ORIENTAL YEAST Co., Ltd.. Male nude and C57BL/6J mice (Charles River Japan) were used as recipients. The cells were stained using PKH26 Red Fluorescent Cell Linker kit for General Cell Membrane Labeling (Sigma-Aldrich) to identify the transplanted cells *in vivo*.

Bolheal (The Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan) was used as clinically available fibrin glue. Fibrinogen solution and thrombin solution were diluted four and two times using DMEM-HAM/F12 (Sigma) respectively before use. Cells were suspended at $1 \times 10^7$ cells/ml by diluted thrombin solution, and injected subcutaneously into the mouse with same volume of diluted fibrinogen solution using injection apparatus included in Bolheal kit. We also transplanted the cells suspended in Matrigel (BD Biosciences, Bedford, MA) at $5 \times 10^6$ cells/ml. In both cases, $5 \times 10^6$ cells were transplanted.

All mice were allowed free access to regular chow and water. Three animals were sacrificed to take serum samples at day 1, 14, 28. In C57BL/6J mice experiments, blood samples were taken from tail without sacrifice to monitor the hLCAT delivery in same animal at day 1, 4, 7, 14 and 28. Transplanted region was taken under fluorescent microscopic observation by SZX16 reflected fluorescence system (OLYMPUS corp. Tokyo, Japan).

### Histological staining

The explanted tissues were fixed in 4% paraformaldehyde following replaced 30% gum-saccarose and embedded in Tissue-Tek O.C.T. Compound (Sakura Finetechnical Co., Ltd, Tokyo, Japan). Immunohistochemical staining was performed using anti-LCAT rabbit monoclonal antibody (250:1; EPITOMICS) and Alexa Fluor 488 goat anti-rabbit IgG (1000:1; Invitrogen) as primary and secondary antibody, respectively. The slides were counterstained with DAPI using VECTASHIELD Mounting Medium with DAPI (Vector Laboratories, Inc., Burlingame, CA). TUNEL staining of the explanted tissues were performed using *In situ* Apoptosis Detection Kit (TaKaRa Bio Inc., Shiga, Japan). Ki67 imuunostaining was performed using anti-mouse Ki67 Rabbit polyclonal antibody (Abcam plc., Cambridge, UK), followed by biotin-conjugated anti-Rabbit Ig/HRP-conjugated streptavidin reaction. Signals were visualized by HRP reaction with DAB and the slides were counterstained with hematoxylin for TUNEL and Ki67 staining.

### Statistical analysis

Data are presented as means ± S.D. Statistical comparison were made by Student's *t*-test or by ANOVA followed by the post hoc Tukey test to compare using SPSS software. In all cases, *P* values of less than 0.05 were considered as significant.

### Acknowledgements

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## References

Asada S, Kuroda M, Aoyagi Y, Bujo H, Tanaka S, Konno S, Tanio M, Ishii I, Aso M, Saito Y. Disturbed apolipoprotein A-I-containing lipoproteins in fish-eye disease are improved by the lecithin:cholesterol acyltransferase produced by gene-transduced adipocytes *in vitro*. Mol Genet Metab 2011;102:229-31

Billings E Jr, May JW Jr. Historical review and present status of free fat graft autotransplantation in plastic and reconstructive surgery. Plast Reconstr Surg 1989;83:368-81

Cho SW, Kim I, Kim SH, Rhie JW, Choi CY, Kim BS. Enhancement of adipose tissue formation by implantation of adipogenic-differentiated preadipocytes. Biochem Biophys Res Commun 2006;345:588-94

Fielding CJ, Fielding PE. Molecular physiology of reverse cholesterol transport. J Lipid Res 1995;36:211-28

Gimble JM, Katz AJ, Bunnell BA. Adipose-derived stem cells for regenerative medicine. Circ Res 2007;100:1249-60

Ito M, Bujo H, Takahashi K, Arai T, Tanaka I, Saito Y. Implantation of primary cultured adipocytes that secrete insulin modifies blood glucose levels in diabetic mice. Diabetologia 2005;48:1614-20

Kimura Y, Ozeki M, Inamoto T, Tabata Y. Adipose tissue engineering based on human preadipocytes combined with gelatin microspheres containing basic fibroblast growth factor. Biomaterials 2003;24:2513-21

Kitagawa Y, Kawaguchi N. De novo adipogenesis for reconstructive surgery. Cytotechnology 1999;31:29-35

Kuramochi D, Unoki H, Bujo H, Kubota Y, Jiang M, Rikihisa N, Udagawa A, Yoshimoto S, Ichinose M, Saito Y. Matrix metalloproteinase 2 improves the transplanted adipocyte survival in mice. Eur J Clin Invest 2008;38:752-9

Kuroda M, Aoyagi Y, Asada S, Bujo H, Tanaka S, Konno S, Tanio M, Ishii I, Machida K, Matsumoto F, Satoh K, Aso M, Saito Y. Ceiling culture-derived proliferative adipocytes are a possible delivery vehicle for enzyme replacement therapy in lecithin:cholesterol acyltransferase deficiency. Open Gene Ther J 2011; In press

Landazuri N, Krishna D, Gupta M, Le Doux JM. Retrovirus-polymer complexes: study of the factors affecting the dose response of transduction. Biotechnol Prog 2007;23:480-7

Malafaya PB, Silva GA, Reis RL. Natural-origin polymers as carriers and scaffolds for biomolecules and cell delivery in tissue engineering applications. Adv Drug Deliv Rev 2007;59:207-33

Mano JF, Silva GA, Azevedo HS, Malafaya PB, Sousa RA, Silva SS, Boesel LF, Oliveira JM, Santos TC, Marques AP, Neves NM, Reis RL. Natural origin biodegradable systems in tissue engineering and regenerative medicine: present status and some moving trends. J R Soc Interface 2007;4:999-1030

Matsumoto F, Bujo H, Kuramochi D, Saito K, Shibasaki M, Takahashi K, Yoshimoto S, Ichinose M, Saito Y. Effects of nutrition on the cell survival and gene expression of transplanted fat tissues in mice. Biochem Biophys Res

Commun 2002;295:630-5

Mizuno H, Itoi Y, Kawahara S, Ogawa R, Akaishi S, Hyakusoku H. *In vivo* adipose tissue regeneration by adipose-derived stromal cells isolated from GFP transgenic mice. Cells Tissues Organs 2008;187:177-85

Neuss S, Apel C, Buttler P, Denecke B, Dhanasingh A, Ding X, Grafahrend D, Groger A, Hemmrich K, Herr A, Jahnen-Dechent W, Mastitskaya S, Perez-Bouza A, Rosewick S, Salber J, Wöltje M, Zenke M. Assessment of stem cell/biomaterial combinations for stem cell-based tissue engineering. Biomaterials 2008;29:302-13

Ning H, Liu G, Lin G, Yang R, Lue TF, Lin CS. Fibroblast growth factor 2 promotes endothelial differentiation of adipose tissue-derived stem cells. J Sex Med 2009;6:967-79

Patrick CW Jr. Adipose tissue engineering: the future of breast and soft tissue reconstruction following tumor resection. Semin Surg Oncol 2000;19:302-11

Patrick CW Jr. Tissue engineering strategies for adipose tissue repair. Anat Rec 2001;263:361-6

Piasecki JH, Moreno K, Gutowski KA. Beyond the cells: scaffold matrix character affects the *in vivo* performance of purified adipocyte fat grafts. Aesthet Surg J 2008;28:306-12

Stashower M, Smith K, Williams J, Skelton H. Stromal progenitor cells present within liposuction and reduction abdominoplasty fat for autologous transfer to aged skin. Dermatol Surg 1999;25:945-9

Torio-Padron N, Baerlecken N, Momeni A, Stark GB, Borges J. Engineering of adipose tissue by injection of human preadipocytes in fibrin. Aesthetic Plast Surg 2007a;31:285-93

Torio-Padron N, Borges J, Momeni A, Mueller MC, Tegtmeier FT, Stark GB. Implantation of VEGF transfected preadipocytes improves vascularization of fibrin implants on the cylinder chorioallantoic membrane (CAM) model. Minim Invasive Ther Allied Technol 2007b;16:155-62

Yamaguchi M, Matsumoto F, Bujo H, Shibasaki M, Takahashi K, Yoshimoto S, Ichinose M, Saito Y. Revascularization determines volume retention and gene expression by fat grafts in mice. Exp Biol Med (Maywood) 2005;230:742-8

Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, Benhaim P, Lorenz HP, Hedrick MH. Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng 2001;7:211-28

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# Adiponectin: A Key Player in Obesity Related Disorders

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**Abstract:** Recent studies revealed that adipose tissue is not only an energy storing organ, but is a kind of endocrine organ which secretes a variety of bioactive substances, so-called adipokines or adipocytokines. Visceral fat accumulation is associated with hypersecretion of adipocytokines such as tumor necrosis factor-alpha and plasminogen activator inhibitor-1 which may regulate inflammatory and atherogenic diseases. Adiponectin is a relatively new adipocytokine which we discovered in 1996 and has anti-diabetic, anti-atherogenic and anti-inflammatory properties. Adiponectin is present in plasma at a very high concentration, but in contrast to other adipocytokines, its production is reduced in subjects with visceral fat accumulation and the plasma levels are negatively correlated with visceral adiposity. Hypoadiponectinemia induced by visceral fat accumulation is closely associated with type 2 diabetes, lipid disorders, hypertension and also certain inflammatory diseases. In this review, the mechanisms of obesity-related diseases including nonalcoholic fatty liver disease will be discussed from the aspect of important roles of adipocytokines, especially adiponectin.

**Keywords:** Visceral fat, adipocytokines, adiponectin, metabolic syndrome.

## 1. INTRODUCTION

Excess body fat, especially intra-abdominal visceral fat accumulation, is associated a number of disease condition including dyslipidemia, type 2 diabetes, hypertension and cardiovascular disease [1]. Therefore visceral fat accumulation estimated by waist circumference is adopted as an essential component of the metabolic syndrome which has been recently noted as a highly atherogenic state. Recent research including ours has shown that adipose tissue secretes various bioactive substances collectively referred to as adipocytokines that may directly contribute to pathogenesis of conditions associated obesity [2]. Thus, adipose tissue seems to be an endocrine organ that can affect the function of other organs, including the vascular walls in the whole body, through secretion of various adipocytokines. These adipocytokines include heparin-binding epidermal growth factor-like growth factor (HB-EGF), leptin, tumor necrosis factor-α(TNF-α), plasminogen activator inhibitor type1(PAI-1) and angiotensinogen [3,4]. The expression and plasma levels of these adipocytokines increase with visceral fat accumulation and are implicated in insulin resistance and atherosclerosis. In addition to the analysis of known genes shown above, we identified adipose most abundant gene transcript-1 which we named apM-1 [5] and the protein encoded by apM-1 adiponectin [6]. In contrast to other adipocytokines, adiponectin levels are inversely correlated to visceral adiposity and low levels have been associated with visceral obesity, type 2 diabetes, hypertension and cardiovascular disease [7].

This article discusses physiological roles of adiponectin in the prevention of obesity-related diseases especially focusing on its anti-immflamatory function.

## 2. DISCOVERY OF ADIPONECTIN AND ITS CLINICAL SIGNIFICANCE

When we started the comprehensive analysis of expressed genes in human adipose tissue, 40% of expressed genes in adipose tissue were unknown, in other words, novel genes. The molecule encoded by apM-1 possesses a signal peptide, collagen-like motif and globular domain, and has notable homology with collagen X, VIII and complement factor C1q. We termed the collagen-like protein adiponectin. The mouse homolog of adiponectin has been cloned as AdipoQ or ACRP30 [8,9]. We established the method for the measurement of plasma adiponectin levels using enzyme-linked immunosorbent assays [10]. The average levels of adiponectin in human plasma are extremely high—up to 5-10 μg/ml. Plasma concentrations are negatively correlated with body mass index (BMI), whereas leptin increases with BMI. The negative correlation of adiponectin levels and visceral adiposity is stronger than between adiponectin levels and subcutaneous adiposity (Fig. 1).

