# 人工骨による骨・関節疾患の治療

大阪大学大学院医学系研究科器官制御外科学(整形外科)　*教授　**病院教授
*吉川秀樹(よしかわひでき)　玉井宣行(たまいのりゆき)　**名井陽(みょういあきら)

整形外科において広く行われている骨移植術は，主として骨腫瘍や感染による骨欠損の補填，難治性骨折や偽関節などの修復促進，関節固定や脊椎固定の骨性癒合，人工関節周辺の母床骨の補填，骨粗鬆症により脆弱化した骨の補強などに用いられる．従来から，患者自身の腸骨，腓骨などから移植骨を採取し，患部に移植するという自家骨移植が広く施行されてきた．自家骨移植は，骨再生に優れるが，採骨部の疼痛，血腫，骨折などの術後合併症の頻度が高く，高侵襲手術である．

近年，自家骨移植に代わり，種々の人工骨が開発され，臨床での使用が急速に普及しつつある[1]．人工骨は，①移植骨採取の侵襲がない，②任意の量・形状を調節できる，③生体適合性が良い，④免疫反応がない，などの利点を有するが，一方では，①力学的強度が弱い，②細胞の侵入が困難である，③高価である，などの問題点も有している．今日まで人工骨として，アルミナ，バイオグラス，ハイドロキシアパタイト，β-リン酸3カルシウムなど様々な素材が使用されてきた．形状も，顆粒状，ブロック状，液体注入型など，種々の人工骨が薬事認可を受け，保険適用されている．

その中で，ハイドロキシアパタイトはヒトの骨の無機質成分に近く，海綿骨以上の力学的強度を有し，その優れた生体親和性，骨伝導能から人工骨として最も適していると考えられている．1980年代より整形外科，歯科口腔外科，脳外科領域において骨補填材料として広く臨床使用されてきた[1]．筆者らは，力学的強度を有し，かつ幹細胞や骨増殖因子の導入が可能な骨補填材料として，気孔間連通構造を有する新規ハイドロキシアパタイトを開発した(図1)[2)~6)]．

本稿では，種々の骨・関節疾患に対し本人工骨(NEOBONE®)を用いて治療した症例を提示し，その有用性について解説する．

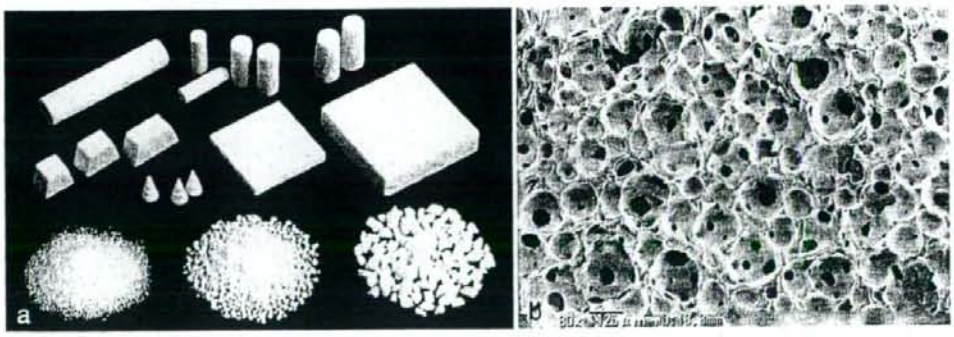
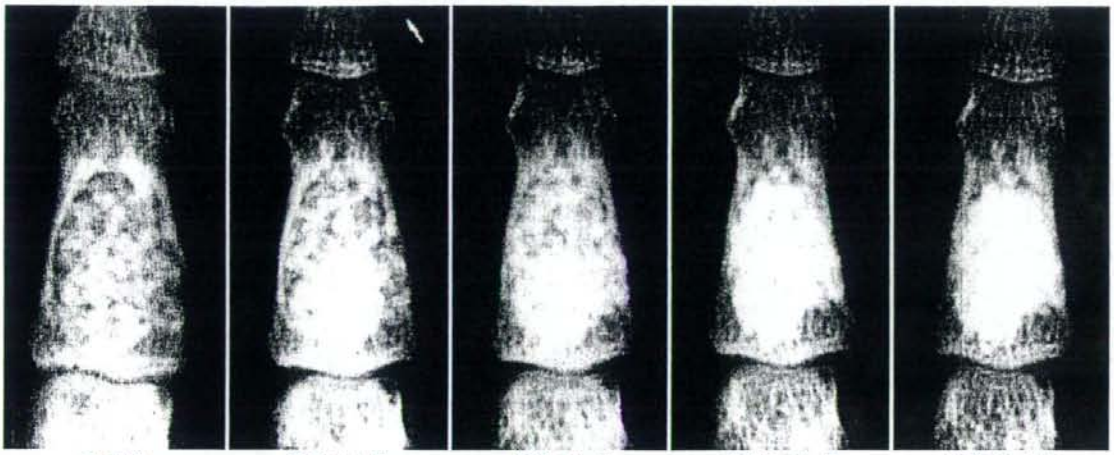



図1　連通多孔体ハイドロキシアパタイト人工骨(NEOBONE)
a：市販の各種NEOBONE，b：走査電顕像での内部微細構造．

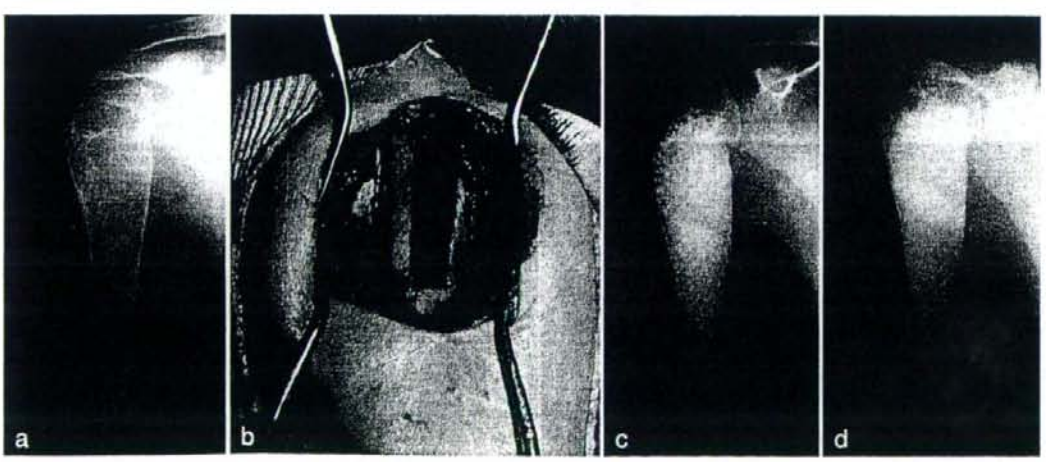
〔症例1(図2)〕28歳，男性．内軟骨腫．
バレーボールにて右手中指を突き指し，受傷した．1週間，疼痛，腫脹が消失しないため受診し，単純X線により中節骨の骨溶解像を認めた．内軟骨腫と診断し，局所麻酔下に病巣掻爬を行い，欠損部に対し顆粒状人工骨（NEOBONE）を充填した．以後経過良好で，術後3，6，12，27カ月の単純X線では人工骨は一体化し，膨隆していた中節骨は，骨リモデリングにより正常化した．



術直後　術後3カ月　術後6カ月　術後12カ月　術後27カ月

図2　右手中指中節骨の内軟骨腫における経時的X線像の変化

〔症例2(図3)〕14歳，男子．単発性骨嚢腫．
野球で投球した後，右肩激痛が出現し，続行不能となった．単純X線により右上腕骨の骨溶解像を認めた(a)．MRIにより，内部は嚢腫様で大部分が液体成分であったため，単発性骨嚢腫と診断した．術中所見では，骨皮質は菲薄化し，内部は漿液性で，骨髄は欠損していた(b)．顆粒状人工骨（NEOBONE）のみを充填し，自家骨移植は行わなかった(c)．術後3カ月の単純X線像では，術直後に見られた顆粒状陰影はほぼ消失し，豊富な骨再生が認められた(d)．術後3年，再発を認めない．




術前　術中　術直後　術後3カ月

図3　右上腕骨の単発性骨嚢腫

〔症例3(図4)〕58歳，女性．変形性膝関節症．
約3年前から，歩行時，左膝関節内側に疼痛が出現し，徐々に痛みが増強した．単純X線では，左膝内側関節裂隙の狭小化を認めた．MRIによると，内側関節軟骨は，菲薄化しているものの残存しており，年齢も考慮して，高位脛骨骨切り術を選択した．骨切り後の骨欠損部には，術前CT三次元構成画像に基づき，切削機により加工したブロック状人工骨(NEOBONE)を移植した(a～g)．術後6カ月で良好な骨癒合が得られた(h)．

