

## Review Article

# Dietary regulation of nuclear receptors in obesity-related metabolic syndrome

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In Western culture, excess visceral fat accumulation or obesity has reached epidemic proportions, resulting in metabolic syndrome. However, more than 10 years of research has shown that adipocytes also function as endocrine cells that release various bioactive substances, so called “adipocytokines or adipokines”, that play a major role in the regulation of food intake, insulin sensitivity, energy metabolism, and the vascular microenvironment. Adiponectin, an adipocytokine, is considered to improve insulin sensitivity. Recently, monocyte chemoattractant protein (MCP)-1 has been reported to be a novel adipocytokine involved in the development of obesity-associated insulin resistance and atherosclerosis. Nuclear receptors, especially peroxisome proliferator-activated receptor- $\alpha$  (PPAR $\alpha$ ) and PPAR $\gamma$  are ligand-activated transcription factors that regulate the metabolism of glucose and lipids. PPAR $\gamma$  is strongly expressed in adipocytes and plays a significant role in the transcriptional activation of adipocytokines including adiponectin. PPAR $\alpha$ , another PPAR isoform, is involved in the control of lipid metabolism in the liver and skeletal muscle. PPAR $\alpha$  activation causes lipid clearance via  $\beta$ -oxidation enhancement. We showed that various dietary terpenoids and other natural ingredients regulate the transcription of PPAR target genes, induces the expression and secretion of adiponectin, and inhibits those of MCP-1 in adipocytes and  $\beta$ -oxidation in liver. These findings indicate that dietary factor acts as an agonist of PPARs and is a valuable medical and food component for the gradual improvement of metabolic syndrome.

**Key Words:** obesity, nuclear receptors, metabolic syndrome, adipocytokine, PPARs

## INTRODUCTION

Since animals are under constant threat of starvation, storage of energy sources inside the body serves for various activities. Therefore, animals exhibit highly sophisticated mechanism of storing energy inside their bodies in adipose tissue. However, in human it has been clarified that fat cell (adipocyte), which compose adipose tissues, differentiation and the extent of subsequent fat accumulation (hypertrophy of cells) are closely associated with the occurrence and advancement of various diseases resulting from obesity. Moreover, progress in biochemical studies with respect to adipocyte in recent years has rapidly clarified new functions and differentiation mechanism of adipocytes. Interesting points, in particular, are the function of white adipocytes as “secreting cells” and molecular mechanism of adipocyte differentiation via the nuclear receptors. Consequently, adipose tissue is being targeted to prevent or treat many common diseases and metabolic syndrome.<sup>1</sup>

Obesity increases the risk for many pathological processes including type 2 diabetes, hypertension, hyperlipidemia, cardiovascular diseases, and certain cancers. The dys-regulation of adipose tissue expansion accompanied by increases in the total number (hyperplasia) and size (hypertrophy) of adipocytes causes obesity. Accumulating body of evidence suggests that adipocytes can secrete

various biologically active molecules referred to as adipocytokines or adipokines, and that adipocyte-derived molecules play a central role in the regulation of energy balance, food intake, insulin sensitivity, lipid and glucose metabolisms, and the vascular environment, and thus modulate obesity-related patho-physiological processes. In this chapter, we will be focused on adipocyte and lipid molecule biology, and their influence on obesity related pathologies such as insulin resistance and atherosclerosis, and metabolic syndrome.

### *The white adipose tissue as an energy storage*

White adipose tissue, which is the representative adipose tissue, is widely distributed throughout the entire body in large quantities. This white adipose tissue is a specified organ which stores surplus energy in the form of neutral fat after the intake of food and resupplies energy in the form of fatty acid and glycerol when necessary. The minimum

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requirement for cells to be defined as white adipose is possession of the ability both to synthesize and decompose neutral fat. It was previously believed that among these cells, the number of mature adipocytes increased only during specific developmental stages, that is, during infancy or adolescence; during these stages the lifetime number of adipocytes, approximately 30 billion, is determined. However, recent detailed studies revealed that the number of adipocytes may increase in adults in the case of excessive energy intake or lack of exercise, reaching as much as 40 to 60 billion cells in obese people.<sup>1</sup> This number of cells accounts for approximately 0.5 to 1% of cells composing the human body. However, the amount of adipocytes accounts for approximately 20% of the weight of healthy people and even 30 to 40% of that of obese people. The diameter of adipocytes varies greatly from 10 to 150  $\mu\text{m}$ . One mature adipocyte normally contains 0.5 to 1  $\mu\text{g}$  of fat up to a maximum amount of 4  $\mu\text{g}$ . White adipocytes not only store fat but also resupply the stored energy in the form of fatty acids to the entire body under the control of the nervous and endocrine systems via  $\beta_3$  adrenergic receptors which maintain an organism's homeostasis.

