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Fat Cell Formation and Obesity-Related Diseases

– Review –

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Abstract

Animals possess a highly sophisticated mechanism of storing energy in adipose tissue inside their bodies. However, in humans it has been clarified that adipocyte (fat cell), which composes the body fat (adipose) tissues, development and the extent of subsequent fat accumulation are closely associated with the occurrence and advancement of various common diseases (e.g., type-2 diabetes, coronary artery disease, and hypertension) resulting from obesity. Recent exciting progress in clinical and biochemical studies of adipocytes has rapidly clarified the functions of adipocytes and adipose tissue. Interesting findings are the function of white adipocytes as "secreting cells" and the molecular mechanism undelying adipocyte differentiation at the transcriptional level in relation to nuclear receptors. Consequently, the adipose tissue is being targeted for the prevention or treatment of many common diseases. In this review, I will focus on recent information on characteristics of adipocytes and the relationship between obesity and common obesity-related diseases.

Key words: adipocyte, obesity, differentiation, common diseases, nutrigenomics, fat, PPARs

OBESITY IN GENERAL

Obesity occurs when a controlled or regulated system fails to maintain energy balance and resulting in excess body fat storage. Americans are even fatter than they think they are, with nearly one-third of all adults, i.e., almost 59 million people, were classified as obese in a disturbing new government survey in 2002 (the American National Health and Nutrition Examination Surveys), based on actual body measurements (1). The surveys have been conducted periodically for several years. Twenty-three percent of adults were obese in 1994, as compared to 15 percent in 1980. The latest survey in 2002 also found that nearly two-thirds of US adults were overweight, or had a body-mass index (BMI) of between 25 and 30. The International Obesity Task Force defines obesity as having a BMI of 30 or above. In recent years, BMI has become the medical standard used to measure overweight and obesity. BMI is obtained using a mathematical formula based on a person's height and weight, i.e., BMI equals weight in kilograms divided by height in meters squared (BMI = kg/m^2). A BMI of 25 to 29.9 indicates a person is overweight. A person with a BMI of 30 or higher is considered obese.

The Japan Society for the Study of Obesity (JASSO) sets the obesity standard at a BMI of 25. In the United States, the government's obesity level is 30, and doctors usually do not recommend obesity treatment for Caucasians unless they have reached this level (2). Although less than 3% of Japanese are obese under standards set by the International Obesity Task Force, the percentage increases to 20% when determined by Japanese standards, which are based on risk factors obesity-related diseases. Using the Japanese definition, the percentage of obese Japanese men increased from 14.7% in 1979 to 24.1% in 1998; for women, it remained constant at 18.7% (a report from the Japanese Ministry of Health, Labor and Welfare).

It is speculated that the genetic background of most Japanese people, i.e., carrying single nucleotide polymorphisms (SNPs), means that even relatively small weight gains substantially increase susceptibility to these diseases. Obesity and it's related diseases tend to run in families and races, suggesting a genetic cause. Genes, however, do not predestine people to a lifetime of obesity. Environment also strongly influences obesity. This includes life style, such as what a person eats and his or her degree of physical activity.

Recent progress in clinical and biochemical studies of obesity has greatly clarified the functions of adipocytes, which compose the adipose tissue. In humans it has been clarified that adipocyte differentiation and the extent of subsequent fat accumulation are closely associated with the development and advancement of various common

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diseases, such as type-2 diabetes, cardiovascular disease and hypertensionand some types of cancer, resulting from obesity.

ADIPOSE TISSUE

We possess body fat, i.e., the adipose tissue, composed of adipocytes (fat cells), but do not know its detail. Adipocytes include white and brown adipocytes. The term sole adipocyte refers to the former. The minimum requirement for cells to be classified as white adipocytes is the ability to both synthesize and decompose fat. Both types of adipocytes develop and differentiate into tissues in characteristic parts of organisms. The white adipose tissue has been considered to merely store fat and to be a metabolically inactive tissue. However, recent findings disclose that the adipose tissue regulates metabolic pathways that are under the control of the sympathetic nervous system or the endocrine system. It has been clarified that the adipose tissue associated with fat metabolism and carbohydrate metabolism is a key to maintaining the energy balance within an organism.