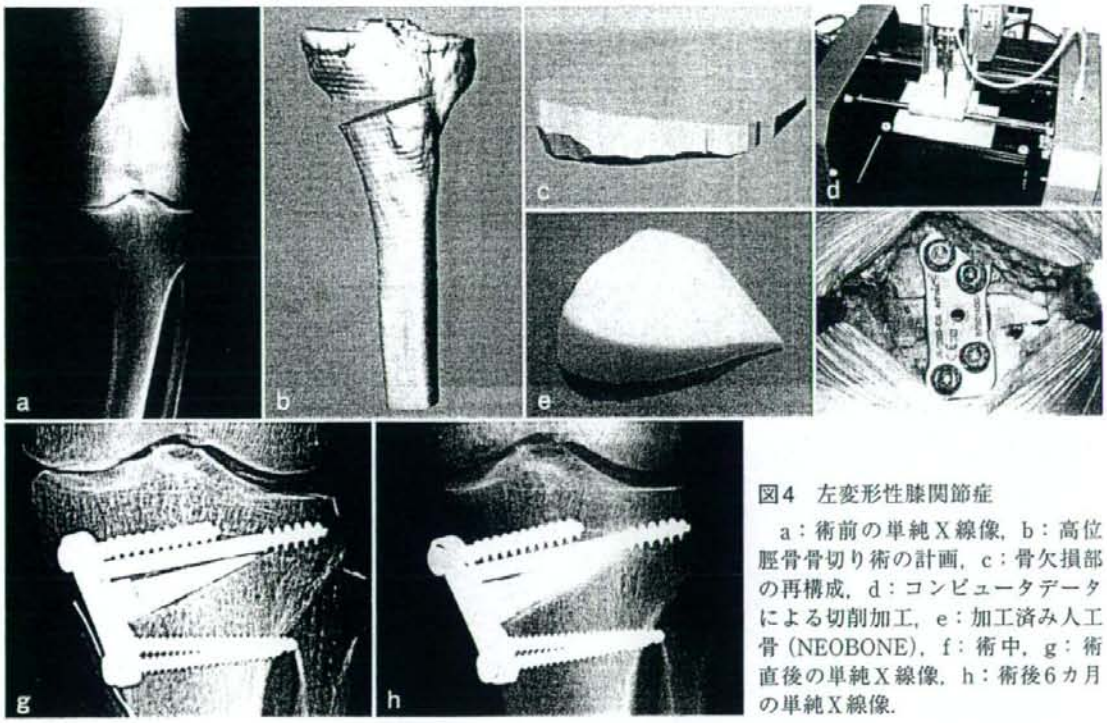
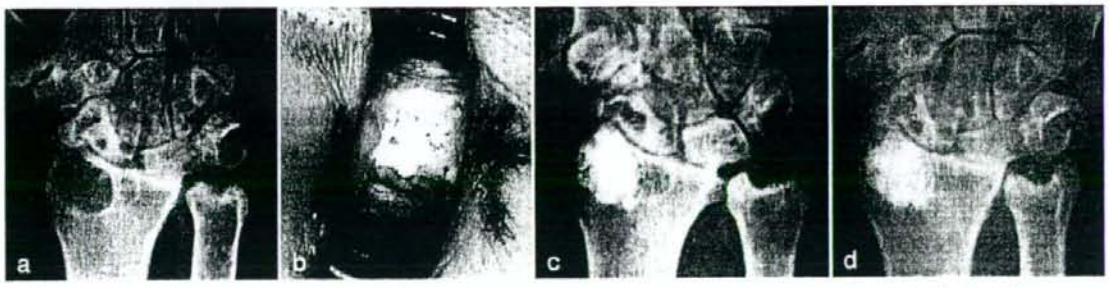



図4　左変形性膝関節症
a：術前の単純X線像，b：高位脛骨骨切り術の計画，c：骨欠損部の再構成，d：コンピュータデータによる切削加工，e：加工済み人工骨(NEOBONE)，f：術中，g：術直後の単純X線像，h：術後6カ月の単純X線像．

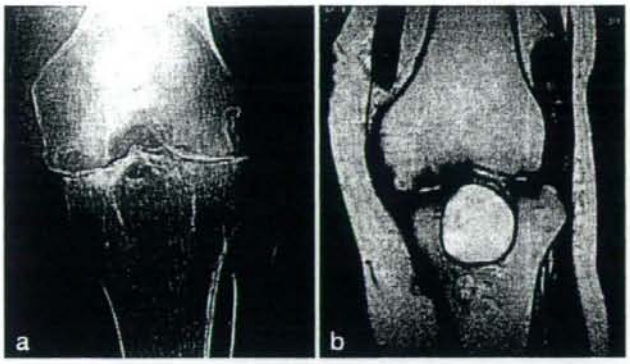
〔症例4(図5)〕58歳，女性．関節リウマチ．
10年前に発症した関節リウマチで治療中に右手関節の疼痛が著明となった．単純X線では，手根骨の骨破壊と橈骨関節近傍の円形の骨溶解像を認めた(a)．関節リウマチによる骨嚢胞と診断し，骨破壊の進行予防，病的骨折予防の目的で，骨嚢胞に対し人工骨移植術を施行した．橈骨骨皮質を小開窓し，嚢胞内を掻爬後，顆粒状人工骨(NEOBONE)を充填した(b・c)．術後1カ月で手関節痛は軽減し，以後徐々に骨形成が進行した(d)．術後2年，疼痛は消失している．



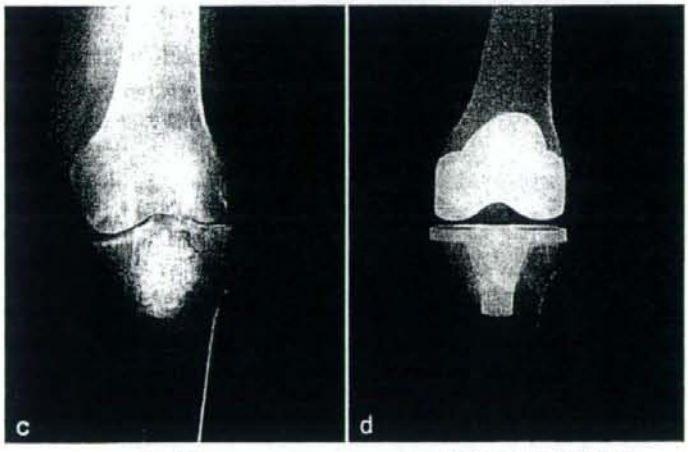

術前　　術中　　術直後　　術後12カ月

図5　右手関節の関節リウマチ

〔症例5(図6)〕65歳，女性．関節リウマチ．
7年前に発症した関節リウマチで治療中に左膝関節の疼痛が著明となった．単純X線では，膝関節の骨破壊，関節裂隙の消失，脛骨近位中央部の巨大な嚢腫様変化を認めた(a・b)．この状態での人工膝関節の設置は不安定であるため，骨破壊の進行予防，病的骨折予防の目的で，まず脛骨骨嚢胞に対し，人工骨(NEOBONE)移植術を施行した(c)．術後6カ月で，良好な骨再生が確認されたため，この時点で人工膝関節全置換術を施行した(d)．術後3年，人工関節のゆるみも生じず，経過良好である．




術前　　術前MRI T1強調像




術直後　　人工膝関節全置換術直後

図6　左膝関節の関節リウマチ

〔まとめ〕

近年，種々の人工骨の開発が進められ，骨の再生医療への臨床応用が期待されている．本稿で述べたように，筆者らが開発したNEOBONEの使用により，骨盤などからの自家骨の採取が不要となり，低侵襲手術が可能となった．また，骨の小病変などに対しては，従来，自家骨を採取してまでは手術適応とならなかった症例があったが，NEOBONEの使用により，そのような症例にも手術適応が拡大した．将来的には，NEOBONEの気孔間連通構造を利用して，骨髄幹細胞や骨形成蛋白(BMP)等の増殖因子を気孔内に導入したり，外科的に血管を導入することにより，さらなる骨再生の促進が期待できる．近い将来，先端医療としての骨組織のtissue engineeringが可能になるものと思われる．

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# Adenovirus-mediated gene transfer of adiponectin reduces the severity of collagen-induced arthritis in mice

Kosuke Ebina [a,b], Kazuya Oshima [a], Morihiro Matsuda [b,c,d], Atsunori Fukuhara [b], Kazuhisa Maeda [b], Shinji Kihara [b], Jun Hashimoto [a], Takahiro Ochi [e], Nirmal K. Banda [f], Hideki Yoshikawa [a], Iichiro Shimomura [a,*]

[a] Departments of Orthopaedics and Metabolic Medicine, Graduate School of Medicine, Osaka University, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan
[b] Metabolic Medicine, Graduate School of Medicine, Osaka University, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan
[c] Division of Analysis for Pathophysiology, Institute of Clinical Research, National Hospital Organization Kure Medical Center, Kure, Hiroshima 737-0023, Japan
[d] Department of Internal Medicine, National Hospital Organization Kure Medical Center, Kure, Hiroshima 737-0023, Japan
[e] Osaka Police Hospital, 10-31 Kitayama-cho, Tennoji-ku, Osaka 543-0035, Japan
[f] Division of Rheumatology B115, University of Colorado at Denver and Health Sciences Center, M-20 3104, 1775 North Ursula St., Aurora, CO 80045, USA

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ABSTRACT

Adiponectin (APN) is a hormone released by adipose tissue with anti-inflammatory properties. The purpose of this study was to examine the therapeutic effects of systemic delivery of APN in murine arthritis model. Collagen-induced arthritis (CIA) was induced in male DBA1/J mice, and adenoviral vectors encoding human APN (Ad-APN) or beta-galactosidase (Ad-β-gal) as control were injected either before or during arthritis progression. Systemic APN delivery at both time points significantly decreased clinical disease activity scores of CIA. In addition, APN treatment before arthritis progression significantly decreased histological scores of inflammation and cartilage damage, bone erosion, and mRNA levels of pro-inflammatory cytokines in the joints, without altering serum anti-collagen antibodies levels. Immunohistochemical staining showed significant inhibition of complement C1q and C3 deposition in the joints of Ad-APN infected CIA mice. These results provide novel evidence that systemic APN delivery prevents inflammation and joint destruction in murine arthritis model.



Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic inflammation of joint synovial tissues, followed by cartilage destruction and bone erosion. Collagen-induced arthritis (CIA) is an established rodent model of autoimmune polyarthritis with many similarities to human RA, and the immunopathological process of CIA has been reported in details [1]. Briefly, injection of chicken type II collagen (CII) in complete Freund's adjuvant (CFA) results in proliferation and differentiation of T-cells into CD4+Th1-cells in the draining lymph nodes. These cells then promote the production of anti-collagen IgG by activated CII-specific B-cells. These antibodies enter the joint and bind to CII, forming an immunocomplex (IC), which activates the complement cascade. Complement enhances the permeability of the vascular endothelium, and facilitates infiltration of monocytes (macrophages) and neutrophils into the joint. In the joint space, macrophages produce tumor necrosis factor (TNF)-α and interleukin (IL)-1. TNF-α enhances vascular permeability and migration of inflammatory cells into the joint space, and IL-1 is the primary trigger of tissue destruction by infiltrating cells and resident synoviocytes.