Since animals are under constant threat of starvation, storage of energy sources inside the body for activities such as capturing or escaping from predators is essential for survival. Therefore, animals exhibit highly sophisticated mechanism of storing energy inside their bodies. Adipose tissues store energy in the form of fat highly efficiently via a series of processes such as adipocyte proliferation and differentiation. It is easy to maintain energy inside the body in the form of fat for survival; however, it is difficult to release it. Such physiologically essential characteristics have been closely associated with the development of obesity in humans.

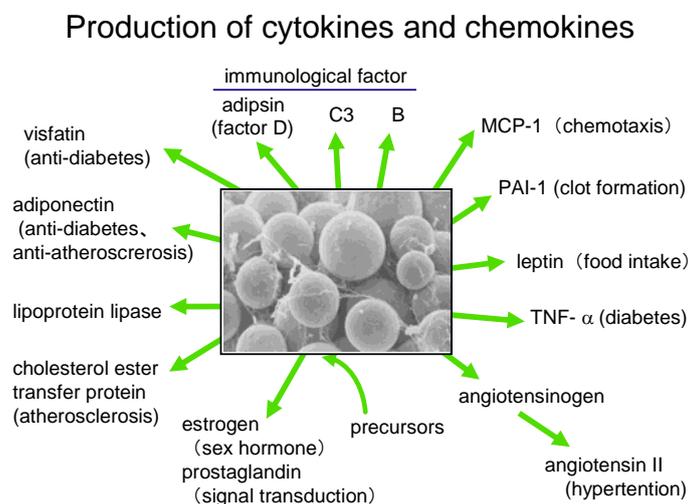
#### Secreting cells and obesity-related pathologies

Moreover, progress in biological and particularly in biochemical studies with respect to adipocytes in recent years has gradually clarified new functions of adipocytes. Adipocytes are well-recognized as endocrine secretory cells as well as fat storage cells, which produce biologically active substances such as hormones, cytokines, and other factors. These molecules, collectively called adipo-

cytokines<sup>2</sup> (recently often called adipokine), are involved in regulating adipocyte functions and metabolism via a network of endocrine, paracrine, and autocrine signals, and thus modulate adipocyte biology. Adipocytokines include tumor necrosis factor $\alpha$  (TNF- $\alpha$ ), interleukin-6 (IL-6), plasminogen activator inhibitor-1 (PAI-1), angiotensinogen, tissue factor, transforming growth factor- $\beta$  (TGF- $\beta$ ), leptin, adiponectin, resistin, and certain chemokines such as monocyte chemoattractant protein-1 (MCP-1) (Figure 1). Most adipocytokines are increasingly produced with increasing adiposity, and are closely associated with obesity-related pathologies (e.g., diabetes and cardiovascular diseases).

#### Nuclear receptors associated with regulation of adipocyte differentiation and function

Recently, methods of analysis of a master regulator for the expression and regulation of adipocyte differentiation genes have been rapidly developed. Such analysis revealed that the CCAAT/enhancer binding protein (C/EBP) family, which is a family of leucine zipper-type transcription factors, and the peroxisome proliferator-activated receptor (PPAR) family, which is a family of ligand-dependent receptor-type transcription factors, interact with each other, form a network and function as a master regulator.<sup>3</sup> PPAR $\gamma$  and C/EBP  $\alpha$  are bound to their counterpart genomic promoter region and activate transcription to maintain each other's activity. PPARs are the so-called orphan receptors with an unidentified ligand when these were discovered. PPARs also present transcription activation ability induced by compounds such as an anti-hyperlipidemia drug (clofibrate) which has an induction effect on the hepatocyte peroxisome. Since an induction agent was not directly bound to PPAR as its ligand, the term "activated" is attached to the name PPAR. In 1990, the  $\alpha$  type was subjected for the first time to cloning from a mouse liver cDNA library. Subsequent cDNA cloning experiment identified several PPAR subtype genes from various animal species and organs. These PPAR subtype genes form a family. In mammals, three subtype genes,  $\alpha$ ,  $\delta$  [called as NUC I in human, fatty acid-activated receptor (FAAR) in mouse, and PPAR  $\beta$  in frog] and  $\gamma$ , were found out. The  $\alpha$  type was expressed



**Figure 1.** Adipocyte secretes variety of biologically active molecules (adipocytokine or adipokine)

mainly in the liver, cardiac muscle and digestive tract, while the  $\delta$  type was expressed not in specific tissues but in all tissues ubiquitously.

