# Vitamin D Decreases Adipocyte Size of Pericardial Adipose Tissue in Obese Female Wistar Rat: A Preliminary Study

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***Abstrac*t—Obesity is also associated with the accumulation of fat mass both in subcutaneous and visceral. Pericardial adipose tissue (PAT) is one of the visceral fat tissue that increases when obesity occurs. In previous study, Vitamin D deficiency in obesity was associated with increased thickness of epicardial adipose tissue (EAT). PAT and EAT are associated in anatomical conjunction. This study wants to prove whether the administration of vitamin D can reduce the size of adipocytes in paracardial fat of obese female wistar rat. In this preliminary study, 3 obese female wistar rat (275, 278, 280 grams) were divided into 3 treatments: control, 800 IU of vitamin D, 2400 IU of vitamin D. Vitamin D were administered by oral gavage for 8 weeks.** All of rats **were sacrificed on week 8 and pericardial fat were extracted for stereology analysis. Our histologis examination showed that the total size of adipocytes pericardial fat were decreased in 2400 IU vit D, in comparison to the 800 IU and control treatment. It showed that vitamin D could decrease the size of adipocyte of pericardial fat in obese female wistar rat.**

***Index Terms*—obesity, PAT, EAT, adipocyte size, vitamin D**

## I. Introduction

Obesity is associated an increased risk of morbidity and cardiovascular diseases such as coronary heart disease, hypertension, stroke, diabetes mellitus. Obesity is also associated with the accumulation of fat mass both in subcutaneous and visceral. Subcutaneous fat is adipose tissue located under the skin, which serves as a protective body against temperature changes, both hot and cold temperatures, while visceral fat is adipose tissue around organs which functions as a protective tissue for the surrounding organs. Research shows that an increase in visceral fat will increase complications from obesity. Increased visceral fat causes lipolysis and the addition of free fatty acid flow in the plasma, especially to portal circulation. Increased intake of free fatty acids into the liver is expected to inhibit insulin clearance and increase lipid synthesis which results in hypersinsulinemia and peripheral hyperlipidemia [1].

Pericardial Adipose Tissue (PAT) is one of the visceral fat tissue that increases when obesity occurs. Adipose epicardial tissue plays an important role in the biochemical and physiological regulation of cardiac homeostasis. The amount of adipose tissue in the heart can cause enlargement of the left ventricular mass and affect contractions, diastolic dysfunction and thickening of the septum wall [2]. This large mass of PAT also causes an unbalanced adipokin secretion such as leptin secretion, adiponectin, resistin, IL-6, etc. This imbalance can activate atherogenic pathways that can cause metabolic syndrome. Increasing in PAT mass also increases the production of pro-inflammatory factors that will worsen endothelial function which can lead to coronary heart disease and insulin resistance in obesity [1].

Pericardial Adipose Tissue (PAT) is also has relationship to EAT in anatomical conjunction. Vitamin D deficiency that occurs in obesity turns out to be associated with increased thickness of Epicardial Adipose Tissue (EAT) in pre-menopausal women [3]. It is also related to the previous case report on vitamin D malefficiency, which will stimulate hypertrophy in cardiomyocytes, which are associated with decreased Vitamin D Receptors (VDR), suppression of cytokine signals and increased expression of inflammatory markers (IL6, TNF α, MCP-1 / CCL2) on the EAT [4]. These data indicate that vitamin D deficiency in EAT plays a role in the causes of pro-inflammatory factors and vice versa vitamin D supplementation as an anti-inflammatory agent and cardioprotective action [5] Although so far vitamin D supplementation has not had the expected effect on the thickness of EAT [3] Recent developments and evidence suggest that vitamin D has a number of physiological functions that play a role in several diseases such as obesity, metabolic syndrome and cardiovascular disease. Recent studies state that adipose tissue is a direct target of vitamin D, which is proven that

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vitamin D affects body fat mass by inhibiting adipogenic transcription factors, and fat accumulation during adipocyte cell differentiation, and releasing anti-inflammatory activity [6]. Vitamin D also affects adipokin production and the inflammatory response in adipose tissue. Therefore vitamin D deficiency can interfere with the normal metabolic function of adipose tissue in addition to the effects of immunity. Based on these research, we want to prove whether the administration of vitamin D can reduce the adipocyte size in pericardial adipose tissue of obese female wistar rats.

