B. 研究方法：

1） GR、PXR の周辺分子の同定
Flag タグ付き GR、PXR 発現系を構築し、それを bait として GR、PXR 結合タンパク質を質量分析法によって網羅的に同定する。

2） 同定した周辺分子の機能解析と GR、PXR のエピジェネティック制御機構の解明
周辺分子の機能は、強制発現系を用いたコトランスフェクション、免疫沈降法、ノックダウン法、などによって解析する。アデノウイルス発現系構築、遺伝子改変動物作成などによって個体における役割の解析を行う。

（倫理面での配慮）
動物実験などは法令、施設基準などを遵守して実施した。

C. 研究結果：

1）①GR の周辺分子の同定
Flag-GR、GST-HEXIM1 を用いた解析などから、核内タンパク HEXIM1 が GR にリガンド非依存性に結合することを見いだした（図 1）。HEXIM1 は脳、肺、肝臓、心筋などにおいて高発現していた（図 2）。その際、興味深いことに、組織を問わずほぼすべての細胞内に豊富に存在する non-coding RNA である 7SK RNA を含む複合体と含まない複合体が存在することがわかった。
一方、骨格筋における GR 機能解析の過程で、栄養センサーmTOR が GR 機能を抑制することを見いだした(図 3)。現在、その分子機構を解明すべく、GR-mTOR 相互作用に介在する分子を探索中である。
②PXR 周辺分子の同定
Flag-PXR を用いた解析から、すでに知られている Small heterodimer partner (SHP/NC0B2)、nuclear receptor corepressor 2 (NCoR2/SMRT) 、 steroid receptor coactivators 1 (SRC1/NCOA1) と 2 (SRC2/GRIP1)、nuclear receptor interacting protein 1 (NRIP1/RIP140) 、 peroxisome proliferator-activated receptor-gamma coactivator (PGC-1)、Forkhead transcription factor FKHR (FOXO1)が PXR に結合することを確認した。さらに、それら以外にも多数の PXR 結合タンパク候補を単離しており、現在その同定を試みている。

2）GR 機能のエピジェネティック制御機構の解明
①HEXIM1 による GR 抑制機構として、HEXIM1 発現アデノウイルスを用いた検討などから、直接のタンパク-タンパク相互作用であることを確認した。現在、7SK RNA の関与について検討中である。一方、個体レベルにおける HEXIM1 の役割を明らかにすべく、臓器特異的 HEXIM1 トランスジェニックマウス作出を試みた。現在、心筋特異的に発現するマウスの作成に成功した。外観、心臓は肉眼的に野生型と差はない（図 4）。現在その機能を解析中である。

D. 健康危険情報：
とくになし。

E. 研究発表：

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F. 知的財産権の出願・登録状況：
なし。

G. 添付資料

なし。

図1　グルココルチコイドレセプターGRはHEXIM1と結合する

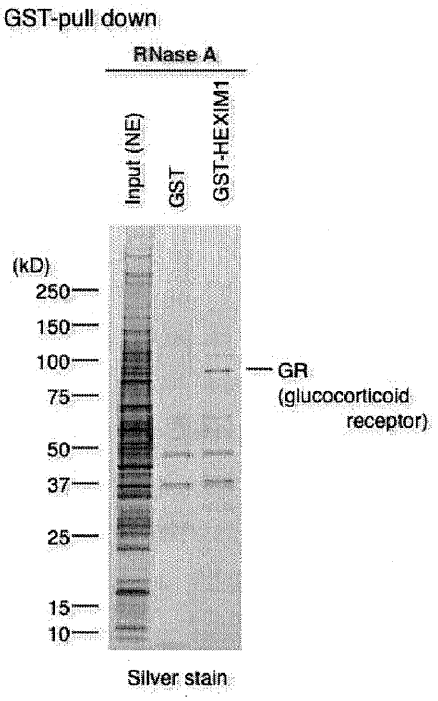


図2　各組織におけるHEXIM1のタンパク発現

図 3　骨格筋におけるグルココルチコイドレセプターGR と mTOR のクロストーク
－mTOR は GR 応答性遺伝子発現を抑制性に制御する

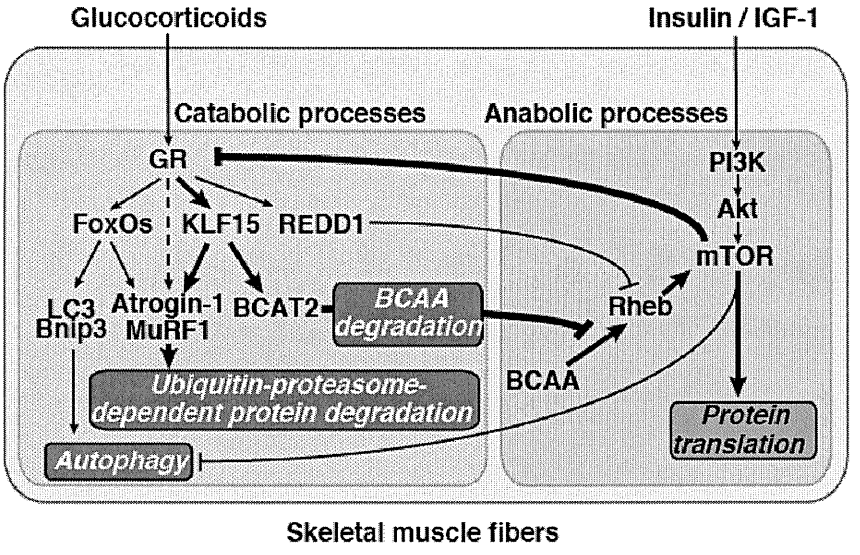
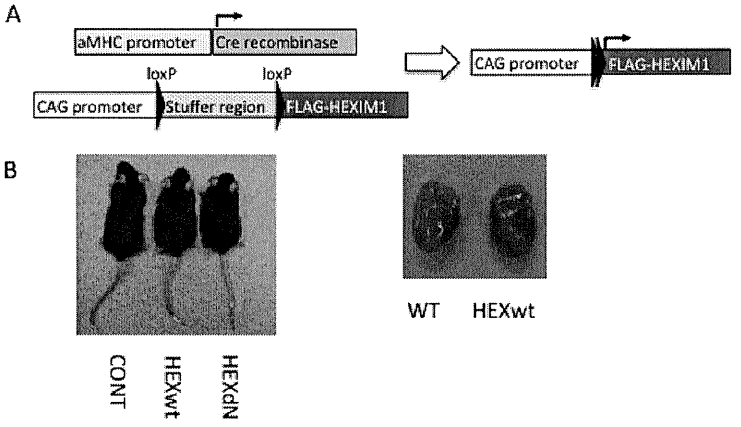


図 4　心筋特異的 HEXIM1 トランスジェニックマウスの作成



Ⅳ. 研究業績

INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Elevated Serum Levels of Resistin, Leptin, and Adiponectin are Associated with C-reactive Protein and also Other Clinical Conditions in Rheumatoid Arthritis

Takumi Yoshino[1], Natsuko Kusunoki[1], Nahoko Tanaka[1], Kaichi Kaneko[1], Yoshie Kusunoki[1], Hirahito Endo[1], Tomoko Hasunuma[1,2] and Shinichi Kawai[1]

Abstract

Objective Body fat is an important source of hormones and cytokines (adipokines) that not only regulate the energy balance, but also regulate the inflammatory and immune responses. This study investigated the association of clinical conditions with serum levels of adipokines in patients with rheumatoid arthritis.

Methods Serum levels of resistin, leptin, and adiponectin were measured by enzyme-linked immunosorbent assay in 141 patients (110 women) who fulfilled the 1987 revised criteria of the American Rheumatism Association for the diagnosis of rheumatoid arthritis and in 146 normal controls (124 women). Then the correlations between adipokine levels and clinical parameters were evaluated.

