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SUMMARY OF DOCTORAL THESIS

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Title: Health science studies on the roles of bioactive lipids in adipogenesis, adipocyte inflammation, and metabolic syndrome
（脂肪細胞形成、脂肪細胞の炎症、及びメタボリックシンドロームにおける生理活性脂質の役割に関する健康科学研究）

Obesity poses a serious health hazard and contributes to the increase morbidity and mortality since it increases an individual's risk to comorbidities like type 2 diabetes, cardiovascular diseases, certain cancers and other related life-style diseases. Obesity is characterized by a state of positive energy balance leading to increase in adipose tissue mass. Adipose tissue mass increase relates to the changes in the size or number of adipocytes. Adipose tissue mass can increase in two ways: hyperplasia, which is characterized by an increase in the cell number or hypertrophy, which is brought about by an increase in the size of the cell. The adipocytes- a capricious cell that has evolved to provide mammalian energy storage- regulates itself depending on the environment. Obesity includes the changes in the functions of adipocytes, such as the onset of insulin resistance, adipocyte inflammation, and the associated metabolic syndrome. One of the earliest events in the adipocyte differentiation is the activation of the peroxisome proliferator-activated receptor (PPAR)γ as a master regulator of adipogenesis. Since PPARγ is a ligand-activated transcription factor, endogenous ligands should be provided for the activation of PPARγ in adipose tissues. The present study was undertaken to conduct health science studies on the novel roles of bioactive lipids and lipid parameters in adipogenesis, adipocyte inflammation, and metabolic syndrome.

The biochemical and molecular biological studies clarified the regulation of gene expression of isoforms of cyclooxygenases (COXs) linked to the prostaglandin (PG)D syntheses at the levels of enzyme activities, proteins, and the transcription upon several external signals. PGD_2 can be produced in adipocytes and dehydrated to PGs of J_2 series including 15-deoxy-$\Delta^{12,14}$-PGJ_2 (15d-PGJ_2) and Δ^{12}-PGJ_2, that are considered as pro-adipogenic prostanoids through the activation of PPARγ. Several lines of evidences suggested the generation of 15d-PGJ_2 in matured adipocytes and its contribution to the upregulation of adipogenesis. However, the quantitative determination of Δ^{12}-PGJ_2 has not been attempted during the life stage of adipocytes. In this study, an enzyme-linked immunosorbent assay using mouse antiserum specific for Δ^{12}-PGJ_2 was developed. According to the standard curve, the amount of Δ^{12}-PGJ_2 can be measured from 0.5 pg to 14.4 ng in an assay. This antiserum did not recognize most other prostanoids including 15d-PGJ_2, while it only showed the cross-reaction of 28% with unstable PGJ_2. This immunological assay was applied to the determination of the endogenous formation of Δ^{12}-PGJ_2 in cultured 3T3-L1 adipocytes during the maturation phase. The ability of cultured adipocytes to form endogenous Δ^{12}-PGJ_2 increased gradually at an earlier stage of the maturation phase and detectable at higher levels than 15d-PGJ_2. Treatment of cultured cells with either aspirin or indomethacin, a general cyclooxygenase inhibitor, significantly reduced the production of endogenous Δ^{12}-PGJ_2 in the maturation medium as expected. Furthermore, the exogenous effects of PGJ_2 series were evaluated individually at various doses on adipogenesis during the maturation phase. Although Δ^{12}-PGJ_2 was slightly less potent than 15d-PGJ_2, each of these PGJ_2 series rescued effectively both the accumulation of fats and the gene expression of typical adipocyte-markers that were attenuated in the presence of aspirin. Taken together, these findings indicate that endogenous Δ^{12}-PGJ_2 contributes substantially to the up-regulation of adipogenesis program through the activation of PPARγ together with 15d-PGJ_2 during the maturation phase of cultured adipocytes.