Adiponectin (APN) is an adipocytokine that shares strong homologies with the complement factor C1q and TNF-α [2]. APN has anti-inflammatory effects, and suppresses TNF-α and IL-6 production by macrophages activated with lipopolysaccharide (LPS) through suppression of nuclear factor-kappa B (NF-κB) signaling [3]. Accumulating evidence suggests a novel link between APN and inflammatory joint diseases. For example, APN concentration in the synovial fluid correlates negatively with synovial fluid leukocyte count in patients with RA, suggesting that APN is an anti-inflammatory molecule in RA [4]. However, APN levels in synovial fluid and serum are elevated in patients with RA compared with healthy controls [4], and APN treatment induces IL-6 production by synovial fibroblasts from RA patients [5], suggesting that APN is a pro-inflammatory molecule in RA. Thus, the effects of APN in RA are controversial. In the present study, we investigated the effects of APN on CIA mice using APN-producing adenovirus.

## Materials and methods

*Materials.* Enzyme-linked immunosorbent assay (ELISA) for murine APN (including all isoforms) was purchased from Otsuka Pharmaceutical (Tokyo, Japan). Anti-APN polyclonal antibody used

* Corresponding author. Fax: +81 6 6879 3739.
*E-mail address:* ichi@imed2.med.osaka-u.ac.jp (I. Shimomura).

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for Western blotting was described previously [6]. Tartrate-resistant acid phosphatase (TRAP) staining kit was purchased from Cell Garage (Tokyo, Japan).

*Induction and assessment of CIA.* To induce CIA, we injected intradermally 100 μl of an emulsion containing 200 μg of chicken CII (Sigma, St. Louis, MO) and 200 μg of *Mycobacterium tuberculosis* in CFA (Chondrex, Redmond, WA) at the base of the tail of 6-week-old male DBA/1J mice (CLEA Japan, Tokyo), twice with a 21-day gap, as described previously [7]. Clinical severity of arthritis was assessed as described previously [8]. Each limb was scored, yielding a maximum possible score of 16 per mouse. Serum was collected from the tail vein at each time point.

*APN adenovirus and systemic delivery in vivo.* Adenovirus producing the full-length mouse APN was prepared as described previously [9]. Then, 200 μl of $2 \times 10^8$ plaque-forming units of adiponectin-producing adenovirus (Ad-APN) or control β-galactosidase-expressing adenovirus (Ad-β-gal) were injected into the jugular vein, on 19 (before arthritis progression) or 27 (during arthritis progression) days after initial injection of CII.

*Determination of IgG, IgG2a, and IgG1 titers against CII and serum complement C1q and C3 levels.* The total IgG anti-collagen antibody titers against chicken CII were determined through ELISA kit (Chondrex). IgG1 and IgG2a anti-collagen antibody titers against chicken CII were determined as described previously, and expressed in optical density (OD) value [10]. Serum C1q and C3 levels were determined by ELISA as described previously [11].

*Histological analysis.* On day 35 after initial injection of CII, joints were harvested and fixed in phosphate-buffered 4% paraformaldehyde, decalcified in 14% ethylenediaminetetraacetic acid (EDTA), and embedded in paraffin. Joint sections were stained with Safranin O and hematoxylin/eosin, and then histologically scored for inflammation, cartilage damage, and pannus formation as described previously [12]. Immunostaining for mouse IgG, C1q, C3, neutrophils, CXC chemokine ligand (CXCL)12, and APN was performed on paraffin-embedded samples with goat anti-mouse IgG (Cappel), rat anti-mouse C1q (Hycult Biotechnology b.v., Uden, Netherlands), rat anti-mouse C3 (Hycult Biotechnology), rat-anti-mouse neutrophils, (Serotec, Oxford, UK), monoclonal antihuman/mouse CXCL12 antibody (R&D Systems Inc., Minneapolis, MN), and rabbit-anti-mouse APN (Otsuka Pharmaceutical), respectively. The other steps were performed according to the instructions provided on the labeling of Vectastain Elite ABC system (Vector Laboratories, Burlingame, CA). Scoring for IgG, C1q and C3 staining on the cartilage, and CXCL12 staining on the synovium was performed as described previously [13]. The average number of infiltrating neutrophils in the synovium was determined using a modified version of the published method [14].

*Quantitative real-time PCR of joint samples.* Total RNA was extracted by pulverizing the frozen individual fore paws with an RNA STAT-60 kit. The first-strand cDNA was synthesized using ThermoScript RT-PCR System (Invitrogen, San Diego, CA). Real-time polymerase chain reaction (PCR) was performed on a Light Cycler using the Fast Start DNA Master SYBR Green I (Roche Diagnostics, Indianapolis, IN). The sequences of primers were designed based on a previous report [13], and other primers are listed in Supplementary Table 1.

*Lymph node cell proliferation assay. In vitro* proliferation of draining lymph node (DLN) cells was examined by Cell Proliferation ELISA Bromodeoxyuridine (BrdU) kit (Roche) using a modified version of the published method [10].

*Cytokine production by cultured splenocytes.* Spleens were removed and cell suspensions ($2 \times 10^6$ cells/well) were distributed to flat bottom 96-well plates. Spleen cells were cultured without or with either 50 μg/ml heat-denatured chicken CII (Sigma–Aldrich) or 5 μg/ml LPS from *Escherichia coli* (Sigma–Aldrich). After 48-h incubation, the supernatants were collected, and TNF-α and IL-1β levels were measured using ELISA kit (Quantikine Mouse ELISA kit, R&D Systems Inc.).

*Skeletal morphology.* Three-dimensional microcomputed tomography (3D-μCT) scan for ankle joints was undertaken and the trabecular bone area (percentage of bone volume [BV] per tissue volume [TV]) of distal tibia was measured using a composite X-ray analysis system (Shimadzu, SMX-100CT-SV, Kyoto, Japan).

*Statistical analysis and ethical considerations.* Data were expressed as means ± standard error of the mean. Differences between groups were examined for statistical significance using Chi-square test, Student's *t* test, or analysis of variance with Fisher's protected least significant difference test. A *P* value less than 0.05 denoted the presence of a statistically significant difference. The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Osaka University School of Medicine.

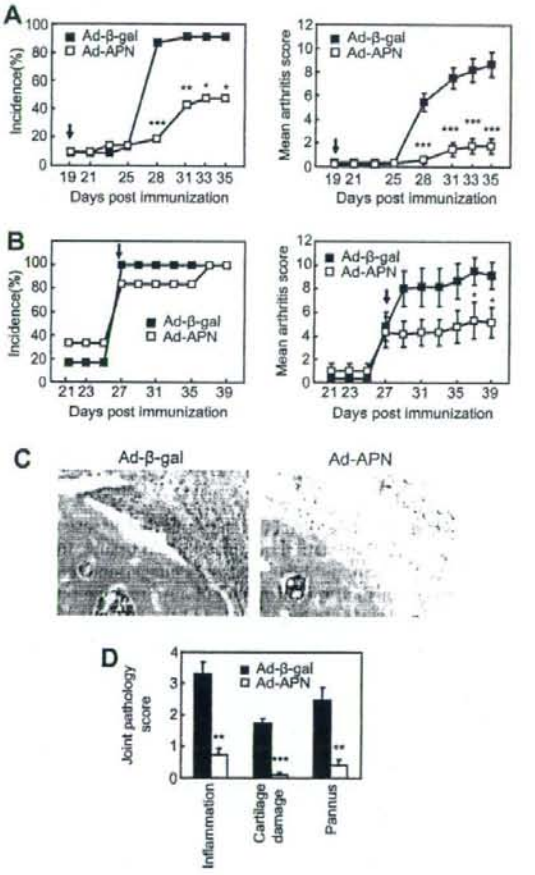


**Fig. 1.** Systemic delivery of APN in collagen-induced arthritis (CIA) mouse model and histological analysis of the joints. (A) On day 19, before arthritis progression, mice were injected with adenoviral vector directing the expression of either *lacZ* gene (Ad-β-gal) or APN (Ad-APN) intravenously ($n = 23$ Ad-β-gal-infected mice, $n = 21$ Ad-APN-infected mice). (B) On day 27, during arthritis progression, mice were injected with the same adenovirus ($n = 6$ mice in each group). (C) Histological features of representative hematoxylin and eosin-stained sections of the ankle joints (original magnification 200×), and (D) mean pathological scores of the joints of adenovirus-infected CIA mice ($n = 36$ joints in each group). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, versus Ad-β-gal-infected CIA mice.

## Results

### *Ad-APN suppresses progression of arthritis in CIA model*

First, we tried two protocols to evaluate the effect of Ad-APN on CIA. When the virus was injected on day 19 (Fig. 1A), the incidence and disease activity on day 35 were significantly suppressed by Ad-APN treatment compared with Ad-β-gal (arthritis score: Ad-APN-infected mice: 1.76 ± 0.63, Ad-β-gal-infected mice: 8.68 ± 1.06; $P < 0.001$). In addition, when the virus was injected on day 27 (Fig. 1B), disease activity was significantly suppressed on day 39 by Ad-APN treatment compared with Ad-β-gal (arthritis score: Ad-APN-infected mice: 5.17 ± 1.25, Ad-β-gal-infected mice: 9.17 ± 1.15; $P < 0.05$). For the rest of this study, we used the former protocol (Fig. 1A). Histological analysis of the ankle joint showed typical features of active arthritis in Ad-β-gal-infected CIA mice, including infiltration of inflammatory cells into the synovium, cartilage damage, and pannus formation. These changes were significantly less pronounced in Ad-APN-infected CIA mice (Fig. 1C and D).

