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<td>タイトル</td>
<td>ステロイドの変動は血液および骨髄液のアドポイキン濃度に影響を与える。</td>
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<td>見出し</td>
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<tr>
<td>発行日</td>
<td>2017-02-01</td>
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<tr>
<td>URL</td>
<td><a href="http://hdl.handle.net/10069/37570">http://hdl.handle.net/10069/37570</a></td>
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<td>©</td>
<td>2016 Biomedical Research Press</td>
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Steroid changes adipokine concentration in the blood and bone marrow fluid

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(Received 8 April 2016; and accepted 26 April 2016)

ABSTRACT
Our previous study has shown that plasminogen activator inhibitor 1 (PAI-1) gene expression and secretion from bone marrow adipocytes increased markedly with dexamethasone administration. The purpose of the present study was to measure the secretion of various adipokines from human bone marrow and blood, and investigate how adipokine secretion changes in a steroid environment. Human blood and bone marrow fluid were collected from a steroid treatment group and a control group during hip replacement surgery, and an enzyme-linked immunosorbent assay (ELISA) was used to measure the adiponectin, leptin, and PAI-1 levels. Adiponectin and leptin showed no significant differences between bone marrow and blood levels, but PAI-1 was significantly higher in bone marrow. The steroid treatment group had higher levels of leptin and PAI-1 in both the blood and bone marrow than the control group. PAI-1 was present at high concentrations in the bone marrow and increased by steroid treatment. High levels of PAI-1 in bone marrow may influence intraosseous hemodynamics and may induce necrotic bone disorders.

Osteonecrosis of the femoral head (ONFH) is a common complication caused by high-dose administration of glucocorticoids. Glucocorticoid hormone has diverse activities in multiple organs throughout the body, and hypercortisolism causes various disorders, including ONFH. Several studies have shown that micro-vascular thrombosis and subsequent local blood flow are principle features in the development of glucocorticoid-induced osteonecrosis (4). Other reports presented that vascular endothelial cell disturbance due to various oxidative stresses or abnormal lipid metabolism was important cause of ONFH (12, 27).

Recent reports have shown that visceral adipocytes secrete various physiologically active substances known as adipokines. In the bone marrow space, there is a large quantity of mature adipocytes that are possible candidates for adipokine secretion. Considering the enclosed nature of the bone marrow space, intramedullary adipocytes may be involved in bone metabolism, as we previously reported that bone marrow adipocytes express receptor activator of nuclear factor kappa-B ligand (RANKL) and support osteoclast differentiation (5, 8).

Plasminogen activator inhibitor-1 (PAI-1), one of the adipokines secreted by adipocytes (1), suppresses fibrinolysis by binding tissue-type plasminogen activator (t-PA), and a relationship between PAI-1 and thrombosis or hypercoagulation has been suggested. Furthermore, blood coagulation in the femoral head is thought to be associated with osteonecrosis. There are several reports regarding increased PAI-1 secretion in serum of patients with ONFH (11, 16, 25, 26). We also previously reported dexamethasone-induced PAI-1 secretion from human bone marrow adipocytes (9). Its secretion with steroid administration was increased about 2.5-fold for 24 h, but inhibited by simvastatin, which is a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase
inhibitors (21). These results imply the possibility that PAI-1 that has been increased by steroids affects intraosseous hemodynamics and is involved in the development of ONFH.

The purpose of the present study was to examine and compare the adipokine concentration in blood and bone marrow fluid from patients with or without steroid treatment.

MATERIAL AND METHODS

The subjects were 35 patients who underwent hip arthroplasty, consisting of 15 cases receiving steroid administration from among patients with idiopathic femoral head necrosis (S group), and 20 cases with hip osteoarthritis without steroid treatment as a control group (C-group). Patients with rheumatoid arthritis or abnormal lipid metabolism, or who were positive for hepatitis B or C virus, or had a C-reactive protein (CRP) of 2.1 mg/dL or higher were excluded from this study. The S-group had 3 men and 12 women, with a mean age of 57 (29 to 80) years, body mass index (BMI) of 22.9 (16.7 to 29.1) kg/m², and mean steroid dosage of 10.5 (2.5 to 22.5) mg prednisolone equivalent; all cases had idiopathic femoral head necrosis. The C-group had 4 men and 16 women, with a mean age of 63 (43 to 76) years and BMI of 23.5 (18.2 to 28.5) kg/m²; all cases had hip osteoarthritis.

