Table 1 Relation between the urinary ratio and metabolic parameters.

Parameter	*r*	*P* value	*n*
BMI (kg/m^2)	−0.27	0.0001^{**}	201 (group A)
Waist circumference (cm)	−0.21	0.0030^{**}	
SBP (mmHg)	−0.081	0.25	
log DBP	−0.060	0.40	
T-CHO (mg/dl)	−0.12	0.084	
log HDL-CHO	0.063	0.37	
LDL-CHO (mg/dl)	−0.086	0.22	
log TG	−0.073	0.30	
log AST	−0.16	0.018^{*}	
log ALT	−0.22	0.0014^{**}	
UA (mg/dl)	−0.073	0.30	
FPG (mg/dl)	−0.068	0.57	72 (group B)
log IRI	−0.074	0.53	
log HOMA-IR	−0.085	0.48	
log HbA1c	−0.14	0.22	
VFA (cm^2)	−0.041	0.73	
SFA (cm^2)	−0.22	0.062	
VFA + SFA (cm^2)	−0.18	0.13	
Ratio of VFA/SFA	0.14	0.24	
log leptin	−0.24	0.057	62 (group C)
Adiponectin (μg/dl)	0.24	0.061	
log hsCRP	−0.30	0.017^{*}	
log TNF-α	−0.087	0.50	
log IL-6	−0.11	0.39	

Pearson's correlation coefficient was used to examine for association between the urinary ratio and each parameters ($^{*}P < 0.05$, $^{**}P < 0.01$ when compare with the urinary ratio).

Abbreviations: logarithm (base e) (log), systolic blood pressure (SBP), and diastolic blood pressure (DBP), total cholesterol (T-CHO), high-density-lipoprotein-cholesterol (HDL-CHO), low-density-lipoprotein-cholesterol (LDL-CHO), triglyceride (TG), aspartate 2-oxoglutarate aminotransferase (AST), alanine 2-oxoglutarate aminotransferase (ALT), uric acid (UA), visceral fat area (VFA), subcutaneous fat area (SFA), fasting plasma glucose (FPG), fasting insulin (IRI), homeostasis model assessment of insulin resistance (HOMA-IR), tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), high sensitivity C-reactive protein (hsCRP)

ratio = 0.21 ± 0.05) was much greater than that in group X (delta urinary ratio = 0.01 ± 0.05) ($P = 0.0086$) (Fig. 4D). It should be noted that no significant changes in BMI were observed among the mild calorie restriction group ($n = 10$, from 30.7 ± 1.3 kg/m^2 to 29.8 ± 1.3 kg/m^2), pioglitazone group ($n = 17$ patients, from 29.1 ± 1.2 kg/m^2 to 27.4 ± 1.3 kg/m^2), group X ($n = 8$, from 27.2 ± 1.4 kg/m^2 to 26.6 ± 1.4 kg/m^2), and group Y ($n = 9$, from 31.0 ± 1.7 kg/m^2 to 29.0 ± 1.7 kg/m^2). Values of transaminases did not change significantly in the treatment of pioglitazone in both group X (=non-responded group) and group Y (=responded group). The initial value of AST in group X was 20 ± 1 U/l and 20 ± 2 U/l in 2 months after the treatment, whereas ALT values were 24 ± 4 U/l and 21 ± 4 U/l, respectively. The initial values of AST in group Y was 34 ± 4 U/l and 32 ± 4 U/l in 2 months after the treatment, whereas ALT values were 51 ± 10 U/l and 43 ± 10 U/l, respectively.

Discussion

The present study provides evidence of the existence of significant associations between the urinary ratio and the metabolic status in the case of obesity, liver function, or glucose homeostasis in Japanese subjects. The ratio of end products of glucocorticoid metabolism in urine samples has been regarded as a compelling index of the systemic balance of intracellular glucocorticoid metabolism [7–9,11]. However, except for a few rare diseases such as AME [16], there are controversies regarding the clinical implications of the urinary ratio in common metabolic diseases [7–11,22]. For example, Valsamakis et al. showed that an ''inverse'' correlation exists between the urinary ratio and BMI [7], whereas Andrew et al. reported that a ''positive'' correlation exists between the urinary ratio and waist circumference in male subjects [8]. Rask et al. also demonstrated that a ''positive'' association exists between the urinary ratio and adiposity

Please cite this article in press as: Kobayashi N, et al. Index of the systemic balance of end products of glucocorticoid metabolism in fresh urine from humans. Its potential usefulness in the evaluation of obesity-related diseases. Obes Res Clin Pract (2009), doi:10.1016/j.orcp.2008.11.002

in females [11]. Recently, they reported that the urinary ratio was elevated in subjects with a BMI ranging from 22 to 26, while it was decreased in subjects with a BMI ranging from 26 to 31, indicating a bell-shape correlation [10]. Thus, the relationship between the urinary ratio and the metabolic status has largely remained obscure.

In the present study, the urinary ratio in 24-h pooled urine samples (1.13 ± 0.7) from 18 healthy subjects (8 males and 10 females; age, 33.7 ± 1.3; BMI, 21.3 ± 0.7) was found to be equivalent to that obtained from patients in another laboratory [9] (urinary ratio, 1.06 ± 0.08; in 12 control subjects (6 males and 6 females) age, 27.9 ± 1.5; BMI, 22.3 ± 0.41). Similarly, the ratio in the case of male subjects in the present study (1.28 ± 0.09) was comparable to that in the case of a previous report [10] (urinary ratio, 1.18 ± 0.28 in 11 control males; age, 46.8 ± 8.7; BMI, 22.9 ± 1.4), validating the accuracy of measurement in the present study. On the basis of these results, using fresh urine samples obtained at 09:00 h, we investigated the clinical implications of the urinary ratio in metabolic diseases. The present study is the first to demonstrate that the urinary ratio in healthy volunteers showed no apparent circadian variation and show that there was no difference between fresh urine and 24-h pooled urine samples, suggesting that the ratio in fresh urine samples is constant and reproducible for the same individual. This is also the first report stating that the urinary ratio showed a normal distribution curve. Our findings raise the possibility that fresh urine sample, as little as 1 ml, can reveal the systemic balance of intracellular glucocorticoid metabolism.

Recent human studies showed a significantly positive association between the 11β-HSD1 mRNA level in subcutaneous adipose tissue and BMI [18–20]. Contrary to our initial prediction, however, the present study demonstrates a significantly inverse correlation between the urinary ratio and BMI or waist circumference in a large Japanese cohort. These results suggest that intracellular glucocorticoid metabolism in the whole body is decreased in the case of human obesity. Furthermore, when adjusted for BMI, there was no gender difference in the urinary ratio (data not shown). This suggests that sexual dimorphism in the urinary ratio is attributable, at least partly, to the gender difference in body fat mass.

As recent studies reported that NASH is critically associated with intra-abdominal obesity and insulin resistance [29], we investigated the urinary ratio in patients with histologically proven NASH. Diagnosis of NASH requires histopathologic evaluation because the lesions of parenchymal injury and fibrosis cannot be detected by imaging studies or laboratory tests [30]. The present study is the first to demonstrate that the urinary ratio was significantly lower in patients with NASH compared to healthy volunteers; this result was in agreement with those of a previous report where the urinary ratio in 24-h pooled urine was significantly lower in patients with non-alcoholic fatty liver diseases diagnosed by magnetic resonance imaging [13]. Therefore, it is reasonable to speculate that decreased activity of 11β-HSD1 as well as increased activity of 5α- and 5β-reductase in a steatotic liver may contribute to the decrease in the urinary ratio in NASH patients [11,13,21]. A higher BMI in patients with NASH may also be associated with the decrease in the urinary ratio. In this context, conducting liver biopsies to measure the activities and expression of 11β-HSDs and 5α- and 5β-reductase would be of considerable interest in future studies.

In previous cross-sectional studies, no significant difference was observed in the urinary ratio between patients with type 2 diabetes and the control subjects [7,22]. However, none of the studies have investigated the urinary ratio during the treatment of type 2 diabetes [7,22]. PPARγ is expressed abundantly in adipose tissue and plays a pivotal role in regulating a variety of adipocyte genes [26,31,32]. Importantly, PPARγ agonists are known to suppress 11β-HSD1 exclusively in adipose tissue [31]. In this context, we assessed the urinary ratio in patients with type 2 diabetes during the 2-month treatment with pioglitazone [26,31]. The present study demonstrates that mild calorie restriction without significant body weight changes did not affect the urinary ratio at all. In contrast, with an equipotent amelioration of HbA1c to calorie restriction therapy, the urinary ratio was slightly but significantly reduced in patients treated with pioglitazone. On the basis of this result, we further explored the impact of pioglitazone treatment on the urinary ratio in relation to drug responsiveness. In a recent report by Satoh et al., pioglitazone responders were defined as those who showed a >1% reduction in HbA1c with 3 months of treatment [33]. Since we evaluated the drug response for 2 months, patients who showed an improvement in glycemic control of 0.7% or more in terms of HbA1c levels over 2 months were defined as drug responders. In terms of glycemic control, our data demonstrates that the decrease in the urinary ratio was increased to a large extent in patients who responded to pioglitazone. Although the underlying mechanism is still obscure, a recent study demon-

Please cite this article in press as: Kobayashi N, et al. Index of the systemic balance of end products of glucocorticoid metabolism in fresh urine from humans. Its potential usefulness in the evaluation of obesity-related diseases. Obes Res Clin Pract (2009), doi:10.1016/j.orcp.2008.11.002

Table 2 Changes in the urinary ratio in diseases/treatments.