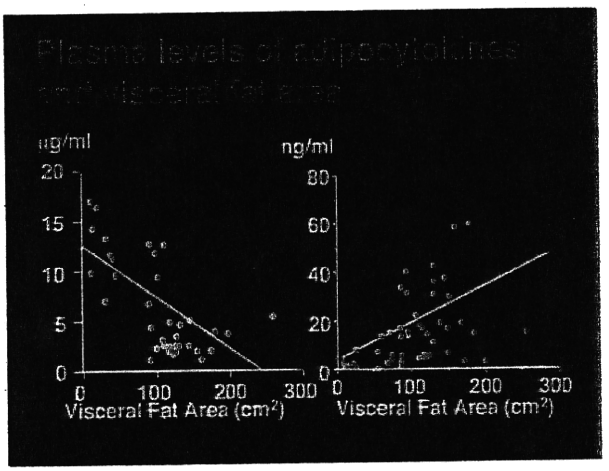


**Fig. (1).** Plasma levels of adiponectin are negatively correlated with visceral adiposity.

The mechanism by which plasma levels are reduced in individuals with visceral fat accumulation is not yet clarified. Co-culture with visceral fat inhibits adiponectin secretion from subcutaneous adipocytes [11]. This finding suggests that some inhibiting factors for adiponectin synthesis or secretion are secreted from visceral adipose tissue. Tumor necrosis factor-α was reported to be a strong inhibitor of adiponectin promoter activity [12]. The negative correlation between visceral adiposity and adiponectin levels might be explained by the increased secretion of this cytokine from accumulated visceral fat as at least one mechanism.

Plasma adiponectin concentrations are lower in people who have type 2 diabetes mellitus than in BMI-matched controls [13]. The plasma concentrations have been shown to correlate strongly with insulin sensitivity, which suggests that low plasma concen-

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trations are related to insulin resistance [14]. In a study of Pima Indians, individuals with high levels of adiponectin were less likely than those with low concentrations to develop type 2 diabetes [15]. High adiponectin concentration was, therefore, a notable protective factor against development of type 2 diabetes.

Studies on adiponectin$^{-/-}$ mice support observations in humans. The knockout mice showed no specific phenotype when they were fed a normal diet but a high-sucrose and high-fat diet induced a marked elevation of plasma glucose and insulin levels. Notable insulin resistance, estimated by insulin tolerance test during the high-sucrose with high-fat diet, also developed in the knockout mice. The supplementation of adiponectin by adenovirus transfection clearly improved this insulin resistance [16]. Although I will not mention the details of molecular mechanism, adiponectin has been shown to exert its actions on muscle fatty acid oxidation and insulin sensitivity by activation of AMP-activated protein kinase [17,18]. Plasma levels of adiponectin are also decreased in hypertensive humans, irrespective of the presence of insulin resistance [19]. Endothelium-dependent vasoreactivity is impaired in people with hypoadiponectinemia, which might be at least one mechanism of hypertension in visceral obesity [20].

Most importantly, plasma concentrations of adiponectin are lower in people with coronary heart disease than in controls even when BMI and age are matched. Kaplan–Meier analysis in Italian individuals with renal insufficiency demonstrated that those with high adiponectin concentrations were free from cardiovascular death for longer than other groups [21]. A case–control study performed in Japan demonstrated that the group with the plasma levels less than 4 μg/ml has been shown to have increased risk of coronary artery disease and multiple metabolic risk factors, which indicates that hypoadiponectinemia is a key factor in the metabolic syndrome [22]. A prospective study by Pischon *et al.* [23] confirmed that high adiponectin concentrations are associated with reduced risk of acute myocardial infarction in men. In addition to hypoadiponectinemia accompanied with visceral fat accumulation, genetic hypoadiponectinemia caused by a missense mutation has been reported, which also exhibit the clinical phenotype of metabolic syndrome [24]. These clinical evidences show that hypoadiponectinemia is a strong risk factor for cardiovascular disease.

## 3. ADIPOSE TISSUE AND INFLAMMATION

Recent studies revealed that inflammatory responses contribute to the development of a variety of common diseases including atherosclerosis and metabolic diseases including diabetes mellitus. On the other hand, adipose tissue has been recognized to secrete bioactive substances which relate to inflammation. TNF-α is a typical cytokine which plays a major role in inflammatory cellular phenomena. Since Hotamisligil first reported that adipose tissue secretes this cytokine and one of the candidates for molecules inducing insulin resistance [25], TNF-α has been recognized as an important adipocytokine. It has been shown that adipose TNF-α mRNA and plasma TNF-α protein are increased in most animal models and humans with obesity and insulin resistance [26,27]. Neutralizing the blood TNF-α in obese rats with a soluble TNF-α receptor-immunoglobulin G fusion protein markedly improves insulin resistance. These results indicate that higher production of TNF-α in accumulated adipose tissue may be causative for obesity-associated insulin resistance. In addition to TNF-α, IL-1β, IL-6, macrophage migration factor, nerve growth factor and haptoglobin have been shown to be secreted from adipose tissue and linked to inflammation and the inflammatory response. More recently one of typical marker for inflammation, C-reactive protein (CRP) itself was found to be produced in adipose tissue and the CRP mRNA expression was found to be enhanced in adipose tissue in adiponectin KO mouse [28].

The elevated production of these inflammation–related adipocytokines is increasingly considered to be important in the development of diseases linked to obesity, particularly type2 diabetes and cardiovascular disease, so-called metabolic syndrome. Namely, adipose tissue is involved in extensive cross-talk with other organs and multiple metabolic systems through the various adipocytokines.

## 4. ADIPONECTIN AS A POTENT ANTI-INFLAMMATION ADIPOCYTOKINE

As already mentioned, adiponectin has plural functions for prevention of metabolic diseases and cardiovascular diseases. More recently adiponectin was shown to prevent liver fibrosis [29] and some kinds of cancer such as endometrial cancer [30], breast cancer [31] and colon cancer [32]. Besides these well characterized biological functions, recent evidences support a strong anti-inflammatory function. We first reported that adiponectin strongly suppresses the production of the potent pro-inflammatory cytokine, TNF-α in macrophages. Treatment of cultured macrophages with adiponectin significantly inhibits their phagocytic activity and their lipopolysaccharide-induced production of TNF-α. Suppression of phagocytosis by adiponectin is mediated by one of the complement C1q receptors, C1qRp, because this function was completely abrogated by the addition of an anti-C1qRp monoclonal antibody [33]. These observations suggest that adiponectin is an important negative regulator in immune and inflammation systems indicating that it may be involved in ending inflammatory responses through its inhibitory functions.

In the process of development of atherosclerosis, macrophages play crucial roles in plaque formation. Adiponectin attenuates cholesterol ester accumulation in macrophages. The adiponectin-treated macrophages contained fewer lipid droplet stained by oil red O [34]. Adiponectin suppresses the expression of the class A macrophage scavenger receptor(MSR) at both mRNA and protein levels by Northern and immunoblot analyses, respectively, without affecting the expression of CD36 which was qualified by the flow cytometry [35]. Adiponectin inhibited TNF-α-induced mRNA expression of monocyte adhesion molecules without affecting the interaction between TNF-α and its receptors in human aortic endothelial cells. Adiponectin suppressed TNF-α-induced IκB-α phosphorylation and subsequent NF-κB activation without affecting other TNF-α-mediated signals, including Jun N-terminal kinase, p38 kinase and Akt kinase [36]. This inhibitory effect of adiponectin is accompanied by cAMP accumulation and is blocked either adenylate cyclase inhibitor or protein kinase A (PKA) inhibitor. These observations suggest that adiponectin, which is naturally present at enormous amount in the blood stream, modulates the inflammatory response of both macrophages and endothelial cells through cross talk between cAMP–PKA and NF-κB signaling pathways (Fig. 2). Anti-inflammatory function of adiponectin may result in the prevention of atherogenic cell phenomena such as monocyte adhesion to endothelial cells, differentiation of monocytes to macrophages and finally foam cell formation.

Current studies have shown that adiponectin also induces various anti-inflammatory cytokines, such as interleukin-10(IL-10) or IL-1 receptor antagonist [37]. Acute coronary syndrome is considered to determine the prognosis of cardiovascular disease in which vulnerability of plaque is the important determinant of plaque rupture. In this process, matrix metalloproteinase (MMP) secreted by macrophages is thought to play an important part in plaque vulnerability. Tissue inhibitor of metalloproteinase (TIMP) is thought to act as protector of plaque rapture by inhibition of MMP activity. Adiponectin increases the expression of mRNA and protein production of TIMP in macrophages. Prior to the induction of TIMP formation and secretion, adiponectin is shown to induce IL-10 synthesis in macrophages, suggesting that adiponectin induces TIMP formation and secretion *via* induction of IL-10 synthesis in autocrine manner in macrophages and may inhibit MMP activity (Fig. 3) [38].

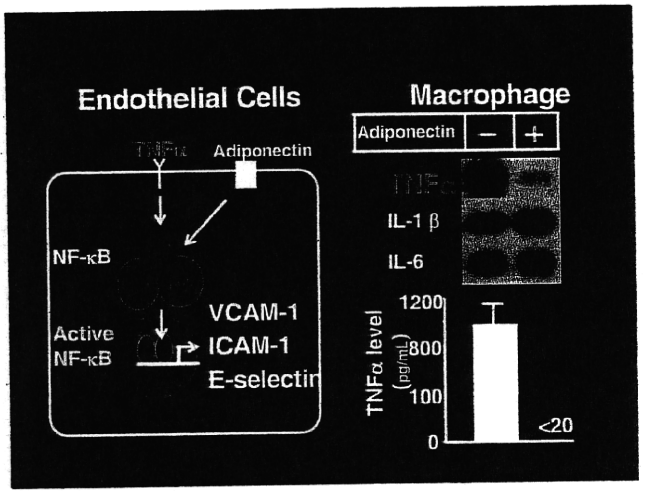


**Fig. (2).** Cell biological mechanism of anti-atherogenic function of adiponectin.

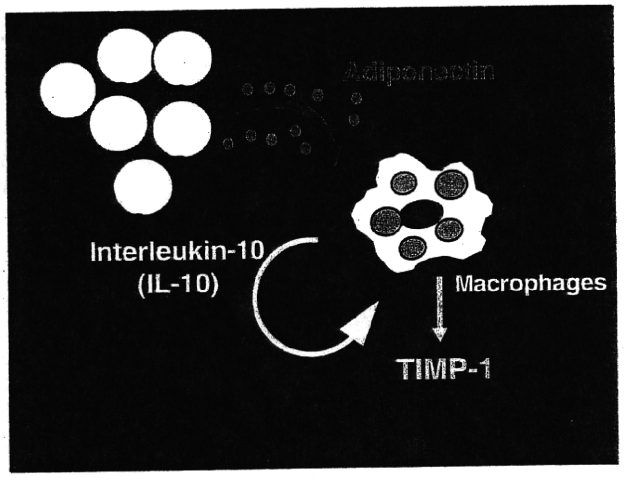


**Fig. (3).** Adiponectin enhances TIMP1 secretion from macrophages through induction of IL-10 expression.

These functions may result in the prevention of acute coronary syndrome. As I mentioned before, CRP is a typical marker of inflammation and high-sensitive CRP is now considered to be a risk factor for atherosclerosis. A number of studies have shown that there is a reciprocal association of adiponectin and C-reactive protein in plasma in healthy subjects and in the subjects with a variety of diseases including type 2 diabetes, metabolic syndrome, end-stage kidney disease [39,40]. The mechanism of this reciprocal association of CRP and adiponectin remains to be clarified. Our group detected the expression of adiponectin mRNA in human adipose tissue and demonstrated a significant inverse correlation between the CRP and adiponectin mRNA. In addition, the CRP mRNA level of white adipose tissue in adiponectin deficient mice was higher than that of wild-type mice [41]. The reciprocal association of adiponectin and CRP levels in both human plasma and adipose tissue might participate in the development of atherosclerosis *via* inflammatory response.