Venous blood and bone marrow fluid were collected at the same time during the surgery, which was performed at a constant time of day; 1.8 mL of blood or bone marrow fluid were then mixed into 0.2 mL of 3.2% sodium citrate. Each specimen was promptly plasma-separated by centrifugation at 4°C (3000 rpm, 15 min) and stored frozen at −80°C. The specimens were thawed when it was time for testing, and an enzyme-linked immunosorbent assay (ELISA) was used to measure the secretion levels of various adipokines (PAI-1, adiponectin, and leptin). PAI-1 was measured using a t-PA/PAI-1 complex (test method: ELIA; kit reagent: LPIA/tPAI test; measurement equipment: ELISA F300, Sysmex Corp., Kobe, Japan) and total PAI-1 (test method: latex agglutination method; kit reagent: LPIA-tPAI test; measurement equipment: STACIA, Mitsubishi Chemical Medience Co., Ltd. Tokyo, Japan). Adiponectin (test method: turbidimetric latex agglutination; kit reagent: human adiponectin latex; measurement equipment: JEOL BM-9030, Mitsubishi Chemical Medience Co., Ltd.) and leptin (test method: double antibody assay; kit reagent: Human Leptin RIA kit; measurement equipment: γ-counter AccuFLEX γ-7010, LINCO RESEARCH, St Charles, MO) were also measured by ELISA. There were 13 cases in the S-group and 19 cases in the C-group where only the t-PA/PAI-1 complex was studied, due to discontinuation of sales of the ELISA kit in the middle of the experiment. Considering the possibility that PAI-1 is affected by inflammation, only CRP-negative cases (< 0.17 mg/dL) in the C (13 cases) and S (6 cases) groups were investigated and compared.

The Wilcoxon signed-rank test was used for comparisons between blood and bone marrow fluid, and the Mann-Whitney U test was used for comparisons between the C- and S-groups. A P-value <0.05 was considered significant.

RESULTS

Adiponectin levels in the C group were 9.3 (2.8–17.3) μg/mL for blood and 8.9 (2.6–19.1) μg/mL for bone marrow fluid, and in the S-group, 9.3 (3.1–22.8) μg/mL for blood and 8.4 (3.0–22.8) μg/mL for bone marrow; no significant differences were found between the blood and bone marrow and between the C- and S-groups (Fig. 1).

Leptin levels were 5.6 (1.1–25.9) ng/mL for blood and 4.8 (1.4–20.8) ng/mL for bone marrow fluid in the C group, and 13.2 (3.4–40.5) ng/mL for blood and 11.2 (4.2–32.8) ng/mL for bone marrow fluid in the S group. The leptin level of the S-group was significantly higher, by two-fold or more, in both blood and bone marrow (Fig. 2). A significant difference was not found between blood and bone marrow in either the C-group or the S-group.

PAI-1 (measured with t-PA/PAI-1 complex) was 12.2 (2.0–23.6) ng/mL for blood and 43.8 (24.6–91.5) ng/mL for bone marrow fluid in the C group, and 21.4 (7.1–45.4) ng/mL for blood and 67.8 (23.3–100) ng/mL for bone marrow in the S group. PAI-1 levels in the bone marrow fluid were markedly higher, by about three-fold, compared to levels in the blood for both the C-group and the S-group. Comparing the C- and S-groups, PAI-1 levels were significantly higher, by about three-fold, compared to levels in the blood for both the C-group and the S-group. Comparing the C- and S-groups, PAI-1 levels were significantly higher in the S-group, with the PAI-1 in the bone marrow fluid of the S-group were the highest (Fig. 3a).

