レプチンの臨床的意義を考える上でも興味深いデータであり、今後その他の摂食関連蛋白を測定しその意義を解明したい。

結論

AN 患者の体重と体組成変化を経時的に測定した。BMI が極度に低値の際は脂肪重量の回復に加え、除脂肪体重の増加が優先される。脂肪の増加量と血清レプチン濃度は相関しない。

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＊健康危険情報

なし

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知的財産権の出願・登録状況

なし

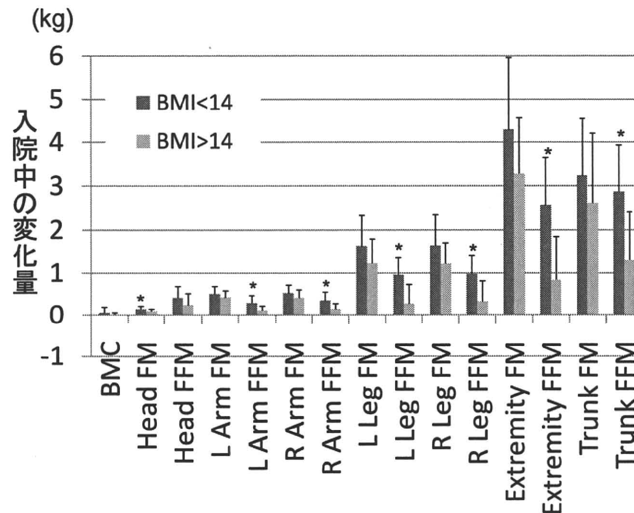
表－1 患者基本データ

	罹病期間 (year)	病型 (AN-R/ AN-BP)	年齢 (year)	入院期間 (day)	入院時BMI (kg/m²)	退院時BMI (kg/m²)	体重増加量 (kg)
BMI＜14 (kg/m²) N=17	2.2 ±3.3	11/6*	21.9 ±9.2*	242 ±71*	12.4 ±2.1*	17.9 ±1.4*	13.6 ±4.3*
BMI≧14 (kg/m²) N=16	3.1 ±4.1	5/11	23.7 ±7.3	189 ±42	16.0 ±1.3	19.3 ±1.0	8.2 ±3.5

平均値±SD

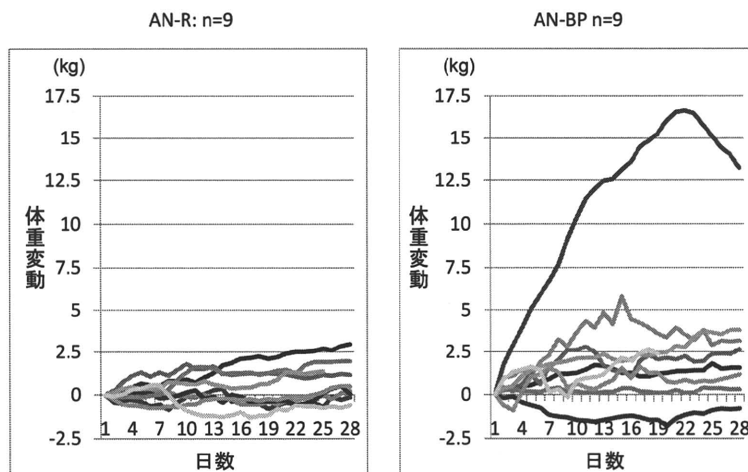
*p<0.05
BMI<14kg/m² VS. BMI≧14kg/m²

研究１－基本データ（被験者 32 人を BMI14kg/m2 未満と BMI14kg/m2 以上の２群に分けた。罹病期間以外はすべて、二群間に差がある。ＢＭＩ１４以下群は、ＡＮ－Ｒ多く、年齢は若く、入院期間が長く、体重増加量も多い。



図－1 各部位の入院中の変化量

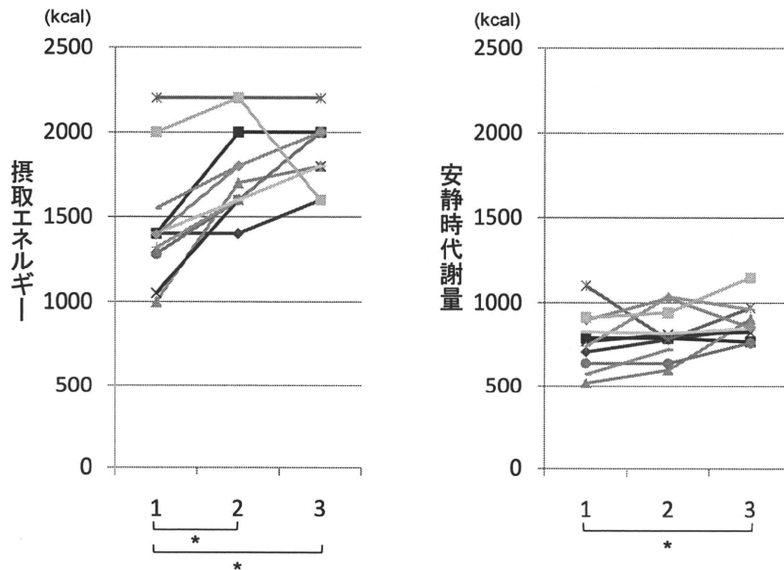
BMC：骨塩、FM：脂肪、FFM：除脂肪
L：左、R：右、Arm：腕、Leg：脚、Trunk：体幹部
Extremity ：四肢=L Arm + R Arm + L Leg + R Leg
* p<0.05



図－2 入院後4週間の体重変動（入院翌日の体重を基準）

研究２

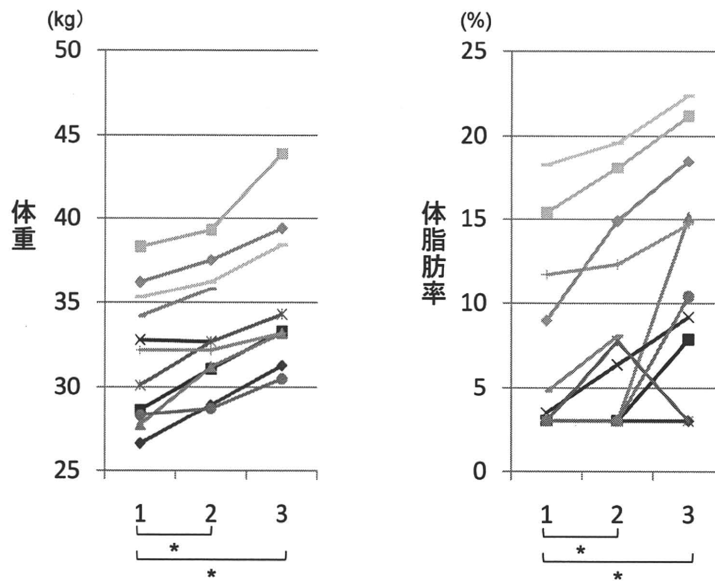
入院直後4週間の体重変化を示す。AN-BP の患者は体重変動が大きいが、1ヶ月後には安定する。