## 5. ADIPONECTIN AND NONALCOHOLIC FATTY LIVER DISEASE

Obesity especially visceral obesity is an independent risk factor in the development of nonalcoholic steatohepatitis (NASH) or nonalcoholic fatty liver disease(NAFLD) which may result in the development of liver cirrhosis and finally hepatic cell carcinoma (HCC) [42, 43]. Two –hit theory has been proposed for the pathogenesis of NAFLD. Namely the first hit of hepatic steatosis is followed by the second hit of oxidative injury leading to inflammation and progression to fibrosis and progression to fibrosis and cancer. In the following paragraphs, I will focus on the roles of adiponectin in each step of the two-hit theory of NAFLD pathogenesis.

### 1). Adiponectin and Hepatic Steatosis

Visceral fat accumulation plays a role in the development of liver steatosis. Increased release of fatty acids and glycerol, products of the lipolysis of visceral adipocyte triglyceride from accumulated visceral fat gets into the liver through portal circulation and enhances triglyceride formation in hepatocytes. In this process, we reported that free fatty acids (FFA) may act as a bioactive substance and enhance transcription acyl CoA synthesis gene, one of key factors for liver triglyceride synthesis [44]. In addition to this direct effect, visceral fat accumulation is closely related to the adiponectin reduction in plasma, which may be relevant to hepatic steatosis. Choline-deficient L-amino acid –defined diet has been known to induce fatty liver in rodent. Kamada et al reported that hepatic steatosis was induced more severely in adiponectin knockout mice than wild type and over-expression of adiponectin protein by adenovirus vector resulted in attenuation of hepatic steatosis [45]. The lack of adiponectin in these mice enhanced the expression of two rate-limiting enzymes in fatty acid synthesis, acetyl-CoA carboxylase(ACC) and fatty acid synthetase(FAS). Adiponectin is also known to stimulate mitochondrial β-oxidation of AMPK and PPAR-α. Activated PPAR-α upregulates carnitine palmitoyltransferase(CPT)-1, a rate –limiting enzyme in fatty acid oxidation. In addition, activated AMPK phosphorylates ACC and attenuates the activity of ACC [46]. Inactivation of ACC leads to decrease in the concentration of its product malonylCoA(a potent inhibitor of CPT-1) and induces fatty acid oxidation in the liver. Adiponectin was also reported to downregulate the expression of SREBP-1c, a master regulator of fatty acid synthesis.

### 2). Adiponectin and Liver Inflammation

As I previously showed, adiponectin is a potent anti-inflammatory protein which suppresses TNF-α-induced NFκB activation and block TNF-α release from endothelial cells and macrophages. [36] In a mouse model of lipopolysaccharide(LPS) – induced acute hepatitis, adiponectin has been shown to protect against hepatic injury through inhibition of production of a pro-inflammatory cytokine, TNF-α and induction of an anti-inflammatory cytokine, IL-10 in Kupffer cells [47]. Lack of adiponectin accelerates LPS-induced liver injury and the survival rate of adiponectin KO mice was significantly lower than that of wild type mice. Pretreatment of these mice with adiponectin reduced LPS-induced TNF-α production and increased IL-10 production by Kupffer cells. [48] In addition, adiponectin was reported to alleviate experimental T-cell mediated hepatitis induced by concanavalin A in mice, and protect primary hepatocytes from TNF-α-induced death. These evidences suggest that hypoadiponectinemia in obese subjects may thus lead in enhanced sensitivity of Kupffer cells to pro-inflammatory mediators like LPS.

A recent study reported that adiponectin promotes clearance of apoptotic cells by macrophages through a receptor-dependent pathway involving calreticulin. This novel function of adiponectin is similar to surfactant proteins and C1q, which serve as anti-inflammatory molecules by promoting the clearance of apoptotic cell debris [49].

### 3). Adiponectin and Liver Fibrosis

In a clinical study in NASH patients, the extent of fibrosis correlates significantly with low adiponectin levels [50]. We

reported that carbon tetrachloride-induced liver fibrosis is much more severe in adiponectin KO mice and adiponectin supplementation attenuate fibrosis [29]. We demonstrated that adiponectin suppressed the proliferation and migration of hepatic stellate cells (HSC) which play central roles in the development if liver fibrosis, and attenuate the effect of transforming growth factor-beta (TGF-β1) on the expression of fibrogenic genes and on nuclear tranlocation of Smad2 in HSC. Other groups reported that adiponectin induces apoptosis of activated HSC and activated AMPK, which modulates the activated phenotype of HSC [51].

### 4). Adiponectin and Oxidative Stress

Recent studies indicate that adiponectin could suppress oxidative stress [52] and that systemic oxidative stress as measured by urinary 8-epi-prostaglandin F2α, correlates strongly with hypoadiponectinemia [53]. Animal experiment also demonstrated that adiponectin KO mice showed enhanced oxidative stress in CDAA diet-induced NASH [45]. Lack of adiponectin is shown to down regulate CYPE2E1 which has been shown to initiate lipid peroxidation *via* production of reactive oxygen species [54], ROS. Moreover, thiobarbituric acid reactive substance (TBARS), a marker of oxidative stress and 8-hydrooxyguanosine, 8-OHdG, a marker of oxidative DNA damage, were also increased in the liver of adiponectin KO mice [55].

Thus, adiponectin attenuates pro-inflammatory cytokine production and inflammatory cell phenomena in the liver, which are considered to be the second hit in NASH. Moreover, adiponectin ameliorates liver fibrosis *via* suppression of activate HSC function and might decelerated the progression of hepatocarcinogenesis (Fig. 4).

## 5. ADIPONECTIN RECEPTOR

Molecular mechanism of adiponectin functions has not been fully clarified and is considered to be very complicated. In contrast to other bioactive substances such as cytokines and hormones, adiponectin is present abundantly in plasma. In addition, bioactivities of adiponectin are displayed more potently in multimerized high molecule form than monomer or trimer type. With respect to the studies on adiponectin receptor, two kinds of concept have been proposed Adiponectin receptors AdipoR1 and AdipoR2 were identified by Kadowaki *et al.* [55] They showed that both receptors have roles in activation of AMP activated kinase, AMPK and PPARγ. Lodish *et al.* suggested that T-cadherin may act as a coreceptor for an-yet –unidentified signaling receptor through which adiponectin transmits metabolic signals [56]. As I mentioned above, the mode of action adiponectin may be different from that of other bioactive substances. It accumulates in injured tissue primarily by binding with extracellular collagens and may bind to some adhesion molecule such as T-cadherin or some signaling receptors such as AdipoRs which are expressed in target cells. Further studies for molecular mechanism of adiponectin action are necessary to know physiological role of this unique protein.

## 6.SUMMARY

Adipose tissue has long been considered to be an organ which stores excess energy preventing starvation at the lack of foods. However recent studies have revealed that adipose tissue secretes a variety of bioactive substances, adipokines, controlling other organs and cells. Substantial proportion of adipokines is involved in inflammatory stimulation and response as either pro-inflammatory adipokines or anti-inflammatory adipokines. As mentioned above, adiponectin is the most potent anti-inflammatory adipocytokine and visceral fat accumulation causes the decrease of adiponectin plasma levels together with the increase of pro-inflammatory adipokines such as TNF-α and PAI-1. Especially the insufficient amount of adiponectin may be the background of enhanced inflammatory response and may result in the development of metabolic and cardiovascular diseases.

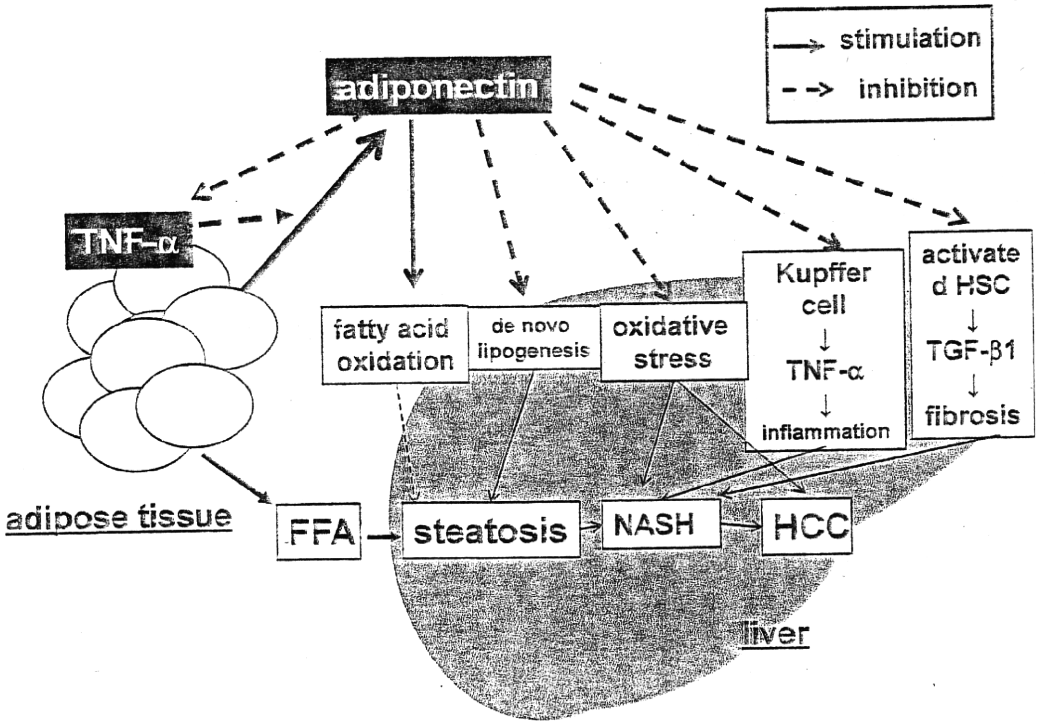


Fig. (4). Adiponectin attenuates steatosis, fibrosis and inflammation of the liver.

## REFERENCES


[1] Matsuzawa Y. Pathophysiology and molecular mechanism of visceral fat syndrome: The Japanese case. Diabetes/Metabol Rev 1997; 13: 3-13.

[2] Maeda K, Okubo K, Shimomura I, Mizuno K, Matsuzawa Y, Matsubara K. Analysis of expression profile of genes in the human adipose tissue. Gene 1997; 190: 227-35.

[3] Shimomura I, Funahashi T, Takahashi M, Maeda K, Kotani K, Nakamura T, *et al.* Enhanced expression of PAI-1 in visceral fat: Possible contribution to vascular disease in obesity. Nat Med 1997; 2: 1-5.

[4] Masumoto S, Kishida K, Shimomura I, Maeda N, Nagaretani H, Matsuda M, *et al.* Increased plasma HB-EGF associated with obesity and coronary artery disease. Biochem Biophys Res Commun 2002; 292: 781-6.

[5] Maeda K, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y, Matsubara K. cDNA cloning and expression of a novel adipose specific collagen- like factor, apM1 (Adipose most Abundant Gene Transcript 1. Biochem Biophys Res Commun 1996: 221: 286-9.

[6] Ouchi N, Kihara S, Arita Y, Maeda K, Kuriyama H, Okamoto Y, *et al.* Novel modulator for endothelial adhesion molecules. Circulation 1999; 100: 2473-6.

[7] Matsuzawa Y. Adiponectin and metabolic syndrome. Atheroscler Thromb Vas Biol 2004; 24: 29-33.

[8] Scherer EP, Williams S, Fogliano M. A novel serum protein similar to C1q, produced exclusively in adipocytes. J Biol Chem 1995; 270: 26746-9.

[9] Hu E, Liang P, Spiegelman BM. .AdipoQ is a novel adipose tissue-specific gene dsegulated in obesity. J Biol Chem 1996; 271: 10697-703.

[10] Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, *et al.* Paradoxisal decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Comm 1999; 257: 79-83.

[11] Halleux CNM, Lee WJ, Delporte ML, Detry R, Funahashi T, Matsuzawa Y, *et al.* Secretion and regulation of apM1 gene expression in human visceral adipose tissue. Biochem Biophys Res Commun 2001; 288: 1102-7.