Diseases/treatments	Change in urinary ratio
Obese subjects	↓
NASH	↓
Mild calorie restriction	→
Pioglitazone	
Responded group (group Y)	↓
Non-responded group (group X)	→

The present study demonstrated an inverse correlation between the urinary ratio and BMI in a large Japanese cohort. Together with that the urinary ratio was also decreased in patients with NASH, decrease in 11β-HSD1 activity in liver of obese individuals may contribute, at least partly, to the fall in the urinary ratio in obesity. Although further studies are warranted, the finding that decrement of the urinary ratio was exaggerated in patients who responded to pioglitazone may be a reflection of 11β-HSD1 inhibition mainly in adipose tissue.

strated that growth hormone supplementation in patients with adult growth hormone deficiency (AGHD) markedly lowered the urinary ratio, reflecting 11β-HSD1 inhibition mainly in adipose tissue [34].

In summary, the present study is the first to provide novel evidence that the urinary ratio in fresh urine reflects a facet of metabolic function in adipose tissue and liver (Table 2), thereby offering a unique method to evaluate the metabolic status and therapeutic effectiveness in human clinics.

Conflicts of interest

None of the authors have any conflict of interest.

Acknowledgments

We thank H. Chusho, N. Saito and H. Aono for their help and discussions. We are grateful to M. Nagamoto for experimental assistance. This work was supported in part by Grants-in-Aid for Scientific Research (B2) and (S2), Research on Measures for Intractable Diseases (Health and Labor Science Research Grant), Special Coordination Funds for Promoting Science and Technology (JST), Research Grant of National Cardiovascular Center, Takeda Medical Research Foundation, Smoking Research Foundation and Sankyo Research Foundation.

References

[1] Kotelevtsev Y, Holmes MC, Burchell A, Houston PM, Schmoll D, Jamieson P, et al. 11beta-Hydroxysteroid dehydrogenase type 1 knockout mice show attenuated glucocorticoid-inducible responses and resist hyperglycemia on obesity or stress. Proc Natl Acad Sci USA 1997;94(26):14924–9.

[2] Masuzaki H, Paterson J, Shinyama H, Morton NM, Mullins JJ, Seckl JR, et al. A transgenic model of visceral obesity and the metabolic syndrome. Science 2001;294(5549):2166–70.

[3] Matsuzawa Y. Therapy insight: adipocytokines in metabolic syndrome and related cardiovascular disease. Nat Clin Pract Cardiovasc Med 2006;3(1):35–42.

[4] Stewart PM. Tissue-specific Cushing's syndrome, 11beta-hydroxysteroid dehydrogenases and the redefinition of corticosteroid hormone action. Eur J Endocrinol 2003;149(3):163–8.

[5] Seckl JR. 11beta-Hydroxysteroid dehydrogenases: changing glucocorticoid action. Curr Opin Pharmacol 2004;4(6):597–602.

[6] Tomlinson JW, Stewart PM. Mechanisms of disease: selective inhibition of 11beta-hydroxysteroid dehydrogenase type 1 as a novel treatment for the metabolic syndrome. Nat Clin Pract Endocrinol Metab 2005;1(2):92–9.

[7] Valsamakis G, Anwar A, Tomlinson JW, Shackleton CH, McTernan PG, Chetty R, et al. 11beta-Hydroxysteroid dehydrogenase type 1 activity in lean and obese males with type 2 diabetes mellitus. J Clin Endocrinol Metab 2004;89(9):4755–61.

[8] Andrew R, Phillips DI, Walker BR. Obesity and gender influence cortisol secretion and metabolism in man. J Clin Endocrinol Metab 1998;83(5):1806–9.

[9] Stewart PM, Boulton A, Kumar S, Clark PM, Shackleton CH. Cortisol metabolism in human obesity: impaired cortisone → cortisol conversion in subjects with central adiposity. J Clin Endocrinol Metab 1999;84(3):1022–7.

[10] Rask E, Olsson T, Soderberg S, Andrew R, Livingstone DE, Johnson O, et al. Tissue-specific dysregulation of cortisol metabolism in human obesity. J Clin Endocrinol Metab 2001;86(3):1418–21.

[11] Rask E, Walker BR, Soderberg S, Livingstone DE, Eliasson M, Johnson O, et al. Tissue-specific changes in peripheral cortisol metabolism in obese women: increased adipose 11beta-hydroxysteroid dehydrogenase type 1 activity. J Clin Endocrinol Metab 2002;87(7):3330–6.

[12] Draper N, Echwald SM, Lavery GG, Walker EA, Fraser R, Davies E, et al. Association studies between microsatellite markers within the gene encoding human 11beta-hydroxysteroid dehydrogenase type 1 and body mass index, waist to hip ratio, and glucocorticoid metabolism. J Clin Endocrinol Metab 2002;87(11):4984–90.

[13] Westerbacka J, Yki-Jarvinen H, Vehkavaara S, Hakkinen AM, Andrew R, Wake DJ, et al. Body fat distribution and cortisol metabolism in healthy men: enhanced 5beta-reductase and lower cortisol/cortisone metabolite ratios in men with fatty liver. J Clin Endocrinol Metab 2003;88(10):4924–31.

[14] Sandeep TC, Andrew R, Homer NZ, Andrews RC, Smith K, Walker BR. Increased in vivo regeneration of cortisol in adipose tissue in human obesity and effects of the 11beta-hydroxysteroid dehydrogenase type 1 inhibitor carbenoxolone. Diabetes 2005;54(3):872–9.

Please cite this article in press as: Kobayashi N, et al. Index of the systemic balance of end products of glucocorticoid metabolism in fresh urine from humans. Its potential usefulness in the evaluation of obesity-related diseases. Obes Res Clin Pract (2009), doi:10.1016/j.orcp.2008.11.002

[15] Paterson JM, Morton NM, Fievet C, Kenyon CJ, Holmes MC, Staels B, et al. Metabolic syndrome without obesity: hepatic overexpression of 11beta-hydroxysteroid dehydrogenase type 1 in transgenic mice. Proc Natl Acad Sci USA 2004;101(18):7088–93.

[16] Ulick S, Tedde R, Mantero F. Pathogenesis of the type 2 variant of the syndrome of apparent mineralocorticoid excess. J Clin Endocrinol Metab 1990;70(1):200–6.

[17] Bujalska IJ, Kumar S, Stewart PM. Does central obesity reflect "Cushing's disease of the omentum"? Lancet 1997;349(9060):1210–3.

[18] Paulmyer-Lacroix O, Boullu S, Oliver C, Alessi MC, Grino M. Expression of the mRNA coding for 11beta-hydroxysteroid dehydrogenase type 1 in adipose tissue from obese patients: an in situ hybridization study. J Clin Endocrinol Metab 2002;87(6):2701–5.

[19] Lindsay RS, Wake DJ, Nair S, Bunt J, Livingstone DE, Permana PA, et al. Subcutaneous adipose 11 beta-hydroxysteroid dehydrogenase type 1 activity and messenger ribonucleic acid levels are associated with adiposity and insulinemia in Pima Indians and Caucasians. J Clin Endocrinol Metab 2003;88(6):2738–44.

[20] Wake DJ, Rask E, Livingstone DE, Soderberg S, Olsson T, Walker BR. Local and systemic impact of transcriptional up-regulation of 11beta-hydroxysteroid dehydrogenase type 1 in adipose tissue in human obesity. J Clin Endocrinol Metab 2003;88(8):3983–8.

[21] Livingstone DE, Jones GC, Smith K, Jamieson PM, Andrew R, Kenyon CJ, et al. Understanding the role of glucocorticoids in obesity: tissue-specific alterations of corticosterone metabolism in obese Zucker rats. Endocrinology 2000;141(2):560–3.

