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SUMMARY OF DOCTORAL THESIS

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Title: **Studies on serum parameters associated with metabolic syndrome and pro-adipogenic effects of prostaglandin D_2 and its stable analogue**
（代謝症候群に関連する血清パラメーターとプロスタグランジンD_2及びその安定類縁体の脂肪細胞形成の促進効果に関する研究）

Adipogenesis is a crucial aspect in regulating body fat mass and energy homeostasis. Recently, there has been a dramatic increase in the incidence of obesity with abnormal storage of fats in white adipose tissue in industrialized and newly developed countries. Obesity is recognized as a major risk factor for the development of insulin resistance and the related metabolic syndrome. In this study, I conducted health science studies regarding serum parameters associated with metabolic syndrome and pro-adipogenic effects of prostaglandin (PG) D_2 and its stable, isosteric analogue.

Metabolic syndrome is a bunch of several hazardous heart attack risk factors: diabetes and raised fasting glucose in plasma, high cholesterol, abdominal obesity and hypertension. About 20 - 25% of adult population is suffering from metabolic syndrome through the world and they are also at the three times greater risk for the development of heart attack as compared with people lack the syndrome. In addition, these patients are five times more prone to risk of developing type-2 diabetes while 230 million people already widely have lived with diabetes, as a result it becomes one of the most widely spread chronic diseases and thus takes fourth or fifth place among narrative disorders causes death in developed countries. On account of this, metabolic syndrome is now considering a new epidemic of cardiovascular disease because its pathological conditions are clustered with cardiovascular disease risk factors that intensified abnormal heart condition. In Pacific and Middle East nations, it causes death to one out of four in adults (35 - 64 years) and also susceptible to the greater risk of developing type-II diabetes. Type-II diabetes comprises about 90% of all diabetes and becomes one of the leading causes of illness, premature death and heart diseases, which in turn responsible for up to 80 percent of deaths globally. Either hampered glucose tolerances or type 2 diabetes status are liable for generating various risk factors that often take place altogether what are now literally denoted under the name of "metabolic syndrome". Mostly in same individual "clustering" of metabolic abnormalities occur and precipitated to confer substantial additional cardiovascular risk with the clustering of the risk consociated with each abnormality. However, it is ascertained that when a person is identified with diabetes, hyperglycemia and related changes in blood lipids including increased triglycerides and decreased cholesterol of high-density lipoprotein, the one is also concurrently showing high blood glucose level which in turn deteriorate the risk of cardiovascular disease. The rate of mortality due to cardiovascular arrest is highly proportional to the presence of components of metabolic syndrome. Diabetes is associated with cardiovascular difficulties and also responsible for blindness, amputation and kidney failure, which aggrandize social and economic burden related to diabetes. The prediction, “the prevalence of diabetes will double by 2025” stipulates an analogous raise in cardiovascular disease following death related to enormous and incorrigible impacts on health systems throughout the world. The total medical costs of all diabetes in 25 countries of the European Union at 20 and 79 years old was estimated up to 64.9 billion dollars, equivalent to 7.2 percent of total health expense in these countries.

In recent years, there has been a notable increase in the number of people with the metabolic syndrome. It has become an epidemic disease and acknowledged globally with other life style