Total PAI-1 in the C-group was 17.4 (10–50) ng/mL for blood and 81.7 (46–118) ng/mL for bone marrow, while it was 35.1 (10–79) ng/mL for blood and 90.8 (42–173) ng/mL for bone marrow in the S group. Similar to the t-PA/PAI-1 complex, the total PAI-1 levels in the bone marrow fluid were markedly higher, by about four-fold, than levels in the blood. Furthermore, PAI-1 levels in the bone mar-
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Adiponectin

![Adiponectin levels in the blood and bone marrow fluid. Significant differences in adiponectin levels are not observed in the blood and bone marrow fluid in the C- and S-groups.](image1)

In the present study, neither adiponectin nor leptin was different between blood and bone marrow, and we infer that there is no major difference between the environments in the blood and in the bone. However, leptin did show clearly higher levels in the S-group, in both blood and bone marrow fluid, implying the possibility that steroids are responsible for some form of systemic effect. In the periphery, leptin acts to promote the production of reactive oxygen species and has a neovascularization effect and an arteriosclerosis promotion effect (2). It has been demonstrated that leptin levels in the blood and vitreous body are elevated in diabetic retinopathy which is one of the representative microangiopathy (24). Though the present study does not represent especially high leptin levels, the possibility cannot be ruled out that, in the bone marrow, which is an enclosed environment similar to the vitreous body, steroid-induced effects and various intercellular interactions are involved in the micro-arterial dynamics. The mechanism of its action will need to be further investigated.

PAI-1 levels, comparing blood and bone marrow fluid, were markedly higher in the bone marrow fluid as compared to other adipokines for both tPA/PAI-1 complex and total PAI-1; they were also higher in the S-group than in the C-group. In obese rats, PAI-

**DISCUSSION**

In recent years, there have been various reports on the physiological effects of various adipokines, such as adiponectin, leptin, and PAI-1. Adiponectin is a secretory protein that is specifically expressed in adipocytes; it inhibits the occurrence of type 2 diabetes and arteriosclerosis (28), and it has been shown to be associated with metabolic syndrome (14, 20). In bone metabolism, adiponectin has been shown to inhibit the differentiation of osteoclasts and promote the differentiation of osteoblasts (18). However, adiponectin also has a negative effect on bone mass, and high levels of adiponectin increase the risk of bone fracture; adipocyte differentiation is enhanced by steroids, and some reports suggest an association between steroids and osteoporotic fractures. Leptin is a typical appetite-regulating hormone that is secreted by adipocytes, and it exhibits an appetite-suppressing effect and energy consumption-increasing effect (6). In bone metabolism, similar to adiponectin, it is thought to promote calcification and growth differentiation by acting on osteoblasts (19). PAI-1 is thought to be associated with thrombosis and cardiovascular disease (7), and as regards its effects on bone, it is a risk factor for ONFH (3); it has been reported that, when glucocorticoid levels are higher, PAI-1 increases and becomes a cause of bone necrosis (12).

In the present study, neither adiponectin nor leptin was different between blood and bone marrow, and we infer that there is no major difference between the environments in the blood and in the bone. However, leptin did show clearly higher levels in the S-group, in both blood and bone marrow fluid, implying the possibility that steroids are responsible for some form of systemic effect. In the periphery, leptin acts to promote the production of reactive oxygen species and has a neovascularization effect and an arteriosclerosis promotion effect (2). It has been demonstrated that leptin levels in the blood and vitreous body are elevated in diabetic retinopathy which is one of the representative microangiopathy (24). Though the present study does not represent especially high leptin levels, the possibility cannot be ruled out that, in the bone marrow, which is an enclosed environment similar to the vitreous body, steroid-induced effects and various intercellular interactions are involved in the micro-arterial dynamics. The mechanism of its action will need to be further investigated.