One characteristic of the PPAR family is that the compounds with various chemical structures can accommodate ligands. The  $\alpha$  and  $\delta$  types accept the clofibrate group or the fatty acid group as a ligand, while the  $\gamma$  type accept thiazolidinedione derivatives, which are discussed subsequently, as ligands. Specificity is observed in ligand binding or activation among PPAR subtypes. Such specificity is also supported by the low homology in the ligand-binding domain among subtypes.

### **Chemokine as new adipocytokine and metabolic syndrome**

Chemokines form a superfamily of structurally related small (8–14 kDa) chemotactic cytokines classified into CC, C, CXC, and CX3C chemokines, which are mostly inflammatory mediators. In general, chemokines function to induce chemotaxis, which is a nonrandom and directional movement of cells from a lower concentration to a higher concentration of chemattractants. It is well documented that chemokines play a critical role in various inflammatory pathophysiological processes (e.g., infectious diseases, rheumatoid arthritis, multiple sclerosis, organ transplant rejection, atherosclerosis, diabetes, and cancer metastasis). Several recent studies have shown that preadipocytes/adipocytes are sources of specific chemokines.<sup>4–7</sup> The constitutive expression of CC chemokines, such as monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein-1 $\alpha$ (MIP-1 $\alpha$ ), IL-8 and macrophage inflammatory protein-related protein 2 (MRP-2), were detected in preadipocytes/adipocytes.<sup>4,5</sup> These chemokines alter lipid accumulation and leptin secretion by adipocytes, or suppress the formation of adipocyte-specific differentiation markers (e.g., PPAR $\gamma$ , aP2, GPDH),<sup>4,5</sup> supporting the hypothesis that chemokines are important regulators of adipocyte biology. Chemokines are also involved in obesity-related pathologies such as insulin resistance and atherosclerosis. For example, the expression of MCP-1 mRNA was found to be 7.2 times more in obese mice than in normal mice.<sup>8</sup> MCP-1 is an insulin-responsive gene and affects insulin sensitivity.<sup>9</sup> The elevation of circulating inflammatory molecules (e.g., C-reactive protein, IL-6 and TNF- $\alpha$ ) in obesity supports the idea that obesity may be a low-grade systemic inflammatory condition and is implicated in insulin resistance or atherosclerosis.<sup>6,7,10</sup> The decreases in weight/adiposity correlate with the decrease in the circulating levels of proinflammatory adipocytokines (e.g., TNF- $\alpha$  and IL-6).

### **Adipose macrophage as a target of metabolic syndrome**

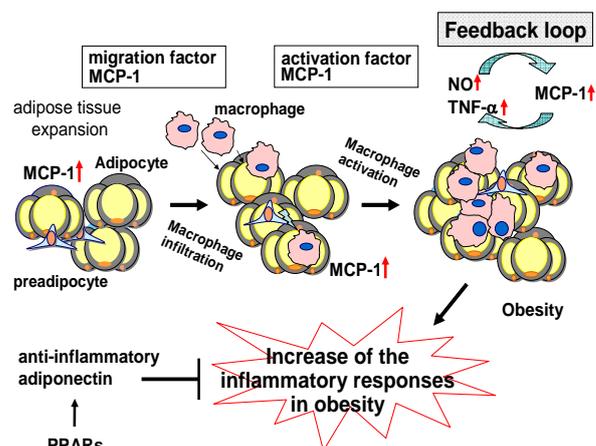
It appears that macrophage infiltration into adipose tissues is characteristic of human obesity.<sup>11</sup> The cause and implication of macrophage infiltration into adipose tissues in obesity are currently unknown. Although it has not yet been explored in detail, chemokines may be involved in macrophage infiltration. When adiposity increases, adipocyte-derived chemokines induce monocyte infiltration into adipose tissues, and adipocyte-derived MCSF may induce monocyte differentiation, leading to the accumula-

tion of macrophages in adipose tissues.<sup>11</sup> The interaction between adipocytes and macrophages through chemokine/cytokine signals may augment the inflammatory reaction in adipose tissues, increasing the levels of circulating inflammatory chemokines/cytokines in obesity. In this context, macrophages may modulate adipocyte biology through chemokine/cytokine signaling and regulate the inflammatory pathway in obesity and obesity-related pathologies.<sup>12</sup> However, the direction of the causal relationship—that is, whether obesity causes inflammation or whether inflammatory condition causes obesity—is still unclear. Further studies are necessary to address the link between adipocytes/macrophages, inflammation, and obesity.