図－3 摂取エネルギーと安静時代謝量の変化

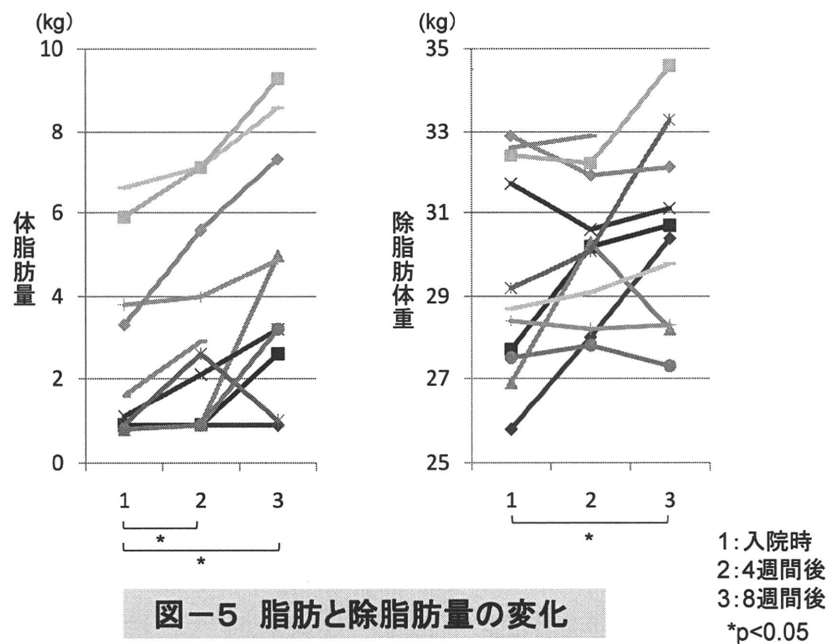
1:入院時
2:4週間後
3:8週間後
*p<0.05

摂取エネルギーは１－２，１－３で有意に増加、代謝は１－２で不変、１－３で増加の傾向。その差は、１－２、１－３で有意に増加



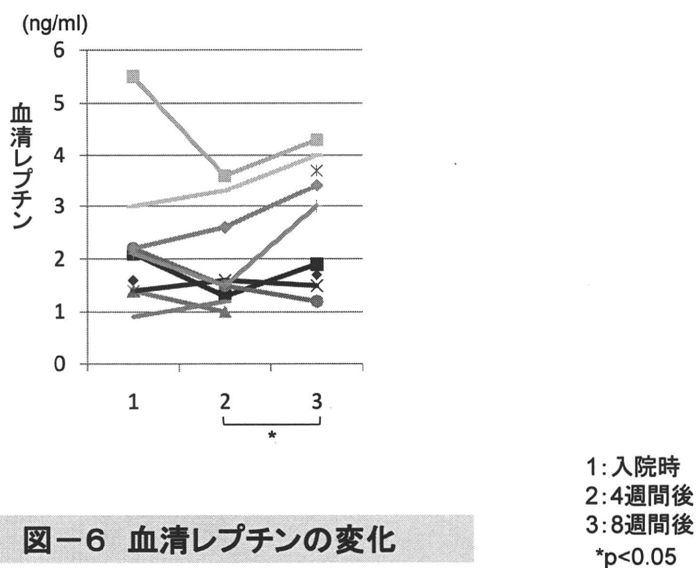
図－4 体重と体脂肪率の変化

1:入院時
2:4週間後
3:8週間後
*p<0.05



図－5　脂肪と除脂肪量の変化

統計学的に検定を行うと、脂肪は１－２、１－３でいずれも有意に増加し、FFMは１－３では有意な増加を示していた。しかし、FFMは１－２で有意な増加はしていなかった。
ただし、今回は症例数が少ないため、細分化した統計処理はできないが、１０例のなかでFFMの重量が極端に少ない４例（入院時FFMが25.8～27.7kg）はFFMが１－２で増加、それ以外の６例は減少あるいは不変であった。



図－6　血清レプチンの変化

厚生労働科学研究費補助金（難治性疾患克服研究事業）
分担研究報告書

神経性食欲不振症の性腺補充療法

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研究協力者　　永井　章　　　国立成育医療センター思春期診療科　医長

研究要旨　本研究では、近年わが国で問題となっている、中枢性摂食異常症(摂食障害)若年化の実態を調査し、予防と早期発見体制の確立を目指すことを目的としている。パイロット研究として、研究協力に同意の得られた首都圏の私立と公立の小･中学校　計 40ー50 校の学童･生徒(小学校 5 年ー中学 3 年)を対象とし、1) 養護教諭への質問紙調査(各校の背景、摂食障害経験数、学年毎の発症数、医療機関受診の実態、2) 過去 2 年間の全生徒の身長体重データ、3) 学童･生徒に対する質問紙調査(EAT26 を使用)を行い、摂食障害やその予備軍の人数を調査する。今年度はそのための質問紙、依頼状の作成と倫理申請、参加校のリクルートを行ってきた。パイロット研究後、同様の調査を全国で実施し、地域性を比較したり日本全体の状況を把握していく予定である。

研究目的

近年、神経性食欲不振症（AN）をはじめとする摂食障害が増加し、なかでも思春期前後の児童・生徒の発症が増えていることが危惧されている。摂食障害の低年齢化は、現在の成長・成熟が妨げられるだけでなく、骨粗鬆症の予備軍になるなど将来にわたり身体の健康が障害され、さらには妊娠率の低下や胎児の成長が妨げられるなど、次世代への影響も懸念される。また、社会性の醸成など、精神的な成熟も不十分となる可能性もあり、社会的に問題である。従って、現在の状況を正確に把握し、予防策を積極的に講じていくことは喫緊の課題である。

現在までに、AN に関する実態調査が何回か行われてきたが、最近の日本における情勢は必ずしも捉えられていないのが実情である。特に、医療機関への調査は、受診に至っていない患者実数を計ることはできない。

そこで本研究では、AN 実数と AN の若年化傾向の有無把握を目的とし、学校現場を対象とした実態調査を行った。

研究方法

対象

日常、児童・生徒の健康状況を把握している小中学校の養護教諭と、小学校 5 年から中学 3 年までの生徒本人を対象とした。今年度の研究では、全国疫学調査に先立ち首都圏の国公立高と私立校に質問紙調査を依頼した。

方法

以下の 3 点を調査項目とした。

1）小学校 5，6 年、中学 1，2，3 年生の、今年度と昨年度の身長体重データ（各学年 4 月のデータ、および可能であれば学期ごとのデータ）

自記式質問紙と照合するためにイニシャルを記入

2）養護教諭への質問紙：上記学年における、過去 5 年間の神経性食欲不振症診断確定者数、現在の疑い例数、現在治療中の例数

3）上記学年生徒への質問紙：EAT26 質問紙調査、自記式身長・体重、身長・体重計測データと照合するためのイニシャルと性別

養護教諭への質問紙により、神経性食欲不振症の実数把握を行い、実測身長体重データより不自然な体重減少や成長停止例数を算出、また、生徒の質問紙調査により摂食障害の心性を有する実数把握と、身長・体重実測値と自己申告値の違いなどを検討することとした。

倫理面への配慮

本研究は、疫学研究に関する倫理指針に則り計画し、国立成育医療研究センター倫理委員会にて承認を得た。調査の性質上、生徒個人・保護者の同意は必要な

いが、保護者への協力依頼の手紙を作成し配布することで、保護者の了解を得ることとした。

研究結果

調査依頼状と質問紙作成

別添の調査依頼状と質問紙を作成した。質問紙を見本として研究対象校に依頼状と共に送付した。

研究対象校と調査同意

調査の依頼は、東京都下の私学女子中学校81校、私学男子中学校27校、私学共学中学校56校、国立中学校8校、国・私立小学校46校と、世田谷区立小学校64校・中学校31校を対象とした。

世田谷区立小中学校は、イニシャルと身長体重データが世田谷区の個人情報保護条例に反することを理由に協力は得られなかったが、それ以外の項目については世田谷区教育委員会と学校長会の同意の下、調査が実施できた。