[12] Maeda N, Takahashi M, Funahashi T, Kihara S, Nisizawa H, Kishida K, *et al.* PPARγ ligands increase expression and plasma concentration of adiponectin, an adipose-derived protein. Diabetes 2001; 50: 2094-9.

[13] Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, *et al.* Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. Arterioscl Thromb Vas Biol 2000; 20: 1595-9.

[14] Hotta K, Funahashi T, Bodkin NL, Ortmeyer HK, Arita Y, Hansen BC, *et al.* Circulating concentrations of the adipocyte protein, adiponectin, are decreased in parallel with reduced insulin sensitivity during progression to type 2 diabetes in rhesus monkeys. Diabetes 2001; 50: 1126-33.

[15] Lindsay RS, Funahashi T, Hanson RL, Matsuzawa Y, Tanaka S, Tetaranni PA, *et al.* Adiponectin protects against development of type 2 diabetes in the Pima Indian population. Lancet 2002; 360: 57-8.

[16] Maeda N, Shimomura I, Kishida K, Nishizawa H, Matsuda M, Nagaetani H, *et al.* Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. Nat Med 2002; 8: 731-7.

[17] Kubota N, Terauchi Y, Yamauchi T, Kubota T, Moroi M, Eto K, *et al.* Disruption of adiponectin causes insulin resistance and neointimal formation. J Biol Chem 2002; 277: 25863-6.

[18] Tomas E, Tsao TS, Saha AK, Murray HE, Zhang CC, Itani SI, *et al.* Enhanced muscle fat oxidation and glucose transport by ACRP30 globular domain: acetyl-CoA carbxylase inhibitor and AMP-activated protein kinase activation. Proc Natl Acad Sci USA 2002; 99: 16309-13.

[19] Iwashima Y, Katsuya T, Ishikawa K, Ouchi N, Ohishi M, Sugimoto K, *et al.* Hypoadiponectinemia is an independent risk factor for hypertension. Hypertension 2004; 43: 1318-23.

[20] Ouchi N, Ohishi M, Kihara S, Funahashi T, Nakamura T, Nagaretani H, *et al.* Association of hypoadiponectinemia with impaired vasoreactivity. Hypertension 2003; 42: 231-4.

[21] Zoccali C, Mallamaci F, Tripepi G, Parlongo S, Malatino L, Bonanno G, *et al.* Adiponectin, the most abundant adipocyte-derived protein, is functionally related to metabolic risk factors and predicts cardiovascular outcomes in end stage renal disease. J Am Soc Nephrol 2002; 13: 134-41.

[22] Kumada M, Kihara S, Sumitsuji S, Kawamoto T, Matsumoto S, Ouchi N, *et al.* Association of hypoadiponectinemia with coronary artery disease in men. Arterioscler Thromb Vasc Biol 2003; 23: 85-89.

[23] Pischon T, Girman CJ, Hotamisligil GS, Rifai N, Hu FB, Rimm EB. Plasma adiponectin levels and risk of myocardial infarction in men. JAMA 2004; 291: 1730-7.

[24] Kondo H, Shimomura I, Matsukawa Y, Kumada M, Takahashi M, Matsuda M, *et al.* Association of adiponectin mutation with type 2 diabetes: a candidate gene for the insulin resistance syndrome. Diabetes 2002; 51: 2325-8.

[25] Ho RC, Davy KP, Hickery MS, Melby CL. Circulating tumor necrosis factor α is higher in non obese, non –diabetic Mexican Americans compared to non-Hispanic white adults. Cytokine 2005; 30: 14-21.

[26] Moon YS, Kin DH, Song DK. Serum tumor necrosis factor –α levels and components of the metabolic syndrome in obese adolescents. Metabolism 2004; 53: 863-7.

[27] Uysal KT, Wiesblock SM, Mario MW, Hotamisligil, GS. Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. Nature 1997; 389: 610-4.

[28] Ouchi N, Kihara S, Funahashi T, Nakamura T, Nishida T, Kumada M, *et al.* Reciprocal association of C-reactive protein with adiponectin in blood stream and adipose tissue. Circulation 2003; 107: 671-4.

[29] Kamada Y, Tamura S, Kiso S, Matsumoto H, Saji Y, Yshida Y, *et al.* Enhanced carbon tetrachrolide-induced liver fibrosis in mice lacking adiponectin. Gastroenterology 2003; 125: 1796-807.

[30] Petridou E, Mantzoros C, Dessypris N, Koukoulomatis P, Addy C, Voulgaris Z, *et al.* Plasma adiponectin concentrations in relation to endometrial cancer: a case-control study in Greece. J Clin Endocrinol Metab 2003; 88: 993-7.

[31] Miyoshi Y, Funahashi T, Kihara S, Taguchi T, Tamaki Y, Matsuzawa Y, *et al.* Association of serum adiponectin levels with breas cancer. Clin Cancer Res 2003; 9: 5699-704.

[32] Otake S, Takeda H, Suzuki Y, Fukui T, Watnabe S, Ishihama K, *et al.* Association of visceral fat accumulation and plasma adiponectin with colorectal adenoma: evidence for participation of insulin resistance. Clin Cancer Res 2005; 11: 3642-6.

[33] Yokota T, Oritani K, Takahashi I, Ishikawa J, Masuyama A, Ouchi N, *et al.* Adiponectin, a new member of the family of soluble defence collagens, negatively regulates the growth of monocytic progenitors and the functions of macrophages. Blood 1999; 96: 1727-32.

[34] Okamoto Y, Kihara S, Ouchi N, Nishida M, Arita Y, Kumada M, *et al.* Adiponectin reduces atherosclerosis in apolipoprotein E-deficient mice. Circulation 2002; 26: 2767-2770.

[35] Ouchi N, Kihara S, Arita Y, Nishida M, Matsuyama A, Okamoto Y, *et al.* Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrophages. Circulation 2001; 103: 1057-63.

[36] Ouchi N, Kihara S, Arita Y, Okamoto Y, Maeda K, Kuriyama H, *et al.* Adiponectin, adiocyte-derived plasma protein, inhibits endothelial NF-κB signaling through c-AMP dependent pathway. Circulation 2001; 102: 1296-301.

[37] Tilg H, Wolf AM. Adiponectin: a key fat-derived molecule regulating inflammation. Exp Opin Ther Targets 2005; 9: 245-51.

[38] Kumada M, Kihara S, Ouchi N, Kbaashi H, Okamoto Y, Ohashi K, *et al.* Adiponectin specifically increases tissue inhibitor of metalloproteinase-1 through interleukin-10 expression in human macrophages. Circulation 2004; 109: 2046-9.

[39] Ridker JE, Shih J, Matias M, Hennekens CH. Prospective study of C-reactive protein and the risk of future cardiovascular events among apparently healthy women. Circulation 1998; 98: 731-3.

[40] Matsushita K, Yatsuya H, Tamakoshi K, Wada K, Otsuka R, Takefuji S, *et al.* Comparison of Circulating adiponectin and proinflammatory markers regarding their association with metabolic syndrome in Japanese men. Arterioscler Thromb Vas Biol 2005; 26: 871-6.

[41] Ignacy WW, Chudek J, Adamczak M, Funahashi T, Matsuzawa Y, Kokot F, *et al.* Reciprocal association of plasma adiponectin and serum C-reactive protein concentration in hemodialysis patients

with end-stage kidney disease- a follow-up study. Nephron Clin Prac 2005; 101:18-24.

[42] Naveau S, Giraud V, Borrotto E, Aubert A, Capron F, Chaput JC. Excess weight is a risk for alcoholic liver disease. Hepatology 2007; 25: 108-11.

[43] Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity and mortality from cancer in a prospectively studied cohort U.S. adults. N Engl J Med 2003; 348, 1625-38.

[44] Kuriyama H, Yamashita S, Shimomura I, Funahashi T, Ishigami M, Aragane K, *et al.* Enhanced expression of hepatic acyl-CoA synthetase and microsomal triglyceride transfer protein mRNAs in the obese and hypertriglyceridemic rat with visceral fat accumulation. Hepatology 1998; 27: 557-62.

[45] Kamada Y, Matsumoto H, Tamura S, Fukushima J, Kiso S, Fukui K. Hypoadiponectinemia accelerates hepatic tumor formation in nonalcoholic steatohepatitis mouse model. J Hepatol 2007; 47: 556-64.

[46] Shklyaev S, Aslanidi G, Tennant M, Prima V, Kohlbrenner E, Kroutov V, *et al.* Sustained peripheral expression of transgene adiponectin offsets the development of diet-induced obesity in rats. Proc Natl Acad Sci USA 2003; 100: 14217-22.

[47] Matsumoto H, Tamura S, Kamada Y, Kiso S, Fukushima J, Wada A. Adiponectin deficiency exerbates lipopolysaccharide /D-galactosamine –induced liver injury in mice. World J Gastroenterol 2006; 12: 3352-8.

[48] Masaki T, Chiba S, Takawa H, Yasuda T, Noguchi H, Seike M. Adiponectin protects LPS-induced liver injury through modulation of TNFα in KK-Ay obese mice. Hepatology 2004; 40: 177-84.

[49] Vandivier RW, Ogden CA, Fadok VA, Hoffmann PR, Brown KK, Botto M, *et al.* Role of surfactant proteins A, D and C1q in the clearance of apoptotic cells *in vivo* and *in vitro*: calreticulin and CD91 as a common collectin receptor complex. J Immunol 2002; 169: 147-52.

[50] Musso G, Gambino R, Biroli G, Carello M, Faga E, Pacini G, *et al.* Hypoadiponectinemia predicts the severity of hepatic fibrosis and pancreatic beta-cell dysfunction in non diabetic nonobese patients with nonalcoholic steatohepatitis. Am J Gastroenterol 2005; 100: 2438-46.

[51] Ding X, Saxena NK, Lin S, Xu A, Srinvasan D, Anania FA. The role of leptin and adiponectin: a novel paradigm in adipocytokine regulation of liver fibrosis and stellite cell biology. Am J Pathol 2005; 166: 1655-69.

[52] Motoshima H, Wu X, Mahadev K, Goldstein BJ. Adiponectin suppresses proliferation and superoxide generation and enhances eNOS activity in endothelial cells treated with oxidized LDL. Biochem Biophys Res Commun 2004; 315: 264-71.

[53] Fujita K, Nishizawa H, Funahashi T, Shimomura I, Shimabukuro M. Systemic oxidative stress is associated with visceral fat accumulation and the metabolic syndrome. Circ J 2006; 70: 1437-42.

[54] Lieber CS. Cytochrome P-4502E1: its physiological and pathological role. Physiol Rev 1997; 77: 517-54.

[55] Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K, Tobe K. Adiponectin and adiponectin receptors in insulin resistance, diabetes and the metabolic syndrome. J Clin Invest 2006; 116: 1784-92.

[56] Hug C, Wang J, Ahmad NS, Bogan JS, Tsao TS, Lodish HF. T-cadherin is a receptor for hexameric and high-molecular-weight forms of Acrp/adiponectin. Proc Natl Acad Sci USA 2006; 101: 10318-3.

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Review

# Establishment of a concept of visceral fat syndrome and discovery of adiponectin

By Yuji MATSUZAWA[*1,*2,†]

(Communicated by Hiroo IMURA, M.J.A.)

**Abstract:** Although obesity is a major background of life style-related diseases such as diabetes mellitus, lipid disorder, hypertension and cardiovascular disease, the extent of whole body fat accumulation does not necessarily the determinant for the occurrence of these diseases. We developed the method for body fat analysis using CT scan and established the concept of visceral fat obesity, in other word metabolic syndrome in which intra-abdominal visceral fat accumulation has an important role in the development of diabetes, lipid disorder, hypertension and atherosclerosis. In order to clarify the mechanism that visceral fat accumulation causes metabolic and cardiovascular diseases, we have analyzed gene expression profile in subcutaneous adipose tissue and visceral adipose tissue. From the analysis, we found that adipose tissue, especially visceral adipose tissue expressed abundantly the genes encoding bioactive substances such as cytokines, growth factors and complements. In addition to known bioactive substances, we found a novel collagen-like protein which we named adiponectin. Adiponectin is present in plasma at a very high concentration and is inversely associated with visceral fat accumulation. Adiponectin has anti-diabetic, anti-hypertensive and anti-atherogenic properties and recent studies revealed that this protein has an anti-inflammatory and anti-oncogenic function. Therefore hypoadiponectinemia induced by visceral fat accumulation should become a strong risk factor for metabolic and cardiovascular diseases and also some kinds of cancers.