Results The serum resistin level did not differ between the patients and controls. However, serum leptin levels were significantly higher in male and female rheumatoid arthritis patients than in the corresponding controls, while the serum adiponectin level was significantly higher in female patients than in female controls. Multivariate analysis revealed that predictors of an elevated resistin level were female sex and C-reactive protein (CRP), while the leptin level was related to the body mass index and CRP. Predictors of an elevated adiponectin level were the use of prednisolone and CRP, however, CRP was negatively associated with adiponectin in patients with rheumatoid arthritis.

Conclusion The serum levels of resistin and leptin were positively associated with CRP level in patients with rheumatoid arthritis, suggesting that these adipokines may act as pro-inflammatory cytokines in this disease. The serum adiponectin level was elevated in the patients, however, it was negatively associated with CRP level. In addition, the serum levels of resistin, leptin, and adiponectin were also associated with female sex, BMI and the use of prednisolone, respectively.

Key words: rheumatoid arthritis, resistin, leptin, adiponectin, C-reactive protein

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Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune inflammatory disease that is characterized by symmetrical synovitis, progressive joint damage, pain, fatigue, and disability. Although the exact cause of this disease is still unknown, investigation of its pathogenesis has confirmed a role for various pro-inflammatory cytokines, including tumor necrosis factor-α (TNFα), interleukin-1 (IL-1), and interleukin-6 (IL-6) (1-3). Accordingly, inhibition of these cytokines has become the new therapeutic strategy for RA.

Recent studies have demonstrated that cytokines secreted by adipocytes (adipokines) have an important physiological role. Adipokines, including resistin, leptin, and adiponectin, have been demonstrated to influence eating behavior and the energy balance, and have also been noted as new mediators of the inflammatory process (4, 5). Recently, we reported

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Table 1. Demographic Profile of the Patients with Rheumatoid Arthritis and Control Subjects

	Sex	RA patients (n=141)	Control subjects (n=146)	p value
Male : Female		31:110	22:124	
Age (years)	M	61.0 ± 12.7	45.6 ± 13.8	<0.001
	F	59.0 ± 14.0	57.5 ± 16.6	0.456
Height (cm)	M	166.8 ± 6.1	170.0 ± 6.2	0.069
	F	154.4 ± 6.6	156.0 ± 6.0	0.091
Weight (kg)	M	64.7 ± 11.1	64.5 ± 9.9	0.952
	F	52.9 ± 9.3	52.8 ± 7.0	0.956
BMI (kg/m^2)	M	23.2 ± 3.2	22.3 ± 2.8	0.29
	F	22.2 ± 3.8	22.2 ± 3.0	0.932
Rheumatoid factor positive, %	M	80.6	–	–
	F	89.1	–	–
Duration of RA (years)	M	7.8 ± 8.6	–	–
	F	11.4 ± 8.9	–	–
DAS28-ESR	M	3.4 ± 1.9	–	–
	F	3.8 ± 1.4	–	–
Stage of RA (I:II:III:IV)	M	12:5:6:8	–	–
	F	11:25:16:58	–	–
CRP (mg/L)	M	10.4 ± 10.5	–	–
	F	8.2 ± 14.4	–	–
ESR (mm/h)	M	23.7 ± 22.2	–	–
	F	33.6 ± 24.9		

Data are shown as the mean±SD; M, Male; F, Female; RA, rheumatoid arthritis; BMI, body mass index; DAS, disease activity score; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein.

Table 2. Current Medications in Patients with Rheumatoid Arthritis

Current medications	Male (n=31)			Female (n=110)		
	n	%	dosage	n	%	dosage
Prednisolone (mg/day)	10	32.3	4.4±2.6	52	47.2	5.2±2.1
Methotrexate (mg/week)	17	54.8	8.0±2.6	67	60.9	8.0±2.3
other DMARDs						
Salazosulfapyridine (g/day)	17	54.8	1.0±0.3	41	37.3	1.0±0.3
Bucillamine (mg/day)	11	35.5	132±64	19	17.3	192±42
Biological agents						
Infliximab (mg/kg/2 months)	1	3.2	3	8	7.3	3.7 ± 0.8
Etanercept (mg/week)	2	6.5	50	13	11.8	44 ± 11

Data are shown as the mean±SD; n, number of samples; DMARDs, disease modifying anti-rheumatic drugs

that adiponectin stimulates the production of IL-8 (6) and prostaglandin E_2 (7) by rheumatoid synovial fibroblasts. These findings suggest that adipokines may contribute to synovial inflammation in RA.

In the present study, we measured the serum concentrations of 3 adipokines (resistin, leptin, and adiponectin) in Japanese patients with RA and in normal controls to further investigate the role of these molecules in the pathogenesis of this disease.

Methods

Subjects

One hundred and forty-one patients with RA diagnosed according to 1987 revised criteria of the American Rheumatism Association (8) were enrolled in this study, and 146 healthy persons were also enrolled as controls. The demographic characteristics of the RA patients and the controls are shown separately for males and females in Table 1. Clinical features of the male and female RA patients are also shown in Table 1. The body mass index (BMI) was calculated as [body weight/height^2] (kg/m^2). Demographic characteristics did not differ between the RA group and the control group, except for the mean age of the males. Medications in the RA patients are shown in Table 2.

Disease activity score 28 (DAS28) was calculated with the following equation (9): $DAS28 = 0.56 \times \sqrt{28TJC} + 0.28 \times \sqrt{28SJC} + 0.7 \times \ln ESR + 0.014 \times GH$, where 28TJC and 28SJC are the tender joint count and swollen

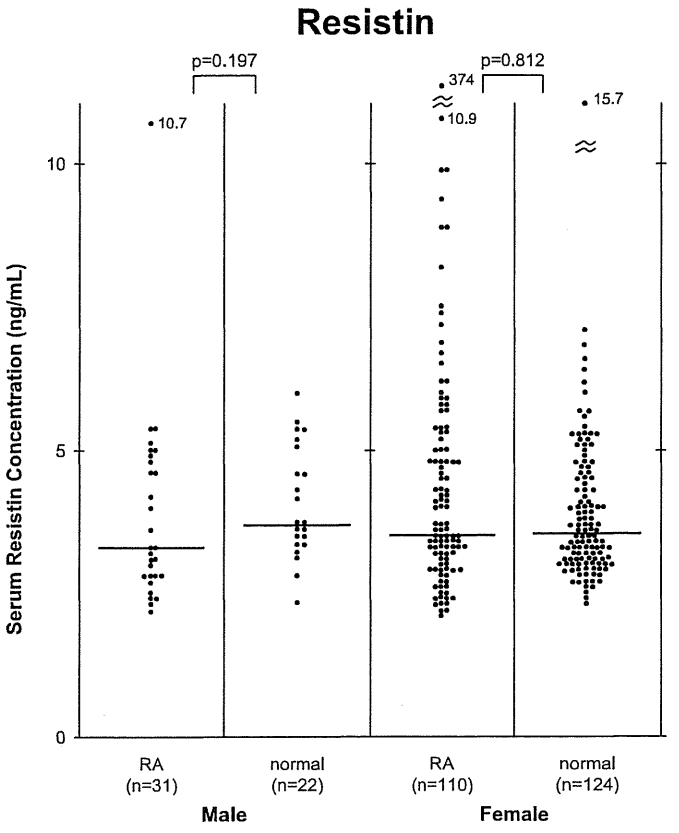

Figure 1. Serum resistin levels in RA patients and control subjects. Horizontal bars indicate median values. Statistical significance was determined by Mann-Whitney test.

joint count from 28 joints and general health (GH) is the patient's global assessment on a 100-mm visual analog scale (VAS).