White adipose tissue functions as an energy server

The white adipose tissue (WAT) specifically stores surplus energy in the form of triacylglyceride (fat) after the intake of food and resupplies energy in the form of fatty acid and glycerol under the control of the nervous and endocrine systems. It is an important system for maintaining energy homeostasis. WAT is widely distributed throughout the body in large quantities. The adipose tissue consists of various cell types, such as adipocytes in which fat droplets are stored, preadipocytes, fibroblasts, endothelial cells of blood vessels and neurocytes.

It was previously believed that 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, a detailed study revealed that the number of adipocytes may increase in adults with excessive energy intake or who lack exercise, reaching as much as 40 to 60 billion cells in obese people (3). The total 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 human adipocytes varies greatly from 10 to 140 (average 70～90) micrometers. One mature adipocyte normally contains 0.5 to 1 microgram of fat up to a maximum amount of 4 microgram.

In adults with mild obesity, the fat content of each adipocyte increases resulting in cell hypertrophy, which has been determined to be closely associated with the occurrence of a common disease. Meanwhile, since the size of adipocytes can only increase up to a certain extent, if food is taken excessively, hyperplasty results and obtained energy is quickly stored without expending it.

Brown adipose tissue function as a heater

The brown adipose tissue (BAT), or brown fat as it is also called, was first described by Conrad Gesner in 1551 (4). BAT has highly developed thermogenic functions such as a heater. One kilogram of tissue possesses 300 to 400 W of thermogenetic ability, which is far higher than that for basal metabolism of mammals in general, i.e., 4.1 W per 1 kg body weight. BAT is known to function in awakening of hibernants or maintaining hyperthermia during periods immediately after parturition of animals including humans. Since this tissue is hardly observed macroscopically, its function in humans tends to be questioned, unlike that in experimental animals. However, a series of genes (UCP 2,3 and 4) similar to the gene encoding the uncoupling protein, UCP 1, which is a thermogenetic molecule in brown adipocytes, were recently cloned. Ever since, it was determined that these genes are present in abundance in the human skeletal muscle. UCP has been the focus of great interest as a target gene for regulating obesity. More detail studies have been published (5-7).

ADIPOGENESIS: DIFFERENTIATION AND PROLIFERATION OF ADIPOCYTES

Does the number of adipocytes remain constant throughout life?

Regarding the number of adipocytes, it has been generally believed that the number is fixed early in life and then remains constant throughout life for both rats (8) and humans (9). Consequently, any expansion of the adipose tissue of adults should only occur through an increase in fat cell size. Since there is a limit to a cell size increase, the magnitude of the fat gain would be limited. A detail examination showed that accurate discrimination between the hypertrophic and hyperplastic forms of obesity is difficult, and that mixed types of obesity are frequently observed in both animals and humans (10). Then, a recent study suggested that the number of adipocytes might increase even in adults (11). Bertrand and colleagues reported the increase in the number of adipocytes in retroperitoneal depots throughout most of the life span (12).

Until recently, it has been believed that the number of adipocytes increases only at the preadipocyte stage. However, Sugihara and colleagues recently reported that the proliferation of adipocytes was observed even at the mature stage (13). Recently, they presented the obesity criteria for cell biological classification based on their experimental data in relation to BMI as shown Fig. 1 (14). They observed three proliferation types of human obese