私学は、全調査協力校は　小・中学校併せて5校のみであり、養護教諭への質問紙のみの協力は現在のところ15校、協力不同意が15校で、他は回答を待っているところである。

考察

2000年以降、本症に関する全国的な臨床疫学調査は施行されていないため、本研究成果はわが国における本症の実態把握のための新規の基盤データとなるとともに、将来の大規模疫学調査のパイロットスタディーの予備調査となる。近年、わが国では若年者に多い中枢性摂食異常症の患者と予備軍は確実に増加していると考えられているが、小学校高学年の実態調査を行うことでそれを明らかにできることが期待される。

パイロット研究後、同様の調査を全国で実施し、地域性を比較したり日本全体の状況を把握していく予定である。本研究成果は本症の早期発見と予防に大きく貢献すると考えられる。

結論

本邦における小児・思春期での AN 有病数実態把握を計画し、参加リクルートまで行った。参加校を増やし、全国疫学調査につなげていき、AN の実態を明らかにすることで、小児期から続き成人期の健康に影響を及ぼす生涯の早期発見・予防が可能となると思われる。

＊健康危険情報

なし

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学会発表

なし

知的財産権の出願・登録状況

なし

平成 22 年度

IV.　研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル	発表誌名	巻号	ページ	出版年
M. Tanaka, T. Suganami, S. Sugita, Y. Shimoda, M. Kasahara, S. Aoe, M. Takeya, S. Takeda, Y. Kamei, Y. Ogawa.	Role of central leptin signaling in renal macrophage infiltration under unilateral ureteral obstruction.	Endocr. J.	57	61-72	2010
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C. Arimura, T. Nozaki, S. Takakura, K. Kawai , M. Takii N. Sudo, C .Kubo.	Predictors of menstrual resumption by patients with anorexia nervosa.	Eat Weight Disord.	109		2010
久保千春、河合啓介	「摂食障害の治療」	こころのりんしょう à•la•carte	29	383-388	2010

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吉松博信	食欲調節メカニズム	門脇孝	糖尿病ナビゲーター　第2版	メディカルレビュー社	2010	192-193
鈴木（堀田）眞理	摂食障害のための理想の治療施設・治療環境論	西園マーハ文	専門医のための精神科治療リュミエール28摂食障害の治療	中山書店	2010	16-28
河合啓介	神経性食欲不振症（分担）		薬局増刊号　病気と薬　パーフェクトBOOK2010	南山堂	2010	1008-1009

平成 22 年度

V. 研究成果の刊行物・別刷

Endocrine Journal 2010, **57** (1), 61-72

Role of Central Leptin Signaling in Renal Macrophage Infiltration

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Abstract. Monocytes/macrophages are key mediators of wound repair, tissue remodeling, and inflammation. However, the molecular mechanisms underlying macrophage recruitment to the site of inflammation is not fully understood. Leptin acts directly on the hypothalamus, thereby regulating food intake and energy expenditure. The leptin receptor, a single transmembrane protein that belongs to the gp130 family of cytokine receptor superfamily, is expressed not only in the hypothalamus but in a variety of peripheral tissues, suggesting the role of leptin as a pro-inflammatory adipocytokine in peripheral tissues. Here, we show that deficiency of leptin signaling reduces renal macrophage infiltration after unilateral ureteral obstruction (UUO). Bone marrow transplantation studies using leptin signaling-deficient *db/db* mice revealed that leptin signaling in bone marrow cells may not play a major role in the UUO-induced renal macrophage infiltration. Interestingly, central leptin administration reverses the otherwise reduced UUO-induced renal macrophage infiltration in leptin-deficient *ob/ob* mice. This is effectively abolished by central co-administration of SHU9119, a melanocortin-3 receptor/melanocortin-4 receptor antagonist. This study demonstrates that central leptin administration in *ob/ob* mice accelerates renal macrophage infiltration through the melanocortin system, thereby suggesting that the central nervous system, which is inherent to integrate information from throughout the organism, is able to control peripheral inflammation.

Key words: Chemotaxis, Inflammation, Leptin, Macrophages, Melanocortin

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THE ADIPOSE tissue is an important endocrine organ that secretes a large number of adipocytokines; pro-inflammatory cytokines such as monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor-α (TNFα) and anti-inflammatory cytokines such as adiponectin [1, 2]. Leptin is such an adipocytokine with multiple regulatory potentials; it acts directly on the hypothalamus, where it activates the melanocortin system [3, 4], thereby regulating food intake and energy expenditure [5, 6]. The leptin receptor (Ob-R), a single transmembrane protein that belongs to the gp130 family of cytokine receptor superfamily, has several alternatively spliced isoforms [7], one of which, a biologically active longest isoform or Ob-Rb, is expressed not only in the hypothalamus but in a variety of peripheral tissues including renal parenchymal cells and macrophages [8]. The peripheral actions of leptin include activation of platelet aggregation, modulation of immune functions, and stimulation of vascular endothelial cell proliferation and angiogenesis [9-12]. These findings, taken together, suggest that leptin acts as a pro-inflammatory adipocytokine in peripheral tissues. Indeed, previous reports have demonstrated that vascular remodeling and neointimal formation are markedly attenuated in leptin-deficient *ob/*

Received Oct. 5, 2009; Accepted Oct. 12, 2009 as K09E-296
Released online in J-STAGE as advance publication Oct. 23, 2009
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ob mice and *db/db* mice with defective Ob-Rb signaling [13, 14], suggesting that leptin may accelerate the development of vascular injury.

Unilateral ureteral obstruction (UUO) is a widely used experimental model for progressive renal fibrosis that encompasses many aspects of renal diseases, which is considered independent of systemic blood pressure and immune disease [15]. In UUO-induced renal injury, the renal tubular epithelium, in response to the mechanical stress produced by ureteral obstruction, produces a number of chemokines, thereby increasing the recruitment of macrophages [16]. Evidence has supported the role of infiltrated macrophages as a mediator of UUO-induced renal injury in wildtype animal models [17]. For instance, transforming growth factor-β, which is derived at least in part from macrophages, may greatly contribute to the progression of renal fibrosis [18].

Using UUO as an experimental model system, this study was designed to investigate how leptin is involved in macrophage infiltration into the site of inflammation. Here, we demonstrate that deficiency of leptin signaling reduces renal macrophage infiltration after UUO. Interestingly, central leptin administration in *ob/ob* mice effectively reverses the otherwise reduced renal macrophage infiltration. Furthermore, this effect of leptin is abolished by central co-administration of SHU9119, a melanocortin-3 receptor (MC3R)/melanocortin-4 receptor (MC4R) antagonist. The data of these studies suggest that leptin increases renal macrophage infiltration in *ob/ob* mice through the activation of central melanocortin system, thereby highlighting central regulation of peripheral monocyte/macrophage function in chronic inflammatory diseases.

Materials and Methods

Materials

All reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA) or Nacalai Tesque (Kyoto, Japan) unless otherwise noted.

Animals

Eight-week-old C57BL/6J-*ob/ob* and C57BL/KsJ-*db/db* and their wildtype mice (C57BL/6J and C57BL/KsJ mice, respectively) were purchased from Charles River Japan (Kanagawa, Japan). The mice were maintained in a temperature-, humidity-, and light-controlled room (12 h light/dark cycles), allowed free access to water and standard chow (Oriental MF, 360 kcal/100g) (Oriental Yeast, Tokyo, Japan) unless otherwise noted. All animal experiments were conducted according to the guidelines of Tokyo Medical and Dental University Committee on Animal Research (No.0080010).