[22] Andrews RC, Herlihy O, Livingstone DE, Andrew R, Walker BR. Abnormal cortisol metabolism and tissue sensitivity to cortisol in patients with glucose intolerance. J Clin Endocrinol Metab 2002;87(12):5587–93.

[23] Vogt B, Dick B, N'Gankam V, Frey FJ, Frey BM. Reduced 11beta-hydroxysteroid dehydrogenase activity in patients with the nephrotic syndrome. J Clin Endocrinol Metab 1999;84(2):811–4.

[24] Yoshizumi T, Nakamura T, Yamane M, Islam AH, Menju M, Yamasaki K, et al. Abdominal fat: standardized technique for measurement at CT. Radiology 1999;211(1):283–6.

[25] Brunt EM. Nonalcoholic steatohepatitis: definition and pathology. Semin Liver Dis 2001;21(1):3–16.

[26] Masuzaki H, Flier JS. Tissue-specific glucocorticoid reactivating enzyme, 11beta-hydroxysteroid dehydrogenase type 1 (11beta-HSD1)—a promising drug target for the treatment of metabolic syndrome. Curr Drug Targets Immune Endocr Metabol Disord 2003;3(4):255–62.

[27] Furuta T, Namekawa T, Shibasaki H, Kasuya Y. Simultaneous determination of tetrahydrocortisol and tetrahydrocortisone in human plasma and urine by stable isotope dilution mass spectrometry. J Chromatogr B Biomed Sci Appl 1998;706(2):181–90.

[28] Shackleton CH. Mass spectrometry in the diagnosis of steroid-related disorders and in hypertension research. J Steroid Biochem Mol Biol 1993;45(1–3):127–40.

[29] Eriksson S, Eriksson KF, Bondesson L. Nonalcoholic steatohepatitis in obesity: a reversible condition. Acta Med Scand 1986;220(1):83–8.

[30] Brunt EM. Nonalcoholic steatohepatitis. Semin Liver Dis 2004;24(1):3–20.

[31] Berger J, Tanen M, Elbrecht A, Hermanowski-Vosatka A, Moller DE, Wright SD, et al. Peroxisome proliferator-activated receptor-gamma ligands inhibit adipocyte 11beta-hydroxysteroid dehydrogenase type 1 expression and activity. J Biol Chem 2001;276(16):12629–35.

[32] Xu C, Wang LL, Liu HY, Ruan CM, Zhou XB, Cao YL, et al. A novel dual peroxisome proliferator-activated receptors alpha and gamma agonist with beneficial effects on insulin resistance and lipid metabolism. Biotechnol Lett 2006;28(12):863–8.

[33] Satoh N, Ogawa Y, Usui T, Tagami T, Kono S, Uesugi H, et al. Antiatherogenic effect of pioglitazone in type 2 diabetic patients irrespective of the responsiveness to its antidiabetic effect. Diabetes Care 2003;26(9):2493–9.

[34] Paulsen SK, Pedersen SB, Jorgensen JO, Fisker S, Christiansen JS, Flyvbjerg A, et al. Growth hormone (GH) substitution in GH-deficient patients inhibits 11beta-hydroxysteroid dehydrogenase type 1 messenger ribonucleic acid expression in adipose tissue. J Clin Endocrinol Metab 2006;91(3):1093–8.

Please cite this article in press as: Kobayashi N, et al. Index of the systemic balance of end products of glucocorticoid metabolism in fresh urine from humans. Its potential usefulness in the evaluation of obesity-related diseases. Obes Res Clin Pract (2009), doi:10.1016/j.orcp.2008.11.002

Am J Physiol Endocrinol Metab 298: E930–E940, 2010.
First published September 23, 2009; doi:10.1152/ajpendo.00320.2009.

Glucocorticoid reamplification within cells intensifies NF-κB and MAPK signaling and reinforces inflammation in activated preadipocytes

Takako Ishii-Yonemoto, Hiroaki Masuzaki, Shintaro Yasue, Sadanori Okada, Chisayo Kozuka, Tomohiro Tanaka, Michio Noguchi, Tsutomu Tomita, Junji Fujikura, Yuji Yamamoto, Ken Ebihara, Kiminori Hosoda, and Kazuwa Nakao

Division of Endocrinology and Metabolism, Department of Medicine and Clinical Science, Kyoto University Graduate School of Medicine, Sakyoku, Japan

Submitted 18 May 2009; accepted in final form 16 September 2009

Ishii-Yonemoto T, Masuzaki H, Yasue S, Okada S, Kozuka C, Tanaka T, Noguchi M, Tomita T, Fujikura J, Yamamoto Y, Ebihara K, Hosoda K, Nakao K. Glucocorticoid reamplification within cells intensifies NF-κB and MAPK signaling and reinforces inflammation in activated preadipocytes. *Am J Physiol Endocrinol Metab* 298: E930–E940, 2010. First published September 23, 2009; doi:10.1152/ajpendo.00320.2009.—Increased expression and activity of the intracellular glucocorticoid-reactivating enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) contribute to dysfunction of adipose tissue. Although the pathophysiological role of 11β-HSD1 in mature adipocytes has long been investigated, its potential role in preadipocytes still remains obscure. The present study demonstrates that the expression of 11β-HSD1 in preadipocyte-rich stromal vascular fraction (SVF) cells in fat depots from *ob/ob* and diet-induced obese mice was markedly elevated compared with lean control. In 3T3-L1 preadipocytes, the level of mRNA and reductase activity of 11β-HSD1 was augmented by TNF-α, IL-1β, and LPS, with a concomitant increase in inducible nitric oxide synthase (iNOS), monocyte chemoattractant protein-1 (MCP-1), or IL-6 secretion. Pharmacological inhibition of 11β-HSD1 and RNA interference against 11β-HSD1 reduced the mRNA and protein levels of iNOS, MCP-1, and IL-6. In contrast, overexpression of 11β-HSD1 further augmented TNF-α-induced iNOS, IL-6, and MCP-1 expression. Moreover, 11β-HSD1 inhibitors attenuated TNF-α-induced phosphorylation of NF-κB p65 and p38-, JNK-, and ERK1/2-MAPK. Collectively, the present study provides novel evidence that inflammatory stimuli-induced 11β-HSD1 in activated preadipocytes intensifies NF-κB and MAPK signaling pathways and results in further induction of proinflammatory molecules. Not limited to 3T3-L1 preadipocytes, we also demonstrated that the notion was reproducible in the primary SVF cells from obese mice. These findings highlight an unexpected, proinflammatory role of reamplified glucocorticoids within preadipocytes in obese adipose tissue.

11β-hydroxysteroid dehydrogenase type 1; preadipocyte; nuclear factor-κB; mitogen-activated protein kinase; adipose inflammation

OBESE ADIPOSE TISSUE IS CHARACTERIZED by low-grade, chronic inflammation (24, 58). In humans and rodents, it has been shown that intracellular glucocorticoid reactivation is exaggerated in obese adipose tissue (38). Two isoenzymes, 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) and type 2 (11β-HSD2), catalyze interconversion between hormonally active cortisol and inactive cortisone (2). In particular, 11β-HSD1 is abundantly expressed in adipose tissue and preferentially reactivates cortisol from cortisone (2). In contrast, 11β-HSD2 inactivates cortisol mainly in tissues involved in water and electrolyte metabolism (60). Transgenic mice overexpressing 11β-HSD1 in adipose tissue display a cluster of fuel dyshomeostasis (61). Conversely, systemic 11β-HSD1 knockouts and adipose-specific 11β-HSD2 overexpressors, which mimic adipose-specific 11β-HSD1 knockouts, are completely protected against diabetes and dyslipidemia on a high-fat diet (14, 30, 31, 42). Interestingly, 11β-HSD1 knockout mice on a high-fat diet showed preferential accumulation of subcutaneous adipose tissue, whereas wild-type mice accumulated considerable fat pads also in visceral (mesenteric) adipose tissue (39). These findings suggest that increased activity of 11β-HSD1 in adipose tissue contributes to dysfunction of adipose tissue and subsequent metabolic derangement.

Adipose tissue is composed of mature adipocytes (∼50–70% of total cells), preadipocytes (∼20–40%), macrophages (∼1–30%), and other cell types (22). Biopsy studies of human adipose tissue demonstrated that the distribution of adipocyte diameter is bimodal, consisting of populations of very small adipocytes ("differentiating preadipocytes") and mature adipocytes (28, 35). Interestingly, the proportion of very small adipocytes was higher in obese people compared with the lean controls (28). Notably, insulin resistance was associated with an expanded population of small adipocytes and decreased expression of differentiation marker genes, suggesting that impairment of adipocyte differentiation may contribute to obesity-associated insulin resistance (35). In this context, a potential link between preadipocyte function and pathophysiology of obese adipose tissue has recently attracted research interest (53, 57).