The role of lipocalin-type PGD synthase (L-PGDS) in adipocytes remains still uncertain due to the related enzymatic or non-enzymatic conversion of PGD_2 as well as the multiple cell-surface membrane

receptors and the nuclear receptors for PGD_2 and PGJ_2 series. Here, to study the role of L-PGDS in adipogenesis, the transfection technology was undertaken to manipulate the expression levels of intracellular L-PGDS in cultured preadipocytes. The resulting stable transfectants were employed to monitor adipogenesis associated with the sustained expression of L-PGDS in adipocytes during the maturation phase. To manipulate the expression levels of L-PGDS in cultured adipocytes, cultured preadipogenic 3T3-L1 cells were transfected stably with a mammalian expression vector having cDNA encoding murine L-PGDS oriented in the sense direction. The isolated cloned stable transfectants with L-PGDS expressed higher levels of the transcript and protein levels of L-PGDS, and synthesized PGD_2 from exogenous arachidonic acid at significantly higher levels. By contrast, the synthesis of PGE_2 remained unchanged, indicating no influence on the reactions of COX and PGE synthase. Furthermore, the ability of these transfectants to synthesize Δ^{12}-PGJ_2 increased more greatly during the maturation phase. The sustained expression of L-PGDS in cultured stable transfectants hampered the storage of fats during the maturation phase of adipocytes, which was accompanied by the reduced gene expression of adipocyte-specific markers reflecting the down-regulation of adipogenesis program. The suppressed adipogenesis was not rescued by either of exogenous aspirin and PPARγ agonists including troglitazone and 15d-PGJ_2. Taken together, the results indicate the negative regulation of adipogenesis program by the enhanced expression of L-PGDS through a cellular mechanism involving the interference of the PPARγ signaling pathway without the contribution of endogenous pro-adipogenic prostanoids.

Alternatively, I investigated whether PGJ_2 derivatives can modulate the gene expression of monocyte chemoattractant protein-1 (MCP-1), a pro-inflammatory chemokine, during the maturation phase of adipocytes. Each of selective or nonselective inhibitors for COX isoforms suppressed significantly the accumulation of fats by interfering the induced expression of the PPARγ gene. Immunological assays of PGJ_2 series revealed higher production of Δ^{12}-PGJ_2 than 15d-PGJ_2 by cultured adipocytes, implicating the contribution of endogenous PGJ_2 series to the stimulated adipogenesis. In addition, the increased transcription of MCP-1 was detectable at later maturation phase of adipogeneis, which was prevented by co-incubation with aspirin. Although 15d-PGJ_2 was more potent than Δ^{12}-PGJ_2, both PGJ_2 series had similar effects to rescue dose-dependently the expression of the MCP-1 gene attenuated by aspirin. These findings suggest that the expression of MCP-1 involved in adipocyte inflammation could be positively regulated by those PGJ_2 series during adipogenesis in adipose tissue. Our current studies should promote further attempts to understand the role of endogenous PGJ_2 series in the regulation of the gene expression of pro-inflammatory factors in adipose tissue in vivo.

Diabetes mellitus is becoming a pandemic worldwide due to the global increase in obesity and sedentary lifestyles. Here, I aimed at to explore the prevalence of metabolic syndrome among persons of different age groups visiting to a diabetic clinic, and living in a coastal area of Bangladesh. Metabolic syndrome is a clustering of metabolic abnormalities that has close association with cardiovascular mortality. Metabolic syndrome has becoming a major challenge for public health. To get insight knowledge about the metabolic syndrome in diabetes, this population-based study was designed. Here I assessed the metabolic syndrome in diabetes from a coastal region of Bangladesh. Metabolic syndrome was estimated by using the modified definition of the National Cholesterol Education Program Adult Treatment Panel III. A total of 500 patients visiting to a diabetic clinic were included in this study. Anthropometric, clinical and biochemical data were recorded. Our result revealed that, about 47.0% patients with type 2 diabetic mellitus were suffering from metabolic syndrome. Female were more prevalent than male (58.6% vs 36.1%) for suffering from metabolic syndrome. Among other risk factors for metabolic syndrome, obesity and hypertriglyceridemia followed by low levels of high-density lipoprotein (HDL) were more prevalent in female. In contrast, male were more likely to have low levels of HDL and hypertriglyceridemia followed by high blood pressure. Since diabetic patients are more prone to suffer from metabolic syndrome, and it becomes a major risk factor for cardiovascular disease, correct measurement should be taken to identify metabolic syndrome among different population to reduce the high abnormalities.

Thus, in light of the roles played by PGJ_2 metabolites and overexpressed L-PGDS in adipogenesis, the manipulation of lipid mediator signaling, through either enzyme manipulation or receptor antagonists and agonists, has potential as a therapeutic approach to the associated metabolic disease. Furthermore, the present study was able to provide the novel results on the facets regarding the roles for bioactive lipids in adipocyte inflammation, and clarified the specific lipid parameters associated with metabolic syndrome in diabetic patients in a coastal region of Bangladesh.