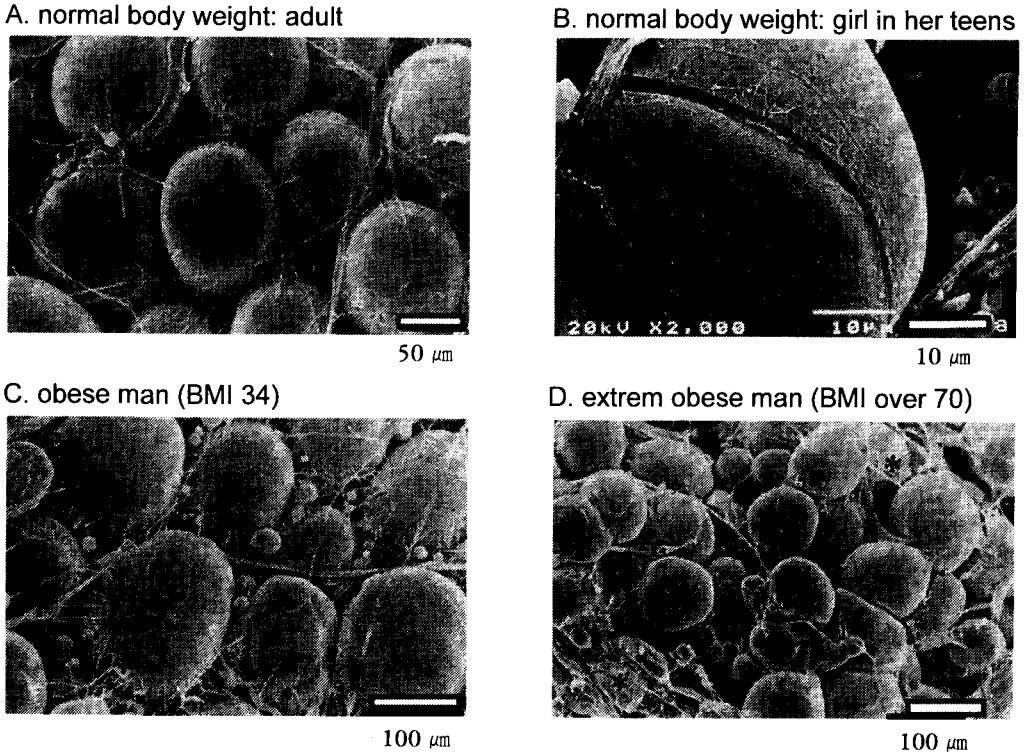

Fig. 1. Obesity criteria for cell biological classification. Based on experimental data on BMI (normal: A, normal, high magnification: B, 34: C, 70 over: D). In B, it is noteworthy that large mature adipocyte was split into two cells. Three developmental types of human obese adipose tissue termed, 1) the hypertrophy predominant type, 2) the hypertrophy and Hyperplasia type, and 3) the hyperplasia predominant type (with permission from J Japan Soc for the Study of Obesity. 2002).

adipose tissue as follows: 1) the hypertrophy predominant type, 2) the hypertrophy and hyperplasia type, and 3) the hyperplasia predominant type. These proliferation types have the ability to double. Therefore, the adipose tissue may physiologically adapt to rapid storing of excessive energy. Therefore, the idea that the number of adipocytes increases in adults is now accepted as a general concept.

On the cell biochemical aspect, the proliferation of preadipocytes or fibroblast-like adipocytes is highly dependent on "competent factor" into "progression factor" as a protein growth factor (15). We previously reported the presence of a protein factor (preadipocyte growth factor: PAGF) in the rat adipose tissue, which specifically permits the proliferation of 3T3-L1 and Ob1771 preadipocytes cultured in a completely defined serum-free medium containing only progression factors (epidermal growth factor and insulin) (16). Furthermore, we found that PAGF in fat depots functions in response to energy intake and contributes to the de novo formation of adipocytes and the growth of adipose tissue (17). PAGF may be a useful tool for further elucidation of the relationship between energy storage in adipose tissue and adipose tissue development. Recently, it has been reported that FGF10 secreted from adipocyte itself functions as an important factor at the terminal adipocyte differentiation (18).

Molecular mechanism of adipocyte differentiation depends on transcription factors and modified factors

A key regulator for the expression and regulation of adipocyte differentiation genes has been recently clarified. A study revealed that the CCAAT/enhancer binding proteins (C/EBPs)and the peroxisome proliferator-activated receptors (PPARs) interact with each other, form a network and function as a master regulator (19). C/EBPs are a family of leucine zipper-type transcription factors. PPARs are a family of ligand-dependent receptor-type transcription factors. PPAR γ and C/EBP α are bound to their corresponding genomic promoter field and activate transcription to maintain each other's activity.

C/EBPs

It was reported that C/EBP α is the dominant candidate as a transcription factor directly associated with adipocyte differentiation. It was clarified that a C/EBP response element exists in fatty acid binding protein (aP2), phosphoenolpyruvate carboxykinase (PEPCK), leptin, and even the promoter region of major adipocyte-differentiation-related proteins such as PPAR γ . The C/EBP and PPAR families function as a master regulator for adipocyte differentiation by forming a network.