### *Ad-APN increases APN protein in serum and bone marrow, and does not alter serum anti-collagen antibodies or complement levels*

In the experiments described in Fig. 1A, injection of Ad-APN resulted in about 5-fold increase on day 21 and about 30-fold increase on day 35 in serum APN levels (Fig. 2A). The high serum APN protein levels in Ad-APN-infected CIA mice were mainly composed of high- and middle-molecular weight forms of APN (Fig. 2B). Moreover, Ad-APN substantially increased APN protein content in knee joints compared with Ad-β-gal (Fig. 2C). Immunohistochemical staining of knee joints with anti-APN antibody indicated accumulation of APN in the bone marrow but not on the cartilage surface in Ad-APN-infected CIA mice (Fig. 2D). Under such conditions, anti-CII IgG, IgG2a, and IgG1 titers, serum C1q and C3 levels were not different between Ad-APN- and Ad-β-gal-infected CIA mice (Fig. 2E and F).

### *Treatment of CIA mice with Ad-APN suppresses local deposition of C1q and C3, infiltration of neutrophil, and changes in mRNAs of pro-inflammatory genes*

Next, we examined the accumulation of IgG, C1q, and C3 on the cartilage of wrist, knee, and ankle joints by immunohistochemical staining (Fig. 3A and B). Under the conditions with minimum background staining, IgG deposition on the cartilage was observed in both adenovirus-infected CIA mice. On the other hand, C1q and C3 deposits on the cartilage were significantly suppressed by Ad-APN treatment compared with Ad-β-gal. Furthermore, neutrophil infiltration and synovium deposition of CXCL12, a chemokine that promotes leukocyte migration, were also significantly decreased in Ad-APN-infected CIA mice (Fig. 3A and B). To assess the inflammatory status, mRNA levels of pro-inflammatory genes, complement factors, and F4/80 (a marker of monocyte/macrophage linage) were measured in isolated forepaws. The expression levels of IL-1β, IL-6, COX-2 IFN-γ, TNF-α, C1q, C3, and F4/80 were all significantly decreased by Ad-APN treatment (Fig. 3C).

### *Effects of Ad-APN on immunocyte activities in lymph nodes and spleen, and bone erosion of CIA mice*

Next, activities of immunocytes were measured. DLN cells from Ad-APN-infected CIA mice showed marginally inhibited proliferation activities (Fig. 4A). Splenocytes from Ad-APN-infected CIA

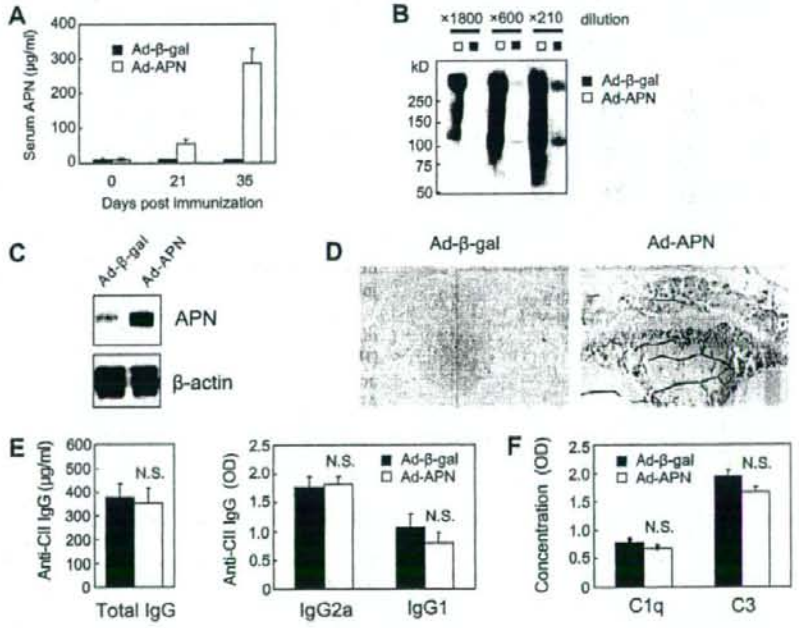


**Fig. 2.** High APN levels in serum and joints do not alter serum levels of anti-CII antibody, complement C1q, or C3 in CIA mice. (A) Serum APN concentrations at each time point were determined by ELISA ($n = 6$ in each group). Representative serum samples (B) and protein lysates prepared from knee joints (C) of adenovirus-infected CIA mice on day 35 were subjected sodium dodecyl sulfate-polyacrylamide gel electrophoresis without reducing reagent, and analyzed by western blot using anti-APN antibody. (D) Representative sections of proximal tibia immunostained with APN (original magnification 40×). Serum samples were obtained on day 35, and anti-CII specific IgG, IgG2a, and IgG1 levels (E), and complement C1q and C3 levels (F) were measured by ELISA ($n = 6$ in each group). NS = not significant.

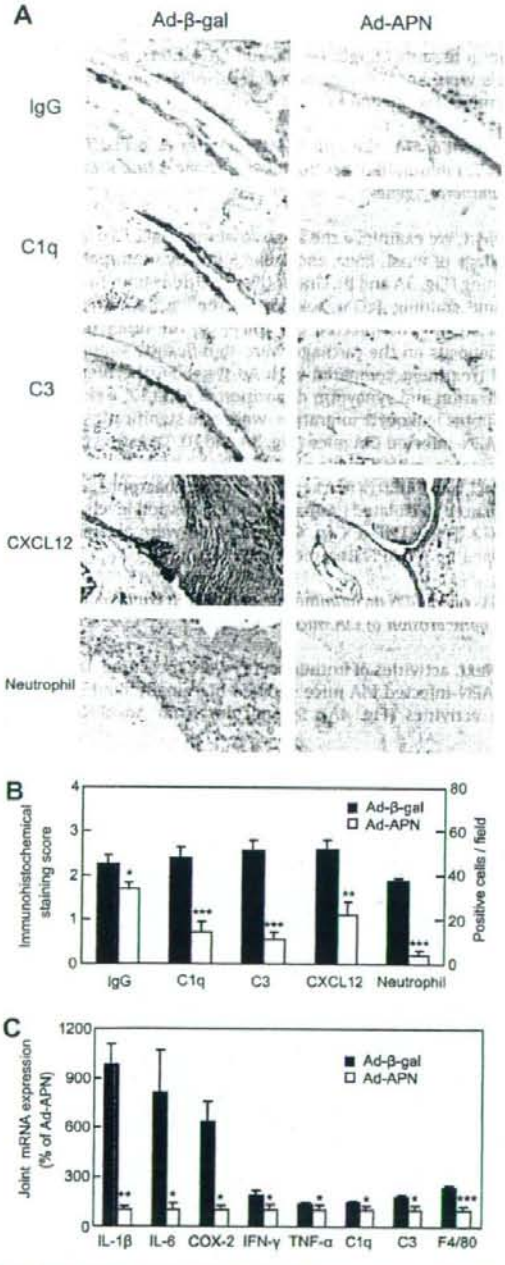


**Fig. 3.** Ad-APN inhibits complement deposition/expression and pro-inflammatory cytokine/enzyme expression in CIA mice. (A) Representative sections immunostained for IgG, C1q, C3, CXCL12, and neutrophil on the cartilage surface and synovium of the ankle joints of adenovirus-infected CIA mice (original magnification 200×). (B) Scoring of immunostained joint sections from adenovirus-infected CIA mice ($n = 16$ joints in each group). (C) mRNA expression levels in forepaws of adenovirus-infected CIA mice. ($n = 6$ joints in each group). Values are normalized to the level of 36B4 mRNA. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, versus Ad-β-gal-infected CIA mice.

mice produced less IL-1β in all conditions, and less TNF-α in response to LPS *in vitro* (Fig. 4B). Finally, analysis of bone erosion of the distal tibia using μCT revealed that trabecular bone volume were significantly decreased in Ad-β-gal-infected CIA mice compared to those in Ad-APN-infected CIA mice (Fig. 4C). In addition, the number of TRAP-positive cells was significantly decreased in ankle joints of Ad-APN-infected CIA mice (Fig. 4D).

## Discussion

In the present study, we demonstrated for the first time that Ad-APN significantly improved joint inflammation and bone erosion in CIA mice. There were no significant differences in anti-CII IgG, C1q, and C3 levels between Ad-APN and Ad-β-gal infected CIA mice (Fig. 2E and F), indicating that Ad-APN has little effect on humoral immunity. On the other hand, C1q and C3 deposition were markedly suppressed on the cartilage surface of Ad-APN-infected CIA mice (Fig. 3A and B). Therefore, we investigated the direct effect of APN on complement activation. APN has a substantial sequence similarity to C1q, and also binds to C1q receptor [15]. In addition, we confirmed the binding between human recombinant APN from mammalian cells and human C1q *in vitro* as reported previously [16]. However, in our preliminary experiments, this recombinant APN did not alter C1q binding to adherent CII-IC [11], CII-IC-induced mouse serum C3 activation (mainly involves classical pathway) [11], or zymosan-induced mouse serum C3 activation (mainly involves alternative pathway) [10] *in vitro* (data not shown). To elucidate the direct effect of APN on the complement activation, further *in vivo* and *in vitro* analyses are required.

We showed marked suppression of C1q and C3 deposition, accompanied by significant downregulation of C1q, C3, and F4/80 mRNAs in the Ad-APN-infected CIA joints (Fig. 3). A previous report demonstrated that APN inhibited the expression of endothelial adhesion molecules induced by TNF-α, and consequent transendothelial migration of monocytes [17]. In addition, TNF-α-induced vascular permeability is required for the migration of inflammatory cells into the joint and development of inflammatory process in mouse arthritis models [1], and APN was reported to inhibit TNF-α-induced hyperpermeability in endothelial cells [18]. Our group demonstrated that APN was protective against murine colitis through inhibition of macrophages infiltration and release of pro-inflammatory cytokines [19]. Considering that C1q is mainly produced by monocyte/macrophage linage [20], and C3 is produced by liver and inflamed synoviocytes [21], reduced accumulation of F4/80 positive cells and consequent C3 production by inflamed synovium should result in suppression of complement deposition and pro-inflammatory cytokine production in the CIA joints.

