



Title	The effect of hibernating phase serum on osteoclastogenic and osteogenic differentiation in-vitro in Japanese black bears [an abstract of dissertation and a summary of dissertation review]
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Citation	北海道大学. 博士(獣医学) 甲第14547号
Issue Date	2021-03-25
Doc URL	http://hdl.handle.net/2115/81653
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Type	theses (doctoral - abstract and summary of review)
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File Information	Alireza_Nasoori_abstract.pdf (論文内容の要旨)



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学位論文内容の要旨
Abstract of the dissertation

博士の専攻分野の名称：博士（獣医学）

Name: Alireza Nasoori

学位論文題名
The title of the doctoral dissertation

The effect of hibernating phase serum on osteoclastogenic and
osteogenic differentiation *in-vitro* in Japanese black bears

(ツキノワグマにおける *in-vitro* での破骨細胞および
骨芽細胞への分化に対する冬眠期血清の効果)

Background:

Bears do not suffer from osteoporosis during hibernation, which is associated with long-term inactivity, lack of food intake, and cold exposure. However, the mechanisms involved in bone loss prevention have scarcely been elucidated in bears.

Materials and methods:

I investigated the effect of serum from hibernating Japanese black bears (*Ursus thibetanus japonicus*) on osteoclastogenic and osteogenic differentiation of peripheral blood mononuclear cells (PBMCs) and adipose derived stem cells (ADSCs), respectively. PBMCs and ADSCs collected from 3 bears were separately cultured with 10% serum of active (n= 4 for PBMCs and n= 7 for ADSCs culture) and 10% serum of hibernating bears (n= 4 for PBMCs and n= 7 for ADSCs culture). Each individual serum type was assessed separately by a bear PBMCs or ADSCs. Osteoclastogenic differentiation was induced by treatment of PBMCs with macrophage colony stimulating factor (M-CSF) and receptor activator of NF- κ B ligand (RANKL) for 11 days. For osteogenic differentiation of ADSCs, the cells were cultured in osteogenic medium for 34 days. PBMCs and ADSCs were incubated at 37°C with 5% CO₂.

Results:

PBMCs that were cultured with the active bear serum containing medium (ABSM) differentiated to multi-nucleated osteoclasts, and were positive for TRAP stain. However, cells supplemented with hibernating bear serum containing medium (HBSM) failed to form OCs, and showed significantly lower TRAP stain ($p < 0.001$). On the other hand, osteogenic differentiation of ADSCs was similar between ABSM and HBSM ($p > 0.05$) in 3 intervals within 34 days.

Conclusions:

It was revealed that osteoclastogenesis of PBMCs is hindered by HBSM, but osteogenic differentiation is ongoing similarly with both ABSM and HBSM, implying underlying mechanisms for bone maintenance during hibernation in bears. In addition, this study for the first time showed the formation of bears' osteoclasts *in-vitro*, and elucidated the effects of bears' serum on cell culture *in-vitro*.