Many of the genes overexpressed in mature adipocytes are associated with metabolic and secretory function, whereas the most representative function of the genes overexpressed in nonmature adipocytes, i.e., stromal vascular fraction (SVF) cells, is related to inflammation and immune response (9). Macrophage infiltration into obese adipose tissue contributes to local and systemic inflammation in subjects with obesity (63, 65). Furthermore, recent research (12, 48) highlights a pathophysiological role of preadipocytes in obese adipose tissue. In the proinflammatory milieu, preadipocytes act as macrophages (11, 13), share in phagocytic activities (11), and secrete an array of inflammatory substances (13).

A pharmacological dose of glucocorticoids is widely used for anti-inflammatory therapies in human clinics (49). On the other hand, recent research is highlighting the stimulatory effects of glucocorticoids on inflammatory response. Such effects are observed at lower concentrations relevant to phys-

---

Address for reprint requests and other correspondence: H. Masuzaki, Division of Endocrinology and Metabolism, Dept. of Medicine and Clinical Science, Kyoto University Graduate School of Medicine, 54, Shogoin Kawaharacho, Sakyoku, Kyoto, 606-8507, Japan (e-mail: hiroaki@kuhp.kyoto-u.ac.jp).

iological stress in vivo (35, 55, 66). Therefore, the potential role of 11β-HSD1 in a variety of inflammatory responses has stimulated academic interest (10, 26). Furthermore, it is known that mature adipocytes abundantly express 11β-HSD1, which is related to adipocyte dysfunction in obese adipose tissue (44, 61). On the other hand, the role of 11β-HSD1 in SVF cells remains largely unclear.

In this context, the present study was designed to explore the expression, regulation, and pathophysiological role of 11β-HSD1 in activated preadipocytes. The results demonstrate that inflammatory stimuli-induced 11β-HSD1 reinforces NF-κB and MAPK signals and results in induction of proinflammatory molecules.

MATERIALS AND METHODS

Reagents and chemicals. All reagents were of analytical grade unless otherwise indicated. TNF-α, IL-1β, LPS, and carbenoxolone (3, 52), a nonselective inhibitor for 11β-HSD1 and 11β-HSD2, were obtained from Sigma-Aldrich (St. Louis, MO). The recently developed 11β-HSD1 selective inhibitors 3-(1-adamantyl)-5,6,7,8,9,10-hexahydro[1,2,4]triazolo[4,3-α]azocine trifluoroacetate salt (WO03/065983, inhibitor A; Merck, Whitehouse Station, NJ; Ref. 23) and 2,4,6-trichloro-*N*-(5,5-dimethyl-7-oxo-4,5,6,7-tetrahydro-1,3-benzothiazol-2-yl) benzenesulfonamide (BVT-3498; Biovitrum, Stockholm, Sweden; Ref. 25) were synthesized according to the patent information.

Polyclonal antibodies against NF-κB p65, phospho-p65, p38 MAPK, phospho-p38, ERK1/2, phospho-ERK1/2, JNK, phospho-JNK, Akt, and phospho-Akt were purchased from Cell Signaling Technology (Beverly, MA). Polyclonal antibodies against SHIP1, PP2A, and MKP-1 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). An antibody against β-actin was purchased from Upstate Biotechnology (Lake Placid, NY). Horseradish peroxidase-conjugated anti-mouse, anti-rat, and anti-rabbit IgG antibodies and ECL Plus Western blotting detection kits were purchased from Amersham Biosciences (Piscataway, NJ).

Cell culture. 3T3-L1 cells (kindly provided by Dr. H. Green and Dr. M. Morikawa, Harvard Medical School, Boston, MA) were maintained in DMEM containing 10% (vol/vol) calf serum at 37°C under 10% CO_2.

Animals. Seventeen-week-old male C57BL/6 and nine-week-old *ob/ob* mice were used for the experiments. Mice were maintained on a standard diet (F-2, 3.7 kcal/g, 12% of kcal from fat, source soybean; Funahashi Farm) or a high-fat diet (Research Diets D12493, 5.2 kcal/g, 60% of kcal from fat, source soybean/lard) under a 14:10-h light-dark cycle at 23°C. The high-fat diet was administered to the diet-induced obese (DIO) mice from 3 to 17 wk of age. Animals were allowed free access to food and water. All animal experiments were undertaken in accordance with the guidelines for animal experiments of the Kyoto University Animal Research Committee.

Isolation of SVF and the mature adipocyte fraction. Subcutaneous (SQ), mesenteric (Mes), and epididymal (Epi) fat deposits were chopped using fine scissors and digested with 2 mg/ml collagenase (Type VIII; Sigma-Aldrich) in DMEM for 1 h at 37°C under continuous shaking (170 rpm). Dispersed tissue was filtered through a nylon mesh with a pore size of 250 μm and centrifuged. Digested material was separated by centrifugation at 1,800 rpm for 5 min. The sedimented SVF and cell supernatant [mature adipocyte fraction (MAF)] were both washed with DMEM. For primary culture experiments, SVF cells from epididymal fat pads were plated in sixwell plates and cultured overnight in DMEM containing 10% (vol/vol) FBS at 37°C under 10% CO_2. After being rinsed with the medium three times, the cells were incubated with or without TNF-α, carbenoxolone, or inhibitor A for 24 h.

Quantitative real-time PCR. Total RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, CA), and cDNA was synthesized using an iScript cDNA synthesis kit (Bio-Rad, Hercules, CA) according to the manufacturer's instruction. The sequences of probes and primers are summarized in Suppl. Table S1 (supplemental data for this article are available at the *Am J Physiol Endocrinol Metab* website). Taqman PCR was performed using an ABI Prism 7300 sequence detection system following the manufacturer's instructions (Applied Biosystems, Foster City, CA). mRNA levels were normalized to those of 18S rRNA.

11β-HSD1 enzyme activity assay. 11β-HSD1 acts as a reductase and reactivates cortisol from cortisone in viable cells (54). In certain substrates, however, such as tissue homogenates or the microsome fraction, 11β-HSD1 acts as a dehydrogenase and inactivates cortisol to cortisone (8). 11β-HSD1 reductase activity in intact cells was measured as reported previously (8). Cells were incubated for 24 h in serum-free DMEM, with the addition of 250 nM cortisone and tritium-labeled tracer $[1,2\text{-}^{3}\text{H}]_2$-cortisone (Muromachi Yakuhin, Kyoto, Japan) for reductase activity and 250 nM cortisol with $[1,2,6,7\text{-}^{3}\text{H}]_4$-cortisol (Muromachi Yakuhin) for dehydrogenase activity. Cortisol and cortisone were extracted using ethyl acetate, evaporated, resuspended in ethanol, separated using thin-layer chromatography in 95:5 chloroform/methanol, and quantified using autoradiography.

To validate inhibitory potency of compounds against 11β-HSD1 with the use of FreeStyle 293 cells transiently transfected with human 11β-HSD1, the enzyme activity assay was carried out with 20 mM Tris · HCl at pH 7.0, 50 μM NADPH, 5 μg protein of microsomal fraction, and 300 nM $[^{3}\text{H}]$cortisone for 2 h. The reaction was stopped by 18β-glycyrrhetinic acid. The labeled cortisol product was captured by mouse monoclonal anti-cortisol antibody, bound to scintillation proximity assay beads coated with protein A, and quantified in a scintillation counter.

ELISA. Monocyte chemoattractant protein-1 (MCP-1) and IL-6 concentrations in the cultured media of 3T3-L1 preadipocytes were measured using ELISA according to the manufacturer's instructions (R&D Systems, Minneapolis, MN).

Western blot analysis. Two days after confluence, 3T3-L1 preadipocytes were stimulated with 10 ng/ml TNF-α in the absence or presence of 11β-HSD1 inhibitors (50 μM carbenoxolone or 10 μM inhibitor A) for 24 h.

For primary culture experiments, SVF from epididymal fat pads were plated in sixwell plates and cultured overnight in DMEM containing 10% (vol/vol) FBS at 37°C under 10% CO_2. After being rinsed with the medium three times, the cells were incubated with or without TNF-α, carbenoxolone, or inhibitor A for 24 h.

After 2-h serum starvation, cells were treated with TNF-α for 10 min to detect NF-κB and MAPK signals. Cells were washed with ice-cold PBS and harvested in lysis buffer (1% wt/vol SDS, 60 mM Tris · HCl, 1 mM Na_3VO_4, 0.1 mg/ml aprotinin, 1 mM PMSF, and 50 nM okadaic acid at pH 6.8) and boiled at 100°C for 10 min. After centrifugation, supernatants were normalized to the protein concentration via the Bradford method and then equal amounts of protein were subjected to SDS-PAGE and immunoblot analysis.