## II. Material and Method

This preliminary study was using the rat obese model with diet induce obese. Three female Wistar rats aged 9 months, weighing 275, 278, 280 grams were used in this study. Nine months old were used because this is similar to the age of adult women. This study was conducted at the Experimental Animal Care Unit, Laboratory of Pharmacology, Faculty of Medicine Udayana University from October 2016 – March 2017. In this preliminary study, we just had taken 3 rats which were assigned to three different treatments. First rat was assigned to 2400 IU vitamin D, second rat was assigned to 800 IU vitamin D, and the third was assigned to placebo. Vitamin D was administrated once daily for 8 weeks.The animals were housed in cages floored with wood shavings that were changed regularly. The animals were placed under a normal 12 hours light/dark diurnal cycle and provided with standard rat pellets and water ad libitum. This research has had ethical review permission by Research Ethical Commission, Faculty of Medical, Udayana University/ Sanglah Hospital, with protocol number: 1058.02.1.2016.

### *A. Hematoxylin and Eosin (H&E) Staining*

Pericardium tissues were dehydrated, cleared and embedded in paraffin. The tissues were sectioned for 5 µm thickness on microtome Jung Histocut 820 (Leica, Germany). The sections were mounted on slides and were stained with H&E following standard procedures. After dehydration, the sections were mounted with Entellan New (Merck, Germany). Images were obtained using a light microscope (CX41, Olympus, Japan) equipped with a digital camera (OptilabPro, Miconos, Indonesia) connected to a PC monitor.

### *B. Measurement of Cell' Diameter*

All measurements were obtained to ensure objectivity in blind conditions (2 observers for each experiment), and control and experimental samples were assayed under the same conditions. The number of H&E in a 250×250-µm square were counted in using an image analyzing system (Image Raster, Miconos, Indonesia). Measurement were obtained by averaging the total diameter from each animal per group.

## III. Results

These pictures below showed adipocytes size of PAT with each treatment as follows:

Fig. 1, Fig. 2 and Fig. 3 show the effect of administration of Vitamin D on adipocyte size of PAT in obese female wistar rats at different dosage. Vitamin D 2400 IU administration has made the smallest size of adipocyte, followed by vitamin D 800 IU, and rat without treatment of vitamin D had the largest with each diameter average of adipocyte are 12.27µm, 26.62 µm, 36.84 µm respectively.

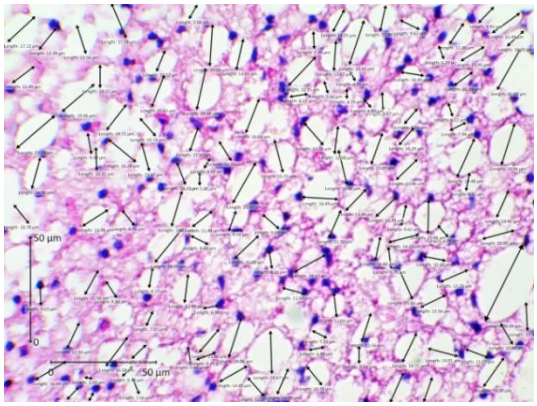


Figure 1. Photomicrograph of the pericardium lipid stained with H & E (x400) in group with Vitamin D 2400 IU.

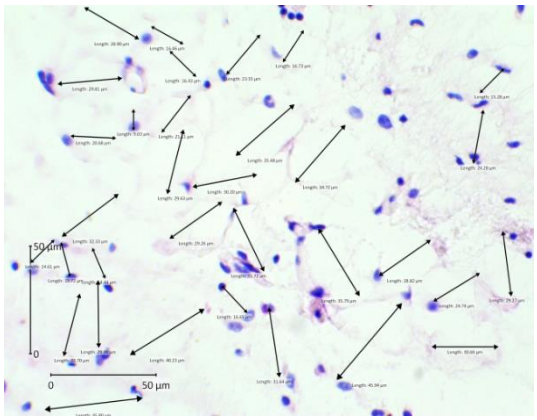


Figure 2. hotomicrograph of the pericardium lipid stained with H & E (x400) in group with Vitamin D 800 IU.