UUO

UUO was performed as described previously [19]. Briefly, in mice under pentobarbital anesthesia (30 mg/kg), the middle portion of the right ureter was ligated with 5-0 silk at two points through a midline abdominal incision and cut between the ligatures to prevent retrograde infection. Three to 14 days after the surgery of UUO, mice were euthanized by anesthesia and blood and kidney were sampled.

Leptin infusion experiments

Continuous subcutaneous (s.c.) or intracerebroventricular (i.c.v.) administration of leptin was performed as described [4, 20]. In brief, 3 days before UUO, recombinant mouse leptin (800 ng/h) or an equal volume of vehicle (phosphate-buffered saline or PBS) was started infused into 8-week-old male mice using a micro-osmotic pump (Model 1002; Durect Corporation, Cupertino, CA, USA) implanted in the dorsal s.c. space. For i.c.v. infusion, mice were anesthetized with isoflurane and a cannula was implanted into the lateral ventricle (Brain infusion kit 3; Durect Corporation). The cannula was connected to the micro-osmotic pump (Model 1002) placed in the dorsal subcutaneous space of mice. The rate of delivery was 5 ng/h of leptin and/or 400 ng/h of SHU9119 or vehicle (artificial cerebrospinal fluid or aCSF: 136.89 mM NaCl, 2.95 mM KCl, 1.23 mM $MgCl_2$, 1.32 mM $CaCl_2$, and 3.55 mM glucose in 0.5 mM phosphate buffer, pH 7.4) [4]. Three days after the i.c.v. cannulation, mice were subjected to UUO.

Food restriction experiments

Four-week-old *ob/ob* mice were divided into 2 groups; those fed *ad lib* (*ad lib-ob/ob* mice) and those provided for 5 weeks with the restricted amount of

food (restricted-*ob/ob* mice) so that the body weight of restricted-*ob/ob* mice might be comparable to that of *ad lib*-wildtype mice.

Bone marrow transplantation

Bone marrow transplantation was performed using bone marrow cells from *db/db* and wildtype mice as described previously [21]. Six-week-old male wildtype mice were used as recipients and lethally irradiated with 8 Gy using a ^{60}Co γ source. Five weeks after the bone marrow transplantation, mice were subjected to UUO for further analysis. The substitution rate of bone marrow transplantation was confirmed by detecting Ob-Rb mRNA expression in bone marrow cells of recipient mice using real-time PCR. In this study, the substitution rate of bone marrow transplantation was more than 90% (data not shown).

Histology and immunohistochemistry

Histological analysis was performed as previously described [21]. In brief, paraffin-embedded horizontal kidney sections were immunostained with rat monoclonal anti-mouse F4/80 antibody. Histological analysis was conducted by two observers without knowledge of the origin of the slides.

Quantitative real-time PCR

Total RNA was extracted from the kidney using Sepasol reagent and quantitative real-time PCR was performed with an ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) as described [22]. Primers used in this study were as follows: MCP-1 (Fw: 5'-CCACTCACCTGCTGCTACTC AT-3'; Rv: 5'-TGGTGATCCTCTTGTAGCTCTCC-3'), macrophage colony-stimulating factor (M-CSF) (Fw: 5'-CCCATATTGCGACACCGAA-3'; Rv: 5'-AAGCAGT AACTGAGCAACGGG-3'), regulated on activation normal T cell expressed and secreted (RANTES) (Fw: 5'-TGCCCACGTCAAGGAGTATTT-3'; Rv: 5'-TCTCTG GGTTGGCACACACTT-3'), F4/80 (Fw: 5'-CTTTGGC TATGGGCTTCCAGTC-3'; Rv: 5'-GCAAGGAGGAC AGAGTTTATCGTG-3'), CD68 (Fw: 5'-CTTCCCACA GGCAGCACAG-3'; Rv: 5'-AATGATGAGAGGCAG CAAGAGG-3'), C-C motif chemokine receptor-2 (CCR2) (Fw: 5'-GCTCAACTTGGCCATCTCTGA-3'; Rv: 5'-AGACCCACTCATTTGCAGCATA-3'), Ob-Rb (Fw: 5'-AGAACGGACACTCTTTGAAGTCTC-3'; Rv: 5'-AACCATAGTTTAGGTTTGTTTC-3'), 36B4 (Fw: 5'-GGCCCTGCACTCTCGCTTTC-3'; Rv: 5'-TGCCAGGACGCGCTTGT-3'). Levels of mRNA were normalized to those of 36B4 mRNA.

Blood glucose, serum leptin and insulin measurements

Blood glucose concentrations were determined by the blood glucose test meter (Glutest PRO R; Sanwa-Kagaku, Nagoya, Japan). Serum leptin and insulin concentrations were determined by a commercially available ELISA kit (R&D Systems (Minneapolis, MN, USA), Morinaga (Tokyo, Japan), respectively).

Statistical analysis

All the experiments were repeated at least 3 times with appropriate controls as indicated. Data are presented as the mean ± SE, and $P < 0.05$ was considered statistically significant. Statistical analysis was performed using a one-way ANOVA followed by the Tukey-Kramer test or Student's *t*-test.

Results

Deficiency of leptin signaling reduces UUO-induced renal macrophage infiltration

To investigate the effect of leptin deficiency on renal macrophage infiltration, we performed UUO using 8-week-old male *ob/ob* mice and wildtype littermates. In this study, *ob/ob* mice were markedly obese and hyperglycemic relative to wildtype mice (Table 1). Interstitial macrophage infiltration, as assessed by F4/80 immunostaining, was markedly inhibited in *ob/ob* mice relative to wildtype mice 2 weeks after the induction of UUO (Fig. 1A). This is consistent with reduced mRNA expression of macrophage markers (F4/80, CCR2, and CD68) in the kidney of *ob/ob* mice relative to wildtype mice (Fig. 1, B and C). In this study, mRNA expression of chemokines (MCP-1, M-CSF, and RANTES) was significantly suppressed in *ob/ob* mice relative to wildtype mice (Fig. 1, B and D). We also found that UUO-induced macrophage infiltration and F4/80 and MCP-1 mRNA expression were suppressed in *db/db* mice relative to wildtype mice (Fig. 1, E and F). These observations, taken to-

Table 1. Body and kidney weight and blood glucose concentrations in *ob/ob* and wildtype mice 14 days after the induction of UUO.

	body weight (g)	blood glucose (mg/dL)	OK (g)	CK (g)	OK/CK
wildtype	27.6 ± 0.3	155.6 ± 2.4	0.136 ± 0.01	0.179 ± 0.01	0.762 ± 0.03
ob/ob	47.0 ± 0.5**	215.3 ± 16.6**	0.184 ± 0.01**	0.181 ± 0.01	1.019 ± 0.06**

OK and CK, obstructed and contralateral kidney, respectively. Values are mean ± SE. $^{**}P < 0.01$ vs. WT, $n = 7$.