This study was approved by the Ethical Committees of Toho University and Kitasato University. The RA patients and normal controls were recruited at Toho University Omori Hospital and the Research Center for Clinical Pharmacology of Kitasato University, respectively. Informed consent was obtained from both the patients and the normal controls. In all subjects, a blood sample was collected in the morning after an overnight fast. We did not provide any special dietary management information to the patients or normal controls.

Measurement of adipokines and other laboratory parameters

The serum concentrations of resistin, leptin, and adiponectin were measured by enzyme-linked immunosorbent assay (ELISA). Resistin and leptin ELISA kits were purchased from B-Bridge International, Inc. (Sunnyvale, CA, USA), while the kit for adiponectin was obtained from R&D Systems, Inc. (Minneapolis, MN, USA). Samples were prepared at the appropriate dilutions and paired samples were assayed together according to the instructions of the manufacturers. The intra- and inter-assay coefficients of variation for resistin, leptin, and adiponectin were: <4% and <7%, <8% and <10%, <5% and <7%, respectively. Rheumatoid factor was measured by nephelometry (Mitsubishi Kagaku Iatron, Tokyo, Japan). C-reactive protein (CRP) was also measured by nephelometry according to the manufacturer's specifications (Dade-Behring Inc., Deerfield, IL, USA). The erythrocyte sedimentation rate (ESR) was measured by the Westergren method.

Statistical analysis

Results are expressed as the mean and/or median. Statistical analysis was performed with StatFlex software (ver. 6; ARTEC Co., Ltd., Osaka, Japan). The significance of between-group differences in serum adipokine concentrations was determined by the Mann-Whitney non-parametric test, while differences of background data were evaluated by Student's *t*-test. Simple linear regression analysis was used to assess correlations between serum adipokine levels and patient characteristics, and stepwise forward multiple regression analysis was also performed. Logarithmic transformation was done for highly skewed variables (resistin, leptin, adiponectin, and CRP) when needed in order to satisfy the requirements of multivariate models. In all analyses, $p<0.05$ was considered to indicate statistical significance.

Results

Serum adipokine concentrations

There were no statistically significant differences in serum resistin levels between the RA patients [males: 3.3 (2.8-4.9) ng/mL, females: 3.5 (2.5-5.0) ng/mL] and normal controls [males: 3.7 (3.4-5.0) ng/mL, females: 3.6 (3.1-4.5) ng/mL] (Fig. 1). However, the resistin levels of female RA patients were broadly distributed. Therefore, we compared CRP levels between patients with resistin levels above the 75th percentile (>4.95 ng/mL) and those with resistin levels below the 75th percentile. We found that the CRP level of the former subgroup was significantly higher than that of the latter subgroup (19.1±21.8 mg/L vs. 4.3±7.7 mg/L, $p<0.001$).

The serum concentration of leptin was significantly ($p<0.001$) higher in male RA patients [median 11.2 (interquartile range, 5.1-20.3) ng/mL] than in normal male control subjects [2.7 (1.8-4.3) ng/mL], and serum leptin level was also significantly ($p<0.001$) higher in female RA patients [15.3 (7.3-26.7) ng/mL] than in normal female control subjects [7.4 (3.9-12.0) ng/mL] (Fig. 2). Serum leptin levels were significantly correlated with BMI in all subjects ($p<0.001$), except male RA patients ($p=0.955$), according to linear regression analysis. Since BMI is closely associated with the serum leptin concentration (10, 11), leptin levels were adjusted by BMI. As a result, the leptin/BMI ratios of RA patients [males: 0.51 (0.21-0.95), females: 0.69 (0.35-1.15)] were significantly ($p<0.001$) higher than those of normal control subjects [males: 0.12 (0.10-0.17), females: 0.33 (0.20-0.55)].

Female RA patients had significantly ($p<0.001$) higher serum adiponectin concentrations [10.1 (4.5-26.8) μg/mL] than

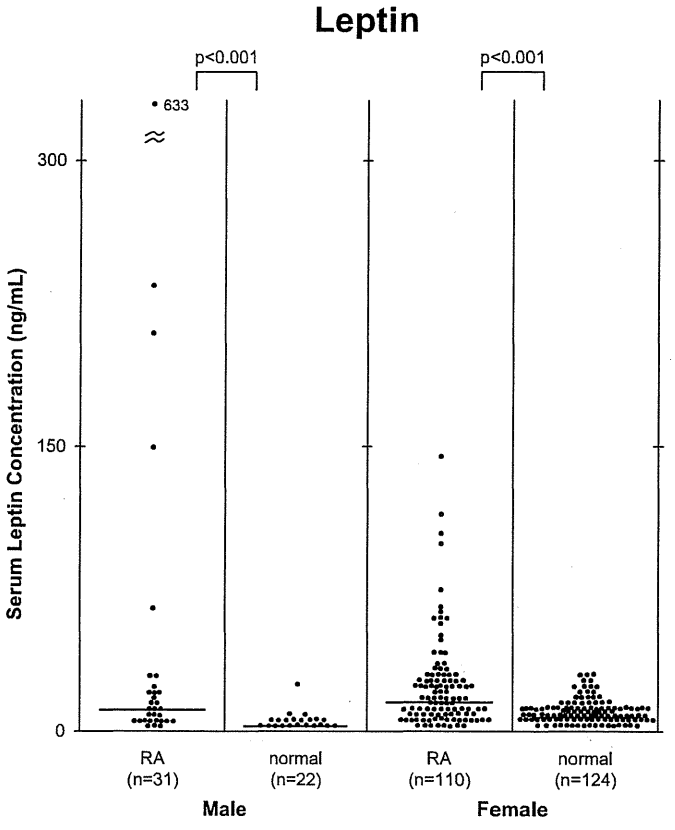

Figure 2. Serum leptin levels in RA patients and control subjects. Horizontal bars indicate median values. Statistical significance was determined by Mann-Whitney test.

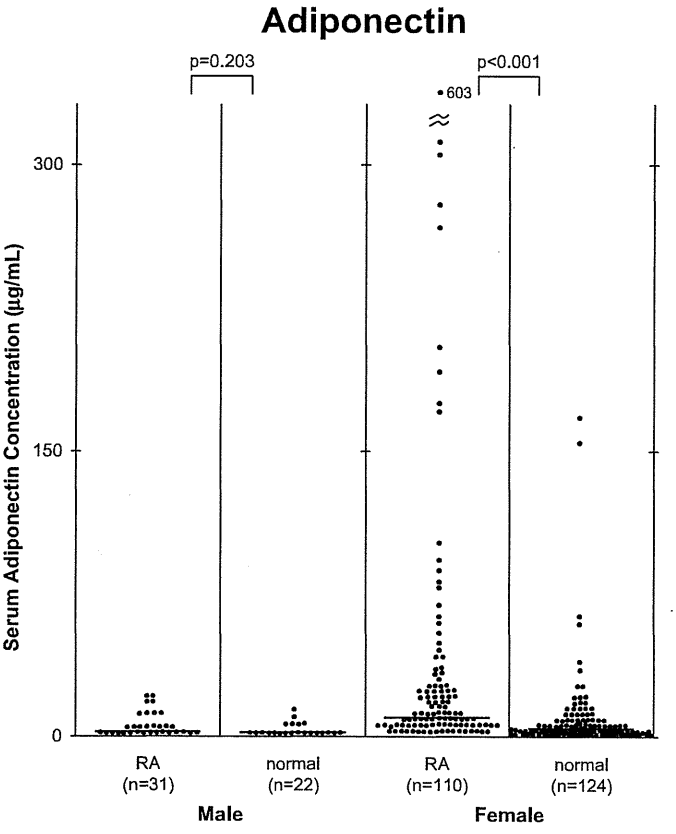

Figure 3. Serum adiponectin levels in RA patients and control subjects. Horizontal bars indicate median values. Statistical significance was determined by Mann-Whitney test.

normal female control subjects [3.6 (2.4-7.4) μg/mL], but no significant difference of adiponectin levels was observed in males (RA males: median 2.6 μg/mL; control males: median 2.3 μg/mL, p=0.203) (Fig. 3).