We also observed that Ad-APN suppressed synovial deposition of CXCL12. CXCL12 is a chemokine anchored to heparan sulfate (HS) proteoglycans on endothelial cells of RA synovium [22], and acts as a critical chemoattractant in the pathogenesis of CIA [23]. APN inhibits the binding of CXCL12 to HS, and alters the distribution of CXCL12 at the site of inflammation [24]. Taken together, Ad-APN may improve joint inflammation through decreased CXCL12 deposition in CIA synovium.

Previous studies showed that the cellular immunity, represented by the activity of immunocytes of lymph nodes or spleen, is causally associated with the disease activity in CIA mice [25]. In this study, Ad-APN marginally reduced DLN cells proliferation (Fig. 4A), and significantly suppressed IL-1β production and TNF-α production from splenocytes (Fig. 4B). These results indicate that Ad-APN could suppress disease activity of CIA partially through inhibition of cellular immunity.

In this study, Ad-APN reduced the number of TRAP-positive cells and resulted in amelioration of bone erosion in the joints of CIA mice (Fig. 4C and D). Previously, we and others reported that

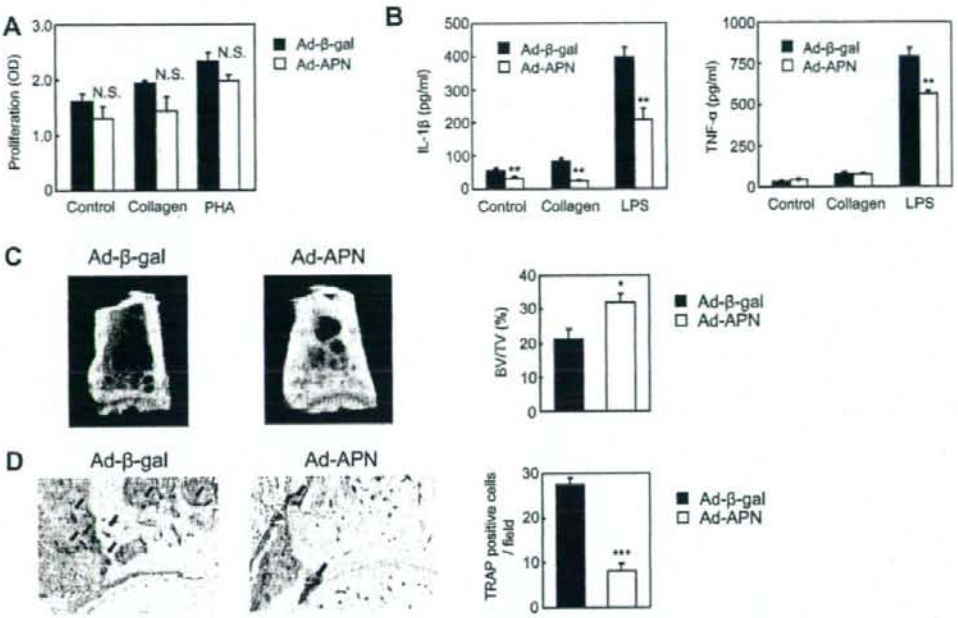


**Fig. 4.** Effects of Ad-APN on DLN cell proliferation and pro-inflammatory cytokine production from splenocytes, and bone erosion in CIA. All samples were obtained on day 35. (A) Proliferative response of DLN cells obtained from adenovirus-infected CIA mice. Isolated cells from lymph nodes were cultured for 72 h without (control) or with either 50 μg/ml heat-denatured chicken CII or 5 μg/ml phytohemagglutinin (PHA). (B) Production of pro-inflammatory cytokine by splenocytes from adenovirus-infected CIA mice. IL-1β and TNF-α levels were measured in supernatants of splenocytes by specific ELISA. (C) Three-dimensional μCT scan of the distal tibia of adenovirus-infected CIA mice. Trabecular bone volume is expressed as percentage of total tissue volume (BV/TV [%]) ($n = 4$ joints in each group). (D) Reduced number of osteoclasts in Ad-APN-infected CIA mice joints. Sections of ankle joints stained with TRAP (original magnification 100×). The number of TRAP-positive cells was counted in 5 randomly selected fields ($n = 8$ joints in each group). NS = not significant. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, versus Ad-β-gal-infected CIA mice.

APN inhibits osteoclasts differentiation in RAW264 cells [26] and mouse bone marrow macrophages [9]. Collectively, besides anti-inflammatory effects in the joints, Ad-APN might directly inhibits bone erosion of CIA mice by inhibiting osteoclasts differentiation.

The present study demonstrates for the first time that systemic APN delivery provides protection against the development of inflammatory arthritis in a murine model, through several anti-inflammatory mechanisms. The results provide new insights on the role of APN in inflammatory arthritis and new strategies for the treatment.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2008.11.005.

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ORIGINAL ARTICLE

# Serum adiponectin concentrations correlate with severity of rheumatoid arthritis evaluated by extent of joint destruction

**Kosuke Ebina · Atsunori Fukuhara · Wataru Ando · Makoto Hirao · Tadashi Koga · Kazuya Oshima · Morihiro Matsuda · Kazuhisa Maeda · Tadashi Nakamura · Takahiro Ochi · Iichiro Shimomura · Hideki Yoshikawa · Jun Hashimoto**

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**Abstract** Adiponectin is a hormone released by adipose tissue with antidiabetic, antiatherogenic, and anti-inflammatory properties. The present observational study focused on the relation between serum adiponectin level and the disease severity of established rheumatoid arthritis (RA). Ninety patients with more than 5-year diagnosis of RA and 42 age- and BMI-matched control were enrolled. The severity of RA was evaluated according to the number of destructed joints of overall 68 joints on plain radiographs (37 patients had mild RA and 53 had severe RA). Serum adiponectin level was significantly higher in the severe RA group (17.7±6.7 μg/ml) than in the control (9.1±3.8 μg/ml) and mild RA groups (13.9±6.5 μg/ml) (control vs. mild RA group, $P<0.001$; mild RA vs. severe RA group, $P<0.01$). These results suggest that increased number of joint destruction is associated with hyperadiponectinemia in established RA patients.

**Keywords** Adiponectin · Disease severity · Number of joint destruction · Rheumatoid arthritis

## Introduction

Adiponectin is a hormone released by adipose tissue and has various biological properties, such as antidiabetic [1], antiatherogenic [2], and anti-inflammatory effects [3]. Part

K. Ebina · W. Ando · M. Hirao · K. Oshima · H. Yoshikawa · J. Hashimoto (✉)
Department of Orthopaedics, Graduate School of Medicine, Osaka University,
2-2 Yamadaoka,
Suita, Osaka 565-0871, Japan
e-mail: junha@ort.med.osaka-u.ac.jp

K. Ebina · A. Fukuhara · M. Matsuda · K. Maeda · T. Nakamura · I. Shimomura
Department of Metabolic Medicine, Graduate School of Medicine, Osaka University,
2-2 Yamadaoka,
Suita, Osaka 565-0871, Japan

T. Koga
Biometrics Department,
Shin Nippon Biomedical Laboratories, Ltd,
2438 Miyanoura,
Kagoshima, Kagoshima 891-1394, Japan

M. Matsuda
Division of Analysis for Pathophysiology,
Institute of Clinical Research,
National Hospital Organization Kure Medical Center,
Kure, Hiroshima 737-0023, Japan

M. Matsuda
Department of Internal Medicine,
National Hospital Organization Kure Medical Center,
Kure, Hiroshima 737-0023, Japan

T. Ochi
Osaka Police Hospital,
10-31 Kitayama-cho, Tennoji-ku,
Osaka 543-0035, Japan

of these effects is mediated by suppressing the production of tumor necrosis factor (TNF)-α and interleukin (IL)-6 by activated macrophage [3]. In addition, it has been reported that adiponectin stimulates the proliferation and differentiation of human osteoblasts [4] and suppresses the differentiation of osteoclasts [5], suggesting that adiponectin may play a role in rheumatoid arthritis (RA).

A recent clinical study showed that serum adiponectin concentrations are higher in RA patients than in healthy control [6, 7]. In addition, adiponectin induces the production of pro-inflammatory IL-6 from RA synovial fibroblasts in vitro [8], suggesting that adiponectin is a potent driving force of arthritis. On the other hand, another report demonstrated that adiponectin concentrations correlated negatively with the number of leukocytes in the synovial fluid of RA patients [7], indicating that adiponectin is a counterpart of the local inflammatory process. Thus, the role of adiponectin in RA is controversial. In a step to define the role of adiponectin in RA, the present study was designed to investigate the correlation between serum adiponectin level and RA disease severity.

## Materials and methods

### Patients

We have previously reported that serum adiponectin level is significantly higher in females than in males and negatively correlates with body mass index (BMI) [9]. In addition, previous reports have demonstrated that most of the progression of joint damage in RA occurs during the first years of the disease and decreases thereafter [10, 11]. Therefore, to investigate the correlation between serum adiponectin level and disease severity and joint destruction in established RA, 90 female patients with more than 5-year history of RA were enrolled in this study. RA was diagnosed based on the 1987 revised American College of Rheumatology (ACR) criteria [12]. The first assessment was carried out from September to November, 2005, and 18 patients were enrolled in the second assessment from March to April, 2008, about 2.5 years after the first assessment. Sixty-five patients (72.2%) were treated with oral prednisolone and 48 patients (53.3%) with methotrexate. All patients were followed-up at Osaka University Hospital.