In this review article, I would like to discuss the mechanism of life style-related diseases by focusing on the dysregulation of adiponectin related to obesity, especially visceral obesity.

**Keywords:** visceral fat, metabolic syndrome, adiponectin, hypoadiponectinemia

## Fat distribution and morbidity of obesity

Contemporary civilized countries provide an increasing number of opportunities for overeating and decreased muscular exercise, where common health problems are closely correlated to this over-nutritional state and its typical consequence, obesity. However, previous studies on the morbidity of obesity have indicated that the severity of obesity-related diseases such as diabetes mellitus, lipid disorders and cardiovascular disease does not necessarily correlate to the extent of body fat accumulation, but it closely related to body fat distribution. Several classifications of obesity concerning body fat distribution have been proposed in order to distinguish the possible mechanisms of obesity-related diseases. An ancient Japanese artist showed great insight into the morbidity of obesity 800 years ago when he painted a picture of an obese woman with the title "A very obese woman who can hardly walk" (Fig. 1) in the old Japanese picture scroll "Yamai Zoshi" which means an illustrated scroll for various diseases. Comparing the body figure of an obese girl painted by famous Renoir, she has marked adiposity in her abdomen.

In the end of 1940s, Prof. Vague noted that "Fat excess is dangerous because of its metabolic complications and a woman normally has twice a man's fat mass, i.e. the mass of an obese man. Though she is as often obese a man or is fatter, she dies later and less often from metabolic complica-

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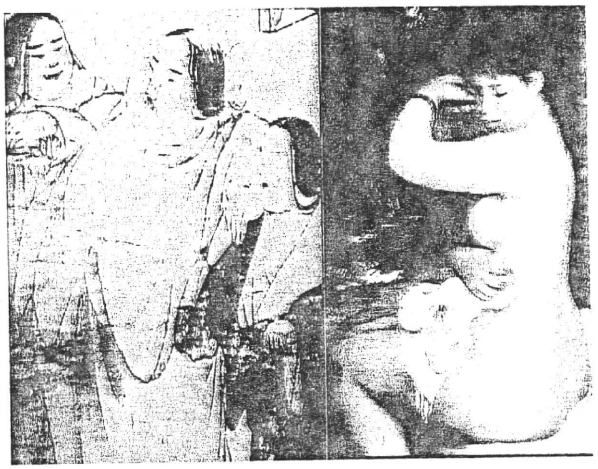

Fig. 1. High risk obesity (left) and low risk obesity (right) in classical paintings.

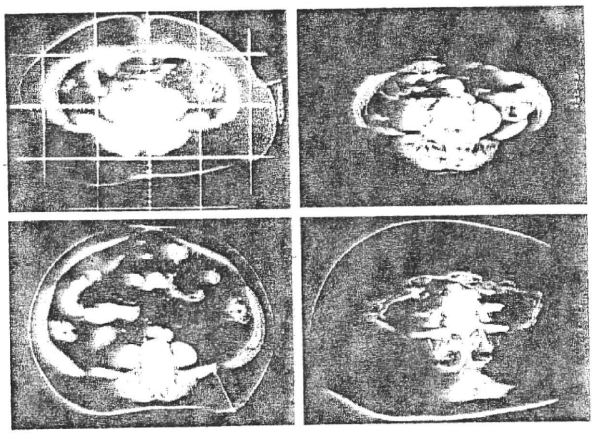

Fig. 2. Marked variation of fat distribution between intra-abdominal cavity and subcutane.[4]

tions of obesity." Then he proposed a classification of obesity into android type and gynoid type in 1947.[1] His classification was based on the brachio-femoral adipomuscular ratio (BFAMR) and the subjects with higher BFAM were designated to be android type in whom metabolic complications were more prevalent. Although his classification is not exactly the same as current one, he is no doubt a pioneer of recognition for high risk obesity based on fat distribution.

In early 1980s, Prof. Björntorp proposed a classification between central obesity and peripheral obesity and Prof. Kissebah proposed a classification between upper body segment obesity and lower body segment obesity based on waist/hip ratio.[2),3)] Our group developed the method for fat analysis using CT scan which enabled us to analyze adipose tissues in body cavity in 1983 and we noticed that there is marked variation in fat distribution between subcutaneous fat and intra-abdominal visceral fat. (Fig. 2)[4)]

## Visceral fat accumulation and metabolic or cardiovascular diseases

Using CT scan method for fat analysis, we demonstrated the contribution of visceral fat accumulation to the development of metabolic disorders, including glucose intolerance and hyperlipidemia. For example, visceral fat area determined by CT correlates significantly with glucose area after oral glucose tolerance test, and with cholesterol and triglyceride levels.[5),6)] Visceral fat accumulation is associated not only with quantitative changes in serum lipids and lipoproteins and but also with qualitative changes in lipoproteins, such as small dense LDL. Studies on muscle glucose uptake reported by Kissebah *et al.*[7)] and the steady-state plasma glucose method by our group,[8)] clearly show that visceral fat obesity has greater insulin resistance than subcutaneous fat obesity.

In addition to these metabolic disorders, we have demonstrated that in premenopausal women visceral fat accumulation correlates closely with systolic blood pressure.[9)] In hypertensive people, we reported a close correlation between the extent of visceral fat reduction, not subcutaneous fat reduction, and a lowering of blood pressure after weight reduction.

Visceral fat accumulation relates to the development of cardiovascular risks mentioned above and might relate directly to the development of cardiovascular disease. Several studies, including ours, have shown that visceral adiposity determined by CT scanning is related to coronary artery disease even in mildly obese individuals.[10)] Visceral fat accumulation is also related to the development of cardiac dysfunction and sleep apnea syndrome.[11),12)] From these evidences, we can conclude that visceral fat accumulation is a major risk of cardiovascular disease as well as metabolic diseases.(Fig. 3)

## Visceral fat syndrome and metabolic syndrome

In the end of 1980s, the concept of multiple risk factor clustering syndrome has been proposed as a

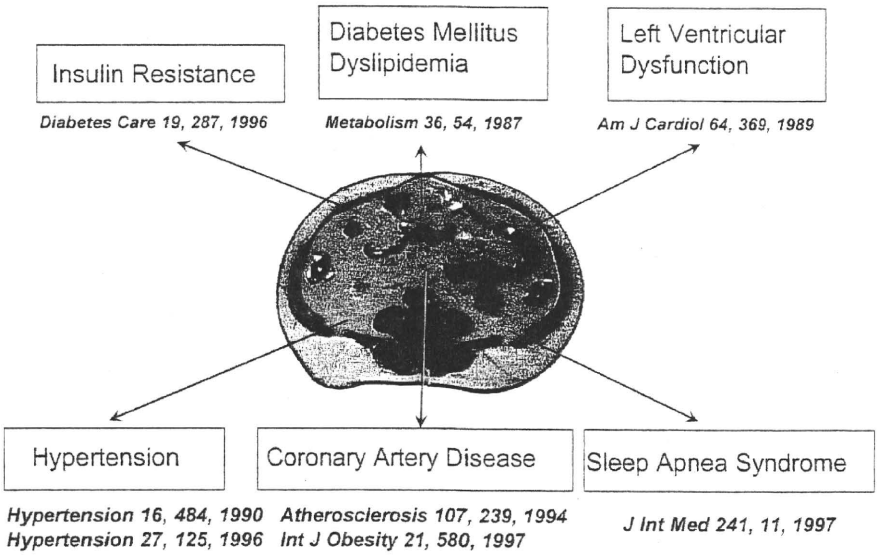


Fig. 3. Visceral fat accumulation is related a variety of diseases.

highly atherogenic state independent from hypercholesterolemia.[13),14)] A variety of common disorders, such as hyperglycemia, hyperlipidemia and hypertension, are seen in individuals with this syndrome, and cardiovascular disease is very prevalent and this syndrome has been called to be the metabolic syndrome. The disorders such as diabetes, dyslipidemia and hypertension are not clustered coincidently, and there is thought to be a key to the simultaneous development within certain individuals along with the associated development of cardiovascular disease. As I showed above, visceral fat accumulation might be present in the upstream of a variety of disorders including cardiovascular disease. Therefore we have proposed the concept of a visceral fat syndrome on the basis of our clinical researches shown above as the same concept of metabolic syndrome.[15)] An important question is, then, why does visceral fat accumulation causes common disorders; more importantly, why is this syndrome so atherogenic? In order to answer these questions, we have investigated the functions of adipose-tissue, which has been traditionally regarded as a tissue passively storing excess energy in the form of triglycerides.

## The concept of adipocytokines

To elucidate the molecular mechanism of visceral fat-related diseases, particularly those in the metabolic syndrome, we have investigated the biological characteristics of visceral adipose tissue and subcutaneous adipose tissue by analysis of the gene-expression profile compared with that of other mesenchymal cells. We systematically analyzed active genes by constructing a 3′-directed complementary DNA library in which the messenger RNA population was faithfully reflected. We found an unexpectedly high frequency of the genes encoding secretary proteins in adipose tissue, most of which are important bioactive substances. Of the gene group classified by functions and subcellular localization, approximately 20% of all genes in subcutaneous adipose tissue encode secretory proteins. This frequency rises to about 30% in visceral adipose tissue. (Fig. 4) We classified these adipose-tissue-derived bioactive substances as adipocytokines. (Fig. 5)

## Adipocytokines and diseases

We found that the genes encoding plasminogen activator inhibitor type 1 (PAI-1) and heparin binding epidermal growth factor-like growth factor are highly expressed in adipose tissue.[16),17)] PAI-1 messenger RNA concentrations increased up to 10-fold in visceral adipose tissue during development of fat accumulation in ventromedial hypothalamic-lesioned rats, which is an experimental animal model of obesity. In subcutaneous adipose tissue, concentrations remained unchanged. In addition to the animal model, we demonstrated that plasma levels of PAI-1 were significantly correlated with visceral adiposity, assessed by CT scanning, in humans. (Fig. 6) Circu-