Correlations between adipokines and patient characteristics

We included various patient characteristics [sex, age, BMI, duration of RA, stage, CRP, ESR, DAS28-ESR, prednisolone, methotrexate, other disease modifying antirheumatic drugs (DMARDs), and biological agents] in a model predicting the serum levels of adipokines (resistin, leptin, and adiponectin) (Table 3-5, respectively).

As shown in Table 3, significant univariate predictors of the serum level of resistin included age, BMI, CRP, ESR, and DAS28-ESR. Inclusion of these univariate predictors in a multivariate model resulted in the final selection of female sex and CRP as significant predictors (Table 3, multivariate model).

Significant univariate predictors of the leptin level included BMI, CRP, and DAS28-ESR (Table 4, univariate model), while multivariate analysis resulted in the final selection of BMI and CRP (Table 4, multivariate model).

For adiponectin, significant univariate predictors included female sex, BMI, RA stage, CRP, and current prednisolone use (Table 5, univariate model). On multivariate analysis, the significant predictors were reduced to CRP and current prednisolone use (Table 5, multivariate model). In addition, a significant positive correlation was found between the serum adiponectin level and the dose of prednisolone in female RA patients by linear regression analysis ($r=0.306$, $p<0.05$). However, we did not find any significant correlation between serum adiponectin levels and the use of methotrexate and/or biological agents.

Discussion

We measured the serum levels of 3 adipokines (resistin, leptin, and adiponectin) in 141 RA patients and 146 normal controls. Most of the previous studies showed the serum levels of several adipokines in only around 50 patients (12-24). They indicated that the serum resistin (12, 13, 25), leptin (14-16, 23, 25) and adiponectin (14, 22-25) levels are higher in RA patients than in healthy controls, while negative results (14, 17-20, 24) were also reported. The present results showed significantly elevated serum levels of leptin and adiponectin, and a trend for an elevated serum resistin level in RA patients. In addition, we found that the serum levels of resistin, leptin and adiponectin in the same samples were all associated with CRP, and they were individually associated with the different clinical conditions of female sex, BMI, and prednisolone use, respectively.

Some previous reports described that the serum levels of these adipokines were associated with dietary supple-

Table 3. Crude and Adjusted Associations of the Serum Resistin Concentration and Patient Characteristics

Characteristic	Resistin*						
	Univariate			Multivariate (complex)		Multivariate (simplified)	
	β	p	R^2	β	p	β	p
Female	0.031	0.352	0.006	0.068	0.061	0.070	0.027
Age	0.004	0.047	0.028	0.002	0.259		
BMI	0.015	0.041	0.030	0.008	0.287		
RA duration	-0.005	0.082	0.022	-0.006	0.077		
Stage	0.029	0.227	0.010	0.022	0.441		
CRP*	0.083	<0.001	0.150	0.075	0.001	0.086	<0.001
ESR	0.004	0.001	0.080	0.001	0.556		
DAS28-ESR	0.043	0.025	0.037	-0.016	0.474		
Prednisolone	0.049	0.080	0.022	0.026	0.369		
Methotrexate	-0.002	0.938	0.000	-0.011	0.699		
Other DMARDs	0.017	0.563	0.002	0.023	0.428		
Biological agents	0.031	0.355	0.006	-0.004	0.911		
R^2				0.163		0.173	

β: regression coefficient; DMARDs: disease modifying anti-rheumatic drugs; Other DMARDs: one or more of sulfasalazine, bucillamine, injectable gold, and/or auranofin; DAS: disease activity score; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; BMI: body mass index; R^2: coefficient of determination.

*Logarithmic transformation was done for highly skewed variables as needed to satisfy the requirements of multivariate models. Significant correlations ($p<0.05$) are underlined.

Table 4. Crude and Adjusted Associations of the Serum Leptin Concentration and Patient Characteristics

Characteristic	Leptin*						
	Univariate			Multivariate (complex)		Multivariate (simplified)	
	β	p	R^2	β	p	β	p
Female	0.037	0.746	0.001	0.150	0.223		
Age	0.008	0.258	0.009	0.004	0.516		
BMI	0.113	<0.001	0.138	0.103	<0.001	0.104	<0.001
RA duration	-0.005	0.621	0.002	-0.003	0.836		
Stage	0.130	0.111	0.018	0.079	0.411		
CRP*	0.216	<0.001	0.087	0.185	0.019	0.187	0.001
ESR	0.004	0.287	0.008	-0.008	0.111		
DAS28-ESR	0.149	0.022	0.038	0.111	0.153		
Prednisolone	0.176	0.064	0.025	0.005	0.959		
Methotrexate	0.067	0.502	0.003	-0.045	0.642		
Other DMARDs	-0.008	0.933	<0.001	0.040	0.678		
Biological agents	0.149	0.186	0.013	0.090	0.500		
R^2				0.162		0.187	

β: regression coefficient; DMARDs: disease modifying anti-rheumatic drugs; Other DMARDs: one or more of sulfasalazine, bucillamine, injectable gold, and/or auranofin; DAS: disease activity score; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; BMI: body mass index; R^2: coefficient of determination.

*Logarithmic transformation was done for highly skewed variables as needed to satisfy the requirements of multivariate models. Significant correlations ($p<0.05$) are underlined.

ments (26-28). The present study did not investigate the relationship between the serum adipokine levels and dietary supplements; no special dietary management was provided for the patients or normal controls.

Tarkowski et al (29) demonstrated that resistin competes with lipopolysaccharide for binding to Toll-like receptor-4 and may act as a pro-inflammatory cytokine in human monocytes. In the present study, we found that the CRP level was higher in the subgroup of high serum resistin levels than in the subgroup of low serum resistin levels. In addition, the CRP level was a significant predictor of the higher serum resistin level according to multivariate analysis. These data suggest that an increased serum level of resistin may contribute to inflammation in RA patients. However, the reason for the gender difference, in which the female sex was associated with high serum resistin levels, is unknown.

Simons et al (30) described that TNFα and IL-1β stimulate leptin production by human preadipocytes. Some reports have described a significant positive correlation between the serum leptin level and the disease activity of RA (14, 15, 21). We also found a significant correlation between the serum leptin level and CRP by multivariate analysis in this study.

Table 5. Crude and Adjusted Associations of the Serum Adiponectin Concentration and Patient Characteristics

Characteristic	Adiponectin*						
	Univariate			Multivariate (complex)		Multivariate (simplified)	
	β	p	R^2	β	p	β	p
Female	0.654	<u>≤0.001</u>	0.152	7.676	0.370		
Age	0.011	0.191	0.012	0.891	0.065		
BMI	-0.068	<u>0.034</u>	0.032	-2.626	0.139		
RA duration	0.025	0.061	0.025	-0.538	0.515		
Stage	0.294	<u>0.003</u>	0.060	3.774	0.572		
CRP*	-0.175	<u>0.024</u>	0.037	-11.144	<u>0.042</u>	-10.453	<u>0.010</u>
ESR	-0.106	0.678	0.001	-0.120	0.728		
DAS28-ESR	0.025	0.755	0.001	4.106	0.448		
Prednisolone	0.283	<u>0.016</u>	0.041	15.485	<u>0.023</u>	17.594	<u>0.005</u>
Methotrexate	0.070	0.572	0.002	-0.428	0.949		
Other DMARDs	-0.061	0.629	0.000	-1.853	0.786		
Biological agents	0.095	0.499	0.003	0.427	0.963		
R^2				0.055		0.090	

β: regression coefficient; DMARDs: disease modifying anti-rheumatic drugs; Other DMARDs: one or more of sulfasalazine, bucillamine, injectable gold, and/or auranofin; DAS: disease activity score; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; BMI: body mass index; R^2: coefficient of determination.