RNA interference. We tested four different small interfering RNA (siRNA) sequences. Stealth RNAi for mouse 11β-HSD1 (MSS205244, MSS205245, and MSS205246) (Invitrogen), and RNA interference (RNAi) for mouse 11β-HSD1 originally designed by an siRNA Design Support System (TaKaRa Bio, Shiga, Japan; sense: 5′-GAAAUGGCAUAUCAUCUGUTT-3′ and antisense: 3′-TTCUUUACCGUAUAGUAGACA-5′). MSS205245 and MSS205246 did not suppress the 11β-HSD1 mRNA level effectively in preliminary experiments. Therefore, we demonstrated the data of MSS205244 [si(1)] and of the originally designed siRNA [si(2)] in this study. According to the manufacturer's protocol, 3T3-L1 preadipocytes were transfected with 10 nM siRNA in antibiotic-free medium using Lipofectamine RNAiMAX (Invitrogen). We assessed the ransfection efficiency using green fluorescent protein (GFP) detection (pmaxGFP), according to the manufacturer's instructions (Amaxa, Cologne, Germany). Fluorescent microscopic observation revealed that more than two-thirds of the cells expressed GFP (data not shown).

Expression vector. A mammalian expression vector encoding Hsd11b1 (Hsd11b1/pcDNA3.1) was constructed by inserting cDNA for mouse 11β-HSD1 into pcDNA3.1 (Invitrogen). 3T3-L1 preadipocytes were detached from culture dishes using 0.25% trypsin. Cells (5×10^6) were mixed with 2 μg plasmid in the solution provided with the cell line Nucleofector Kit V (Amaxa). pcDNA3.1/11β-HSD1 or a control vector was introduced into the cells using electroporation with a Nucleofector (Amaxa) instrument according to the manufacturer's instructions.

Statistical analysis. Data are expressed as the means ± SE of triplicate experiments. Data were analyzed using one-way ANOVA, followed by Student's *t*-tests for each pair of multiple comparisons. Differences were considered significant if $P < 0.05$.

RESULTS

Expression of 11β-HSD1 was elevated in the MAF and in SVF isolated from fat depots in ob/ob mice and DIO mice. Genetic (*ob/ob*) and dietary (DIO) obese models were analyzed. Expression of iNOS, MCP-1, and IL-6, all of which are obesity-related proinflammatory mediators (19, 29, 45, 56), was elevated in the MAF and SVF from both *ob/ob* mice and DIO mice compared with lean littermates (Fig. 1, *A* and *B*). Levels of 11β-HSD1 mRNA in the MAF from obese mice were substantially elevated compared with their lean littermates (*ob/ob*: SQ, 5-fold; Mes, 62-fold) (DIO: SQ, 24-fold; Mes, 460-fold; Fig. 1, *A* and *B*). On the other hand, levels of 11β-HSD1 mRNA in SVF from *ob/ob* mice and DIO mice were also elevated compared with their lean littermates (*ob/ob*: SQ, 3-fold; Mes, 3-fold; and DIO: SQ, 8-fold, Mes, 4-fold; Fig. 1, *A* and *B*).

TNF-α, IL-1β, and LPS augmented 11β-HSD1 mRNA expression and reductase activity in 3T3-L1 preadipocytes. When 3T3-L1 preadipocytes were treated with TNF-α (10 ng/ml) for 24 h, mRNA levels of 11β-HSD1 markedly increased (~4-fold; Fig. 2*iv*). Levels of iNOS, MCP-1, and IL-6 mRNA were concomitantly increased (50-, 70-, and 200-fold, respectively; Fig. 2, *i–iii*). IL-1β (1 ng/ml) and LPS (1,000 ng/ml) substantially augmented 11β-HSD1 mRNA expression in 3T3-L1 preadipocytes (10- and 3-fold vs. control, respectively) (Fig. 2*iv*). Reductase activity of 11β-HSD1 was augmented by TNF-α, IL-1β, and LPS compared with the control (2-, 9-, and 6-fold vs. control, respectively; $P < 0.05$; Fig. 2*v*). Based on the results of 11β-HSD1 activity, TNF-α was used at 10 ng/ml in subsequent experiments. On the other hand, 11β-HSD2 mRNA and the corresponding dehydrogenase activity were undetected not only at the baseline condition but with TNF-α, IL-1β, and LPS treatments (data not shown).

Dexamethasone decreased iNOS, MCP-1, and IL-6 mRNA and protein levels in TNF-α-treated 3T3-L1 preadipocytes. The effects of glucocorticoid on proinflammatory gene expression in TNF-α-treated 3T3-L1 preadipocytes were examined over a wide range of concentrations (10^{-10}, 10^{-9}, 10^{-8}, and 10^{-7} M), representing physiological to therapeutical levels in vivo (5). Dexamethasone (10^{-7} M) decreased mRNA levels of iNOS, MCP-1, and IL-6 (iNOS: 85 ± 2%, MCP-1: 40 ± 16%, and IL-6: 97 ± 1% reduction vs. TNF-α-treated cells) and protein levels in the media (MCP-1: 48 ± 5% and IL-6: 83 ± 1% reduction) in TNF-α-treated 3T3-L1 preadipocytes (Suppl. Fig. S1).

Pharmacological inhibition of 11β-HSD1 attenuated iNOS, MCP-1, and IL-6 mRNA and protein levels in TNF-α-treated 3T3-L1 preadipocytes. The effects of pharmacological inhibition of 11β-HSD1 on proinflammatory gene expression were examined in TNFα-treated 3T3-L1 preadipocytes. In previous in vitro studies, carbenoxolone (CBX), a nonselective inhibitor of 11β-HSD1 and 11β-HSD2, was used at concentrations from 5 to 300 μM (16, 17, 26). To date, an 11β-HSD1-specific

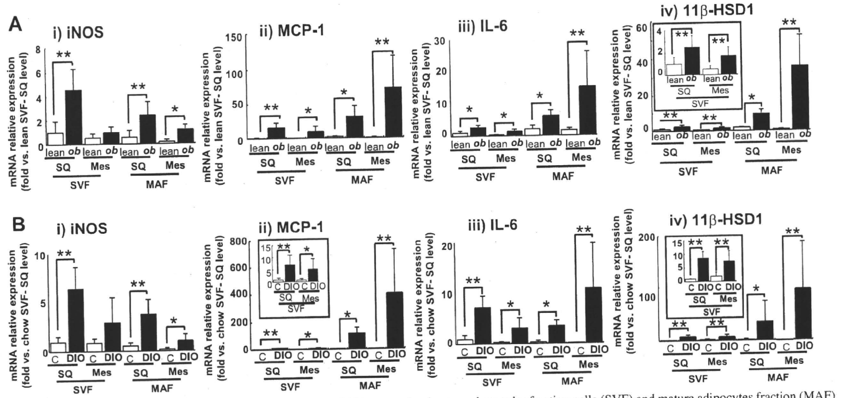

Fig. 1. 11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) mRNA expression in stromal vascular fraction cells (SVF) and mature adipocytes fraction (MAF) isolated from obese adipose tissue of *ob/ob* mice and diet-induced obese (DIO) mice. *A*: *ob/ob* and lean littermates [control (C) 9 wk of age; $n = 6$]. *B*: DIO and littermates on a chow diet (17 wk of age; $n = 6$). Levels of inducible nitric oxide synthase (iNOS; *i*), monocyte chemoattractant protein-1 (MCP-1; *ii*), IL-6 (*iii*), and 11β-HSD1 (*iv*) mRNA in SVF and MAF in subcutaneous abdominal fat depots (SQ) and mesenteric fat depots (Mes). $^*P < 0.05$, $^{**}P < 0.01$ compared with lean littermates.