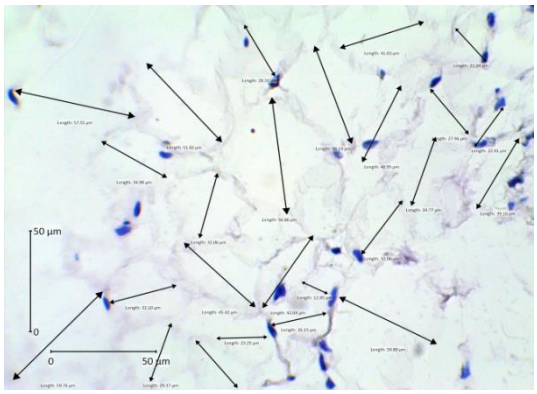


Figure 3. Photomicrograph of the pericardium lipid stained with H & E (x400) in control group without Vitamin D.

## IV. Discussion

The scope of the current study was to evaluate the possible beneficial or adverse effect of vitamin D on pericardial adipose tissue of rat obese model, especially in size of adipocyte. We found that vitamin D administration orally could change the size of adipocyte in pericardial adipose tissue.Vitamin D is the hormone secosterol which is a derivative of 7-dehydrocholesterol (provitamin D) a direct precursor of cholesterol. Vitamin D is fat soluble, and therefore to be transported in the blood requires specific vitamin D-binding proteins. Vitamin D is present in the daily diet, and synthesized in the skin (especially vitamin D3) by ultraviolet radiation from the sun. Ultraviolet B (290-315 nm) absorbed by the skin converts 7-dehydrocholesterol into an unstable previtamin D3 and is rapidly converted to vitamin D3 (cholecalciferol). Vitamin D3 then exits the skin cells, enters the skin capillaries, and is bound with vitamin D binding protein (DBP). Vitamin D in food is absorbed in the small intestine and with the help of bile acids, is converted to vitamin D2 (ergocalciferol). Vitamin D3 enters the lymph vessels after it is absorbed and then enters the circulation and binds with DBP and lipoprotein. Vitamin D3 is then metabolized in the liver by calciol-25-hydroxylase to become a 25-OH D3 (calcidiol) pre-hormone that enters the blood and circulates in binding to DBP. The 25-OH D3 form has a half-life of two weeks and the levels reflect overall vitamin D levels, normal levels of 15-50 ng / mL. Concentrations of less than 25 ng / mL cause an increase in parathyroid hormone and bone resorption. Pre-hormone 25-OH D3 is released from its binding with DBP in the kidneys, binds to megalin tubular cells, enters tubular cells and undergoes hydroxylase in the mitochondria. Calcidiol-1-hydroxylase produces an active form of vitamin D, 1,25-dihydroxyvitamin D3 (calcitriol) while calcidiol-24-hydroxylase produces an inactive form, 24.25-dihydroxy vitamin D (24-hydroxycalcidol). Calcitriol performs its function by binding to vitamin D receptors (VDR) in the small intestine. The calcitriol-VDR complex binds again with the retinoic acid X receptor (RXR) in the nucleus and the calcitriol-VDR-RXR complex is then bound to the epithelial vitamin D responsive element (VDRE) of calcium epithelium. Besides having a classic function in mineral homeostasis, 1.25 (OH) 2D also has a non-skeletal function. It has been reported that organs such as the brain, prostate, mammary, colon, pancreas, and immune cells have vitamin D receptors and respond to the active form of vitamin D [7]. More than 200 types of genes controlled by 1 , 25 (OH) 2D, directly or indirectly, to regulate proliferation, differentiation, apoptosis and angiogenesis [8].

The role of vitamin D in the pathophysiology of obesity is still a pros and cons among scientists. Although many studies show a negative relationship between obesity and serum vitamin D levels, the cause and effect are still unclear [9]. There is a relationship between increased BMI with a low serum concentration of vitamin D and also reported that high body fat content is inversely proportional to serum 25 (OH) D levels, and this relationship is stronger than the relationship of 25 (OH) D concentration with BMI and body weight [10].