*Logarithmic transformation was done for highly skewed variables as needed to satisfy the requirements of multivariate models. Significant correlations ($p<0.05$) are underlined.

Previous reports have shown that the serum leptin level is positively correlated with BMI, (10, 11) as observed in this study, except for male RA patients. We also found that leptin/BMI ratio of RA patients was significantly higher than that of normal control subjects. Based on these results, the absence of correlation between the serum leptin level and BMI in male RA patients might be explained by the influence of inflammation. Moreover, it was suggested that leptin may act as a pro-inflammatory cytokine in this disease.

Rho et al (25) suggested that leptin was associated with reduced radiographic joint damage as estimated by the Larsen score (31). In the present study, leptin as well as other adipokines were not associated with the Steinbrocker stage of RA. In general, high disease activity in RA patient is correlated with joint damage. The relationship between the serum leptin level and radiographic joint damage should be studied in the future.

The serum adiponectin level was significantly higher in female RA patients than in normal female controls. We also found the same trend in male RA patients, although the difference was not statistically significant. However, the serum CRP level was negatively associated with the adiponectin level in RA patients. Schäffler et al (32) reported that adiponectin was increased in the synovial fluid of RA patients compared with osteoarthritis patients, but they found no statistically significant correlations between adiponectin and ESR or CRP in RA patients. Our previous *in vitro* studies (6, 7) have suggested that adiponectin might be a pro-inflammatory cytokine for rheumatoid synovial fibroblasts. The discrepancies in the adiponectin studies between *in vitro* pro-inflammatory effects and various facets in clinical inflammatory conditions in RA patients remain to be studied.

In the present study, the serum adiponectin level was significantly correlated with current prednisolone use by multiple regression analysis, and was also significantly correlated with the dose of prednisolone by linear regression analysis. Maeda et al (33) reported the reciprocal suppression of adiponectin and TNFα production in adipose tissue. Corticosteroids inhibit the production of pro-inflammatory cytokines such as TNFα (34). Thus, the reduction of TNFα by prednisolone might be the cause of the increased serum adiponectin level in the present RA patients.

Laurberg et al (35) found that the plasma adiponectin level was increased by 13% in RA patients who received methotrexate treatment. Nishida et al (36) reported that serum adiponectin levels showed an increase during infliximab (TNFα inhibitor) therapy in RA patients. However, we did not find significant correlations between serum adiponectin levels and the use of methotrexate and/or biological agents in the present study. The reason for the absence of correlation between serum adiponectin levels and TNFα inhibitor therapy might be explained by the small number of patients receiving TNFα inhibitors, comparing with those receiving prednisolone.

In summary, the serum levels of resistin and leptin were positively associated with CRP level in patients with rheumatoid arthritis, suggesting that these adipokines may act as pro-inflammatory cytokines in this disease. The serum adiponectin level was elevated in the patients, however, it was negatively associated with CRP level. In addition, the serum levels of resistin, leptin, and adiponectin were also associated with female sex, BMI and the use of prednisolone, respectively.

The authors state that they have no Conflict of Interest (COI).

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ORIGINAL ARTICLE

Serum adipokine profiles in Kawasaki disease

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Abstract Adipokines are cytokines derived from adipose tissue. Recently it has been established that adipokines are closely linked to the pathophysiology of not only metabolic diseases, such as diabetes mellitus, obesity, and atherosclerosis, but also to inflammation and immune diseases. In this study we measured serum levels of adipokines in patients with acute Kawasaki disease to investigate the role of adipokines in the pathophysiology of Kawasaki disease. Serum resistin, high-molecular-weight (HMW) adiponectin, leptin, and visfatin levels were measured by enzyme-linked immunosorbent assay in a total of 117 subjects: 56 patients with acute Kawasaki disease, 30 healthy children, and 31 patients with acute infectious diseases. Serum resistin levels in patients with Kawasaki disease were significantly higher than those of healthy children and patients with acute infectious diseases. In contrast, mean serum HMW adiponectin, leptin, and visfatin levels in patients with Kawasaki disease exhibited no statistically significant differences compared with those in healthy children and patients with infectious diseases. Serum resistin levels decreased significantly after administration of intravenous immune globulin. Serum resistin levels on admission were significantly higher in nonresponders compared with responders to intravenous immune globulin therapy. A multivariate model revealed that C-reactive protein was a factor that was significantly related to elevated serum resistin level in patients with Kawasaki disease. In patients with Kawasaki disease, serum resistin levels were elevated, but decreased to nearly normal after intravenous administration of immune globulin. In contrast, serum HMW adiponectin, leptin, and visfatin levels showed no statistically significant changes. These findings suggest that resistin plays an important role, while other adipokines do not play a major role, in the pathogenesis of Kawasaki disease.

Keywords Adipokines · Resistin · C-reactive protein · Kawasaki disease

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Introduction

Kawasaki disease is a systemic vasculitis of childhood that was first reported by Tomisaku Kawasaki in 1967 [1]. Patients manifest with fever, bulbar conjunctival injection, changes of the oropharyngeal mucosa, changes of the peripheral extremities, cervical lymphadenopathy, and polymorphous rash [2]. In Japan there are approximately 10,000 new patients annually [3]. The most important complication of this disease is development of coronary lesions that result in acute myocardial infarction. Intravenous immune globulin is a standard therapy that is effective in about 70% of patients, but the cause of the disease has been unclear.

Adipokines or adipocytokines including resistin, adiponectin, leptin, and visfatin are bioactive molecules that are produced and secreted by adipose tissue [4]. Adipokines have various actions in the human body that regulate metabolic conditions, and may have a central role in regulation of insulin resistance [5, 6].

Resistin is an amino acid peptide that belongs to a cysteine-rich secretory protein family [7]. Circulating resistin levels are elevated in humans by obesity and diabetes [8]. Resistin levels are also associated with increasing coronary artery calcification and are predictive of coronary atherosclerosis [9]. Adiponectin is a 244-amino-acid polypeptide that has three isoforms: low molecular weight, middle molecular weight, and high molecular weight (HMW). Decreased levels of HMW adiponectin are associated with coronary artery disease and type 2 diabetes [10, 11]. Leptin is a protein of 167 amino acids. Circulating leptin levels reflect adipose tissue mass, and hyperleptinemia is associated with obesity and other metabolic diseases [12, 13]. Visfatin is one of the adipokines identified in 2004, being predominantly produced and secreted in visceral fat; its expression level in plasma increases during development of obesity [14].

These adipokines show obviously links to metabolic diseases, however recent studies have also suggested that some adipokines might play a role in inflammation and immune diseases [15]; for instance, we have previously shown that serum levels of resistin, leptin, and adiponectin were all associated with C-reactive protein (CRP) level in patients with rheumatoid arthritis, suggesting that these adipokines may act as proinflammatory cytokines in this disease [16].

The object of this study is to clarify serum levels of resistin, HMW adiponectin, leptin, and visfatin in patients with Kawasaki disease during treatment with intravenous immune globulin, and to evaluate the relationships between serum adipokines and their clinical measures.

Methods

Patients

Fifty-six patients (36 males and 20 females, mean age 29.8 ± 1.7 months) with acute-phase Kawasaki disease who were admitted to our university hospital participated in this study. All patients met American Heart Association diagnostic criteria for Kawasaki disease [2]. These patients were treated with oral aspirin and 1 or 2 g/kg intravenous immune globulin after admission. As controls, we collected serum samples from 30 healthy children and 31 patients with acute infectious diseases (14 patients with pharyngitis, 9 with bronchitis, 5 with gastroenteritis, and 3 with exanthema subitum). The protocol for the study was approved by the Ethics Committee of Toho University Hospital. Informed consent was obtained from the parents of all patients.