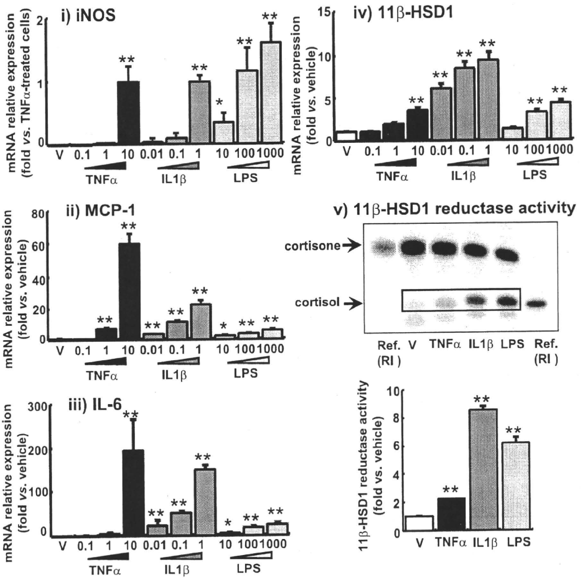

Fig. 2. TNF-α, IL-1β, and LPS augment the expression of proinflammatory mediators and 11β-HSD1 in 3T3-L1 preadipocytes. Cells were treated with TNF-α (0.1, 1, and 10 ng/ml), IL-1β, (0.01, 0.1, and 1 ng/ml) or LPS (10, 100, and 1,000 ng/ml) for 24 h. Levels of iNOS (*i*), MCP-1 (*ii*), IL-6 (*iii*), and 11β-HSD1 (*iv*) mRNA were quantified using real-time PCR. Values were normalized to that of 18S rRNA. *v*: 11β-HSD1 reductase activity (expressed as conversion ability of cortisone to cortisol) was assessed in the medium of 3T3-L1 cells treated with 10 ng/ml TNF-α, 1 ng/ml IL-1β, or 1,000 ng/ml LPS for 24 h. A reference of [^{3}H]cortisone or [^{3}H]cortisol was used as a size marker. A representative autoradiograph of thin-layer chromatography in 11β-HSD reductase activity assay (*top*) and quantification (*bottom*). Intensities of cortisol signals correspond to the enzyme activity of reductase. Ref. (RI), reference samples of [^{3}H]cortisone or [^{3}H]cortisol as size marker. Data are means ± SE of triplicate experiments. *$P < 0.05$, **$P < 0.01$, compared with vehicle (V)-treated group.

inhibitor, inhibitor A, has not been used for in vitro studies; however, another 11β-HSD1-specific inhibitor (compound 544) sharing almost the same structure as inhibitor A was used at a concentration of 5 μM (62). Therefore, in the present study, 10–50 μM CBX and 2.5–10 μM inhibitor A were used.

Before using these inhibitors in intact cells, we validated inhibitory potency of compounds against 11β-HSD1 in the microsome fraction assay. We verified that inhibitor A (10 nM) and CBX (1 μM) inhibited 11β-HSD1 activity as little as 25% vs. control, respectively, and that both of the 11β-HSD inhibitors suppressed 11β-HSD activity in a dose-dependent manner (Suppl. Fig. S2).

In 3T3-L1 preadipocytes, although CBX and inhibitor A did not change the level of 11β-HSD1 reductase activity, both of them suppressed TNF-α-induced reductase activity of 11β-HSD1 in a dose-dependent manner (Fig. 3*A*). CBX (50 μM) and inhibitor A (10 μM) markedly attenuated 11β-HSD1 activity (78 and 60% reduction vs. TNF-α-treated cells, respectively; Fig. 3*A*).

Without TNF-α-treatment, CBX and inhibitor A did not affect mRNA or protein levels of iNOS, MCP-1, and IL-6. On the other hand, in TNF-α-treated cells, these inhibitors reduced the mRNA and protein levels of proinflammatory genes. CBX decreased iNOS, MCP-1, and IL-6 mRNA levels (50 μM; iNOS: 83 ± 5%, MCP-1: 27 ± 4%, and IL-6: 47 ± 10% reduction vs. TNF-α-treated cells without compounds) and protein levels in the media (MCP-1: 17 ± 1% and IL-6: 34 ± 6% reduction) in TNF-α-treated 3T3-L1 preadipocytes (Fig. 3*B*). Similarly, inhibitor A reduced iNOS, MCP-1, and IL-6 mRNA (10 μM; iNOS: 47 ± 13%, MCP-1: 32 ± 12%, and IL-6: 33 ± 9% reduction) and protein levels in the media (MCP-1: 47 ± 3% and IL-6: 14 ± 3% reduction) (Fig. 3*C*).

Effect of 11β-HSD1 knockdown on proinflammatory properties in 3T3-L1 preadipocytes. To explore the potential role of 11β-HSD1 in cytokine release from activated preadipocytes, 11β-HSD1 was knocked down using siRNA. We tested four different siRNA sequences as described in MATERIALS AND METHODS; however, two of them did not suppress 11β-HSD1 mRNA level significantly in the preliminary experiments. Thus we demonstrated the data on si(1) and si(2).

When 3T3-L1 preadipocytes were transfected with 11β-HSD1 siRNA, TNF-α-induced expression of 11β-HSD1 was markedly attenuated [si(1): 60 ± 9% and si(2): 88 ± 7% reduction vs. negative control siRNA; Fig. 4*A*, *i*]. 11β-HSD1 reductase activity was also decreased by 11β-HSD1 siRNA [si(1): 81 ± 9% and si(2): 84 ± 3% reduction vs. negative control siRNA; Fig. 4*A*, *ii*]. 11β-HSD2 mRNA levels and the corresponding dehydrogenase activity were under detectable with or without siRNA treatments in 3T3-L1 preadipocytes (data not sown). Negative control RNAi did not influence the expression of 11β-HSD1. Knockdown of 11β-HSD1 in TNF-α-treated 3T3-L1 preadipocytes effectively reduced iNOS, MCP-1, and IL-6 mRNA levels [si(1): IL-6: 32 ± 7% reduction; and si(2): iNOS: 37 ± 8%, MCP-1: 22 ± 5%, and IL-6: 59 ± 3% reduction] and protein levels in the media [si(1):

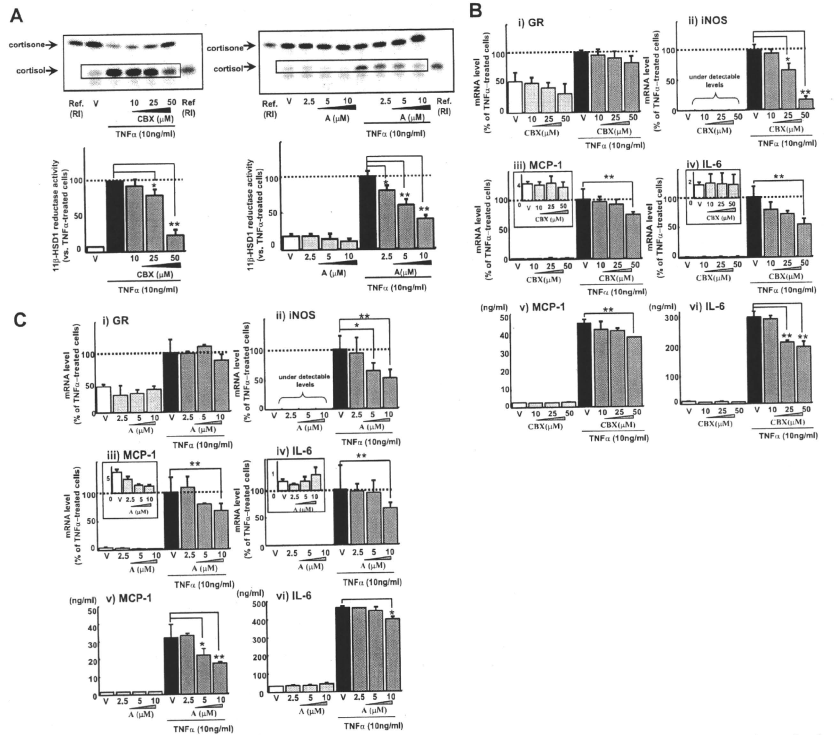

Fig. 3. Effects of pharmacological inhibition of 11β-HSD1 on glucocorticoid receptor (GR), MCP-1, IL-6, and iNOS expression in and secretion from TNF-α-treated 3T3-L1 preadipocytes. *A*: 11β-HSD1 activity assay for validation of 11β-HSD1 inhibitors. 3T3-L1 preadipocytes were incubated for 24 h in serum-free DMEM, adding 250 nM of cortisone with tritium-labeled cortisone. A representative autoradiograph of TLC for the 11β-HSD1 activity assay (*top*) and quantification of 11β-HSD1 activities (*bottom*). Intensities of cortisol signals correspond to the reductase activity. The *y*-axis shows percent 11β-HSD1 reductase activity compared with TNF-α (10 mg/ml)-treated cells. carbenoxolone (CBX; 10–50 μM) and inhibitor A (A; 2.5–10 μM) substantially reduced 11β-HSD1 activity in 3T3-L1 preadipocytes. CBX (*B*; 10–50 μM) and inhibitor A (*C*; 2.5–10 μM) 3T3-L1 preadipocytes were treated with TNF-α (10 ng/ml) or cotreated with CBX and inhibitor A for 24 h. GR (*i*), iNOS (*ii*), MCP-1 (*iii*), and IL-6 (*iv*) mRNA levels were determined using real-time PCR. Values were normalized to that of 18S rRNA and expressed relative to TNF-α-treated cells. Concentrations of MCP-1 (*v*) and IL-6 (*vi*) in the medium were measured with ELISA. Data are means ± SE of triplicate experiments. *$P < 0.05$, ** $P < 0.01$, compared with TNF-α-treated cells.

