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SUMMARY OF DOCTORAL THESIS

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Title: **Control of adipogenesis program by arachidonic acid and prostanoids during the differentiation phase of adipocytes**

（脂肪細胞の分化期におけるアラキドン酸およびプロスタノイドによる脂肪細胞分化プログラムの制御）

Obesity is a chronic, recurrent condition marked by an excessive amount of body fat. The prevailing opinion is that excessive food and insufficient exercise are responsible for the energy imbalance that defines obesity. The prevalence of obesity is becoming alarming given that excess adipose tissue has been connected to the emergence of serious medical conditions like non-insulin-dependent diabetes mellitus, hypertension, coronary artery disease, hyperlipidemia, gallbladder disease, atherosclerosis, and specific cancers.

Confluent 3T3-L1 preadipocytes are incubated in a medium containing a combination of 3-isobutyl-1-methylxanthine (IBMX), dexamethasone, and insulin (MDI) to begin adipogenesis in vitro. In order to force cells to exit the cell cycle and undergo terminal differentiation, MDI causes the post-confluence mitotic clonal proliferation of cells at G0/G1 growth arrest. During the differentiation phase, CCAAT/enhancer-binding protein-β (C/EBPβ) and C/EBPδ are quickly expressed by IBMX and dexamethasone respectively. The main adipogenic transcription factors C/EBPα and peroxisome proliferator-activated receptor-γ (PPARγ) are further stimulated by these transcription factors, creating a positive feedback loop between PPARγ and C/EBPα. The expression of the genes necessary to achieve the adipocyte phenotype is then triggered.

Majority of the total PUFAs consumed in Western diets are n-6 PUFAs, primarily linoleic acid (LA). Arachidonic acid (AA), an n-6 polyunsaturated fatty acid (n-6 PUFA), is produced from dietary LA and functions in the cyclooxygenase (COX) isoforms COX-1 and COX-2-mediated metabolism to produce pro- or anti-adipogenic prostaglandins (PGs). Pro-adipogenic PGs include PGI_2 and the PGJ_2 derivatives, 15-deoxy-$\Delta^{12,14}$-PGJ_2 (15d-PGJ_2) and Δ^{12}-PGJ_2. Additionally, the effects of n-6 PUFAs on adipose tissue assessed based on the proportion of dietary proteins and carbohydrates reveal that a higher carbohydrate/protein proportion in the diet results in a higher plasma insulin/glucagon ratio, an environment in which dietary n-6 PUFAs promote adipose tissue expansion. On the other hand, a diet with a higher protein to carbohydrate proportion raises the glucagon to insulin ratio in the blood and encourages cAMP-dependent signaling. In this environment, the COX-mediated production of PGE_2 and $PGF_{2\alpha}$ is elevated, and these PGs decrease the mass of white adipose tissue. Incubating 3T3-L1 cells with IBMX throughout the differentiation phase may imitate the high protein/carbohydrate ratio in the diet because IBMX is a cAMP-elevating agent. Incubation of 3T3-L1 cells with AA, dexamethasone, and insulin during the differentiation phase, but not IBMX, promotes adipogenesis. In these cell culture conditions, PGE_2 and $PGF_{2\alpha}$ production is lowered but PGI_2 biosynthesis is increased. In contrast, introducing MDI and AA to 3T3-L1 cells during the differentiation phase upregulates the biosynthesis of PGE_2 and $PGF_{2\alpha}$ and reduces MDI-induced adipogenesis. The present study attempted to examine the effect of linoleic acid (LA) in the presence of IBMX during the differentiation phase of adipogenesis and to clarify the action of PGs produced by AA other than PGE_2 and $PGF_{2\alpha}$ as well as how adipogenesis is affected by the crosstalk between AA and the PGs produced during the differentiation phase.