Adipokine measurements

Blood samples were collected from the patients with acute-phase Kawasaki disease upon admission (before intravenous immune globulin) and at 24–48 h after intravenous immune globulin treatment. Serum resistin, HMW adiponectin, leptin, and visfatin were measured using enzyme-linked immunosorbent assay (ELISA) kits. Serum resistin and leptin levels were measured in all 56 patients, but HMW adiponectin and visfatin were measured in 38 patients because the volume of serum samples was too small to perform all four analyses. Resistin and leptin ELISA kits were both purchased from B-Bridge International, Inc. (Sunnyvale, CA, USA). ELISA kits for HMW adiponectin and visfatin were obtained from Fujirebio, Inc. (Tokyo, Japan) and Phoenix Pharmaceuticals, Inc. (Burlingame, CA, USA), respectively.

Biochemical measurements

All of the patients with Kawasaki disease were examined for complete blood cell counts and serum chemistry, including CRP and electrolytes, before immune globulin therapy. Latex nephelometry (Sekisui Medical Co., Tokyo, Japan) was used for CRP measurement.

Statistical analysis

Comparisons between the three groups were made using the Kruskal–Wallis test. Serum adipokine levels before and after intravenous immune globulin were compared by the Wilcoxon matched-pairs signed-rank test. Correlations between serum adipokines and laboratory data were analyzed by simple linear regression analysis. Multiple regression analysis was used for studying multivariable models. Statistical significance was determined at $p < 0.05$. Statistical analyses of the data were conducted using the StatMate III software program (ATMS, Tokyo, Japan).

Results

Characteristics of the study population

The characteristics of the 3 groups are shown in Table 1. There were no statistically significant differences in age, gender or body weight among the 3 groups of children. In patients with Kawasaki disease, mean ± SD age was

Table 1 Background characteristics of the three patient groups

	Age (months)	Gender (M/F)	Body weight (kg)
Patients with Kawasaki disease ($n = 56$)	29.8 ± 21.7	36/20	12.1 ± 3.6
Patients with acute infectious diseases ($n = 31$)	29.2 ± 17.5	20/11	12.1 ± 3.5
Healthy children ($n = 30$)	26.9 ± 13.0	19/11	11.7 ± 2.4

Values are mean ± SD

Table 2 Clinical characteristics of the patients with Kawasaki disease

	Age (months)	Days on IVIG	Serum CRP conc. (mg/dl)	WBC counts ($\times 10^3/\mu l$)	Sodium conc. (mEq/l)	IVIG responder*	CAL
Male 36	26 ± 18	4.5 ± 1.8	7.2 ± 5.2	14.2 ± 5.2	131.5 ± 2.8	21 (58.3%)	3 (8.3%)
Female 20	37 ± 25	4.9 ± 1.9	4.7 ± 4.6	7.5 ± 3.1	135.5 ± 2.3	17 (85.0%)	1 (5.0%)

Values are mean ± SD or cases (percentages)

IVIG intravenous immune globulin therapy, *CRP* C-reactive protein, *conc.* concentrations, *WBC* white blood cell, *CAL* coronary arterial lesion

* Patients who had cessation of fever (<37.5°C) after IVIG and needed no additional therapy

29.8 ± 21.7 months. The clinical profiles of patients with Kawasaki disease are presented in Table 2. Thirty-eight patients (67.9%, 21 males, 17 females) responded to intravenous immune globulin infusion. Four patients had coronary lesions detected by echocardiography at discharge, even after immune globulin therapy.

Serum adipokine levels in patients with Kawasaki disease

Serum adipokine levels are shown in Fig. 1. Serum resistin levels were significantly higher in patients with Kawasaki disease (mean 31.5 ± 20.0, median 27.5 ng/ml) compared with healthy controls (mean 5.0 ± 6.8, median 3.3 ng/ml, $p < 0.001$) and patients with acute infectious diseases (mean 10.6 ± 9.2, median 6.9 ng/ml, $p < 0.001$). However, serum HMW adiponectin, leptin, and visfatin levels in patients with Kawasaki disease (HMW adiponectin: mean 10.8 ± 5.1, median 10.1 μg/ml; leptin: mean 2.4 ± 4.0, median 1.6 ng/ml; visfatin: mean 11.1 ±5.5, median 9.5 ng/ml) showed no statistically significant differences compared with those in healthy controls (HMW adiponectin: mean 23.5 ±9.9, median 22.7 μg/ml; leptin: mean 2.0 ±0.7, median 1.9 ng/ml; visfatin: mean 14.9 ±15.7,

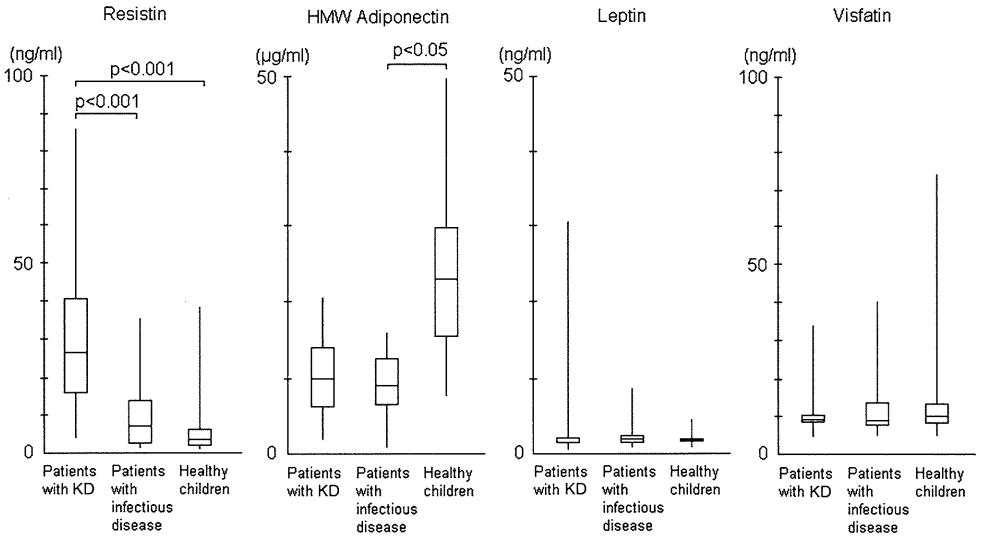

Fig. 1 Serum adipokine levels in the three groups. In the *box plots*, *horizontal lines* indicate median values, and the *lower* and *upper ends* of *boxes* represent the 25th and 75th percentiles. In patients with Kawasaki disease, serum resistin levels were significantly higher than in patients with infectious diseases and in healthy children ($p < 0.001$). Serum high-molecular-weight adiponectin, leptin, and visfatin levels in patients with Kawasaki disease exhibited no statistically significant differences compared with those in healthy controls and patients with acute infectious diseases. *KD* Kawasaki disease, *HMW* high molecular weight

median 11.0 ng/ml) and patients with infectious diseases (HMW adiponectin: mean 9.8 ±4.0, median 8.8 μg/ml; leptin: mean 2.2 ±1.3, median 1.9 ng/ml; visfatin: mean 11.7 ±8.0, median 9.1 ng/ml).

Adipokine levels before and after intravenous immune globulin therapy

Figure 2 shows the changes in serum adipokine levels after intravenous immune globulin treatment. Serum resistin levels decreased significantly after treatment (mean 28.7 ± 18.4 to 9.2 ± 8.3, median 24.5 to 6.7 ng/ml, $p < 0.001$). However, there were no significant changes in serum HMW adiponectin (mean 11.7 ± 5.5 to 11.3 ± 4.8, median 11.9 to 11.1 μg/ml), leptin (mean 3.0 ± 5.9 to 2.2 ± 2.4, median 1.7 to 1.7 ng/ml) or visfatin (mean 11.0 ± 5.3 to 10.9 ± 3.0, median 9.0 to 10.7 ng/ml) levels after intravenous immune globulin.