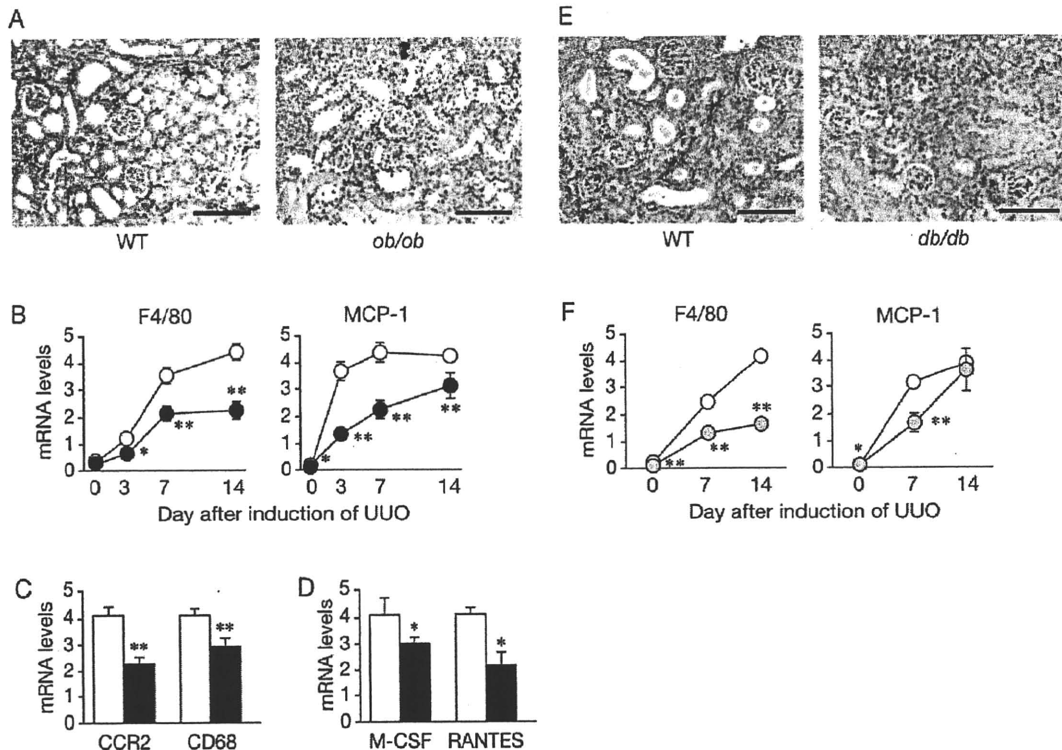

Fig. 1. Deficiency of leptin signaling reduces UUO-induced renal macrophage infiltration.
F4/80 immunostaining of the obstructed kidneys from *ob/ob* (A) and *db/db* mice (E) at day 14. F4/80-positive macrophages were stained in brown. Original magnification, x 200; Scale bars, 100 μm. Time course of mRNA expression of F4/80 and MCP-1 in the obstructed kidneys from *ob/ob* (B) and *db/db* mice (F). Expression of mRNAs for monocyte/macrophage markers at day14 (C) and cytokines at day 7 (D) in the obstructed kidneys from *ob/ob* and wildtype (WT) mice. Open circle and bar, WT mice; closed circle and bar, *ob/ob* mice; gray circle, *db/db* mice. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. WT, $n = 5\text{-}7$.

gether, indicate that deficiency of leptin signaling reduces renal macrophage infiltration after UUO.

Peripheral leptin administration promotes renal macrophage infiltration in ob/ob mice

To determine the effect of exogenous leptin administration on renal macrophage infiltration after UUO, *ob/ob* mice were continuously infused with recombinant mouse leptin s.c. using a micro-osmotic pump. Continuous administration of leptin in *ob/ob* mice decreased significantly body weight, blood glucose concentrations, and serum insulin concentrations relative to vehicle-treated *ob/ob* mice (Fig. 2, A and B, and data not shown). Serum leptin concentrations increased up to 30 ng/mL (Fig. 2C), which are roughly comparable to those in markedly obese subjects [23]. Seven days after the induction of UUO, leptin-treated *ob/ob* mice exhibited significant increases in F4/80 immunostaining and mRNA expression relative to vehicle-treated *ob/ob* mice (Fig. 2, D and E). Expression of MCP-1 mRNA tended to be increased in leptin-treated *ob/ob* mice rela-

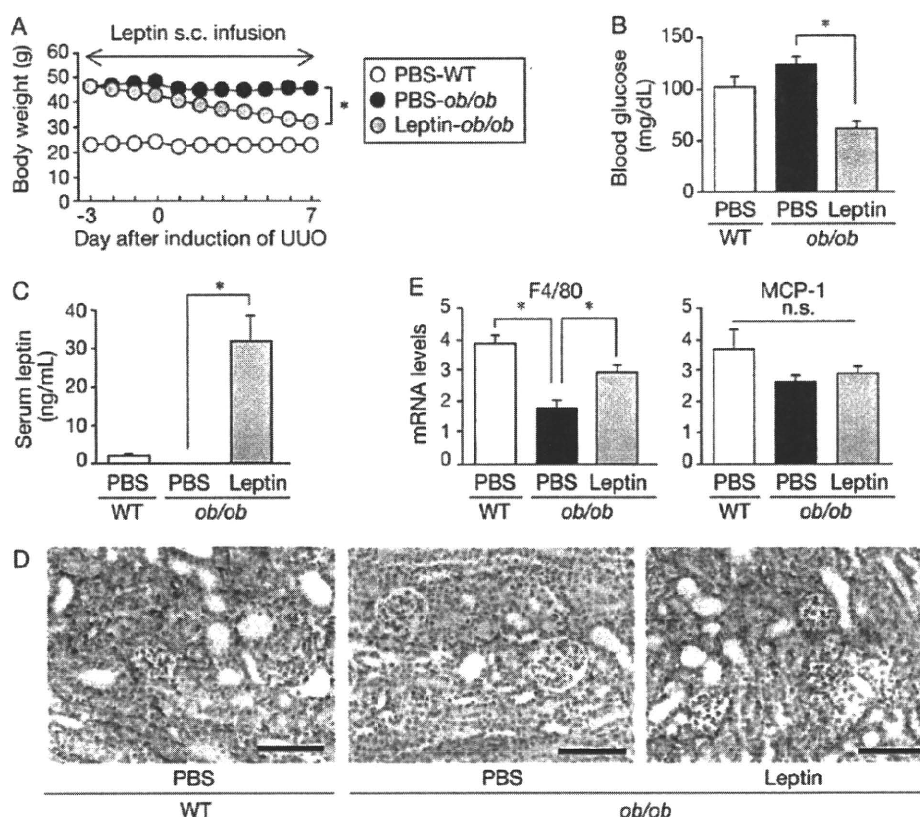

Fig. 2. Peripheral leptin administration promotes renal macrophage infiltration in *ob/ob* mice.
Mice were infused s.c. with leptin (800 ng/h) or PBS 3 days prior to UUO. A, Time course of body weight change. Blood glucose (B) and serum leptin (C) concentrations 7 days after the induction of UUO. F4/80 immunostaining (D) and F4/80 and MCP-1 mRNA expression (E) in the obstructed kidneys 7 days after the induction of UUO. Original magnification, x 200; Scale bars, 100 μm. n.s., not significant. $*P < 0.05$. $n = 6\text{-}8$.

tive to vehicle-treated *ob/ob* mice, although this apparent effect was not statistically significant (Fig. 2E).

Food restriction does not promote renal macrophage infiltration in ob/ob mice

Exogenous leptin administration in *ob/ob* mice resulted in the marked weight decrease (Fig. 2A). To examine the involvement of body weight change in renal macrophage infiltration, we next performed food restriction with 4-week-old *ob/ob* mice for 5 weeks so that body weight of restricted-*ob/ob* mice might be comparable to that of *ad lib*-wildtype mice (Fig. 3A). Food restriction decreased significantly the blood glucose concentrations in *ob/ob* mice relative to *ad lib-ob/ob* mice (Fig. 3B). Seven days after the induction of UUO, restricted-*ob/ob* mice exhibited no appreciable difference in F4/80 immunostaining and mRNA expression relative to *ad lib-ob/ob* mice (Fig. 3, C and D). These observations, taken together, suggest that UUO-induced renal macrophage infiltration is not related to the leptin-induced reduction of food intake and body weight.