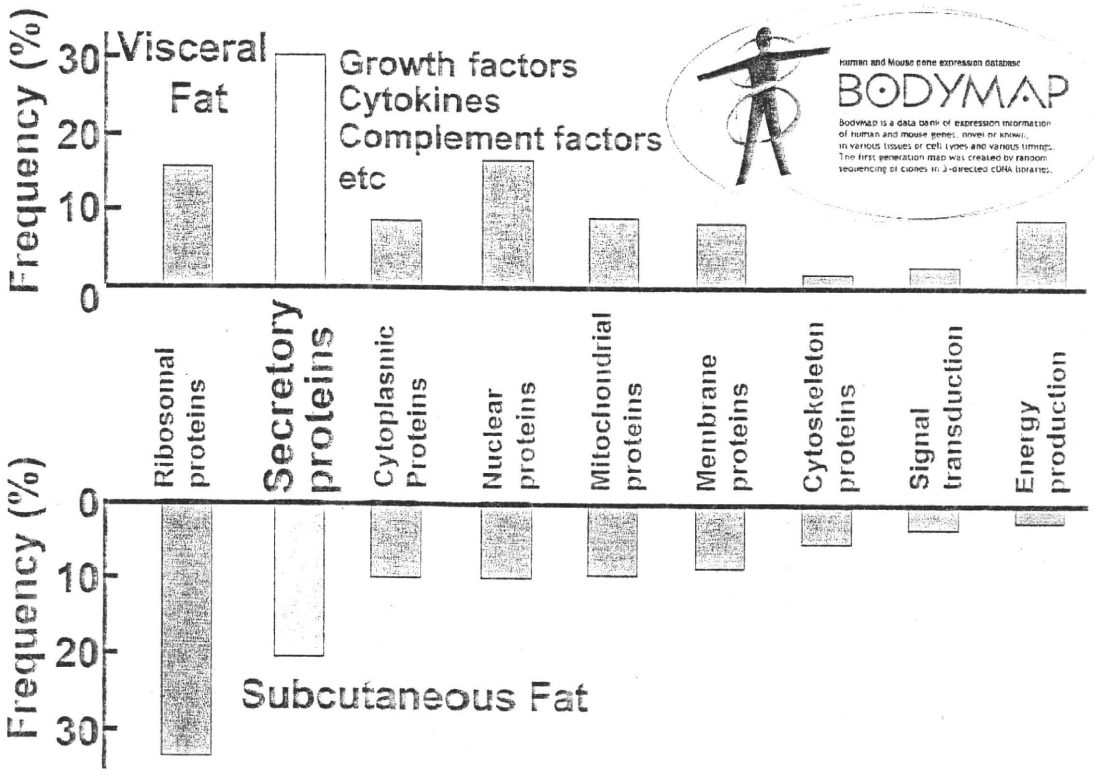


Fig. 4. Distribution profile of gene groups expressed in visceral fat and subcutaneous fat.[44]

lating PAI-1 is deemed as a strong risk factor for thrombotic diseases, including acute myocardial infarction, in metabolic syndrome.[18] Heparin binding epidermal growth factor-like growth factor, a potent factor for smooth-muscle-cell proliferation, secreted from accumulated adipose tissue could also have some significance for vascular remodeling in obesity. Tumor necrosis factor-α was also reported to be secreted from adipose tissue and to induce insulin resistance by Dr. Hotamisligil.[19]

## Discovery of adiponectin and its clinical significance

When we started the comprehensive genetic analysis of human adipose tissue, 40% of the expressed genes were previously unknown genes. The gene expressed most abundantly in adipose tissue, which we named adipose most abundant gene transcript-1, apM-1, was a novel gene.[20] The molecule encoded by apM-1 possesses a signal peptide, collagen-like motif and globular domain, and has notable homology with collagen X, VIII and complement factor C1q. This protein is present in plasma in a unique multimer form, which is more active than low molecule weight form. (Fig. 7) We termed this collagen-like protein adiponectin. The mouse homolog of adiponectin has been cloned as ACRP30.[21] We established the method for the measurement of plasma adiponectin levels using enzyme-linked immunosorbent assay. The average levels of adiponectin in human plasma are extremely high-up to 5–10 μg/ml.[22] Plasma concentrations are negatively correlated with visceral adiposity, whereas PAI-1 increases with visceral fat accumulation as mentioned previously. (Fig. 6)

The mechanism by which plasma levels are reduced in individuals with visceral fat accumulation is not yet clarified. Co-culture with visceral fat inhibits adiponectin secretion from subcutaneous adipocytes. This finding suggests that some inhibiting factors for adiponectin synthesis or secretion are secreted from visceral adipose tissue.[23] Tumor necrosis factor-α was reported to be a strong inhibitor of adiponectin promoter activity.[24] The negative correlation between visceral adiposity and adiponectin levels might be explained by the increased secretion

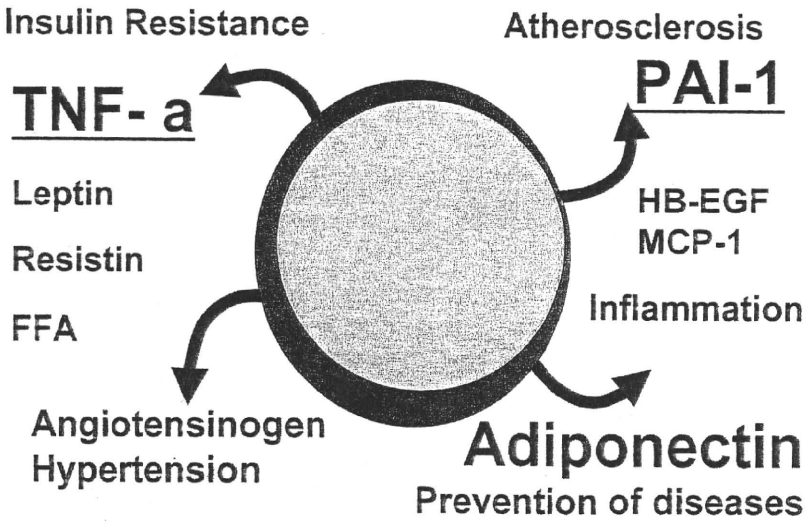


Fig. 5. Concept of adipocytokines.

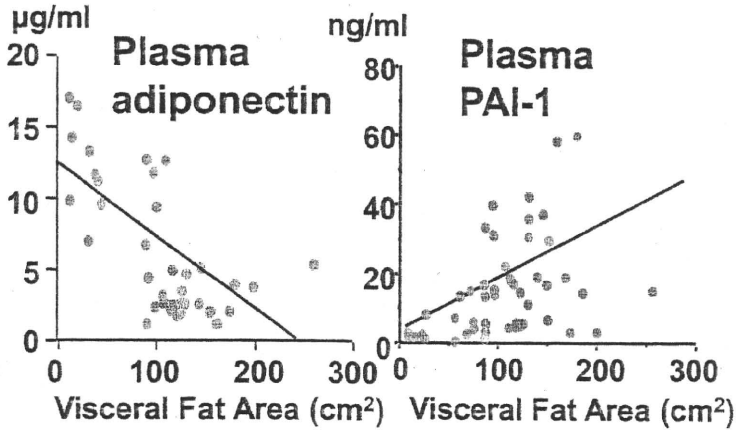


Fig. 6. Correlation between visceral adiposity and plasma levels of adiponectin and PAI-1.[44]

of this cytokine from accumulated visceral fat as at least one mechanism.

Plasma adiponectin concentrations are lower in people who have type 2 diabetes mellitus than in BMI-matched controls.[25] The plasma concentrations have been shown to correlate strongly with insulin sensitivity, which suggests that low plasma concentrations are related to insulin resistance.[26] In a study of Pima Indians, individuals with high levels of adiponectin were less likely than those with low concentrations to develop type 2 diabetes. High adiponectin concentration was, therefore, a notable protective factor against development of type 2 diabetes.[27]

Studies on adiponectin knockout mice support observations in humans. The KO mice showed no specific phenotype when they were fed a normal diet but a high-sucrose and high-fat diet induced a marked elevation of plasma glucose and insulin levels. Notable insulin resistance, estimated by insulin tolerance test during the high-sucrose with high-fat diet, also developed in the knockout mice. The supplementation of adiponectin by adenovirus transfection clearly improved this insulin resistance.[28] Adiponectin has been shown to exert its actions on muscle fatty acid oxidation and insulin sensitivity by activation of AMP-activated protein kinase.[29]

Plasma levels of adiponectin are also decreased in hypertensive humans, irrespective of the presence of insulin resistance.[30] Endothelium-dependent vasoreactivity is impaired in people with hypoadiponecti-

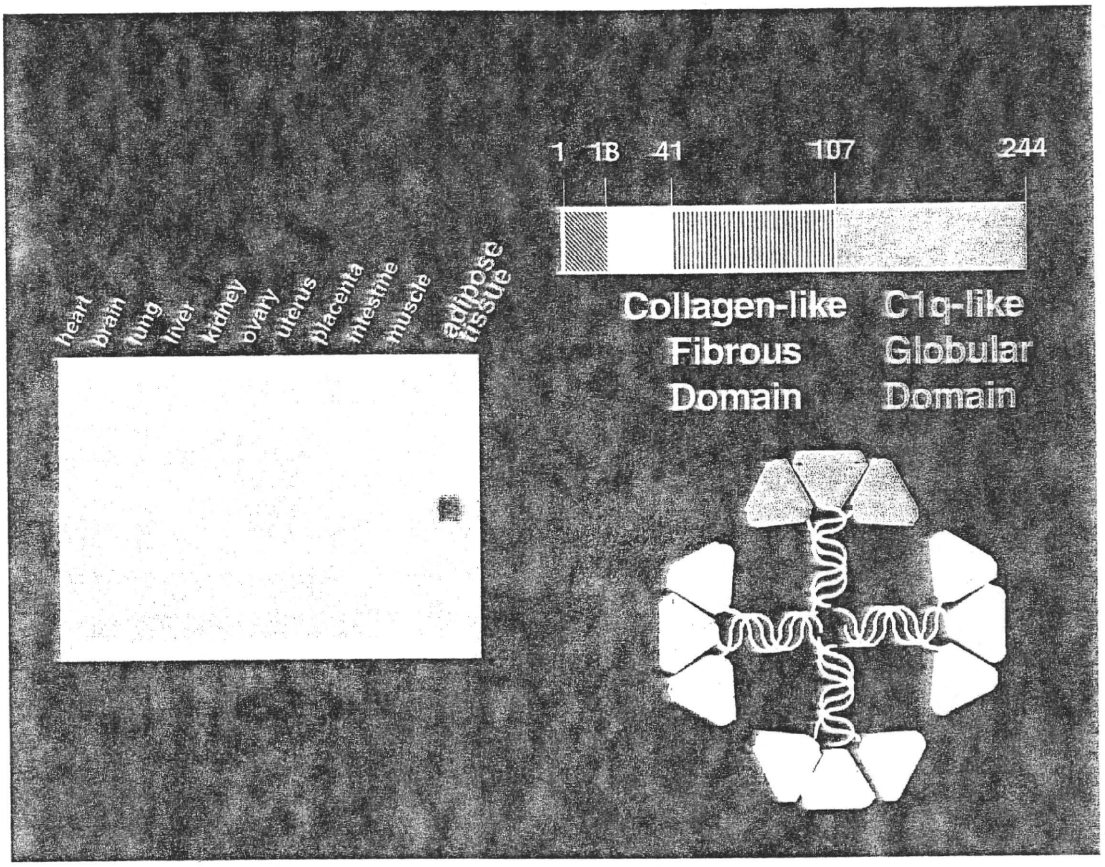


Fig. 7. Adipose-specific collagen-like protein, adiponectin.

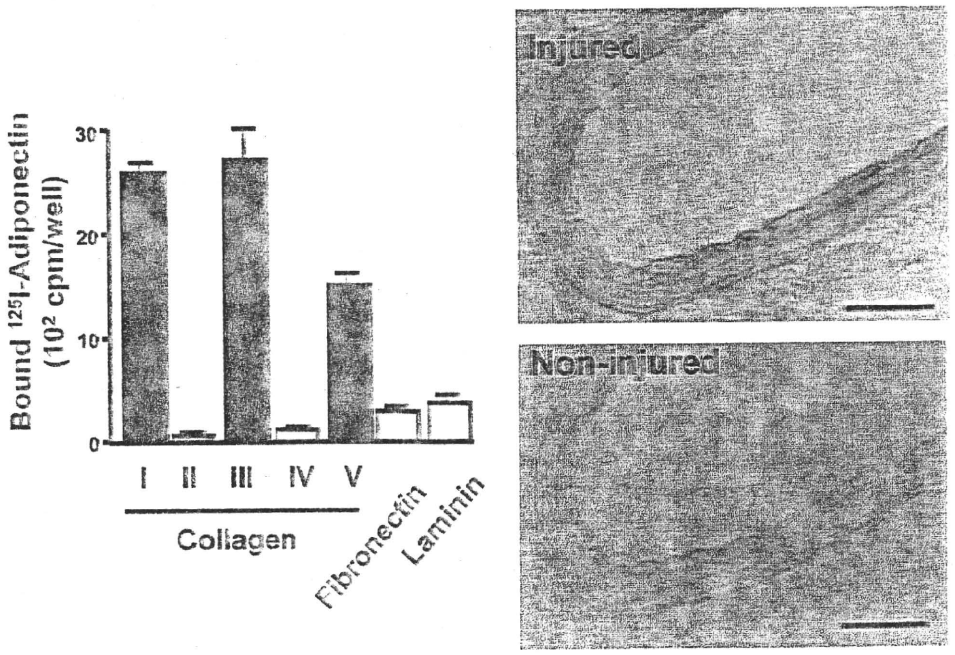


Fig. 8. Adiponectin accumulate injured vascular walls by binding subendothelial collagens.[38]

nemia, which might be at least one mechanism of hypertension in visceral obesity.[31]

Most importantly, plasma concentrations of adiponectin are lower in people with coronary heart disease than in controls even when BMI and age are matched.[32] A case-control study performed in Japan demonstrated that the group with hypoadiponectinemia with the plasma levels less than 4μg/ml has been shown to have increased risk of CAD and multiple metabolic risk factors, which indicates that hypoadiponectinemia is a key factor in the metabolic syndrome.[33] A prospective study by Pischon *et al.*[34] confirmed that high adiponectin concentrations are associated with reduced risk of acute myocardial infarction in men. In addition to hypoadiponectinemia accompanied with visceral fat accumulation, genetic hypoadiponectinemia caused by a missense mutation has been reported, which also exhibit the clinical phenotype of metabolic syndrome.[35]

These clinical evidences show that hypoadiponectinemia is a strong risk factor for cardiovascular disease.

