

Creatine, pyruvate and glucose influence viability, metabolic activity and collagen content of normal and osteoporotic human osteoblast-like cells *in vitro*

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BACKGROUND. For the development of normal bone and during bone repair, cells require energy to survive, proliferate, differentiate and synthesize extracellular matrix, which then mineralizes. During these processes, ATP is generated by glycolysis, oxidative phosphorylation, as well as by the creatine kinase/phosphocreatine system that is present in these cells (1, 2); for review see (3). We have shown that external supplementation with the energy precursor, creatine, had clear stimulatory effects on the differentiation and mineral deposition by primary rat osteoblast-like cells (4). Bone cell bioenergetics may be a key element to understand not only bone formation as such, but also the pathological changes observed in bone diseases, e.g. osteoporosis. We therefore wanted to study the effects of creatine (Cr), pyruvate (pyr) and glucose (glc) supplementation on normal (nHOB) and osteoporotic human osteoblast-like cells (oHOB).

MATERIALS AND METHODS. HOB were isolated from bone chips of patients undergoing hip replacement surgery. HOB were inoculated as monolayer and micromass cultures. Micromass cell culture, representing an organoid system, promote the differentiation of rat osteoblast-like cells (5, 6).

The growth medium contained 5.55, 26.5, and 55.5 mM glc as well as 1 mM pyr and 5.55 mM glc. Osmotic effects of 55.5 mM glucose were compensated by the addition of 49.5 mM mannitol (man) to 5.55 mM glucose. Cr was added to the medium at a concentration of 10 mM. After 2 weeks, viability (neutral red uptake; NR), metabolic activity (MTT) and collagen content were measured.

RESULTS nHOB. Statistical analysis revealed that donors responded individually and differentially to monolayer and micromass culture in all parameters analyzed. Values for monolayer were higher than the ones for micromass cultures concerning viability and metabolic activity.

Viability. Addition of 1 mM pyr to glc 5.55 significantly promotes cell viability as compared to increasing glc concentrations. An increase in glc concentrations reduced the viability, but only glc 55.5 was significantly lower than glc 5.55. There was no osmotic effects of glc 55.5 as both glc 5.5 and glc 5.5; man were significantly are higher than glc 55.5. In addition, high osmotic pressure (glc 5.5;man) significantly increased number of viable cells over glc 5.5.

Metabolic activity. Under all conditions, 10 mM Cr significantly reduced metabolic activity as compared to Cr-free media. Addition of 1 mM pyr to glc 5.5 significantly promoted metabolic activity as compared to increasing glc concentrations and glc 5.5;man 49.5. There was an osmotic effect of glc 55.5 because glc 5.5 was significantly higher than both glc 55.5 and glc 5.5;man .

Collagen. Statistical analysis showed there were significant interaction effects of both Cr*medium and donor*medium. This means that the effects of Cr were dependent on the media used, and the donors respond individually to the media used. Addition of either 1 mM pyr or 49.5 mM mannitol to glc 5.5 seemed to reduce the values of cellular collagen production as compared to the glc groups. Increasing concentrations of glc had hardly any effects. There were no osmotic effects of glc 55.5 as glc 5.5; man 49.5 is lower than both glc 5.5 and glc 55.5

PRELIMINARY RESULTS oHOB. Monolayer and micromasses were usually rather similar. NR and MTT were much lower in oHOB than in nHOB, but cellular collagen content was not negatively affected. Cr had beneficial effects on cellular collagen content, which seems to be more pronounced in the glc groups than in the other groups.

CONCLUSIONS. Pyruvate, glucose and osmotic pressure influence proliferation, metabolic activity and collagen content in HOB. Cell proliferation might be reduced in osteoporotic cells. Cell-cell contacts, as well as Cr supplementation, may have greater beneficial effects in oHOB compared to nHOB.

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