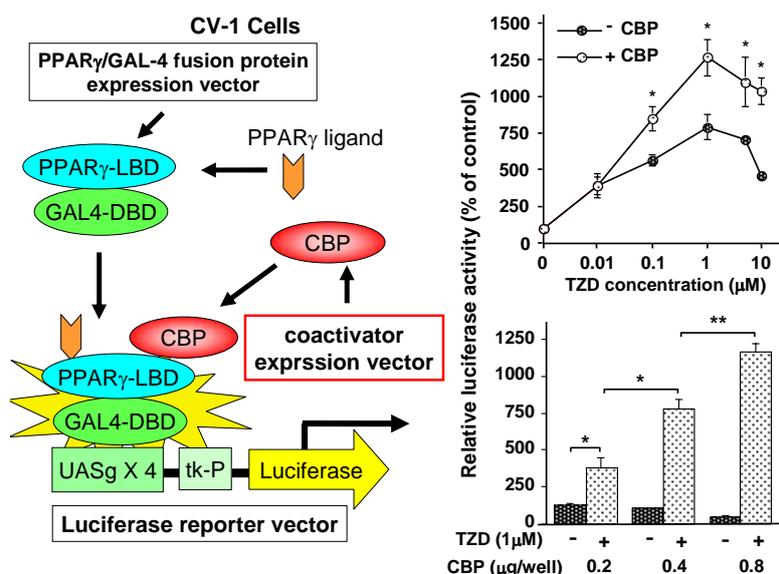
### **Regulation of adipocytokine and inflammation by dietary factors**

Chemokines, such as MCP-1, a member of the CC chemokine superfamily, play a pivotal role in monocyte/macrophage trafficking and activation. The major source of MCP-1 is immune cells such as monocytes and macrophages, but endothelial cells and adipocytes are also reported to produce MCP-1.<sup>5</sup> MCP-1 expression level in adipose tissue is higher in obese animals than in nonobese animals and mesenteric adipose tissue produced the highest level of MCP-1 among several different fat pads. These results suggest that MCP-1 plays a crucial role in adipose tissue inflammatory responses by activating and inducing the infiltration of macrophages into adipose tissues. It was reported that the pacrine loop involving adipocyte-derived free fatty acid (FFA) and macrophage-derived TNF- $\alpha$  establishes a vicious cycle that augments the inflammatory changes and insulin resistance in obese adipose tissue. Therefore, to prevent obesity-related inflammation, it is important to decrease the production of MCP-1, which induces the migration of macrophages, and other proinflammatory factors, including TNF- $\alpha$ , NO, and FFA, in adipose tissue (Figure 2).

Several herbal medicines improve hyperlipidemia, diabetes and cardiovascular diseases. We found that several isoprenols, common components of herbal plants, activate human PPARs as determined using the novel highly sensitive GAL4 ligand-binding domain chimera assay system with coactivator, cAMP-response element binding protein



**Figure 2.** Adipose tissue macrophages and MCP-1 in obesity



**Figure 3.** Cotransfection of coactivator CBP improved the sensitivity of PPAR $\gamma$  ligand assay. CBP; cAMP response element binding protein binding protein, TZD; thiazolidinediones

(CREB)-binding protein (CBP) coexpression (Figure 3).<sup>13,14</sup> Farnesol and geranylgeraniol that are typical isoprenols in herbs and fruits activated not only PPAR $\gamma$  but also PPAR $\alpha$  as determined using the chimera assay system. These compounds also activated full-length human PPAR $\gamma$  and PPAR $\alpha$  in CV1 cells. These isoprenols up-regulated the expression of some lipid metabolic target genes of PPAR $\gamma$  and PPAR $\alpha$  in 3T3-L1 adipocytes and PPAR $\alpha$  expressing HepG2 hepatocytes, respectively. Obese diabetic KK-Ay mice that fed farnesol for 35 days significantly reduced plasma glucose and hepatic triglyceride contents. Moreover farnesol up-regulated the expression of genes involved in fatty acid oxidation in liver. These results suggest that herbal medicines containing isoprenols with dual action on both PPAR $\gamma$  and PPAR $\alpha$  can be of interest for the amelioration of metabolic disorders associated with diabetes.

Furthermore, we showed that citrus auraptene activates PPAR $\alpha$  and PPAR $\gamma$ .<sup>15</sup> Auraptene up-regulated adiponectin expression and increased the ratio of the amount of high-molecular weight multimers of adiponectin to the total adiponectin. However, auraptene down-regulated MCP-1 expression in 3T3-L1 adipocytes. Interestingly, these effects were caused by both PPAR $\gamma$  and PPAR $\alpha$  activations. The results suggest that the consumption of citrus fruits, which contain auraptene, an agonist of PPAR $\alpha$  and PPAR $\gamma$  can lead to a partial prevention of lipid and glucose metabolism abnormalities.

