mM	4.8	4.328
136 mM	4.51	6.198
127 mM	3.988	9.428
121 mM	3.64	11.62
115 mM	3.288	13.844
100 mM	2.86	19.308
90 mM	2.402	22.952

Fig 2: Growth media with low [Na^+].
We used 2x DMEM without sodium and glucose to prepare growth media with different [Na^+]: 153 (i.e., [Na^+] of standard DMEM), 127, 115 and 90 mM. Other parameters were maintained identical to the standard DMEM and medium osmolality was adjusted to reach 307.8 mOsm/Kg H_2O by adding appropriate amounts of mannitol.

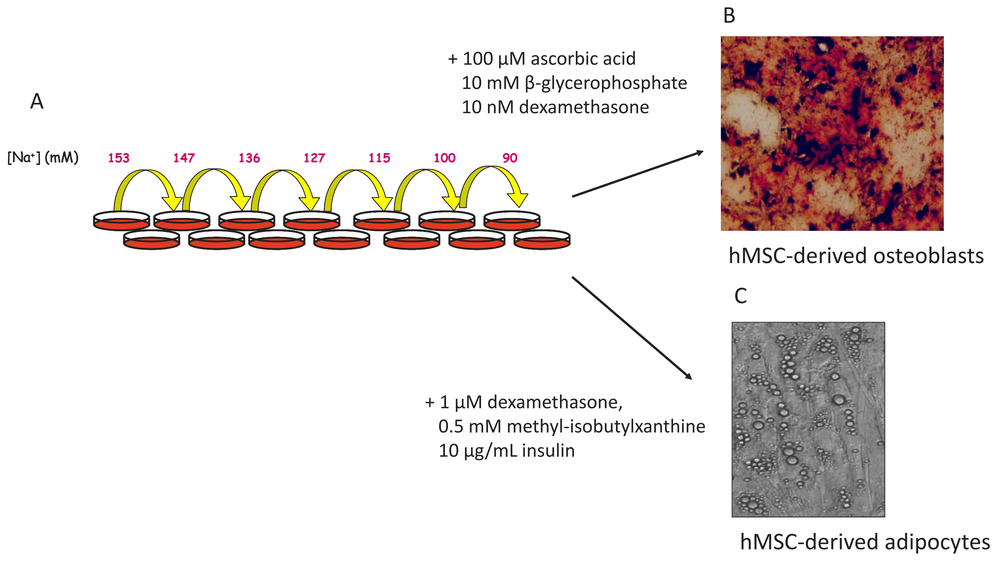

Fig 3: *In vitro* model of chronic hyponatremia.
Extracellular [Na^+] was progressively lowered by daily medium changes, in order to adapt cells to electrolytic variations (A). After exposure to selected [Na^+] for 7 days, cells were treated with specific differentiating cocktails (B, C).