Leptin does not induce renal macrophage infiltration via Ob-Rb in bone marrow cells

To examine whether leptin regulates renal macrophage infiltration via Ob-Rb expressed in bone marrow-derived cells *in vivo*, we produced bone marrow-specific Ob-Rb-deficient (Ob-Rb(-) BM) and control (Ob-Rb(+) BM) mice through the bone marrow transplantation technique (Fig. 4A). There was no appreciable difference in body weight and blood glucose concentrations between Ob-Rb(-) BM and Ob-Rb(+) BM

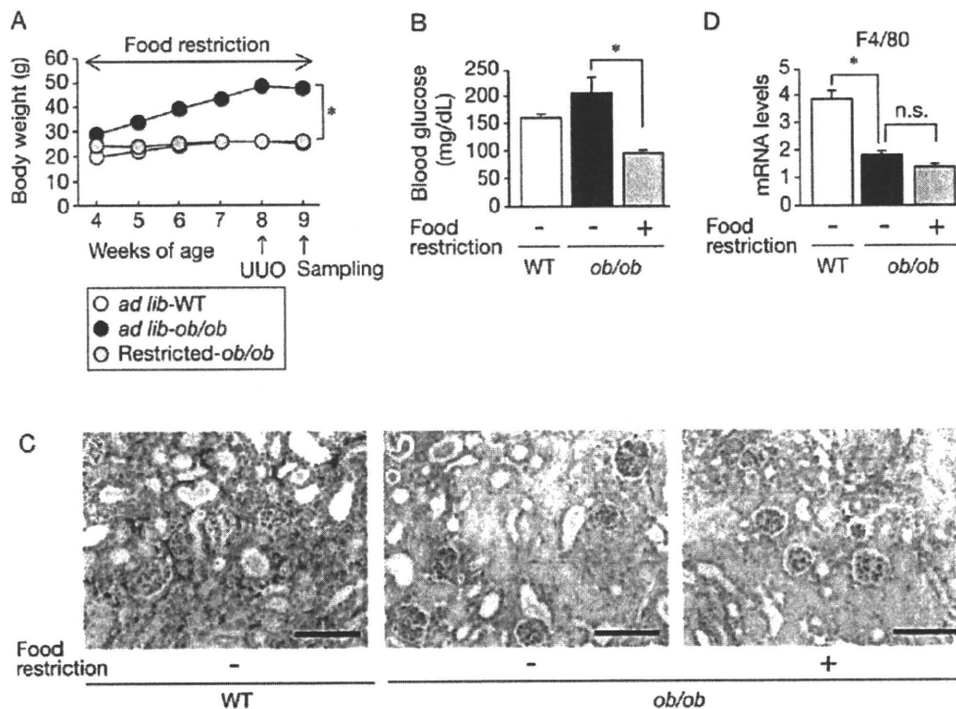

Fig. 3. Food restriction does not affect renal macrophage infiltration in *ob/ob* mice.
Ob/ob mice were divided into 2 groups: *ad lib* group and food restriction group. A, Time course of body weight change in *ad lib*-WT, *ad lib-ob/ob*, and restricted-*ob/ob* mice. Blood glucose concentrations (B), F4/80 immunostaining (C) and F4/80 mRNA expression (D) in the obstructed kidneys 7 days after the induction of UUO. Original magnification, x 200; Scale bars, 100 μm. n.s., not significant. $*P < 0.05$. $n = 6\text{-}8$.

mice (Fig. 4, B and C). We also found no significant difference in F4/80 and CD68 mRNA expression in the obstructed kidneys between Ob-Rb(-) BM and Ob-Rb(+) BM mice (Fig. 4D). These observations, taken together, suggest that Ob-Rb expressed in bone marrow-derived cells (monocytes/macrophages) is not involved in UUO-induced renal macrophage infiltration.

Central leptin signaling regulates renal macrophage infiltration

The biological actions of leptin may be largely mediated through the activation of Ob-Rb expressed in the central nervous system [5]. To address whether leptin regulates macrophage infiltration via a central mechanism, we examined the effect of continuous i.c.v. administration of recombinant mouse leptin on UUO-induced renal macrophage infiltration in *ob/ob* mice. Central administration of leptin in *ob/ob* mice resulted in marked reduction of body weight and blood glucose concentrations relative to vehicle-treated *ob/ob* mice (Fig. 5, A and B). Serum leptin concentrations were unaffected (Fig. 5C), suggesting that there is no substantial leakage of exogenous leptin into the systemic circulation. Central administration of leptin induced marked increases in F4/80 immunostaining and mRNA expression in the obstructed kidneys from *ob/ob* mice (Fig. 5, D and E). In this study, MCP-1 mRNA expression was unchanged in *ob/ob* mice that received central leptin administration (Fig. 5E). These observations, taken together, suggest that leptin affects renal macrophage infiltration via a central mechanism.

There is evidence that satiety effect of leptin is mediated at least partly through the activation of central melanocortin system [3, 4]. To explore the involvement of central melanocortin in renal macrophage infiltration, we examined the effect of central co-administration of leptin and SHU9119 on UUO-induced renal macrophage infiltration in *ob/ob* mice. Central co-administration of SHU9119 abolished the lep-

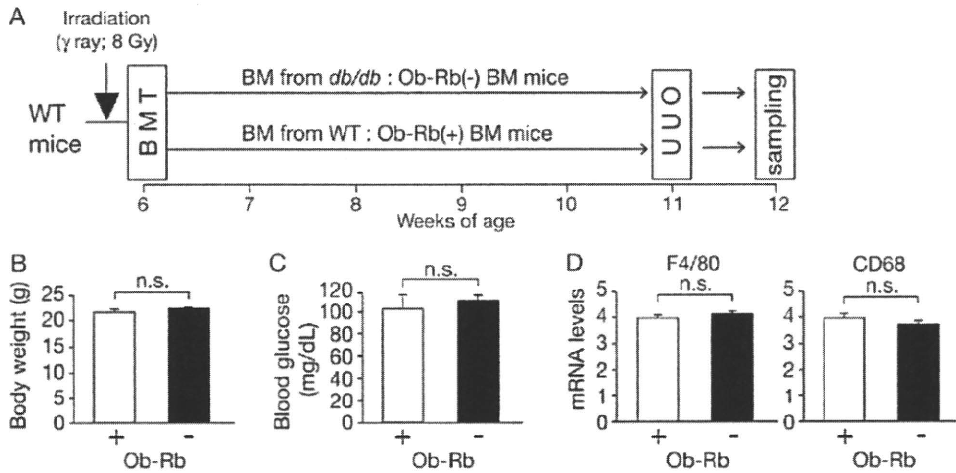

Fig. 4. Leptin does not induce renal macrophage infiltration via Ob-Rb in bone marrow cells.
A, Experimental protocol of bone marrow transplantation (BMT). Bone marrow-specific Ob-Rb deficient mice (Ob-Rb(-) BM) and their control mice (Ob-Rb(+) BM) were subjected to UUO. Body weight (B), blood glucose concentrations (C), and F4/80 and CD68 mRNA expression in the obstructed kidneys (D) 7 days after the induction of UUO. n.s., not significant. n = 6-8.