Comparison of serum adipokine levels in responders and nonresponders to intravenous immune globulin therapy

We compared serum adipokine levels in patients who responded to intravenous immune globulin infusion and in those who did not respond to the treatment, and found that serum resistin levels on admission were significantly ($p < 0.05$) higher in nonresponders (mean 37.2 ± 17.0, median 33.4 ng/ml) compared with responders (mean 28.9 ± 21.0, median 22.9 ng/ml). Serum HMW adiponectin, leptin, and visfatin levels were not significantly different between responders (HMW adiponectin: mean 11.1 ± 9.9, median 10.0 μg/ml; leptin: mean 1.7 ± 0.7, median 1.6 ng/ml; visfatin: mean 11.5 ±6.3, median 9.4 ng/ml) and nonresponders (HMW adiponectin: mean 10.0 ±5.8, median 10.1 μg/ml; leptin: mean 3.7 ± 1.7, median 1.7 ng/ml; visfatin: mean 10.2 ± 2.0, median 10.0 ng/ml).

Correlations between serum resistin levels and clinical data in patients with Kawasaki disease

Since significant elevation of only serum resistin levels was observed in Kawasaki disease, we further analyzed the relationship between serum resistin levels and clinical conditions. Table 3 presents the correlations between serum resistin levels and clinical data considered to be related to disease severity of Kawasaki disease before intravenous immune globulin therapy. Significant univariate predictors of severity included age, CRP, peripheral white blood cell count, and serum sodium level. Simultaneous inclusion of univariate predictors into a multivariate model revealed that high CRP level was a predictor of elevated serum resistin level.

Discussion

In this study, we found that serum resistin levels were elevated in patients with Kawasaki disease compared with in healthy children and in patients with acute infectious diseases. However, there were no significant differences in serum levels of HMW adiponectin, leptin, and visfatin between the various patient groups. Nozue et al. [17] recently reported that serum resistin levels in patients with Kawasaki disease were significantly higher than in healthy controls; however, they did not measure the levels of other adipokines.

Human resistin is produced and released mainly in mononuclear cells (monocytes/macrophages) rather than adipocytes [18, 19]. Mononuclear cells are also important in the pathogenesis of Kawasaki disease, and histopathological

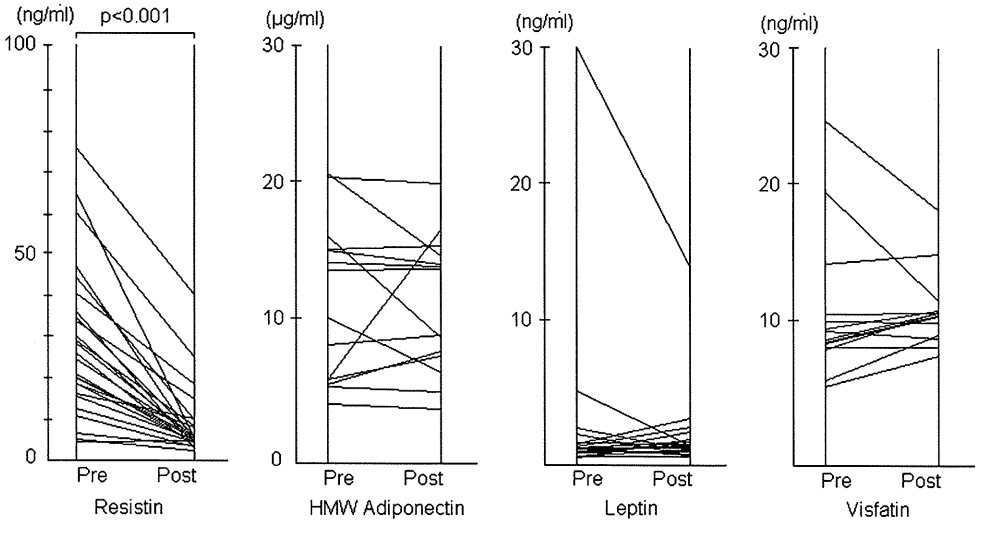

Fig. 2 Adipokine changes after treatment with intravenous immune globulin. Serum resistin levels decreased significantly after administration of intravenous immune globulin ($p < 0.001$). However, serum high-molecular-weight adiponectin, leptin, and visfatin levels did not exhibit any statistically significant changes after intravenous immune globulin treatment. *HMW* high molecular weight

Table 3 Correlations between serum resistin levels and clinical data in patients with Kawasaki disease

Characteristic	Univariate			Multivariate	
	β	p	R^2	β	p
Female	4.887	0.387	0.014	4.013	0.471
Age	0.266	*0.034*	0.081	0.146	0.677
Weight	1.334	0.077	0.057	−0.327	0.871
CRP	1.609	*0.001*	0.205	1.151	*0.022*
WBC	0.001	*0.041*	0.075	0.000	0.373
Sodium	−2.062	*0.019*	0.098	−1.163	0.199
				R^2 0.276	

β regression coefficient, *CRP* C-reactive protein, *WBC* white blood cell

Italics mean significant p values

findings in Kawasaki disease include panvasculitis with infiltration of mononuclear cells [20]. It has been reported that CD14+ monocytes/macrophages play an important role in cytokine production during acute Kawasaki disease [21]. The elevated serum resistin levels in Kawasaki disease shown in our study might have been caused by overproduction by monocytes/macrophages. It was recently shown that resistin competes with lipopolysaccharide for binding to toll-like receptor 4 (TLR4) on peripheral blood mononuclear cells. Torkowski [22] suggested that resistin may partly act as a proinflammatory cytokine via TLR4. It has also been reported that expression of TLR4 is upregulated during the acute phase of Kawasaki disease [23]. These reports and our clinical data of the present study suggest that resistin may be a key cytokine involved in the pathophysiology of Kawasaki disease, possibly as a ligand for TLR4.

After administration of intravenous immune globulin, which is a standard therapy for acute Kawasaki disease, serum resistin levels decreased significantly to nearly normal levels. This suggests that high resistin levels indicate high disease activity. We then examined the correlations between serum resistin level and laboratory parameters considered to be related to disease activity of Kawasaki disease [24, 25].

On simple regression analysis, inflammatory markers (CRP and peripheral white blood cell count) had significant positive correlations with serum resistin levels, while serum sodium levels had a negative correlation with serum resistin levels. Hyponatremia is a common finding in patients with severe Kawasaki disease [26]. Simultaneous inclusion of univariate predictors into a multivariate model resulted in a final parsimonious model with CRP in our study. In contrast, Nozue et al. [17] showed that the only variable significantly associated with resistin concentrations before intravenous immune globulin therapy was body mass index. There were no obvious differences in the background characteristics of patients, including age, gender, weight, and CRP levels on admission, between their study and our present study. We were not able to identify any reasons for the differences in the studies other than the different cohorts of patients.

In our present study, we also compared serum resistin levels in responders and nonresponders to intravenous immune globulin treatment. Serum resistin levels were significantly higher in patients who did not respond to intravenous immune globulin therapy. This result suggests that high serum resistin level may be a predictor of nonresponsiveness to intravenous immune globulin therapy. There were only four patients with coronary arterial lesions, and they had no trend for increased serum resistin levels compared with patients without coronary lesions [serum resistin levels in the four patients with coronary artery lesions: 19.8, 25.8, 29.2, 54.2 ng/ml, patients without coronary lesions ($n = 52$): mean 31.5 ± 20.3, median 28.8 ng/ml].