disease such as type-2 diabetes and cardiovascular disease. It is with an increasing prevalence and leading cause of death in adults and children world widely and considered as the one of the most health squeezing disease for 21st century. Not much work has been done to find out the possible reasons for the development of metabolic syndrome, which is counted to be very significant for the treatment of this syndrome. Thus an attempt has been made to examine serum antioxidant levels like vitamin C, macro minerals (Ca, Na, K), and trace element concentrations (Zn, Fe), and to clinch the correlation between the serum levels of these components of this metabolic patient. This study was undertaken to elucidate the levels of serum antioxidant (vitamin C), macro - minerals (Ca, Na, and K), and trace elements (Zn, Fe) of in patients with metabolic syndromes. Metabolic syndrome was examined by following the definition developed by the National Cholesterol Education Program Adult Treatment Panel III. Serum vitamin C was estimated by phenyl-hydrazine spectrophotometry method, while Macro-minerals and trace elements were determined under coordination of two methods these was flame atomic absorption spectrometryand graphite furnace. This study found that vitamin C was significantly lower ($p < 0.05$) in patients with metabolic syndrome as compared with control subjects. Analysis of serum trace elements (Zn, Fe) and macro minerals (Ca, Na, K) explored lower serum concentrations in examined patients than the control group at the significant levels ($P < 0.05$). Pearson's correlation analysis revealed negative correlations between blood glucose and Fe, and between triglyceride and Zn level were found statistically significant. On the basis of our present study it can be asserted that depletion of Vitamin C, Zn, Fe, Ca, K and Na levels is strongly associated with the metabolic syndrome pathogenesis. Dietary supplementation with antioxidants, macro-minerals and trace elements may drive the treatment of metabolic syndrome and thus reduce its complications. Moreover, changes in lifestyle and therapeutics may reduce adiposity and could provide the benefit of preventing obesity-related morbidity and mortality.

The differentiation of preadipocytes into mature adipocytes is regulated by a cascade of transcriptional factors including the CCAAT enhancer-binding proteins (C/EBPs) and peroxisome proliferator-activated receptors (PPARs). Among these nuclear factors, PPARγ, a member of the nuclear hormone superfamily, is abundantly expressed in adipocytes and regarded as a master regulator of adipogenesis. The activation of PPARγ requires active ligands because this nuclear hormone receptor is a ligand-dependent transcription factor. PPARγ can be activated by a variety of lipophilic ligands such as polyunsaturated fatty acids and their metabolites as natural ligands. Although the true endogenous ligands for this receptor have not yet been established with certainty in vivo, one of natural potent activators for PPARγ is 15-deoxy-$\Delta^{12,14}$- PGJ_2 (15d-PGJ_2), which is formed by non-enzymatic dehydration of unstable PGD_2. Therefore, PGD_2 and the related PGJ_2 derivatives including 15d-PGJ_2 and Δ^{12}-PGJ_2 serve as pro-adipogenic factors in cultured adipocytes expressing PPARγ. PGD_2 is relatively unstable and dehydrated non-enzymatically into PGJ_2 derivatives, which are known to serve as pro-adipogenic factors by PPARγ, a master regulator of adipogenesis. 11-Deoxy-11-methylene-PGD_2 (11d-11m-PGD_2) is a novel, chemically stable, isosteric analogue of PGD_2 in which the 11-keto group is replaced by an exocyclic methylene. Here we attempted to investigate pro-adipogenic effects of PGD_2 and 11d-11m-$PGD_2$and to compare the difference in their ways during the maturation phase of cultured adipocytes. The dose-dependent study showed that 11d-11m-PGD_2 was significantly more potent than natural PGD_2 to stimulate the storage of fats suppressed in the presence of indomethacin, a cyclooxygenase inhibitor. These pro-adipogenic effects were caused by the up-regulation of adipogenesis as evident with higher gene expression levels of adipogenesis markers. Analysis of transcript levels revealed the enhanced gene expression of two subtypes of cell-surface membrane receptors for PGD_2, namely the prostanoid DP_1 and DP_2 (chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2)) receptors together with lipocalin-type PGD synthase during the maturation phase. Specific agonists for DP_1, CRTH2, and PPARγ were appreciably effective to rescue adipogenesis attenuated by indomethacin. The action of PGD_2 was attenuated by specific antagonists for DP_1 and PPARγ. By contrast, the effect of 11d-11m-PGD_2 was more potently interfered by a selective antagonist for CRTH2 than that for DP_1 while PPARγ antagonist GW9662 had almost no inhibitory effects. These results suggest that PGD_2exerts its pro-adipogenic effect principally through the mediation of DP_1 and PPARγ, whereas the stimulatory effect of 11d-11m-PGD_2 on adipogenesis occurs preferentially by the interaction with CRTH2.