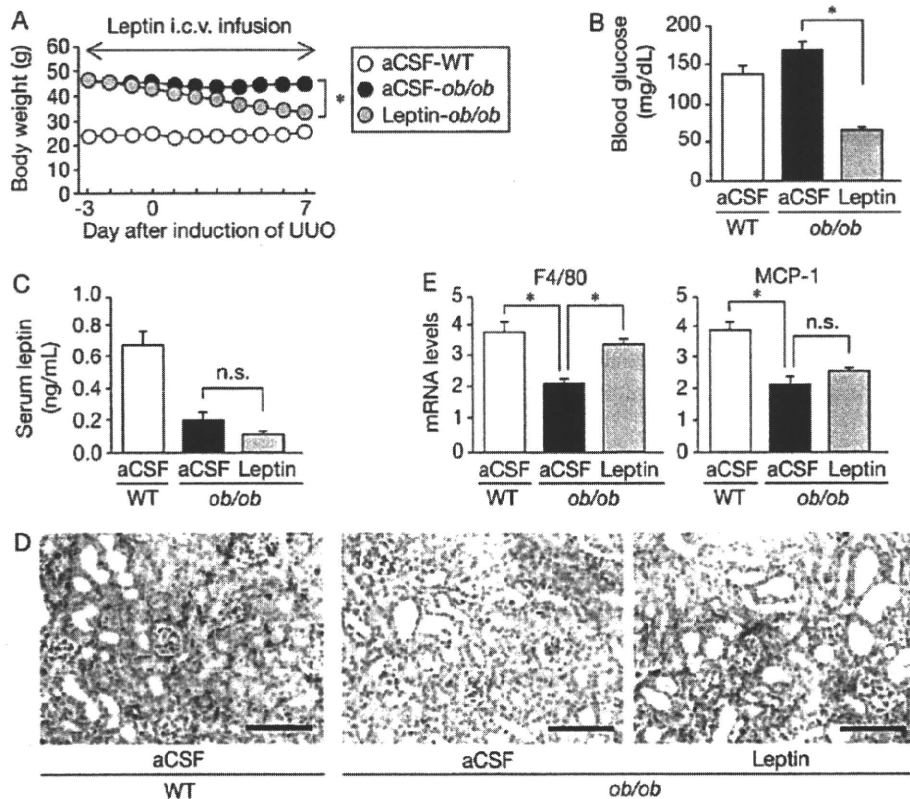

Fig. 5. Central leptin administration promotes UUO-induced renal macrophage infiltration in *ob/ob* mice.
Mice were infused i.c.v. with leptin (5 ng/h) or aCSF 3 days prior to UUO. A, Time course of body weight change. Blood glucose (B) and serum leptin (C) concentrations 7 days after the induction of UUO. F4/80 immunostaining (D) and F4/80 and MCP-1 mRNA expression (E) in the obstructed kidneys 7 days after the induction of UUO. Original magnification, x 200; Scale bars, 100 μm. n.s., not significant. $^{*}P < 0.05$. n = 6-8.

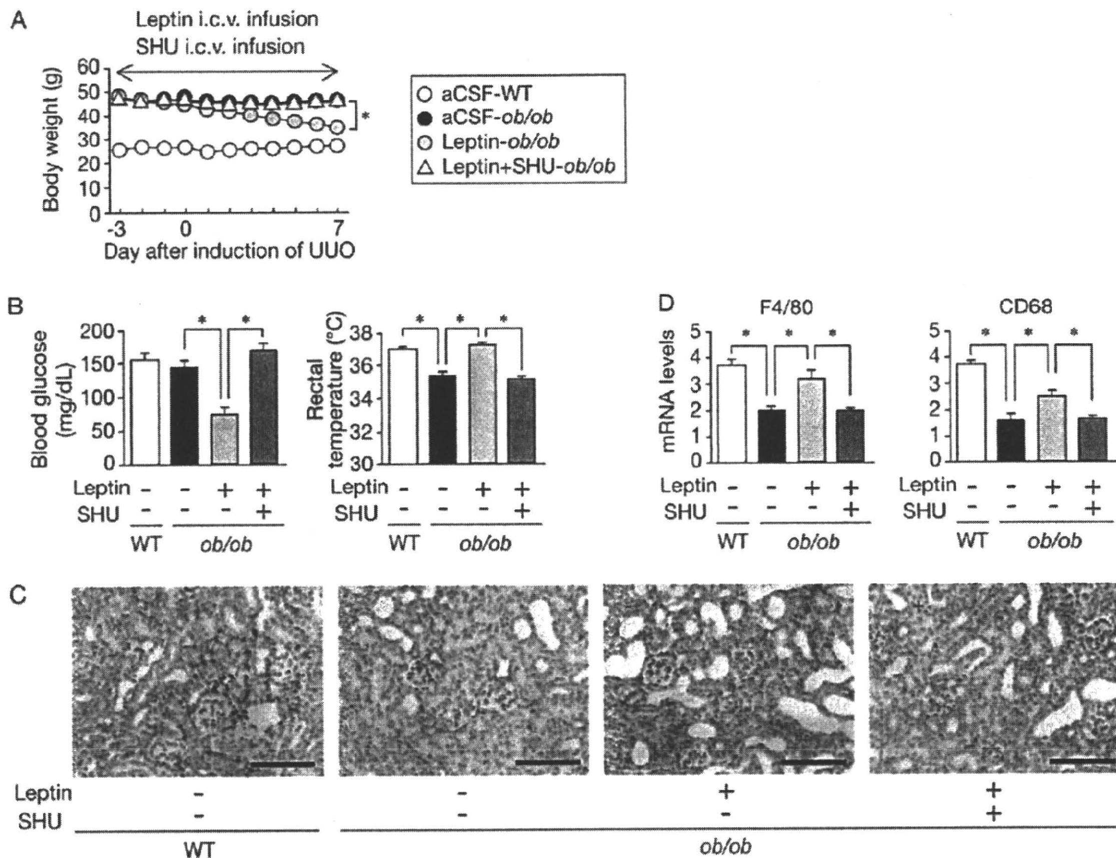

Fig. 6. Central leptin signaling promotes UUO-induced renal macrophage infiltration in *ob/ob* mice through the melanocortin system.

Mice were infused i.c.v. with leptin (5 ng/h) and/or SHU9119 (400 ng/h) or aCSF 3 days prior to UUO. A. Time course of body weight change. B. Blood glucose concentrations and rectal temperature 7 days after the induction of UUO. F4/80 immunostaining (C) and F4/80 and CD68 mRNA expression (D) in the obstructed kidneys 7 days after the induction of UUO. Original magnification, x 200; Scale bars, 100 μm. $^*P < 0.05$, $n = 6\text{-}8$.

tin-induced reduction of body weight, blood glucose concentrations, and serum insulin concentrations and leptin-induced increase in rectal temperature in *ob/ob* mice (Fig. 6, A and B, and data not shown). Moreover, the leptin-induced increase in macrophage infiltration in *ob/ob* mice was effectively inhibited by co-administration of SHU9119 (Fig. 6, C and D). These observations, taken together, suggest that the central melanocortin system is involved in leptin-induced renal macrophage infiltration.