For non-RA controls, 42 age- and BMI-matched women who underwent health examination at the institutions that participated in the Japanese Visceral Fat Syndrome (J-VFS) Study Committee of the Ministry of Health and Welfare of Japan and subjects who visited Osaka University Hospital for health check were enrolled in the present study [13]. Patients treated with antihypertensive, antidiabetic, or antihyperlipidemic regimen or patients who met the definition of each disease indicated in the relevant guidelines were defined as having hypertension, diabetes, and hyperlipidemia, respectively. Patients treated with drugs influencing serum adiponectin levels, such as anti-TNF-α [14, 15], insulin [16], thiazolidinediones [17], telmisartan [18], glimepiride [19], and all other biologics were excluded in this study. The study was approved by the Ethical Committee of Osaka University School of Medicine and written informed consent was obtained from each patient.

### Assessment of disease severity and disease activity

The severity of RA was evaluated by the number of joints with erosions among 68 joints of whole body using plain radiographs, as described previously [20]. Joint erosion was defined as changes equal to or more severe than stage II according to the criteria of Steinbocker et al. [21]. Patients were classified according to disease severity as described previously [22]. Briefly, the least erosive subset (LES) group exhibited erosions in less than 20 joints and erosive articular changes limited to the small peripheral joints of hands or feet. The more erosive subset (MES) group had erosions in more than 21 joints and erosive articular changes in large axial joints. The most erosive subset with mutilating disease (MUD) group, that had erosions in more than 46 joints, and almost all joints were extensively damaged in the early period of RA. In this study, we categorized LES patients as the "mild RA group" ($n$=37), and MES/MUD patients as the "severe RA group" ($n$=53). Disease activity score including a 28 joint count/CRP (DAS28-CRP) was evaluated as described previously [23].

### Measurement of serum adiponectin concentrations

Total serum adiponectin level (including all isoforms) was measured with an enzyme-linked immunosorbent assay (ELISA) kit (Otsuka Pharmaceutical, Tokyo, Japan), as reported previously [13].

### Statistical analysis

Data are expressed as mean ± standard deviation (SD). Differences in variables between the mild and severe RA groups were assessed by the Mann–Whitney $U$ test and the chi-square test. Changes in serum adiponectin levels between the first and second assessment was examined by the Wilcoxon's signed rank test. The influence of serum adiponectin level on other variables was investigated by calculating Spearman's correlation coefficients. The correlation between BMI and disease severity was investigated by logistic regression analysis. Conditional multivariate logistic regression models were constructed and odds ratios (ORs) and 95% confidence intervals (95% CI) were cal-

culated to investigate the association of serum adiponectin level on disease severity, with adjustment for BMI. To investigate the cutoff value for serum adiponectin, a value yielding 80% correspondence to the severity of RA was estimated by a logistic regression model and statistical significance was estimated by Fisher's exact test. Probability values of less than 0.05 were considered statistically significant. All statistical analyses were carried out with SAS software version 9.1.3 (SAS Institute, Cary, NC, USA).

## Results

### Clinical and biochemical characteristics of the study subjects

There were no significant differences between mild and severe RA groups in age (60.8±11.0 vs. 61.7±11.7 years), disease duration (15.5±6.9 vs. 17.3±6.8 years), body mass index (22.1±3.4 vs. 20.8±3.0 kg/m$^2$), and prevalence of hypertension (18.8% vs. 28.3%), diabetes (16.2 vs. 18.9%), and hyperlipidemia (21.6 vs. 24.5%). The age and BMI of subjects of the control group were 61.0±11.4 years and 21.9±3.2 kg/m$^2$, respectively. The prevalence of each

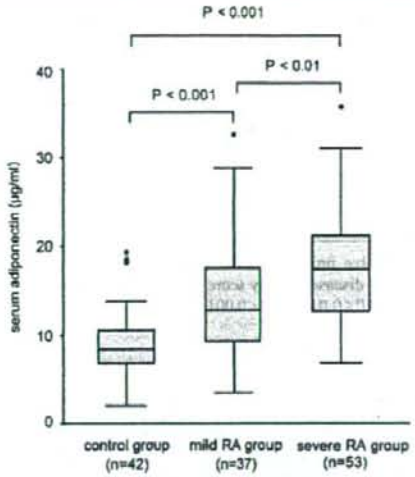


**Fig. 1** Box-and-whisker plots of serum adiponectin levels in the control group, mild RA group, and severe RA group evaluated by the number of joint destruction in 68 joints on plain radiograph. The mean serum level of adiponectin was significantly higher in the severe RA group (17.7±6.7 μg/ml) than in the control (9.1±3.8 μg/ml) or mild RA group (13.9±6.5 μg/ml) (control vs. mild RA group: $P<0.001$, mild RA vs. severe RA group: $P<0.01$, control vs. severe RA group: $P<0.001$)

**Table 1** Baseline demographic, laboratory, and clinical characteristics of the two RA groups

| | mild RA group ($n$=37) | severe RA group ($n$=53) | $P$[a] value |
|---|---|---|---|
| Age, years | 60.8±11.0 | 61.7±11.7 | NS |
| Duration of disease, years | 15.5±6.9 | 17.3±6.8 | NS |
| Body mass index, kg/m$^2$ | 22.1±3.4 | 20.8±3.0 | NS |
| CRP, mg/l | 0.9±1.3 | 1.9±2.0 | 0.003 |
| MMP-3, ng/ml | 146.5±150.9 | 222.8±177.6 | 0.047 |
| IL-6, pg/ml | 14.5±36.0 | 18.1±26.2 | NS |
| RF titer, IU/ml | 158.1±417.0 | 235.7±366.9 | NS |
| RF positivity, % patients | 77.1% | 82.7% | NS[b] |
| BAP, U/l | 24.0±13.5 | 23.9±9.5 | NS |
| iOC, ng/ml | 6.9±3.5 | 6.7±7.3 | NS |
| 1CTP, ng/ml | 4.9±1.9 | 6.5±3.1 | 0.010 |
| uDPD, nmol/mmol creatinine | 6.6±2.2 | 7.9±3.2 | NS |
| DAS28-CRP | 2.2±1.0 | 3.1±1.5 | 0.001 |
| Prednisolone dosage, mg/day | 2.2±2.5 | 4.3±3.6 | 0.001 |
| Methotrexate dosage, mg/week | 4.3±3.2 | 4.4±3.6 | NS |
| Adiponectin, μg/ml | 13.9±6.5 | 17.7±6.7 | 0.008 |

Data are mean ± SD

*RA* rheumatoid arthritis, *NS* not significant, *CRP* C-reactive protein, *MMP-3* matrix metalloproteinase-3, *IL-6* interleukin-6, *RF* rheumatoid factor, *BAP* bone-specific alkaline phosphatase, *iOC* intact osteocalcin, *1CTP* pyridinoline cross-linked carboxyterminal telopeptide of type 1 collagen, *uDPD* urinary deoxypyridinoline, *DAS28-CRP* disease activity score including a 28-joint count/CRP

[a] Except where otherwise indicated, determined by Mann–Whitney *U* test

[b] Except where otherwise indicated, determined by chi-square test

**Table 2** Spearman's correlation analysis of the relation between serum adiponectin and other variables in all RA patients

| Variable | $r$ value | $P$ value |
|---|---|---|
| Age, years | 0.046 | NS |
| Duration of disease, years | 0.068 | NS |
| Body mass index, kg/m$^2$ | −0.269 | 0.011 |
| CRP, mg/liter | 0.078 | NS |
| MMP-3, ng/ml | 0.098 | NS |
| IL-6, pg/ml | 0.120 | NS |
| RF titer, IU/ml | −0.033 | NS |
| BAP, U/l | −0.193 | NS |
| iOC, ng/ml | −0.075 | NS |
| 1CTP, ng/ml | 0.033 | NS |
| uDPD, nmol/mmol creatinine | −0.002 | NS |
| DAS28-CRP | 0.096 | NS |
| Prednisolone dosage, mg/day | 0.040 | NS |

*r value* Spearman's rank correlation coefficient, *NS* not significant, *CRP* C-reactive protein, *MMP-3* matrix metalloproteinase-3, *IL-6* interleukin-6, *RF* rheumatoid factor, *BAP* bone-specific alkaline phosphatase, *iOC* intact osteocalcin, *1CTP* pyridinoline cross-linked carboxyterminal telopeptide of type 1 collagen, *uDPD* urinary deoxypyridinoline, *DAS28-CRP* disease activity score including a 28-joint count/CRP

**Table 3** Results of Spearman's rank correlation analysis of the relation between adiponectin and other variables with a significant difference between the severe and mild RA groups

| | CRP | MMP-3 | 1CTP | DAS28-CRP | Prednisolone | Adiponectin |
|---|---|---|---|---|---|---|
| CRP | | 0.574*** | 0.486*** | 0.717*** | 0.335** | 0.078 |
| MMP-3 | | | 0.331** | 0.532*** | 0.510*** | 0.098 |
| 1CTP | | | | 0.389*** | 0.220* | 0.033 |
| DAS28-CRP | | | | | 0.372** | 0.096 |
| Prednisolone | | | | | | 0.040 |