### Cell biological functions of adiponectin

Antiatherogenicity of adiponectin is also demonstrated in animal experiments. Adiponectin knockout mice developed more-severe intimal thickening by endothelial injury than did wild-type mice.[36] In addition, overexpression of human adiponectin by adenovirus transfection attenuated plaque formation in apolipoprotein E-KO mice.[37]

A large amount of adiponectin flows with the blood stream and, therefore, comes into contact with the vascular walls all over the body. The ways in which adiponectin interacts with vascular cells would be important to know. Immunohistochemical examination with antibodies to adiponectin showed no adiponectin protein in the untreated normal vascular walls in rabbits. Markedly positive immunohistochemical staining was detected, however, in balloon-injured vascular walls. Since adiponectin has the ability to bind subendothelial collagens such as collagen I, III, and V, endothelial injury may induce the adiponectin from entering into the subendothelial space through binding to these collagens. (Fig. 8)[38]

Cell biological studies have demonstrated that adiponectin has multiple, potent antiatherogenic functions. When the endothelial barrier is injured by attacking factors such as oxidized LDL, chemical substances and mechanical stress, adiponectin accumulates in the subendothelial space of vascular walls by binding to subendothelial collagen, at which point antiatherogenic properties of adiponectin become apparent.[38] The protein suppresses monocyte attachment to vascular endothelial cells by inhibiting the expression of adhesion molecules, such as vascular cell adhesion molecule 1, intracellular-adhesion molecule 1 and E-selectin via the inhibition of NF-$\kappa$B activation.[39] Adiponectin also attenuates growth-factor-induced proliferation of vascular smooth-muscle cells by the inhibition of mitogen-activated protein kinase.[40] Adiponectin suppresses foam-cell formation by the inhibition of expression of scavenger receptor class A. (Fig. 9)[41]

Acute coronary syndromes are considered to determine the prognosis of cardiovascular disease in which vulnerability of plaque is the important determinant of plaque rupture. In this process, matrix metalloproteinase secreted from macrophages is thought to play an important part in plaque vulnerability. Tissue inhibitor of metalloproteinase is thought to act as a protector of plaque rapture by inhibition of matrix metalloproteinase. Adiponectin increases the expression of messenger RNA and protein production of tissue inhibitor of metalloproteinase in macrophages via the induction of interleukin-10 synthesis. This finding suggests that adiponectin protects plaque rapture by the inhibition of matrix metalloproteinase function, through the induction of interleukin-10-dependent production of tissue inhibitor of metalloproteinase.[42] Shibata *et al.* have demonstrated that adiponectin-deficient mice shows enhanced concentric hypertrophy and increased mortality under pressure overload. These phenomena were associated with increased extracellular signal-regulated kinase and diminished AMP-activated protein kinase signaling in the myocardium.

Adenovirus-mediated supplementation of adiponectin attenuated cardiac hypertrophy in response to pressure overload.[43]

Molecular mechanism of adiponectin functions has not been fully clarified and is considered to be very complicated. Not like other bioactive substances such as cytokines and hormones, adiponectin is present abundantly in plasma. In addition, bioactivities of adiponectin are displayed more potently in multimerized high molecule form than monomer or trimer type. With respect to the studies on adiponectin receptor, two kinds of concept have been proposed

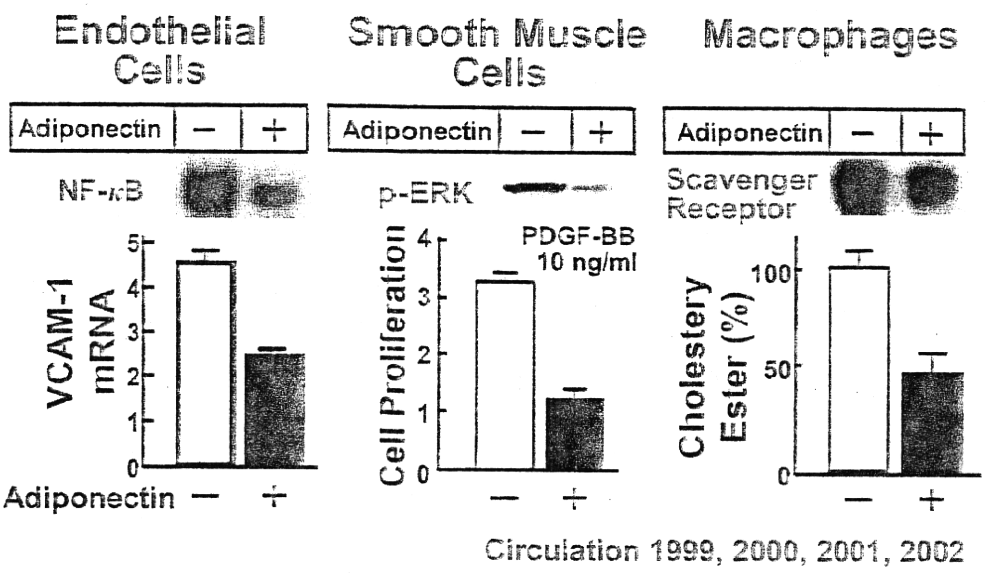


Fig. 9. Cell biological mechanism of anti-atherogenicity of adiponectin.

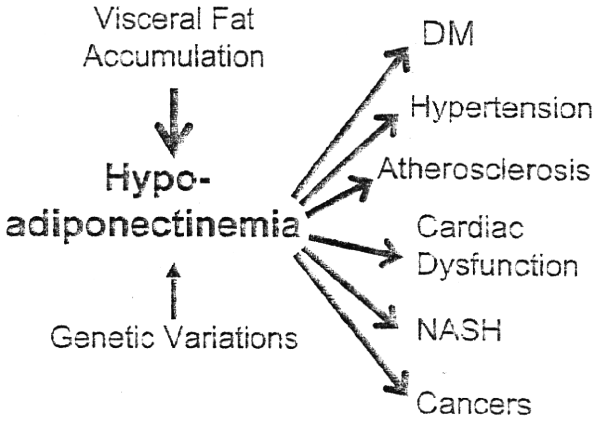


Fig. 10. A disease entity of hypoadiponectinemia

adiponectin receptors, AdipoR1 and AdipoR2 were identified by Kadowaki *et al.*[44] They showed that both receptors have roles in activation of AMP activated kinase, AMPK and PPARγ. Lodish *et al.* suggested that T-cadherin may act as a coreceptor for an-yet-unidentified signaling receptor through which adiponectin transmits metabolic signals.[45] As I mentioned above, the mode of action adiponectin may be dierent from that of other bioactive substances. It accumulates in injured tissue primarily by binding with extracellular collagens and may bind to some adhesion molecule such as T-cadherin or some signaling receptors such as AdipoRs which are expressed in target cells Further studies for molecular mechanism of adiponectin action are necessary to know physiological role of this unique protein.

### Establishment of a disease entity-hypoadiponectinemia

As shown above. it is no doubt that adiponectin is the most important adipocytokine which prevent cardiovascular disease as well as metabolic diseases including type 2 diabetes. In other words, hypoadiponectinemia has been demonstrated to be related to a variety of major diseases such as cardiovascular disease and metabolic disease, namely metabolic syndrome which may threaten life.[46] In addition to the metabolic syndrome, recently hypoadiponectinemia has been reported to be related to non-alcoholic steatohepatitis and some kinds of cancer such as breast cancer and endometrial cancer. (Fig. 10) Therefore I would like to propose a disease entity named hypoadiponectinemia. Hypoadiponectinemia may be classified into two types; one is primary hypoadiponectinemia which may be caused by genetic disorders and the other is secondary hypoadiponectinemia which is caused by visceral fat accumulation. The later is corresponding to metabolic syndrome and much more frequent than primary one. Then I expect the development of therapeutic strategy which can elevate plasma levels of adiponectin, as statin was developed for hypercholesterolemia.

## Conclusion

Adipocytes secrete various adipocytokines to control the functions of other organs and cells. Production and secretion of adipocytokines are considered to be dynamically regulated mainly by the nutritional condition. Lifestyle factors, such as overeating and physical inactivity, induce visceral fat accumulation, which results in the dysfunction of adipocytes. Oversecretion of oensive adipocytokines, such as PAI-1, tumor necrosis factor-α and hyposecretion of defensive adipocytokines, such as adiponectin, might be major mechanisms of lifestyle-related diseases, including diabetes mellitus, hyperlipidemia, hypertension and atherosclerosis, comprising the so-called metabolic syndrome. The reduction of visceral fat might be, therefore, an essential preventive measure for metabolic syndrome and its consequence, cardiovascular disease. The regulation of key adipocytokines such as adiponectin might be considered as an efficient therapeutic procedure.[46]