PPAR gene cloning and subtypes

Peroxisome proliferator-activated receptors (PPARs), a

family of nuclear receptors, are the so-called orphan receptors with an unidentified ligand when these were discovered. PPARs also have transcription activation ability induced by compounds such as an anti-hyperlipidemia drug (clofibrate), which induces the hepatocyte peroxisome, and an anti-diabetes drug (thazolidinedione), which ameliorate insulin sensitivity. Since an induction agent is not directly bound to PPAR as its ligand, the term "activated" is attached to the name PPAR. The α type was cloned for the first time from a mouse liver cDNA library in 1990 (20). After that, several PPAR subtype genes were identified from various origins. These PPAR subtype genes form a family (Fig. 2).

In mammals, three PPAR subtype genes, namely α, δ [called as NUC I in humans, fatty acid-activated receptor (FAAR) in mice, and PPARβ in frogs] and γ, were identified. The α type is expressed mainly in the liver, cardiac muscle and digestive tract, while the δ type is expressed not in specific tissues but ubiquitously in all tissues. Meanwhile, the γ type has two isoforms, the γ 1 and γ 2. They are formed from different mRNAs using the 5′ terminal by selecting the promoter of the PPAR γ gene. The γ 2 isoform specifically expressed in adipocytes is closely associated with adipocyte differentiation which will be discussed subsequently (21). The γ 1 isoform is expressed in organs of the immune system, the adrenal glands and the small intestine. Moreover, γ 3 was found in the human adipose tissue whereas γ 6 and γ 7 isoforms in monkey adipose tissue (22).

PPAR target genes

Similar to nuclear receptors such as the retinoic acid receptor (RAR), the thyroid hormone receptor (TR) and the vitamin D receptor (VDR), each PPAR forms a stable heterodimer with the retinoide x receptor (RXR), whose ligand is 9-cis retinoic acid. Each PPAR is then bound to an element encoded by a specific DNA sequence (AGGTCA-X-AGGTCA; peroxisome proliferator response element (PPRE)) of a target gene (23). Many PPAR target genes are associated with carbohydrate and lipid metabolism in the liver, muscle and adipose tissue.

Nutrigenomics on activation factors for PPARs

An important characteristic of the PPARs is that the natural and chemical synthetic compounds with various chemical structures can accommodate ligands as shown in Fig. 2. The α and δ types accept the clofibrate group or the fatty acid group as a ligand, while the γ type accepts thiazolidinedione derivatives (TZDs), 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 (Fig. 2).

On the nutritional aspect, it is interesting that long chain fatty acids such as linoleic acid, arachidonic acid and eicosapentaenoic acid (EPA) in food directly act as ligands for PPAR α, γ and δ (24,25). Moreover, several natural food components act as PPAR ligands (26). They function not only as adipocyte differentiation regulators but also as PPAR molecular sensors in lipid metabolism to play a significant role in maintaining the obese state. This interesting finding is the focus of several recent nutritional studies or nutrigenomics (26-28).