MCP-1: 13 ± 1% and IL-6: 17 ± 1% reduction; and si(2): MCP1: 19 ± 7% and IL-6: 30 ± 1% reduction; Fig. 4*B*].

Overexpression of 11β-HSD1 augmented iNOS, MCP-1, and IL-6 in TNF-α-treated 3T3-L1 preadipocytes. We examined whether overexpression of 11β-HSD1 is relevant to the augmentation of proinflammatory molecules in activated preadipocytes. The extent of 11β-HSD1 overexpression in 3T3-L1 preadipocytes was assessed by 11β-HSD1 mRNA levels and reductase activity (Fig. 5*A*). As expected, 11β-HSD1 mRNA level was increased by treatment of the 11β-HSD1 vector (~20-fold) or 10 ng/ml TNF-α (~300-fold) compared with the vehicle. TNF-α-induced expression of 11β-HSD1 was further augmented by the introduction of the 11β-HSD1 vector (1.6-fold vs. empty vector). Reductase activity of 11β-HSD1 was also increased by the introduction of the vector (2-fold) or 10 ng/ml TNF-α (10-fold). Notably, TNF-α-induced enzyme activity was further augmented by the vector (1.3-fold vs. empty vector).

Expression of iNOS, MCP-1, and IL-6 did not differ between the 11β-HSD1 vector and the empty vector. On the other hand, TNF-α-induced expression of iNOS, MCP-1, and IL-6 was augmented in 11β-HSD1 transfectants (MCP-1: 172 ±

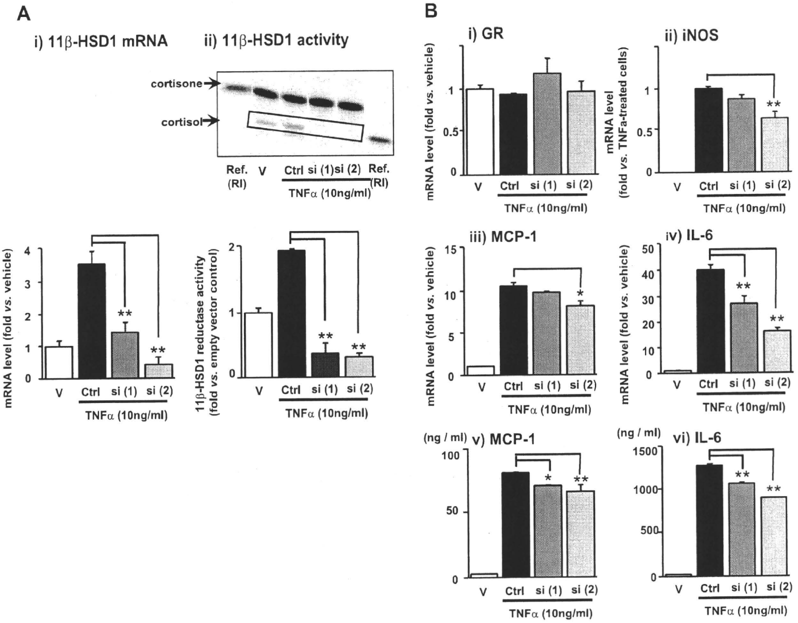

Fig. 4. Effects of 11β-HSD1 knockdown on TNF-α-induced expression of 11β-HSD1 in 3T3-L1 preadipocytes. Cells were transfected with either RNA interference for mouse 11β-HSD1 or a negative control (Ctrl). After 12 h incubation, cells were treated with 10 ng/ml TNF-α for 24 h. *A*: efficiency of 11β-HSD1 knockdown by small-interfering RNA. 11β-HSD1 mRNA (*i*) and reductase activity (*ii*). *B*: effects of knockdown of 11β-HSD1 on MCP-1, IL-6, and iNOS expression in and secretion from TNFα-treated 3T3-L1 preadipocytes. 11β-HSD1 (*i*), GR (*ii*), iNOS (*iii*), MCP-1 (*iv*), and IL-6 mRNA (*v*) levels were determined using real-time PCR. Values were normalized to that of 18S rRNA and expressed as a relative level vs. vehicle control (V). Concentrations of MCP-1 (*vi*) and IL-6 (*vii*) in the medium were measured with ELISA. Data are means ± SE of triplicate experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with TNF-α-treated cells. siRNA for mouse 11β-HSD1: si(1): MSS205244 (Invitrogen) and si(2): sense: 5′-GAAAUGGCAUAUCAUCUGUTT-3′ and antisense: 3′-TTCUUUACCGUAUAGUAGACA-5′ (Takara).

88%, IL-6: 194 ± 64%, and iNOS: 187 ± 47% vs. the empty vector; Fig. 5*B*, *ii-iv*). Similarly, protein levels of MCP-1 and IL-6 in the media were increased in transfectants (MCP-1: 206 ± 32% and IL-6: 156 ± 17% vs. the empty vector; Fig. 5*B*, *v* and *vi*).

Pharmacological inhibition of 11β-HSD1 attenuated TNF-α-induced NF-κB and MAPK signaling in 3T3-L1 preadipocytes. We examined the possible involvement of 11β-HSD1 in proinflammatory signaling pathways. 3T3-L1 preadipocytes were incubated with TNF-α (10 ng/ml), with or without CBX (50 μM) and inhibitor A (10 μM) for 24 h. After a 2-h serum starvation, the cells were incubated with TNF-α (10 ng/ml), with or without CBX (50 μM) and inhibitor A (10 μM) for 10 min. TNF-α-induced p-65 phosphorylation was markedly attenuated by CBX (30 ± 12% decrease vs. TNF-α-treated cells) and inhibitor A (51 ± 11% decrease vs. TNF-α-treated cells; Fig. 6*A*). Regarding MAPK signaling, augmented phosphorylation of p-38, JNK, and ERK with the TNF-α treatment was substantially attenuated by CBX (p-38: 26 ± 8% decrease and JNK: 48 ± 3% decrease vs. TNF-α-treated cells) and inhibitor A (p-38: 51 ± 9% decrease, JNK: 72 ± 5% decrease, and ERK: 36 ± 11% decrease vs. TNF-α-treated cells; Fig. 6*B*).

Pharmacological inhibition of 11β-HSD1 attenuated iNOS, MCP-1, and IL-6 mRNA levels in SVF cells from ob/ob mice. We examined the effects of pharmacological inhibition of 11β-HSD1 on proinflammatory gene expression in primary cultured SVF cells isolated from epididymal fat depots in obese *ob/ob* mice or lean control mice.

CBX (50 μM) and inhibitor A (10 μM) did not change the expression level of 11β-HSD1 (Fig. 7*i*). CBX decreased mRNA level of iNOS, MCP-1, and IL-6 in both the basal state (iNOS: 69 ± 4%, MCP1: 42 ± 7%, and IL-6: 56 ± 14% reduction vs. vehicle control) and TNFα-stimulated state (iNOS: 58 ± 11%, MCP-1: 63 ± 5%, and IL-6: 53 ± 8% reduction vs. TNFα-treated cells without compounds) in SVF cells from *ob/ob* mice.