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![Leptin levels in the blood and bone marrow fluid. A significant difference is not observed in leptin levels in the blood and bone marrow fluid for either the C- or S-group. Leptin levels are higher in the S-group than in the C-group for both the blood and the bone marrow fluid.](image2)
Fig. 3  PAI-1 levels in the blood and bone marrow fluid. a) tPA/PAI-1 complex levels. tPA/PAI-1 complex levels are higher in bone marrow fluid than in blood for both the C- and S-groups, which both show significant differences. t-PA/PAI-1 complex levels are higher for the S-group in both the blood and bone marrow fluid, and values are highest for the S-group bone marrow fluid. b) Total PAI-1 levels. Total PAI-1 levels in bone marrow fluid are higher than blood for the C-group and S-group. Though comparison of bone marrow fluid between the C- and S-groups does not show a significant difference, the highest values are those for bone marrow fluid in the S-group.

Fig. 4  PAI-1 levels in blood and bone marrow fluid in the CRP-negative group. a) tPA/PAI-1 complex levels; b) Total PAI-1 levels. In the CRP-negative group, tPA/PAI-1 complex levels and total PAI-1 levels both show significant differences in comparisons between blood and bone marrow fluid in the C-group and the S-group. Comparison between the C- and S-groups does not reveal a significant difference, but the highest values are exhibited by the S-group bone marrow fluid.
1 levels in the blood are reportedly more reflective of visceral fat accumulation than subcutaneous fat (22), but the results of the present study demonstrated that PAI-1 levels were markedly higher in bone marrow than blood, suggesting that PAI-1 from bone marrow adipocytes has a major effect on bone metabolism. It has been reported that glucocorticoid increases the size of bone marrow adipocytes (13), induces a high level of intraosseous pressure and capillary compression (29), and leads to the development of ONFH (17). Considering the enclosed nature of the bone marrow space, intramedullary adipocytes may be involved in bone metabolism. A high level of PAI-1 in bone marrow, which is mainly secreted from bone marrow adipocytes, is closely associated with the onset of ONFH. The results of the present study are consistent with this hypothesis.

Ibrahim et al. investigated active PAI-1 levels after irradiation in the bone marrow and plasma of mice, and they showed that the bone marrow had a markedly higher rate of increased PAI-1 than the plasma (10); this also supports our hypothesis.

The limitations of the present study include the differences of sex, BMI, diurnal variation, and inflammation values because of the small sample size. However, large differences were not observed between specimens due to sex or BMI. Diurnal variation was reduced as much as possible by collecting blood and bone marrow fluid at substantially the same time in all cases.

Inflammatory diseases are also known to affect PAI-1 levels, and the involvement of inflammation was excluded as much as possible by excluding patients with rheumatoid arthritis, those who were positive for hepatitis B or C virus, those with a CRP of 2.1 mg/dL or higher, and those with bone marrow edema on preoperative MRI. Furthermore, PAI-1 levels in the bone marrow were highest in the S-group in a comparison of only the CRP-negative groups (<0.17 mg/dL) (Fig. 4a, b). Therefore, though inflammation is involved in elevated PAI-1 levels, we believe that this does not rule out our hypothesis that PAI-1 is susceptible to the effects of steroids in bone marrow, which is an enclosed space.

In terms of technical issues regarding the collection of bone marrow fluid, blood may have been mixed into the bone marrow fluid due to bleeding from other tissue. However, if blood had diluted the bone marrow fluid concentration, pure PAI-1 levels in bone marrow should be higher. PAI-1 is also secreted from vascular endothelial cells and other sources (15, 23), not only from bone marrow adipocytes. However, considering the presence of fat in the bone marrow and several previous studies, the primary origin of PAI-1 is considered to be from bone marrow adipocytes.

In conclusion, levels of various adipokines were compared between human bone marrow and blood. In humans, adiponectin and leptin had equivalent levels in the blood and bone marrow, but PAI-1 levels were significantly higher in the bone marrow. We think that an increase of PAI-1 levels caused by steroids is an important factor that is associated with the development of ONFH.

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