*CRP* C-reactive protein, *MMP-3* matrix metalloproteinase-3, *1CTP* pyridinoline cross-linked carboxyterminal telopeptide of type 1 collagen, *DAS28-CRP* disease activity score including a 28-joint count/CRP
*$P<0.05$; **$P<0.01$; ***$P<0.001$

disease in the control group was 0% for hypertension, 2.3% for diabetes, and 9.5% for hyperlipidemia. Patients of the severe RA group had a significantly higher serum C-reactive protein (CRP) ($P=0.003$), matrix metalloproteinase (MMP)-3 ($P=0.047$), pyridinoline cross-linked carboxyterminal telopeptide of type 1 collagen (1CTP) ($P=0.010$), disease activity score including a 28-joint count/CRP (DAS28-CRP) ($P=0.001$), and prednisolone dose ($P=0.001$) than the mild RA group (Table 1), reflecting high inflammatory state and bone resorption level in this group as described previously [24]. The mean serum level of adiponectin was significantly higher in the total RA group (16.1±6.8 μg/ml) than in the control group (9.1±3.8 μg/ml) ($P<0.001$). Moreover, the mean serum level of adiponectin was significantly higher in the severe RA group (17.7±6.7 μg/ml) than in the control (9.1±3.8 μg/ml) or mild RA group (13.9±6.5 μg/ml) (control vs. mild RA group, $P<0.001$; mild RA vs. severe RA group, $P<0.01$, control vs. severe RA group, $P<0.001$) (Fig. 1). Univariate analysis of the relationship between serum adiponectin level and other variables showed that adiponectin correlated negatively with BMI ($r=-0.269$, $P=0.011$), but did not correlate with other variables such as inflammatory markers, bone metabolism markers, DAS28-CRP, or the dose of prednisolone (Table 2). Calculation of Spearman's rank correlation coefficients for the variables with a significant difference between the mild and severe RA groups showed that CRP correlated with MMP-3 ($r=0.574$, $P<0.001$), 1CTP ($r=0.486$, $P<0.001$), DAS28-CRP($r=0.717$, $P<0.001$), and dose of prednisolone ($r=0.335$, $P<0.01$), while there was no significant correlation with adiponectin ($r=0.078$, $P>0.05$) (Table 3). In addition, the dose of prednisolone correlated with CRP, MMP-3, 1CTP, and DAS28-CRP, but not with adiponectin ($r=0.040$, $P>0.05$) (Table 3). Multivariate logistic regression analyses revealed that even when the odds ratios were adjusted for BMI, serum adiponectin level significantly correlates with disease severity of RA ($P=0.031$) (Table 4).

## Cutoff point of serum adiponectin for severe RA

Figure 2 shows the histogram of serum adiponectin levels of patients of the mild and severe RA groups. For clinical translation, the cutoff levels were selected. The cutoff value for serum adiponectin level was estimated at 18 μg/ml, yielding 80% correspondence with the severity of RA. Among the patients with serum adiponectin level of ≥18 μg/ml, 81.3% (26/32) belonged to the severe RA group and 18.8% (6/32) belonged to the mild RA group. This cutoff line showed significant correlation with disease severity ($P<0.01$). The specificity of this cutoff value was 53.4% (31/58) (Table 5).

## Changes in serum adiponectin levels and severity of RA during follow-up

To further investigate the time-course changes of serum adiponectin levels and severity of RA, 18 patients underwent a second assessment 2.5 years later (Fig. 3). The mean serum adiponectin level of all patients did not change significantly, although it showed tendency to increase (14.0±5.5 to 15.2±5.2 μg/ml; $P=0.07$). Furthermore, the mean serum adiponectin level did not change significantly within the RA group (10.8±5.8 to 12.4±5.8 μg/ml in the mild RA group, $P=0.122$ and 17.2±2.7 to 18.0±2.4 μg/ml in the severe RA group, $P=0.372$). Assessment of RA severity revealed that none of the mild RA patients progressed to severe RA (data not shown).

**Table 4** Adjusted ORs of serum adiponectin level and BMI for disease severity of RA

| | Adjusted OR | 95% CI | *P* value |
|---|---|---|---|
| Adiponectin, μg/ml | 1.085 | 1.007–1.168 | 0.031 |
| BMI, kg/m$^2$ | 0.907 | 0.785–1.048 | NS |

*ORs* odds ratios, *95% CI* 95% confidence interval, *NS* not significant

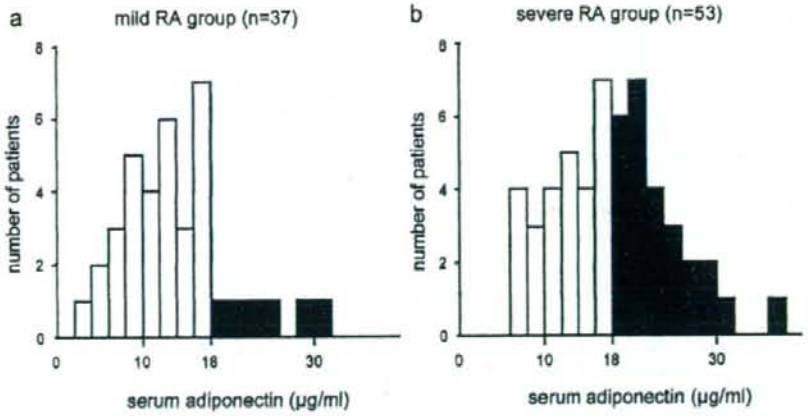


**Fig. 2** Histograms showing the distribution of serum adiponectin levels in mild RA group (**a**) and severe RA group (**b**). Each column covers a serum adiponectin range of 2 μg/ml. When the cutoff value for adiponectin was set at 18 μg/ml, there was 80% correspondence with the severity of RA. Among patients with serum adiponectin levels ≥18 μg/ml, 81.3% (26/32) belonged to the severe RA group and 18.8% (6/32) belonged to the mild RA group

## Discussion

The long-term functional prognosis of RA patients in daily life is mainly determined by the extent of damage in large joints such as the hip, knee, ankle, subtalar, shoulder, and elbow joints, rather than in small joints of the hands or feet. A previous report using Ochi's method demonstrated that MES and MUD groups underwent higher frequency of total knee or hip replacement than LES group (54.7% vs. 0.5%) [25], suggesting that Ochi's method offers some advantages for assessing large joint destruction [20, 25–27]. Therefore, we used Ochi's method to evaluate the severity of RA, to investigate the factors associated with the extent of overall joint destruction, especially in large joints [20]. Evaluation using this method revealed that markers associated with RA activity, such as CRP, MMP-3, 1CTP, and DAS28-CRP were all significantly higher in the severe RA group than in the mild RA group. These results were in agreement with previously published reports evaluated by the modified Sharp/van der Heijde method and Larsen's method (CRP [28], MMP-3 [29], 1CTP [30], and DAS [31]).

We showed for the first time that serum adiponectin levels were higher in the severe RA group than in control and mild RA groups. Interestingly, while other disease severity-related variables, such as MMP-3, 1CTP, DAS28-CRP, and dose of prednisolone correlated with CRP, serum adiponectin levels did not, in both the mild and severe RA groups (Table 3). It has been reported that serum TNF-α and CRP levels are elevated in RA patients [32, 33], and TNF-α, CRP, and corticosteroid markedly inhibit adiponectin gene expression in cultured adipocytes [16, 34]. Furthermore, anti-TNF-α therapy restored serum adiponectin level in RA patients [14, 15, 35]. On the other hand, despite elevated CRP levels and higher dose of treated oral prednisolone (corticosteroid), serum adiponectin levels were elevated in the severe RA group than in the mild

**Table 5** Separation of mild and severe RA using a cutoff value for serum adiponectin of 18 μg/ml

| | Serum adiponectin level | |
|---|---|---|
| | <18 μg/ml | ≥18 μg/ml |
| Mild RA group (n) | 31 | 6 |
| Severe RA group (n) | 27 | 26 |
| % with severe RA | 46.6% | 81.3% |

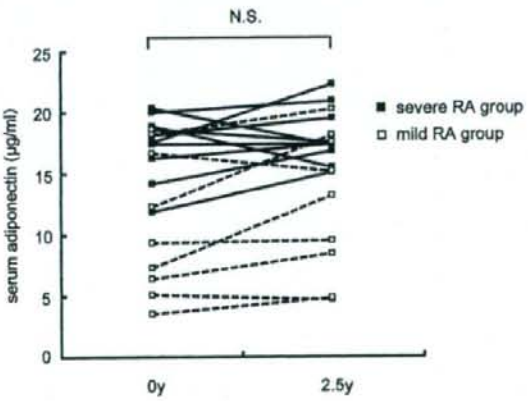


**Fig. 3** Changes in serum adiponectin levels of 18 RA subjects in 2.5-year interval. The mean serum adiponectin level of the whole group was 14.0±5.5 μg/ml at baseline (0 years) and 15.2±5.2 μg/ml at follow-up (2.5 years, $P$=0.07); 10.8±5.8 vs. 12.4±5.8 μg/ml in mild RA group ($P$=0.122) and 17.2±2.7 vs. 18.0±2.4 μg/ml in severe RA group ($P$=0.372) and the change was not significant in either groups. None of the mild RA patients showed worsening to severe RA category during this period

RA group in the present study (Table 3). Considered together, serum adiponectin should be induced by unknown factors associated with the number of destructed joints, especially in large joints, and their effects should exceed the inhibitory effects of TNF-α, CRP, and prednisolone in RA patients. Recently, Fantuzzi [36] suggested that adiponectin promotes survival during periods of catabolism secondary to malnutrition and that hyperadiponectinemia may be the result of response to catabolic state in RA. Consequently, a catabolic state accompanied by joint destruction, especially in large joints, may be one of the strong inducer of serum adiponectin level.

It has been reported that a low BMI is a sensitive and independent predictor of radiographic progression of joint damage assessed by Larsen's method in RA [37, 38]. In this study, there was no significant difference in BMI between control, mild RA, and severe RA group (21.9±3.2 vs. 22.1± 3.4 vs. 20.8±3.0 kg/m$^2$). In addition, in total RA group, BMI showed only tendency of positive correlation with disease severity ($P$=0.059). However, under such condition, serum adiponectin levels were significantly higher in severe RA group than in the control and mild RA groups (Table 1, Fig. 1). Furthermore, multivariate logistic regression analyses revealed that even when the odds ratios were adjusted for BMI, serum adiponectin level significantly correlates with disease severity of RA (Table 4). Therefore, we speculate that serum adiponectin levels can be a better sensitive indicator of the expansion of joint destruction than BMI in RA patients. To further investigate the time course changes in serum adiponectin levels and severity of RA, 18 patients were assessed 2.5 years later. The results showed no significant change in serum adiponectin levels and none of the mild RA patients progressed to severe RA. The lack of change in the severity category during this period is compatible with previous reports indicating that most of the progression of joint damage in RA occurs during the first years of the disease and decrease thereafter [10, 11, 20]; and under such conditions, serum adiponectin level is relatively stable in established RA (disease duration ≥5 years).

