**UNIVERSITI TEKNOLOGI MARA**

# UNDERLYING MECHANISM IN OXIDISED HIGH DENSITY LIPOPROTEIN INDUCED VASCULAR CALCIFICATION AND OSTEOBLAST DYSFUNCTIONS, AND THEIR PREVENTION BY ADIPONECTIN

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**MSc**

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# AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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# ABSTRACT

Vascular calcification is a late stage event in the development of atherosclerosis and its occurrence is commonly coupled with bone loss or disruption of bone turnover, leading to osteoporosis. Atherosclerosis, obesity and osteoporosis are epidemics in Malaysia and worldwide. A connection between the three diseases has been suspected for a long time. Epidemiological studies have shown that atherosclerosis and osteoporosis are linked in many patients and that obesity is a common risk factor. Oxidised lipoproteins such as oxidised HDL (oxHDL) seems to be a good candidate to identify connections of these three conditions. The oxidation of lipoproteins in various diseases has been well established and many researchers could show the detrimental effects on various tissues including bone where oxlipoproteins are associated with increased apoptosis and reduced mineralisation. In contrast to that, vascular tissue shows the opposite where expression of bone markers and calcification is observed. The mechanism where oxHDL can cause these dual effects is unkown. It is postulated that oxHDL exhibits opposing effects on the same process, namely mineralisation. Adiponectin is decreased in obesity and therefore its protective role is lost. It is hypothesised that adiponectin could rescue the bone and vascular cells from the detrimental effect of oxHDL. This study aims to investigate the effects of oxHDL towards biomarkers of inflammation and mineralisation in human aortic vascular smooth muscle cells (HAoVSMCs) and human osteoblast cells (HOBs) and to determine the protective role of adiponectin in attenuating the detrimental effects of oxHDL. HDL was oxidised by copper sulphate and the degree of oxidation was measured by TBARs assay. Cytotoxicity, mineralisation and calcification assays were conducted to optimise the concentration of oxHDL. Then, both HAoVSMCs and HOBs were incubated with HDL, oxHDL, adiponectin or co-incubated with oxHDL and adiponectin for 24 hours. Proteins and gene expressions of IL-6, TNF-α, Osterix, Runx2, ALP, COL1, OPN, OCN, Wnt-5a, NF-κβ (p65), cAMP, STAT-3 and PPAR-α were measured by ELISA and quantitative real time PCR (qPCR). In HAoVSMCs, oxHDL (100µg/ml protein) promoted the formation of mineral nodules and calcium deposits in HAoVSMCs. This was accompanied by an increased protein expression of pro-inflammatory markers, IL-6 and NF-κβ (p65). Protein secretion of Wnt-5a, an important ligand in osteoblast activity, and osteoblast's transcription factor, Osterix were also elevated. Induction of oxHDL also promoted *Runx2*, and subsequently increased *ALPL* and *SPP1* gene expression. Interestingly, these detrimental effects of oxHDL were suppressed by adiponectin. Contradicting effect was observed in HOBs, in which the production of mineral nodules and calcium deposition were suppressed by oxHDL (100µg/ml protein). This effect was accompanied by high protein and gene expression of pro-inflammatory markers [IL-6, TNF-α and NF-κβ (p65)] causing a reduction in protein secretion of Wnt-5a, followed by the reduction of ALP, COL1 and OPN secretion. During co-incubation, adiponectin suppressed the secretion of pro-inflammatory markers [IL-6, TNF-α and NF-κβ (p65)] followed by an increase gene expression of bone associated markers: *RUNX2, ALPL, SPP1, OCN and COL1A2* including *WNT5A*, during co-incubation of adiponectin (5µg/ml) and oxHDL. oxHDL induces contrariwise effects in HAoVSMCs and HOBs though inflammation pathways, which could be the possible link on why atherosclerotic patients are prone to have lower bone mass density. This study also shows the ability of adiponectin in suppressing the detrimental effects of oxHDL.

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