RESULTS

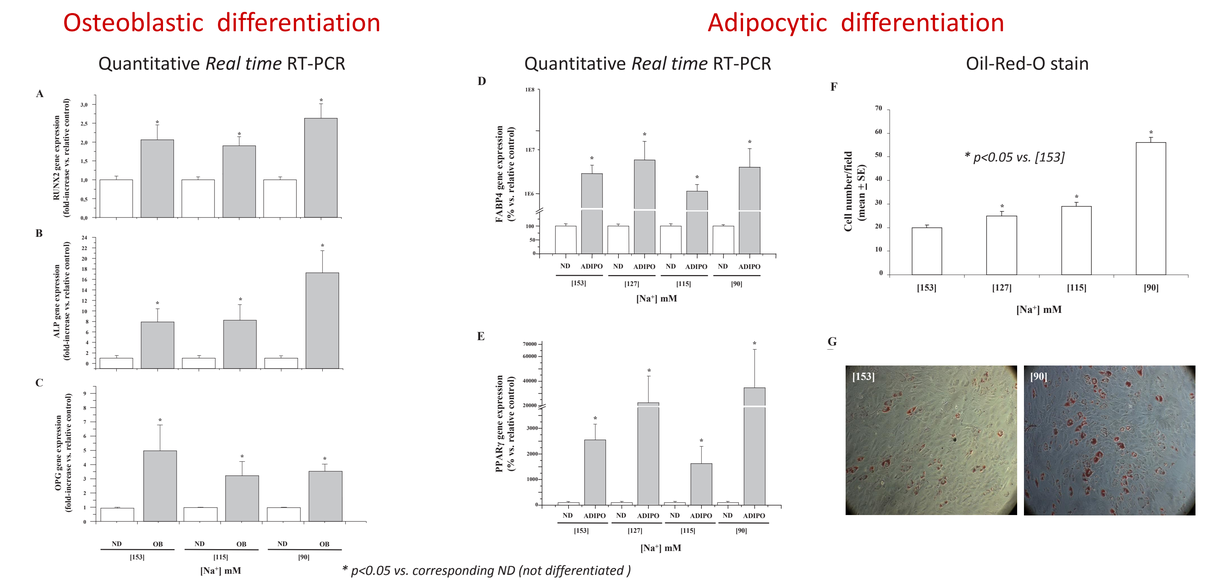

Fig 4: Effects of low extracellular [Na^+] on hMSC commitment toward the osteogenic and adipogenic phenotype. At all [Na^+] tested the gene expression of the osteogenic markers RUNX2 (A), ALP (B) and OPG (C) significantly increased after induction of hMSC to osteogenic differentiation. In differentiated cells, the expression of the three markers was not altered by exposure to low [Na^+]. Similar results were obtained for the markers FABP4 (D) and PPARγ (E) after adipogenic induction. This finding indicates that chronic hyponatremia does not alter the differentiation potential of these cells. In addition, we performed a functional evaluation of the effect of reduced [Na^+] on adipogenic differentiation of hMSC by Oil-Red-O stain (G) and adipose cell count by optical microscopy (F). The dose-dependent increase in the number of adipocytes as a function of reduced extracellular [Na^+] suggests a preferential commitment toward the adipogenic phenotype. Magnification 5x.

Cytoskeleton analysis by immunofluorescence

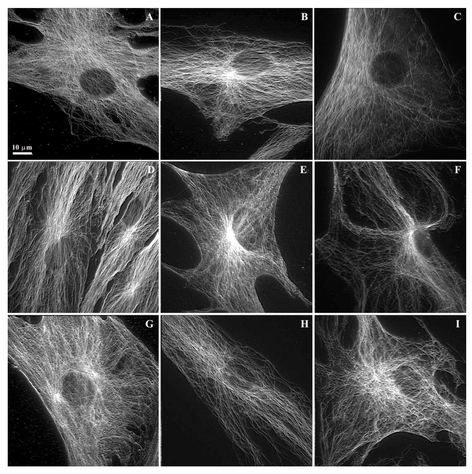

Fig 5: Effects of low extracellular [Na^+] on hMSC cytoskeletal remodeling.
In control cells microtubules (MTs) originated from the MTs organization centre, close to the nucleus, and pointed towards the plasma membrane (A). Cells cultured at low [Na^+] (115 and 90 mM) had a tangled, cage-like MTs network with tortuous MTs (B and C). hMSC induced to differentiate towards osteoblasts at a normal [Na^+] showed a radial arrangement of MTs from the organization centre to the plasma membrane (D). In cells cultured at low [Na^+] before osteoblastic induction, MTs dynamics was significantly altered: at both [Na^+] of 115 and 90 mM a cage-like organization of MTs was observed (E and F). Moreover, at the lowest [Na^+] the MTs decreased significantly, leaving large gaps at the cell periphery. Since it has been reported that MTs disruption allows osteoblast differentiation but altered MTs architecture may affect cell function (in particular the secretion of matrix molecules), these alterations might affect bone quality. Also in hMSC-derived adipocytes differentiated in low [Na^+] (H and I) an alteration and disruption of MTs was observed. However, considering that MTs disruption during adipogenesis is needed for lipid droplets growth and that mature adipocytes present a reduced MTs network, we can hypothesize that the consequences of low [Na^+]-induced MTs alteration might not have an effective impact on adipogenesis. Magnification 100x.