AA added during the differentiation phase of 3T3-L1 cells reduced the accumulation of intracellular TAG in MDI-induced mature adipocytes in a dose-dependent manner. Unlike AA, LA

added during the differentiation phase did not inhibit adipogenesis. The addition of AA during the differentiation phase resulted in the increased production of PGE_2 and $PGF_{2\alpha}$, unchanged level of Δ^{12}-PGJ_2, and reduced PGI_2 production. With the decreased PGI_2 production being reflected in decreased CCAAT/enhancer-binding protein-β (C/EBPβ) and C/EBPδ expression, we anticipated that the coincubation of PGI_2 with AA would suppress the anti-adipogenic effects of AA. However, the coincubation of PGI_2 with AA did not abolish the anti-adipogenic effects of AA. Additionally, the coincubation of pro-adipogenic PG, Δ^{12}-PGJ_2 with AA could not also rescue AA-induced suppression of adipogenesis. RT-qPCR analysis of the established adipocyte-specific marker genes, showed a downregulation in the expression of lipoprotein lipase (Lpl), glucose transporter 4 (Glut4), and Leptin. According to these findings, AA added during the differentiation phase may reduce the IP receptor's activation by PGI_2 and prevent the early adipogenesis phase from progressing by downregulating the expression of C/EBPβ and C/EBPδ. These results indicated that the metabolism of ingested LA to AA is essential to inhibit adipogenesis and that exposure of AA to adipocytes during only the differentiation phase is adequate. As further mechanisms for suppressing adipogenesis, AA was found not only to increase PGE_2 and $PGF_{2\alpha}$ and decrease PGI_2 production but also to annul the pro-adipogenic effects of PGI_2 and Δ^{12}-PGJ_2.

AA is converted to PGH_2 via COXs. PGH_2 is then converted to PGD_2 via lipocalin-type PGD synthase (L-PGDS), which is preferentially expressed in adipocytes. The addition of PGD_2 during the maturation phase of cultured 3T3-L1 cells promotes MDI-induced adipogenesis by binding and activating D-prostanoid 1 (DP1) and/or 2 receptor (DP2, also known as a chemoattractant receptor-homologous molecule expressed on TH 2 cells) in addition to the increased transcriptional activity of PPARγ. Based on these reports, PGD_2 is considered to be pro-adipogenic. Moreover, PGD_2 undergoes nonenzymatic dehydration and is readily converted to PGJ_2 derivatives, whereas the chemically stable PGD_2 analog, 11-deoxy-11-methylene-PGD_2 (11d-11m-PGD_2) with an exocyclic methylene is considered to resist spontaneous conversion to PGJ_2 derivatives. Based on previous reports, we considered 11d-11m-PGD_2 to facilitate the analysis of the mechanism through which PGD_2 affects MDI-induced adipogenesis. We previously reported that the addition of PGD_2, and 11d-11m-PGD_2 in the presence of indomethacin, a well-known COX inhibitor during the maturation phase of cultured 3T3-L1 cells promotes adipogenesis. Here we aimed to elucidate the effects of the addition of PGD_2 or 11d-11m-PGD_2 to 3T3-L1 cells during the differentiation phase on adipogenesis and to analyze the crosstalk between PGD_2 or 11d-11m-PGD_2 and IP signaling that promotes their pro-adipogenic effect.

We evaluated the adipogenic differentiation of 3T3-L1 cells when cultured with PGD_2 or 11d-11m-PGD_2 during the differentiation phase and found that both attenuated the MDI-induced intracellular TAG accumulation through the downregulation of PPARγ expression. The decreased expression of PPARγ also affected the expression levels of its regulated genes, adiponectin and LPL. However, 11d-11m-PGD_2 suppressed adipogenesis more potently than PGD_2, most likely because of its higher resistance to spontaneous transformation into PGJ_2 derivatives. Furthermore, anti-adipogenic effects of PGD_2 or 11d-11m-PGD_2 was attenuated by the coexistence of an IP receptor agonist, MRE-269, suggesting that their effect depends on the intensity of the signaling from the IP receptor. The selective DP1 agonist, BW245C, did not affect the anti-adipogenic effects of PGD_2 or 11d-11m-PGD_2 but, the selective DP2 agonist, 15*R*-15m-PGD_2, slightly but significantly attenuated the inhibitory effects of PGD_2 and 11d-11m-PGD_2. Furthermore, the addition of PGD_2 and 11d-11m-PGD_2 during the differentiation phase reduced the DP1 and DP2 expression during the maturation phase. These results showed that the culture of 3T3-L1 cells with PGD_2 or 11d-11m-PGD_2 during the differentiation phase exerts anti-adipogenic effects, by desensitizing DP1 and DP2. The desensitizing effects may be mediated by the preferential binding of PGD_2 to unidentified receptor(s), other than DP1 and DP2, which causes the dysfunction of DP1 and DP2 during the differentiation phase, leading to the suppression of adipogenesis in the maturation phase.