## References


1) Vague, J. (1947) La differenciation sexuelle, feateur determinant des formes de l'obesite. Presse Med. **55**, 339–340.
2) Kissebah, A.H., Vydelingum, N., Murray, R., Evans, D.J., Hartz, A.J., Kalkhoff, R.K. *et al.* (1982) Relation of body fat distribution in metabolic complication of obesity. J. Clin. Endocrinol. Metab. **54**, 254–260.
3) Björntorp, P. (1987) Classification of obese patients and complications related to the distribution of surplus fat. Am. J. Clin. Nutr. **45**, 112–125.
4) Tokunaga, K., Matsuzawa, Y., Ishikawa, K. and Tarui, S. (1983) A novel technique for the determination of body fat by computed tomography. Int. J. Obes. **7**, 437–445.
5) Després, J.P., Nadeau, A., Tremblay, A., Ferland, M., Moorjani, S., Lupien, P.J. *et al.* (1989) Role of deep abdominal fat in the association between regional adipose tissue distribution and glucose tolerance in obese women. Diabetes **38**, 304–309.
6) Fujioka, S., Matsuzawa, Y., Tokunaga, K., Tarui, S. (1987) Contribution of intra-abdominal fat accumulation to the impairment of glucose and lipid metabolism. Metabolism **36**, 54–59.
7) Evans, D.J., Murray, R. and Kissebah, A.H. (1984) Relationship between skeletal muscle insulin resistance, insulin-mediated glucose disposal and insulin binding: Effect of obesity and body fat topography. J. Clin. Invest. **74**, 1515–1525.
8) Yamashita, S., Nakamura, T., Shimomura, I., Nishida, M., Yoshida, S., Kotani, K. *et al.* (1996) Insulin resistance and body fat distribution. Diabetes Care **19**, 287–291.
9) Kanai, H., Matsuzawa, Y., Kotani, K., Keno, Y., Kobatake, T., Nagai, Y. *et al.* (1990) Close correlation of intra-abdominal fat accumulation to hypertension in obese women. Hypertension **16**, 484–490.
10) Nakamura, T., Tokunaga, K., Shimomura, I., Nishida, M., Yoshida, S., Kotani, K. *et al.* (1994) Contribution of visceral fat accumulation to the development of coronary artery disease in non-obese men. Atherosclerosis **107**, 239–246.
11) Nakajima, T., Fujioka, S., Tokunaga, K., Matsuzawa, Y. and Tarui, S. (1989) Correlation of intraabdominal fat accumulation and left ventricular performance in obesity. Am. J. Cardiol. **64**(5), 369–373.
12) Shinohara, E., Kihara, S., Yamashita, S., Yamane, M., Nishida, M., Arai, T. *et al.* (1997) Visceral fat accumulation as an important risk factor for obstructive sleep apnoea syndrome in obese subjects. J. Intern. Med. **241**, 11–18.
13) Reaven, G.M. (1988) Role of insulin resistance in human disease. Diabetes **37**, 1595–1607.
14) Kaplan, N.M. (1989) The deadly quartet. Arch. Intern. Med. **149**, 1514–1520.
15) Matsuzawa, Y. (1997) Pathophysiology and molecular mechanism of visceral fat syndrome: The Japanese experience. Diabetes Metab. Rev. **13**, 3–13.
16) Shimomura, I., Funahashi, T., Takahashi, M., Maeda, K., Kotani, K., Nakamura, T. *et al.* (1997) Enhanced expression of PAI-1 in visceral fat: Possible contribution to vascular disease in obesity. Nature Med. **2**, 800–803.
17) Matsumoto, S., Kishida, K., Shimomura, I., Maeda, N., Nagaretani, H., Matsuda, M. *et al.* (2000) Increased plasma HB-EGF associated with obesity and coronary artery disease. Biochem. Biophys. Res. Commun. **292**, 781–786.
18) Matsuzawa, Y. (2005) Adipocytokines and metabolic syndrome. Semin. Vasc. Med. **5**, 34–39.
19) Uysal, K.T., Wiesbrock, S.M., Marino, M.W. and Hotamisligil, G.S. (1997) Protection from obesity-induced insulin resistance in mice lacking TNF-α function. Nature **389**, 610–614.
20) Maeda, K., Okubo, K., Shimomura, I., Funahashi, T., Matsuzawa, Y. and Matsubara, K. (1996) cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (AdiPose Most abundant Gene transcript 1). Biochem. Biophys. Res. Commun. **221**, 286–289.
21) Scherer, P.E., Williams, S., Fogliano, M., Baldini, G. and Lodish, H.F. (1995) A novel serum protein similar to C1q produced exclusively in adipocytes. J. Biol. Chem. **270**, 26746–26749.
22) Arita, Y., Kihara, S., Ouchi, N., Takahashi, M., Maeda, K., Miyagawa, J. *et al.* (1999) Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem. Biophys. Res. Commun. **257**, 79–83.
23) Halleux, C.M., Takahashi, M., Delporte, M.L., Detry, R., Funahashi, T., Matsuzawa, Y. *et al.* (2001) Secretion of adiponectin and regulation of apM1 gene expression in human visceral adipose tissue. Biochem. Biophys. Res. Commun. **288**, 1102–1107.
24) Maeda, N., Takahashi, M., Funahashi, T., Kihara, S.,

Nishizawa, H., Kishida, K. *et al.* (2001) PPARγ ligands increase expression and plasma concentration of adiponectin, an adipose-derived protein. Diabetes **50**, 2094–2099.

25) Hotta, K., Funahashi, T., Arita, Y., Takahashi, M., Matsuda, M., Okamoto, Y. *et al.* (2000) Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. Arterioscler. Thromb. Vasc. Biol. **20**, 1595–1599.

26) Stefan, N., Vozarova, B., Funahashi, T., Matsuzawa, Y., Weyer, C., Lindsay, R.S. *et al.* (2002) Plasma adiponectin concentration is associated with skeletal muscle insulin receptor tyrosine phosphorylation and low plasma concentration precedes a decrease in whole body insulin sensitivity in humans. Diabetes **51**, 1884–1888.

27) Lindsay, R.S., Funahashi, T., Hanson, R.L., Matsuzawa, Y., Tanaka, S., Tataranni, P.A. *et al.* (2002) Adiponectin and development of type 2 diabetes in the Pima Indian population. Lancet **360**, 57–58.

28) Maeda, N., Shimomura, I., Kishida, K., Nishizawa, H., Matsuda, M., Nagaretani, H. *et al.* (2002) Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. Nat. Med. **8**, 731–737.

29) Tomas, E., Tsao, T.S., Saha, A.K., Murrey, H.E., Zhang, Cc. C., Itani, S.I. *et al.* (2002) Enhanced muscle fat oxidation and glucose transport by ACRP30 globular domain: acetyl-CoA carboxylase inhibition and AMP-activated protein kinase activation. Proc. Natl. Acad. Sci. U.S.A. **90**, 16309–16313.

30) Iwashima, Y., Katsuya, T., Ishikawa, K., Ouchi, N., Ohishi, M., Sugimoto, K. *et al.* (2004) Hypoadiponectinemia is an independent risk factor for hypertension. Hypertension **43**, 1318–1323.

31) Ouchi, N., Ohishi, M., Kihara, S., Funahashi, T., Nakamura, T., Nagaretani, H. *et al.* (2003) Association of hypoadiponectinemia with impaired vasoreactivity. Hypertension **42**, 231–234.

32) Ouchi, N., Kihara, S., Arita, Y., Maeda, K., Kuriyama, H., Okamoto, Y. *et al.* (1999) Novel modulator for endothelial adhesion molecules. Circulation **100**, 2473–2476.

33) Kumada, M., Kihara, S., Sumitsuji, S., Kawamoto, T., Matsumoto, S., Ouchi, N. *et al.* (2003) Association of hypoadiponectinemia with coronary artery disease in men. Arterioscler. Thromb. Vasc. Biol. **23**, 85–89.

34) Pischon, T., Girman, C.J., Hotamisligil, G.S., Rifai, N., Hu, F.B. and Rimm, E.B. (2004) Plasma adiponectin levels and risk of myocardial infarction in men. JAMA **291**, 1730–1737.

35) Kondo, H., Shimomura, I., Matsukawa, Y., Kumada, M., Takahashi, M., Matsuda, M. *et al.* (2002) Association of adiponectin mutation with type 2 diabetes: a candidate gene for the insulin resistance syndrome. Diabetes **51**, 2325–2328.

36) Matsuda, M., Shimomura, I., Sata, M., Arita, Y., Nishida, M., Maeda, N. *et al.* (2002) Role of adiponectin in preventing vascular stenosis: the missing link of adipo-vascular axis. J. Biol. Chem. **277**, 37487–37491.

37) Okamoto, Y., Kihara, S., Ouchi, N., Nishida, M., Arita, Y., Kumada, M. *et al.* (2002) Adiponectin reduces atherosclerosis in apolipoprotein E-deficient mice. Circulation **26**, 2767–2770.

38) Okamoto, Y., Arita, Y., Nishida, M., Muraguchi, M., Ouchi, N., Takahashi, M. *et al.* (2000) An adipocyte-derived plasma protein, adiponectin, adheres to injured vascular walls. Horm. Metab. Res. **32**, 47–50.

39) Ouchi, N., Kihara, S., Arita, Y., Okamoto, Y., Maeda, K., Kuriyama, H. *et al.* (2000) Adiponectin, adiocyte-derived plasma protein, inhibits endothelial NF-κB signaling through c-AMP dependent pathway. Circulation **102**, 1296–1301.

40) Arita, Y., Kihara, S., Ouchi, N., Maeda, K., Kuriyama, H., Okamoto, Y. *et al.* (2002) Adipocyte-derived plasma protein adiponectin acts as a platelet-derived growth factor-BB-binding protein and regulates growth factor-induced common postreceptor signal in vascular smooth muscle cell. Circulation **105**, 2893–2898.

41) Ouchi, N., Kihara, S., Arita, Y., Nishida, M., Matsuyama, A., Okamoto, Y. *et al.* (2001) Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrophages. Circulation **103**, 1057–1063.

42) Kumada, M., Kihara, S., Ouchi, N., Kobayashi, H., Okamoto, Y., Ohashi, K. *et al.* (2004) Adiponectin specifically increases tissue inhibitor of metalloproteinase-1 through interleukin-10 expression in human macrophages. Circulation **109**, 2046–2049.

43) Shibata, R., Ouchi, N., Ito, M., Kihara, S., Shiojima, I., Pimentel, D.R. *et al.* (2004) Adiponectin-mediated modulation of hypertrophic signals in the heart. Nat. Med. **10**, 1384–1389.

44) Yamauchi, T., Kamon, J., Ito, Y., Tsuchida, A., Yokomizo, T., Kita, S. *et al.* (2003) Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature **423**, 762–769.

45) Hug. C., Wang, J., Ahmad, N.S., Bogan, J.S., Tsao, T.S. and Lodish, H.F. (2004) T-cadherin is a receptor for hexameric and high molecular-weight forms of Acrp30/adiponectin. Proc. Natl. Acad. Sci. USA **101**, 10308–10313.

46) Matsuzawa, Y. (2006) Therapy Insight: adipocytokines in metabolic syndrome and related cardiovascular disease. Nature Clin. Prac. Cardiovasc. Med. **3**, 35–42.

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## Profile

Yuji Matsuzawa was born in 1941 in Tanabe City, Wakayama Prefecture. He graduated Osaka University Medical School in 1966 and received PhD in 1977. He became Professor of the Second Department of Internal Medicine in 1993 and had been Director of Osaka University Hospital from 2000 to 2002. Since 2003, he is Director of Sumitomo Hospital. He has been working on lifestyle-related diseases such as obesity, hyperlipidemia and atherosclerosis. He established a concept of visceral fat syndrome which is corresponding to so-called metabolic syndrome and discovered adiponectin, a key molecule of lifestyle-related diseases. By these achievements, he received the medical award of Japan Medical Association in 2000, Takeda Award in 2004 and Willendorf's Award from International Association for the Study of Obesity in 2006. He is now President of Asian Pacific Atherosclerosis Federation and also the President of Asia Oceania Association for the Study of Obesity.

Original Article

# Obesity as a Risk Factor for Coronary Events in Japanese Patients with Hypercholesterolemia on Low-Dose Simvastatin Therapy

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*Aim*: We previously reported that obesity (defined as a body mass index (BMI) ≥25 kg/m$^2$) was not an independent risk factor for coronary heart disease (CHD) in hypercholesterolemic patients without a history of CHD from the Japan Lipid Intervention Trial (J-LIT). In this study, the obese J-LIT subgroup was further analyzed to assess CHD risk.

*Methods*: In the J-LIT study, patients received simvastatin treatment (usually at 5 mg/day) for 6 years. A total of 38,385 patients (mean age: 57.7±7.9, 12,111 men) without prior CHD and/or stroke were analyzed.

*Results*: In this cohort, 181 CHD (acute myocardial infarction or sudden cardiac death) were observed. Obesity ($n$=12,929) was not an independent risk factor for CHD (relative risk; 1.18, 95% confidence interval; 0.87–1.59) after adjustment for the major risk known factors, such as age, sex, hypertension, diabetes mellitus (DM), and smoking. However, blood pressure, triglycerides, and fasting plasma glucose all increased, while high-density lipoprotein-cholesterol decreased, with increased BMI. The percentage of patients having two or three risk factors (such as dyslipidemia, hypertension, and DM) also increased with increased BMI.

*Conclusions*: Obesity was not an independent risk factor for CHD in hypercholesterolemic patients on statin therapy; however, it is important to control obesity, a condition in which CHD risks accumulate, in order to improve associated risk factors along with the treatment of each risk factor, thus further reducing the risk of CHD.

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**Key words;** Obesity, Body mass index, Risk factors for coronary events, Simvastatin

## Introduction

According to the World Health Organization (WHO)[1], the prevalence of both overweight (body mass index (BMI) ≥25 kg/m$^2$) and obesity (BMI ≥30 kg/m$^2$) is increasing worldwide at an alarming rate in both developing and developed countries. The United States national survey showed that adults who were overweight or obese increased from 56% to 66% between 1988–94 and 2003–4[2, 3]. Almost 108 million adults in the USA are either overweight or obese and their weight increases the risk of various major

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