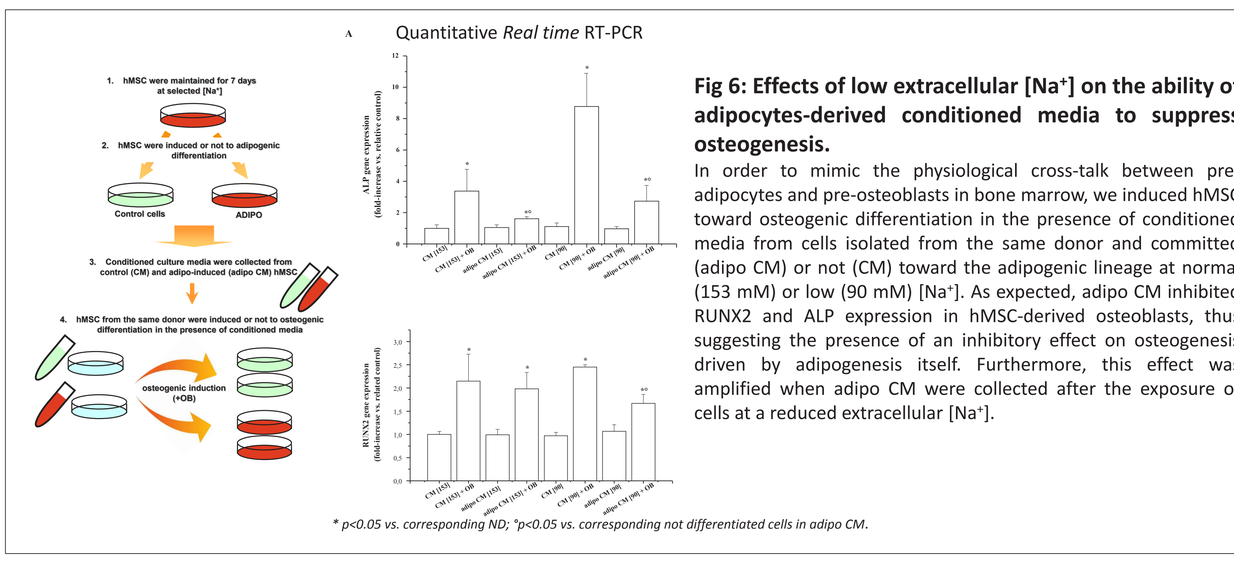

Fig 6: Effects of low extracellular [Na^+] on the ability of adipocytes-derived conditioned media to suppress osteogenesis.
In order to mimic the physiological cross-talk between pre-adipocytes and pre-osteoblasts in bone marrow, we induced hMSC toward osteogenic differentiation in the presence of conditioned media from cells isolated from the same donor and committed (adipo CM) or not (CM) toward the adipogenic lineage at normal (153 mM) or low (90 mM) [Na^+]. As expected, adipo CM inhibited RUNX2 and ALP expression in hMSC-derived osteoblasts, thus suggesting the presence of an inhibitory effect on osteogenesis driven by adipogenesis itself. Furthermore, this effect was amplified when adipo CM were collected after the exposure of cells at a reduced extracellular [Na^+].

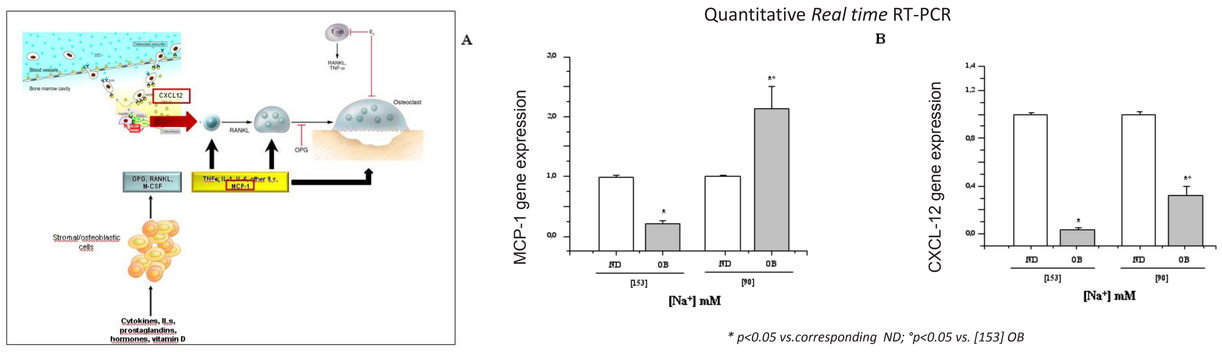

Fig 7: Effects of low extracellular [Na^+] on osteoblast production of molecules able to modulate osteoclastogenesis and osteoclast activity. Low extracellular [Na^+] reverted the inhibition of MCP-1 expression in hMSC-derived osteoblasts and attenuated the inhibition of the expression of the CXCL-12/SDF-1 gene. These data suggest that chronic hyponatremia promotes osteoclastogenesis and osteoclast activation at least in part by modulating the osteoblastic secretion of chemokines involved in these processes.

CONCLUSIONS

We have demonstrated that in our *in vitro* model low [Na^+] does not alter the intrinsic ability of hMSC to differentiate along the osteogenic and the adipogenic lineages, even if it associated with a cytoskeleton disorganization that could affect cell function. *In vivo*, mesenchymal precursors are exposed to conflicting stimuli, which determine a fine equilibrium between adipogenesis and osteogenesis. Our data suggest that reduced extracellular [Na^+] alter this balance and promotes preferentially adipogenesis, by increasing both the number of precursors able to differentiate into adipocytes, and the production of soluble factors which inhibit osteoblastogenesis and induce osteoclastogenesis. These findings add new evidence suggesting that hyponatremia should be carefully taken into account by clinicians because of its negative effects on bone, in addition to the known neurological effects, and indicate for the first time that impaired osteogenesis appears to be involved, in addition to increased osteoclastogenesis and resorptive activity.

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