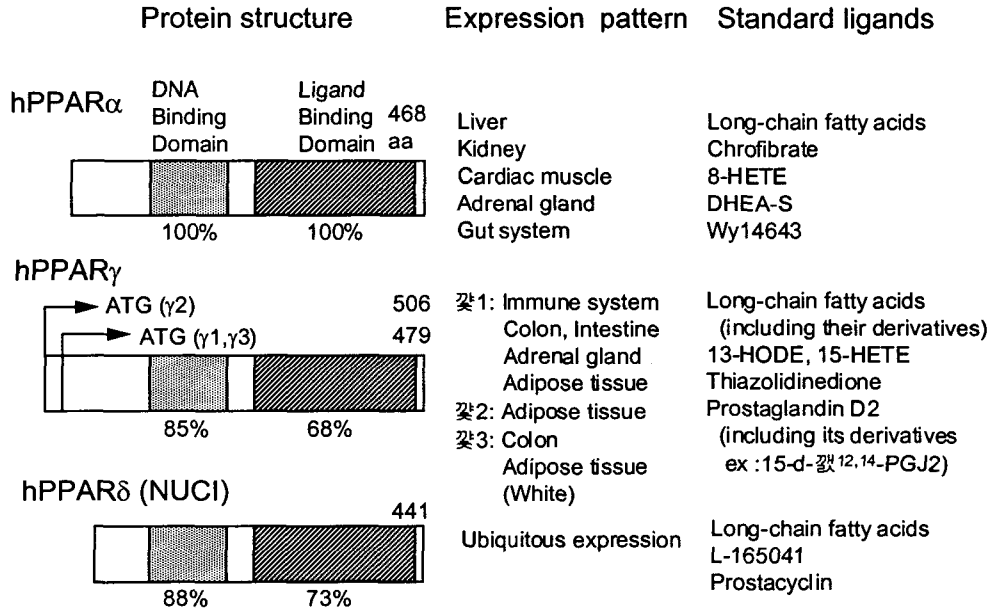

Fig. 2. Biochemical and pharmacological properties of human PPAR α, γ and δ subtypes. Natural and synthetic compounds with various chemical structures that can accommodate ligands should be developed in the future.

Anti-diabetic drugs, such as TZDs, which improve insulin resistance, are compounds originally developed on the basis of their effect of decreasing blood glucose levels as an index for genetically obese animals. The mechanism of action of TZDs was initially unknown. Subsequently, further clarified that TZDs do not only have the effect of decreasing glucose and lipid levels in the blood, but also strongly induce adipocyte differentiation (29). What is interesting is that, according to some recent reports, TZDs and 15-deoxy-$\Delta^{12,14}$-prostaglandine J2, which is a prostaglandin D_2 metabolite, bind directly to PPAR γ as a ligand essential for induction of adipocyte differentiation (30,31).

Another interesting point is that oxidized metabolites of linoleic acid (9- and 13-hydroxy octadecadienoic acid) produced from oxidized LDL were identified as endogenous ligands of PPAR γ, and that PPAR γ is also viewed as a key regulator of gene expression in foam cells (32). PPARs are considered as a significant factor in cardiovascular diseases.

Nutritional behavior and gene mutation determining the activation of PPAR γ

Some reports on nutritional behavior determining the expression of PPAR γ revealed no relationship between the extent of obesity in mice, the intake of fat-enriched food and the PPAR γ mRNA expression level. The expression level of PPAR γ 2 is reduced by fasting and taking vitamins A and D, and the expression level of PPAR γ 2 mRNA is enhanced by insulin (33). Adipocyte differentiation is controlled by cell growth factors. PPAR γ is phosphorylated and inactivated by a mitogen-activated protein (MAP) kinase induced by a growth factor stimulus in cultured adipocytes (34). Furthermore, in a recent study in which proline adjacent to the 114th serine, which is the phosphorylated site of human PPAR γ, was subjected to missense mutation and transformed into glutamine, Pro115Gln mutation was observed. This mutation was closely associated with the occurrence of human obesity (35). Accordingly, it is assumed that human PPAR γ subjected to the aforementioned phosphorylation becomes inactive and controls adipocyte differentiation in a normal state. Furthermore, some reports state that Pro12Ala mutation results in a high BMI (36), while Pro467Leu and Val290Met mutations result in a normal BMI and strong insulin resistance (37).

Cofactors associated with PPAR function and adipocyte differentiation

Lately, characteristics of coactivators or corepressors, called cofactors, which promote interactions between nuclear receptors and proteins, and control activation of transcription positively or negatively, have been clarified. In 1993, it was reported that the CREB (cAMP response element binding-protein) binding-protein (CBP) is a coactivator (integrator) essential for activation of transcription by nuclear receptors. CBP exhibits a high amino acid sequence similarity with p300, which has the same transcription factor coactivator function; therefore, CBP is called CBP/p300. CBP is a 265-KD nuclear protein with histone acethylase (HAT) activity that induces chromatin remodeling. CBP forms a protein complex with various signal transduction systems, such as Jun, Fos, C/EBPβ, NF-KB, MyoD, and SRC-1, and functions as an integrator.

Recently, we reported the direct evidence of the requirement for CBP/p300 as a coactivator of PPAR γ for adipocyte differentiation and the mechanism of action of TZDs and other ligands using 3T3-L1 preadipocytes stably expressing CBP- or p300-specific ribozymes (38). Our results suggest that both CBP and p300 are indispensable for the full activation of PPAR γ and adipocyte differentiation, and that they do not mutually complement in the process.