There has been one previous report dealing with adipokines other than resistin in Kawasaki disease. Takeshita et al. [27] reported that plasma total adiponectin levels in patients with acute Kawasaki disease were significantly lower than in those with convalescent Kawasaki disease or acute febrile disease or in healthy children. In our study, HMW adiponectin in patients with Kawasaki disease had a trend toward being lower than the serum levels in healthy children, but the difference was not statistically significant. Since the HMW fraction of adiponectin is associated more strongly with coronary artery disease than other fractions [10, 11], the trend toward a lower levels might not be highly attributable to the complications of coronary lesions in Kawasaki disease. This may be because of the differences in the pathogenesis of coronary lesions. Coronary lesions in Kawasaki disease are generally panarteritis with acute inflammatory cell infiltrations, in contrast to the progressive atherosclerotic changes associated with adult coronary lesions [28].

We have previously suggested that serum adiponectin level is elevated in adult patients with rheumatoid arthritis [16]. It was also shown that adiponectin stimulates the production of interleukin-8 [29] and prostaglandin E_2 [30] by rheumatoid synovial fibroblasts, suggesting that adiponectin might act as a proinflammatory cytokine in rheumatoid inflammation. Adiponectin is secreted by not only adipocytes, but also in synovial fibroblasts in patients with rheumatoid arthritis [31]. Therefore, the differences in serum adiponectin levels between patients with Kawasaki disease and rheumatoid arthritis may be related to differences in the major affected organs or cells. The different adiponectin levels between Kawasaki disease and rheumatoid arthritis may also be related to their acute and chronic inflammatory condition, respectively.

There have been no previous reports about leptin and visfatin in Kawasaki disease. We found that serum levels of these adipokines in patients with Kawasaki disease exhibited no statistically significant differences compared with in healthy children and patients with acute infectious diseases. In our previous study, significant elevation in serum leptin level was observed in patients with rheumatoid arthritis, and a significant correlation between serum leptin and CRP levels was shown by multivariate analysis in these patients [16]. It was reported that serum levels of visfatin are higher in patients with rheumatoid arthritis, but not in patients with systemic lupus erythematosus and systemic sclerosis [32]. The differences in serum levels of these adipokines in different inflammatory diseases remain to be studied.

In conclusion, we demonstrate herein that serum resistin levels were elevated in patients during the acute phase of Kawasaki disease and decreased to nearly normal after intravenous immune globulin treatment. In contrast, serum HMW adiponectin, leptin, and visfatin levels showed no significant changes. Although further investigations are needed to better understand the detailed roles of adipokines in Kawasaki disease, our data suggest that, among these four adipokines, only resistin participates in the pathogenesis of this disease.

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Conflict of interest None.

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Special Issue "Lipid mediator and Inflammation"

Mini Review

Potential roles of microsomal prostaglandin E synthase-1 in rheumatoid arthritis

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Rheumatoid arthritis (RA) is a chronic autoimmune disease which primarily affects the synovial joints leading to inflammation, pain and joint deformities. Nonsteroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids, both of which inhibit cyclooxygenase (COX), have been extensively used for treating RA patients. Prostaglandin E synthase (PGES) is a specific biosynthetic enzyme that acts downstream of COX and converts prostaglandin (PG) H_2 to PGE_2. Among PGES isozymes, microsomal PGES-1 (mPGES-1) has been shown to be induced in a variety of cells and tissues under inflammatory conditions. The induction of mPGES-1 in the synovial tissue of RA patients is closely associated with the activation of the tissue by proinflammatory cytokines. Although selective mPGES-1 inhibitors have not yet been widely available, mice lacking mPGES-1 (mPGES-$1^{-/-}$ mice) have been generated to evaluate the physiological and pathological roles of mPGES-1 in vivo. Recent studies utilizing mPGES-$1^{-/-}$ mice have demonstrated the significance of mPGES-1 in the process of chronic inflammation and evocation of humoral immune response in autoimmune arthritis models. These recent findings highlight mPGES-1 as a novel therapeutic target for the treatment of autoimmune inflammatory diseases, including RA. Currently, both natural and synthetic chemicals are being tested for inhibition of mPGES-1 activity to produce PGE_2. The present review focuses on the recent advances in understanding the role of mPGES-1 in the pathophysiology of RA.

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Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune inflammatory disease characterized by synovial inflammation, excessive synovial proliferation and progressive joint destruction. However, the etiological factors underlying RA are not yet fully understood. During active RA, inflammatory cells such as monocytes/macrophages, mast cells, T cells and B cells infiltrate the synovial joints. The immune cells interact in a complex and intricate manner leading to the release of pro-inflammatory mediators [1]. High levels of proinflammatory cytokines including tumor necrosis factor-α (TNFα), interleukin (IL) -1 and IL-17 are detectable in the joint fluids and synovium of RA patients. These proinflammatory mediators have been demonstrated to play a vital role in the initiation and development of RA [2, 3]. In addition, these cytokines are closely associated with the production of biologically active lipid mediators, including prostaglandin (PG) E_2.

PGE_2 is highly detectable in the synovial fluid of patients with RA [4]. Proinflammatory cytokine-activated cells, including synovial cells, chondrocytes, macrophages and monocytes, are the primary sources of PGE_2 in the inflamed joints of RA patients. Biosynthesis of the PGE_2 is dependent on sequential enzymatic processes beginning with the release of arachidonic acid from membrane phospholipids by phospholipase. The arachidonic acid derived from the first step is further metabolized to PGH_2 by cyclooxygenase (COX), which consists of two major isozymes, COX-1 and COX-2, as shown in Fig. 1. Under basal conditions, COX-1 is constitutively expressed in most tissues, while COX-2 is significantly upregulated in specific types of cells and tissues, including the synovial tissue. Since PGE_2 production coincides with the up-regulation of COX-2 expression in cells of inflamed joint tissues [5], COX-2 has been targeted for the treatment of RA by using nonsteroidal anti-inflammatory drugs (NSAIDs) including selective COX-2 inhibitors [6].

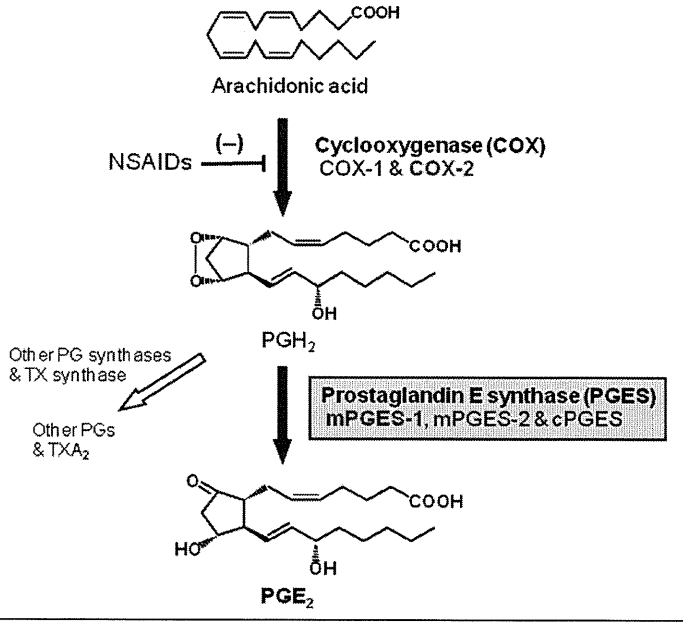

Fig. 1 Pathway of prostaglandin E_2 biosynthesis

Arachidonic acid is converted to the unstable metabolite, prostaglandin (PG) H_2 by two isozymes of cyclooxygenase (COX), constitutive COX-1 and inducible COX-2. PGH_2 is then converted to PGE_2 by prostaglandin E synthase (PGES). There are at least 3 isozymes of PGES, including microsomal PGES (mPGES) -1, mPGES-2 and cytosolic PGES (cPGES). PGH_2 is also metabolized to other PGs by each specific PG terminal synthases (PGDS for PGD_2, PGFS for $PGF_2\alpha$ and PGIS for PGI_2) and to thromboxane (TX) A_2 by TXS. Nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit PGs and TXA_2 production by inhibiting COXs.