## CONCLUSION

It is becoming clear that adipocytokines play an important role in adipocyte biology and obesity-related pathologies such as insulin resistance and cardiovascular diseases, although the mechanism underlying the associations among adipocytes, adipocytokines and pathologies is not yet fully understood. It appears that proinflammatory adipocytokines such as TNF- $\alpha$  and MCP-1 exhibit an adverse effect on adipocyte functions and thus cause obesity-related pathologies such as insulin resistance, while anti-inflammatory adipocytokines such as adiponectin

seem to be protective against insulin resistance and atherosclerosis. Adipocytokines attractive therapeutic targets in obesity and obesity-related pathologies.

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## AUTHOR DISCLOSURES

Teruo Kawada, Tsuyoshi Goto, Shizuka Hirai, Min-Sook Kang<sup>1</sup>, Taku Uemura<sup>1</sup>, Rina Yu and Nobuyuki Takahashi, no conflicts of interest.

## REFERENCES

- Bray GA, Bouchard C, James WPT. Definitions and proposed current classification of obesity. Merce Dekker, Inc. New York. 1998. p.31-40.
- Shimomura I, Funahashi T, Takahashi M, Matsuzawa Y. Enhanced expression of PAI-1 in visceral fat: Possible contributor to vascular disease in obesity. *Nat Med.* 1996;2:800-3.
- Cornelius P, MacDougald A, Lane MD. Regulation of adipocyte development. *Ann Rev Nutr.* 1994;14: 99-129.
- Gerhardt CC, Romero IA, Cancellato R., Camoin L, Strosberg AD. Chemokines control fat accumulation and leptin secretion by cultured human adipocytes. *Mol Cell Endocrinol.* 2001;175:81-92.
- Xu H., Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, Chen H. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest.* 2003;112:1821-30.
- Wellen KE, Hotamisligil GS. Obesity-induced inflammatory changes in adipose tissue. *J Clin Invest.* 2003; 112:1785-8.
- Kim CS, Kawada T, Yoo H, Kwon BS, Yu R. Macrophage inflammatory protein-related protein-2, a novel CC chemokine, can regulate preadipocyte migration and adipocyte differentiation. *FEBS Lett.* 2003;548:125-30.

8. Takahashi K, Mizuarai S, Araki H, Mashiko S, Ishihara A, Kanatani A, Itadani H, Kotani H. Adiposity elevates plasma MCP-1 levels leading to the increased CD11b-positive monocytes in mice. *J Biol Chem.* 2003;278: 46654-60.
9. Sartipy P, Loskutoff DJ. Monocyte chemoattractant protein 1 in obesity and insulin resistance. *Proc Natl Acad Sci. USA* 2003;100:7265-70.
10. Dandona P, Aljada A, Bandyopadhyay A. Inflammation: the link between insulin resistance, obesity and diabetes. *Trends Immunol.* 2004;25:4-7.
11. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel, RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest.* 2003;112:1796-1808.
12. Yu R, Kim CS, Kwon BS, Kawada T. Mesenteric adipose tissue-derived monocyte chemoattractant protein-1 plays a crucial role in adipose tissue macrophage migration and activation in obese mice. *Obesity.* 2006;14:1353-62.
13. Takahashi N, Kawada T, Yamamoto T, Goto T, Taimatsu A, Aoki N, Kawasaki H, Taira K, Yokoyama K, Kamei Y, Fushiki T. Overexpression and ribozyme-mediated targeting of transcriptional coactivators CBP and p300 revealed their indispensable roles in adipocyte differentiation through the regulation of peroxisome proliferator-activated receptor- $\gamma$ . *J Biol Chem.* 2002;277:16906-12.
14. Takahashi N, Kawada T, Goto T, Yamamoto T, Taimatsu A, Matsui N, Kimura K, Saitoh M, Hosokawa M, Miyashita K, Fushiki T. Dual action of isoprenols from herbal medicines on both PPAR $\gamma$  and PPAR $\alpha$  in 3T3-L1 adipocytes and HepG2 hepatocytes. *FEBS Letters.* 2002;514: 315-22.
15. Kuroyanagi K, Kang M-S, Goto T, Hirai S, Ohyama K, Kusudo T, Yu R, Yano M, Sasaki T, Takahashi N, Kawada T. Citrus auraptene acts as an agonist for PPARs and enhances adiponectin production and MCP-1 reduction in 3T3-L1 adipocytes. *Biochem. Biophys. Res. Commun.* 2007 Dec 4; [Epub ahead of print]