ADIPOCYTOKINES AND COMMON OBESITY-RELATED DISEASES

Since animals are under constant threat of starvation, storage of energy sources inside the body for activities such as capturing preys or escaping from predators is essential for survival. Therefore, animals possess a highly sophisticated mechanism of storing energy inside their bodies. The adipose tissue highly efficiently stores energy in the form of fat via a series of processes, namely, 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. Moreover, progress in biological studies and particularly in biochemical studies of adipocytes in recent years has gradually clarified the functions of adipocytes.

The adipose tissue is significant in the regulation of common diseases such as obesity, type-2 diabetes, cardiovascular disease and hypertension (3,39). This is because, during their differentiation and maturation, adipocytes release many bioactive molecules (called "adipocytokines"), including adipsin, angiotensinogen, leptin, tumor necrosis factor-α (TNF-α) and adiponectin (39) (Fig. 3). TNF-α is a negative factor released from mature adipocytes, that is, it suppresses glucose uptake into adipose tissues or skeletal muscles (40). On the other hand, adiponectin, a novel adipocyte derived hormone, is a positive factor released from nonmature adipocytes, that is, it enhances insulin sensitivity (41,42). Thus, under-

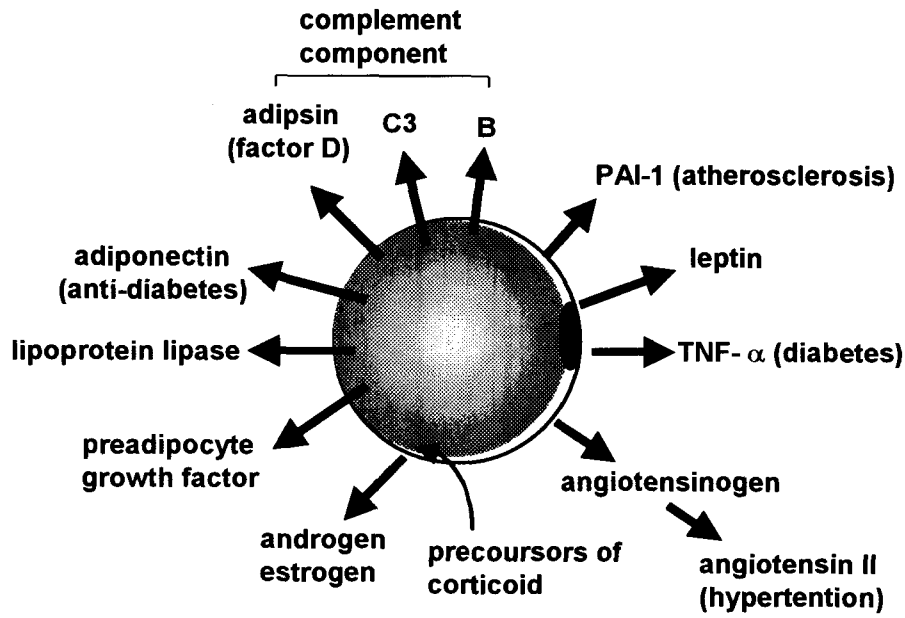

Fig. 3. An adipocyte acts as a secretary cell. Adipocytes release many bioactive molecules (called "adipocytokines"), including adipsin, angiotensinogen, leptin, tumor necrosis factor- α (TNF- α), adiponectin and others. These adipocytokines are associated with the development of common obesity-related diseases.

standing the mechanism underlying adipocyte differentiation is essential to managing common diseases. Furthermore, it is significant to clarify the role of factor groups secreted from adipocytes in the development of common obesity-related diseases.

CONCLUSION AND FUTURE ASPECTS

The function of adipocytes as "secreting cells" may yield some benefits, such as adiponectin acting as an anti-diabetes hormone or applied to tissue enginering and transplant in the field of medicine. The framework of the physiological response of the adipose tissue involves a regulatory mechanism by ligand-requiring PPAR and coactivators. Physiologically, nutritional ligands such as fatty acids or their derivative prostanoids should be supplied. As has been pointed out, obesity-related diseases easily caused by excessive intake of high-energy food, such as type 2 diabetes mellitus, are a result of breakdown of this system (43). It is obvious that the signal-transmitting network via the PPAR-coactivator system is involved in the regulatory mechanism. Further clarification of this system will lead to the development of new nutritional and diet therapies from the viewpoint of nutrigenomics or of new drugs against common diseases associated with obesity.

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