For clinical translation of these findings, we determined the cutoff level of serum adiponectin level. The estimated cutoff levels estimated by the histogram of serum adiponectin level showed relatively high sensitivity (81.3%) but low specificity (53.4%) in this study (Fig. 2, Table 5), indicating that increased number of destructed joints may be one of the additive, but not a specific factor of high serum adiponectin level in RA. Consequently, prospective studies in early stage of RA (disease duration <5 years) and in large number of RA patients are needed to determine the cutoff level of adiponectin to be used as an indicator or predictor of destructed joints. In addition, to elucidate the effect of hyperadiponectinemia on the severity of RA, further animal experiments are needed.

Despite the limitation of observational study, we demonstrated that the severity of RA, evaluated by the number of destructed joints on plain radiographs detected in the whole skeleton, correlated with serum adiponectin concentrations. This finding should encourage further research to investigate the role of adiponectin in RA and design new adiponectin-based treatment strategies for RA.

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**Disclosures** None.

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トピックス

# 肥満と関節リウマチの奇妙な関係

*Curious relation between obesity and rheumatoid arthritis*

蛯名 耕介[*1*3] 福原 淳範[*3]
*EBINA Kosuke* *FUKUHARA Atsunori*
下村伊一郎[*4] 橋本 淳[*2]
*SHIMOMURA Iichiro* *HASHIMOTO Jun*

特集 一般医に必要なリウマチ診療の知識

Key words 関節リウマチ 内臓脂肪型肥満 筋肉量減少 BMI(Body Mass Index)低下

関節リウマチ(Rheumatoid Arthritis：RA)では，その経過中にしばしば体重減少等の身体組成の変化が認められることは以前より広く知られていたが，その詳細なメカニズムは不明であった．一方，発症時や経過中の肥満度(Body Mass Index：BMI)の違いが，その後のRA病態の進行や合併症に影響を与えることが近年明らかとなってきた．今回は，このような肥満度(BMI)・身体組成の変化とRA病態の関連について，最新の知見を踏まえて報告する．

## RA発症率と肥満度(BMI)の関係

脂肪細胞は，TNF-αやIL-6などの炎症性サイトカインを産生することが知られている．また，肥満患者では健常者と比較して，血中のCRPなどの炎症マーカーが上昇していることが報告されている．そこで，過去には炎症の観点から肥満がRA発症のリスクの一つであると考えられてきた．しかし，近年のEBM (evidence based medicine)に基づいた調査の結果，肥満度(BMI)がRAの発症率と相関しないことが複数の施設より報告されている[1)2)]．

## RA関節破壊進行と肥満度(BMI)の関係

一方，RAにおける関節破壊進行とBMIの関係について，いくつかの興味深い報告がなされている．われわれは以前に，RA患者の関節破壊進行因子の一つとして低BMIを報告した．また，発症早期のRA患者のうち，BMI高値の患者において関節破壊の進行速度が有意に抑制されていることが報告されている[1)3)4)]．つまり，肥満度(BMI)の高いRA患者は関節が破壊されにくいと言い換えることができる．

このような現象が起きる一つの可能性として，BMIと骨密度との関係があげられる．健常人において，BMIと骨密度が有意に相関することは広く知られている．RA患者においてもBMIの上昇が骨密度の増加と相関し，また関節破壊の重症化と逆相関がすることが報告されている[5)]．こ

*1大阪大学大学院医学系研究科器官制御外科学 *2同 准教授
*3内分泌代謝内科学 *4同 教授

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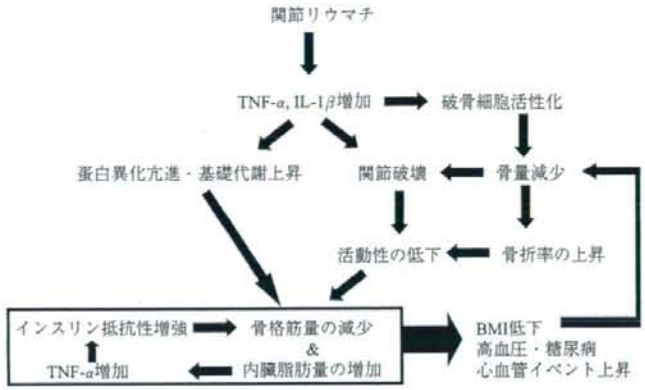


図1 関節リウマチにおける身体組成の変化とその合併症について(文献8より引用改変)

れはBMI高値の患者においては骨への力学的負荷の増加により骨量が増加し，関節破壊の進行が抑制されるためと考えられる．このように，関節破壊抑制の観点からRA患者においてBMIの維持は重要であると考えられる．

## RA発症後のBMI・身体組成の変化

一方，RA患者では発症後に体重やBMIが減少していくことが報告されている[6]．RA発症後の体重減少の特徴として，全身の筋肉と皮下脂肪量の減少と，腹部周囲径の増大(内臓脂肪蓄積を示唆する)が報告されている[7]．骨格筋の減少についてはRA患者の約2/3に，また健常者と比較して約13～14%の減少が認められるとの報告もある[8]．体重およびBMIの減少は，これらの骨格筋と皮下脂肪量の減少と，相反する中心性肥満(内臓脂肪型肥満)の結果と考えられる．

ではなぜRAにおいてこのような身体組成の変化が起きるのであろうか．RA患者における筋肉量減少の原因として，いくつかの可能性が報告されている．RA患者では，血中の炎症性サイトカイン(TNF-αやIL-1β)が上昇していることが知られているが，これらは直接的に筋肉の蛋白合成を阻害し，また蛋白変性を促進させることが報告されている[8]．また，インスリンは筋肉の合成(蛋白同化)に必須で筋肉の変性(蛋白異化)を抑制するため，インスリン抵抗性は筋肉量の減少の原因となると考えられているが，TNF-αはインスリンレセプターシグナルを阻害してインスリン抵抗性を惹起する[8]．実際にRA患者において血中TNF-α濃度とインスリン抵抗性が強く相関することが報告されている[9]．このように，RA患者において血中のTNF-αやIL-1βは，直接・間接的に筋肉量を減少させると考えられる．

内臓脂肪型肥満の原因の一つとしてステロイドの使用が想定されるが，内臓脂肪の増加はステロイドの使用量と相関しないとの報告がある一方，筋肉量の低下による運動量・活動性の低下が脂肪量増加につながる可能性が報告されている[8]．RA患者においては，基礎代謝量が増加しているにも関わらず総エネルギー代謝量の減少が認められ，それは主に活動量の減少が原因との報告がある[8]．以上より，RA患者において炎症性サイトカインによる筋肉量の低下や関節破壊が運動量・活動性の低下を招き，総エネルギー代謝量の減少による内臓脂肪蓄積に関与していると考えられる．

では，このような身体組成の変化はどのような結果をもたらすのであろうか．一般的に筋肉量の減少は筋力低下・身体機能障害・代謝異常などを惹起し，内臓脂肪の増加は心血管疾患・高血圧・糖尿病のリスクを増大させることが知られてい

る[10]．RA 患者は健常者と比較して心筋梗塞の罹患率が2倍以上であり，また約50％が高血圧を，約10～20％が糖尿病を合併するとの報告もある[11]．また，前述のように RA 患者においてしばしば認められるインスリン抵抗性は，糖尿病や動脈硬化の危険因子であることが知られている．RA 患者においてインスリン抵抗性が腹部周囲径(内臓脂肪蓄積の指標)と強い相関を示すことより[11]，筋肉量の減少とそれに伴う内臓脂肪の増加が，これらの代謝異常や合併症の要因となっている可能性が示唆されている．

以上のように RA の発症後さまざまな要因で BMI は低下(内臓脂肪は蓄積)し，関節破壊・代謝異常などの RA の病態に促進的に作用することで，さらに BMI が低下するという悪循環に陥ると考えられる．このような変化は RA 発症初期より認められるため，早期からの適切な治療が必要であると考えられる．ラットのアジュバント関節炎モデルにおいて，抗 TNF-$\alpha$抗体と抗 IL-1$\beta$受容体抗体を投与すると，体重減少と骨格筋減少が抑制されたとの報告がある一方，RA 患者において etanercept による治療が筋肉量を増加させたとの報告もある[12]．

このように RA における身体組成の変化に対しては，①TNF-$\alpha$抗体や IL-1$\beta$受容体抗体などの生物学的製剤の投与，②適切な運動療法とエネルギー摂取，などの有用性が報告されている[8]．生物学的製剤は，炎症性サイトカインを抑制することにより関節破壊を抑制することに加えて，筋肉の蛋白異化を抑制し筋肉量と活動性を維持することで，内臓脂肪蓄積に伴う代謝異常を改善するという両面の効果により，RA 病態を改善すると考えられる．

## まとめ

近年 RA において，BMI を維持すること(高 BMI)が関節破壊の進行速度・心血管イベントによる死亡率・大腿骨頸部骨折の罹患率を低下させるなどの positive な側面をもつことが明らかとなってきた．しかし，RA 発症後は炎症性サイトカインなどの影響で，筋肉量の減少とそれに伴う BMI 低下が促進されることが報告されている．生物学的製剤などによる適切な治療や運動による筋肉量(BMI)の維持が，内臓脂肪型肥満に伴う代謝異常などの合併症の予防，および QOL 向上につながることが示唆されており，今後この分野のさらなる発展が望まれる．


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