One hypothesis explained that low bioavalaibality of vitamin D in obesity because the high content of body fat acts as a reservoir for lipid soluble vitamin D and increases its sequestration [11]. It has also been reported that fat content is inversely related to serum 25(OH)D concentration and that this association is stronger than that between 25(OH)D and BMI. Researchers are now focusing on the interplay between fat mass and vitamin D. Adipocytes are able to rapidly expand in size (hypertrophy) and number (hyperplasia). In obesity, adipocytes become enlarged with increased macrophage infiltration and a switch towards the pro-inflammatory phenotype. The ability to both recruit and differentiate new adipocytes is impaired in individuals with hypertrophic adipose tissue [12]. Differentiation into adipocytes requires key transcription factors like the nuclear receptor peroxisome proliferator-activated receptor $\gamma$ (PPAR $\gamma$) and the CCAAT-enhancer-binding proteins [13]. It has been clearly shown that adipose tissue may both regulate and be regulated by vitamin D [14].The expression of the vitamin D receptor, 25-hydroxyvitamin D 1$\alpha$-hydroxylase (CYP27B1) genes, and 24-hydroxylase enzyme has been shown in human adipocytes [15].

Vitamin D supplementation have cardioprotective effects than previously suggested. Gurses et al, showed a negative correlation between serum 25-(OH) Vit D3 and EAT thickness, but they did not evaluate the epicardial tissue with the adipocyte/pre adipocyte ratio and adipocyte size [3]. In our study, we have found the result that vitamin D supplementation have a correlation to adipocyte size of PAT. The size of mouse adipocyte in treatment of Vitamin D 2400 IU are smaller than those with treatment of Vit D 800 IU and without vitamin D (Fig. 1 and Fig. 2). This showed that the treatment of vitamin D has an impact on adipocyte size among these obese rats. The nuclei of adipocyte in adipose tissue of rats without vitamin D appears to be pressured by adipose cavities filled with lipid fluid so that only a few of nuclei are present (Fig. 3)

Growing evidence suggest that the vitamin D system has a range physiological functions, with vitamin D deficiency contributing to the pathogenesis of obesity, metabolic syndrome and cardiovascular disease. Fat-soluble vit D is stored in adipose tissue, and the enzyms require to produce the active form of Vit D are expresses within the tissue [6]. High levels of Vit D have been found in the adipose tissue of obese person, despite these persons being considered Vit D deficient as a result of blood testing of 25 (OH)D [16]. The exact mechanism for that is still unknown and debatable, there are conflicting reports on the effect of weight loss on levels of vit D [17]-[19]. Several studies that used 3T3-L1 preadipocyte cell cultures showed that 1,25 (OH)2 D supresses adipogenesis [20]. and decreases lipid accumulation [21], [22]. The effect is dose and time dependent [23], [24], because it is only seen in the early stages of adipogenesis and appears to occur through several pathways [22], [25].

Vitamin D suppresses adipogenesis by inhibiting expression of C/EBPα, PPARγ, C/EBPβ, and Adipocyte Protein 2 (AP2) [22], [26]. Inhibition of these pathways suggest a role for Vit D in suppressing terminal differentiation of 3T3-L1 adipocytes. Vitamin D decreases lipid accumulation by suppressing expression of SREBP1c and Lipoprotein Lipase (LPL) [22]. There is evidence of 1,25(OH)2D influencing triglyceride accumulation in 3T3-L1 mouse preadipocytes [27, 28]. Most studies found a greater decrease in lipid accumulation with increasing doses of 1,25(OH)2D.

Our study use rats model with diet induce obesity proved that effect of supplementation vitamin D will get same result with the previous study that using 3T3-L1 mouse (suppresses adipogenesis, and decreases lipid accumulation) [26]. The result is showed in Fig.1 and Fig. 2. It also supported by Duque, which is providing a continuous dose of 1,25(OH)2 D (18pmol/day) for 6 weeks to SAM-P/6 mice for 6 weeks significantly decrease adiposity, suggesting an inhibitory effect on adipogenesis [29].

## V. Conclusion and Suggestion

Based on the result, we could describe that vit D plays a role in adipogenesis and adipocyte size of PAT. But there is still insufficient evidence, because this is a preliminary research that just a descriptive study that should be followed by next research with larger sample and more parameter.

## Conflict of Interest

The authors declare no conflict of interest.

## Author Contributions

Luh Putu Ratna Sundari conducted the research and wrote the paper; IGKN Arijana made histology specimen and analyzed the data and Ni Made Linawati read histology specimen and also wrote the paper and all authors had approved the final version.

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