Discussion

The adipose tissue is capable of communicating with multiple organs or tissues by virtue of a large number of adipocytokines, thereby influencing a variety of physiologic and pathophysiologic conditions [1, 2]. With respect to their inflammatory properties, most adipocytokines may be classified as either pro-inflammatory or anti-inflammatory. Leptin has been considered as a pro-inflammatory adipocytokine, as 1) leptin itself is structurally related to the interleukin-6 family [24], 2) Ob-Rb is a member of the class I cytokine receptor family [5, 25], 3) like other pro-inflammatory adipocytokines, adipose tissue expression of leptin is increased during the course of obesity [1]. Indeed, recent evidence has suggested that deficiency of leptin signaling attenuates the development of experimentally-induced inflammatory changes in

rodents [13, 14, 26]. Here, we examined the effect of leptin on macrophage infiltration into the kidneys, using UUO as an experimental model system.

In this study, we demonstrated that leptin deficiency reduced UUO-induced renal macrophage infiltration into the kidneys in leptin-deficient *ob/ob* mice. We also found that UUO-induced macrophage infiltration was attenuated in Ob-Rb-deficient *db/db* mice. These observations suggest that leptin accelerates UUO-induced renal macrophage infiltration in *ob/ob* mice largely through Ob-Rb. This is reminiscent of the marked attenuation of vascular remodeling and neointimal formation in *ob/ob* and *db/db* mice [13, 14]. Furthermore, thioacetamide-induced hepatic fibrosis is also suppressed in Zucker fatty *fa/fa* rats with leptin signaling defect as a result of leptin receptor missense mutation [26]. On the other hand, there is a report showing that UUO-induced renal infiltration of lymphocytes, rather than that of macrophages, is significantly reduced in *ob/ob* and *db/db* mice [27]. The reason for distinct impact of leptin signaling defect on renal macrophage infiltration is currently unclear. Nevertheless, the above discussion supports the concept that leptin acts as a pro-inflammatory adipocytokine *in vivo*, thereby suggesting that the adipose tissue can modulate the inflammatory status throughout the body.

Given the ubiquitous distribution of Ob-Rb, it is important to differentiate central *vs.* peripheral sites of action of leptin *in vivo*. Previous studies with mice with selective deletion of leptin receptor in hypothalamic neurons have demonstrated that central leptin receptors are essential for the regulation of food intake and energy expenditure [28]. However, whether central leptin signaling is involved in its pro-inflammatory effect *in vivo* has never been addressed. In this study, we demonstrate that central administration of leptin reverses the otherwise reduced UUO-induced macrophage infiltration in *ob/ob* mice. This study represents the first demonstration that leptin is capable of inducing peripheral inflammation via a central mechanism. Indeed, studies with bone marrow-specific leptin receptor-deficient mice revealed that leptin signaling in bone marrow-derived cells does not play a major role in leptin-induced renal macrophage infiltration. We also found no inflammatory responses in cultured renal tubular epithelial cells and macrophages treated with recombinant mouse leptin *in vitro* (Tanaka *et al.* unpublished observations). On the other hand, there is *in vitro* evidence showing that leptin can activate inflammatory pathways in a variety of cell types [12, 29, 30]. Thus, our data do not rule out the possibility that leptin acts directly on the kidney to induce inflammatory responses after UUO.

This study demonstrates that central co-administration of leptin and SHU9119, a non-selective MC3R/MC4R antagonist, reverses leptin-induced renal macrophage infiltration in *ob/ob* mice. These observations suggest that central melanocortin signaling contributes to the leptin-induced renal macrophage infiltration in *ob/ob* mice after UUO. This is in contrast to a recent observation that central regulation of bone metabolism is not mediated through central melanocortin signaling [31]. Importantly, there is no significant difference in F4/80 immunostaining and mRNA expression between MC4R-deficient and wildtype mice, suggesting that MC4R does not play a dominant role in renal macrophage infiltration (Tanaka *et al.* unpublished observations). In this regard, Ellacott *et al.* have shown that obesity-induced adipose tissue inflammation (increased macrophage infiltration and elevated expression of pro-inflammatory adipocytokines) is attenuated in obese MC3R-deficient mice relative to wildtype mice, which is in contrast with those in obese MC4R-deficient mice [32]. We, therefore, postulate that leptin administration in *ob/ob* mice increases renal macrophage infiltration via non-MC4R, possibly MC3R, in the central nervous system. On the other hand, it has been reported that melanocortin peptides such as α-melanocyte-stimulating hormone (α-MSH) and γ-MSH have anti-inflammatory activity *in vivo* and *in vitro* [33, 34]. Since MC3R is expressed in a variety of central and peripheral tissues including monocytes/macrophages [35], future studies with mice with selective disruption of MC3R in hypothalamic neurons or a selective MC3R antagonist would provide insight into the role of central MC3R in the leptin-induced peripheral inflammation.

How the central nervous system regulates peripheral inflammatory status still remains to be elucidated. It is known that central leptin signaling can regulate body weight and glucose and lipid metabolism via both humoral and neural mechanisms. Because monocyte/macrophage mobilization from the bone marrow into the site of inflammation is regulated through a complex interaction among multiple chemokines and chemokine receptors, it is important to know how chemokine-chemokine receptor interac-

tion is affected by the central nervous system. In this study, we observed that UUO-induced renal MCP-1 mRNA expression was significantly attenuated in *ob/ob* mice, which was not reversed by central leptin administration. Nevertheless, central leptin administration reversed the otherwise reduced UUO-induced renal macrophage infiltration in *ob/ob* mice, suggesting the modulation of monocyte/macrophage function by central leptin signaling. This concept is supported by our preliminary data with a matrigel plug assay that increased macrophage migration by formyl-methionyl-leucyl-phenylalanine, a synthetic chemoattractant, is reduced in *ob/ob* mice relative to wildtype mice, which is partly reversed by central leptin administration (Tanaka *et al.* unpublished data). Recent evidence has suggested the role of neuronal network in the regulation of energy expenditure and renal function [36, 37]. Further studies with renal denervation and neuron-specific melanocortin receptor deletion would be helpful to explore the downstream mechanism specific for the regulation of peripheral inflammation.

In this study, we demonstrate that leptin increases UUO-induced renal macrophage infiltration in *ob/ob* mice via a central mechanism. Interestingly, we also found that continuous i.c.v. leptin administration to wildtype mice did not affect renal expression of F4/80 and MCP-1 mRNAs relative to aCSF-administered wildtype mice 7 days after the induction of UUO (Tanaka *et al.* unpublished observations). This is reminiscent of the effect of leptin on the development of B lymphocytes [38]; leptin administration in *ob/ob* mice increased the otherwise reduced number of B lymphocytes, whereas it failed to affect B lymphocyte development in wildtype mice. In this regard, it is interesting to note that leptin administration can regulate bone metabolism in both *ob/ob* and wildtype mice [31]. Further studies are needed to understand the mechanism underlying the differential effect of leptin on peripheral inflammation in *ob/ob* and wildtype mice.

In conclusion, this study demonstrates that central leptin administration in *ob/ob* mice accelerates renal macrophage infiltration through the melanocortin system, thereby highlighting the role for the central nervous system in the regulation of peripheral macrophage function. The data of this study also suggest that the central nervous system, which is inherent to integrate information from throughout the organism, may control the peripheral inflammatory status.

Acknowledgements

We thank Masataka Sata, Goro Katsuura, and Masahiko Miura for technical advice. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, and research grants from Mitsubishi Pharma Research Foundation, Salt Science Research Foundation, CKD Award 2007, and Takeda Science Foundation. M. Tanaka has been supported by Mishima Kaiun Memorial Foundation (2005-2006) and Tokyo Biochemical Research Foundation (2008). S. Sugita is Research Fellow of the Japan Society for the Promotion of Science.

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