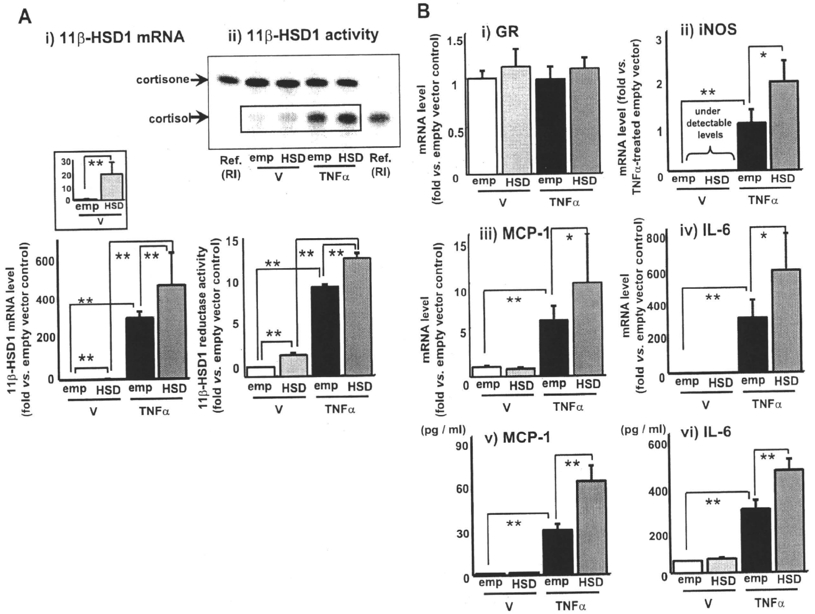

Fig. 5. Effects of overexpression of 11β-HSD1 on MCP-1, IL-6, and iNOS expression in and secretion from TNF-α-treated 3T3-L1 preadipocytes. *A*: efficiency of electroporation-mediated gene transfer. 3T3-L1 preadipocytes were transfected with the expression vector for 11β-HSD1 or a corresponding empty vector using electroporation. After 48 h, cells were treated with or without 10 ng/ml TNF-α for 24 h. Cells were assayed for 11β-HSD1 mRNA (*i*) and reductase activity (*ii*). *B*: effects of overexpression of 11β-HSD1 on MCP-1, IL-6, and iNOS expression in and secretion from TNF-α-treated 3T3-L1 preadipocytes. 3T3-L1 preadipocytes were transfected as above, and 48 h after the infection, cells were treated with or without 10 ng/ml TNF-α for 24 h. Levels of mRNA for GR (*i*), iNOS (*ii*), MCP-1 (*iii*), and IL-6 (*iv*) were determined using real-time PCR. Values were normalized to those of 18S rRNA and expressed as a relative level vs. the vehicle control (V). Concentrations of MCP-1 (*v*) and IL-6 (*vi*) in the medium were measured with ELISA. Data are means ± SE of triplicate experiments. $^{*}P < 0.05$, $^{**}P < 0.01$.

Without TNF-α-treatment, CBX did not change mRNA levels of iNOS, MCP-1 and IL-6 in SVF cells from lean control mice. However, CBX reduced the mRNA levels of iNOS, MCP-1, and IL-6 (iNOS: 64 ± 18%, MCP-1: 67 ± 14%, and IL-6: 58 ± 12% reduction vs. TNF-α-treated cells without compounds) in TNF-α-treated SVF cells from lean control mice (Fig. 7).

Pharmacological inhibition of 11β-HSD1 attenuated NF-κB and MAPK signaling in SVF cells from ob/ob mice. SVF cells from *ob/ob* or lean control mice were incubated with TNF-α (10 ng/ml), with or without CBX (50 μM) and inhibitor A (10 μM) for 24 h. After a 2-h serum starvation, the cells were incubated with TNF-α (10 ng/ml), with or without CBX (50 μM) and inhibitor A (10 μM) for 10 min. Activation of NF-κB (p65) and MAPK (p38, JNK, and ERK) signaling did occur in SVF cells from *ob/ob* mice compared with lean control (Suppl. Fig. S3). In *ob/ob* mice, phosphorylation of these signaling without TNF-α treatment was attenuated by CBX and inhibitor A. TNF-α-induced p-65, p38, JNK, and ERK phosphorylation was also attenuated by CBX and inhibitor A in SVF cells from both *ob/ob* and lean control mice (Suppl. Fig. S3).

DISCUSSION

Here we provide novel evidence that inflammatory stimuli-induced 11β-HSD1 in activated preadipocytes intensifies NF-κB and MAPK signaling pathways and the resultant augmentation of proinflammatory molecules. Not limited to 3T3-L1 preadipocytes, we also demonstrated the notion was reproducible in the primary SVF cells from obese mice. Previous works focused on the metabolically beneficial impact of 11β-HSD1 deficiency on adipose tissue distribution, fuel homeostasis, and insulin sensitivity. On the other hand, clearly distinct from previous works, our present study is the first to highlight an unexpected, proinflammatory role of reamplified glucocorticoids within activated preadipocytes in obese adipose tissue.

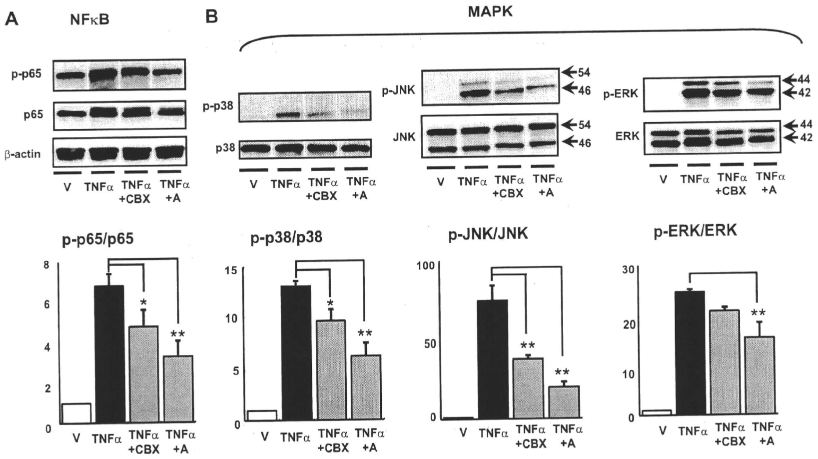

Fig. 6. Effects of inhibition of 11β-HSD1 on TNF-α-induced NFκB and MAPK signaling. NFκB (*A*) and MAPK (*B*) signaling pathways. 3T3-L1 preadipocytes were treated with 10 ng/ml TNF-α for 24 h in the presence or absence of 11β-HSD1 inhibitors (CBX or inhibitor A). After 2-h serum starvation, cells were treated with TNF-α in the presence or absence of 11β-HSD1 inhibitors for 10 min to assess the activation of NFκB and MAPK signaling pathways. Western blot analyses were performed using antibodies against β-actin and NFkB-p65 (*A*), phospho-p65 (*B*), p38-MAPK (*B, left*), phospho-p38 (*B, center*) JNK, phospho-JNK (*B, right*) ERK 1/2, and phospho-ERK1/2. A representative Western blot (*top*) and quantification of p65, p38, JNK, and ERK phosphorylation (*bottom*). Data are means ± SE of triplicate experiments. $*P < 0.05$, $**P < 0.01$ compared with TNF-α-treated cells.

Suppression and overexpression experiments with 11β-HSD1 in activated preadipocytes demonstrate that TNF-α-induced 11β-HSD1 further augments the expression of proinflammatory genes including iNOS, MCP-1, and IL-6. Elevation of iNOS, MCP-1, and IL-6 in adipose tissue is commonly observed in obese subjects, linking to dysfunction of adipose tissue (19, 29, 45, 56). For example, iNOS-deficient mice are protected against obesity-induced insulin resistance and glucose intolerance (45). Moreover, transgenic mice overexpressing MCP-1 in adipose tissue exemplify insulin resistance and exaggerated infiltration of macrophages into adipose tissue (29). Previous studies (20, 36) showed that adipose tissue is a primary production site for IL-6 in humans. In fact, circulating IL-6 levels are shown to elevate in patients with insulin-resistance (19, 56), impaired glucose tolerance (40), and type 2 diabetes (47). Taken together, the present study provides novel evidence for proinflammatory role of 11β-HSD1 in activated preadipocytes.

To optimize experimental condition, the present study was designed to eliminate possible toxic effects and nonspecific effects of 11β-HSD1 inhibitors. Because 11β-HSD2 mRNA and corresponding dehydrogenase enzyme activity (8, 27) were undetected in 3T3-L1 preadipocytes even after the treatment with TNF-α (unpublished observations), CBX virtually serves as a specific inhibitor against 11β-HSD1 in the present study. To further verify the effect of 11β-HSD1 inhibition on activated preadipocytes, we confirmed that an 11β-HSD1-specific inhibitor A exerted similar effects to CBX (Fig. 3). Of note, the expression level of the glucocorticoid receptor did not vary by the treatment with 11β-HSD1 inhibitors (unpublished observations). The notion that TNF-α-induced 11β-HSD1 would reinforce the expression of proinflammatory genes was endorsed by the results of RNAi experiments (Fig. 4) and overexpression experiments (Fig. 5). It should be emphasized that forced overexpression of 11β-HSD1 per se did not influence the expression level of proinflammatory genes in nonactivated preadipocytes (Fig. 5*B*). These findings led us to speculate that 11β-HSD1-mediated active glucoorticoids within cells reinforce inflammation under proinflammatory conditions commonly seen in obese adipose tissue.

The present study demonstrated that 11β-HSD1 was highly expressed in SVF cells from obese adipose tissue (Fig. 1). Although mature adipocytes abundantly express 11β-HSD1 (44, 61), a considerable amount of 11β-HSD1 expression was detected in SVF from adipose tissue (Fig. 1). Potential link between preadipocyte function and pathophysiology of obese adipose tissue has recently attracted research interest (53, 57). A recent study (14) using 11β-HSD1 knockout mice provided evidence that 11β-HSD1 in preadipocytes may affect fat distribution under overnutrition. In 3T3-L1 cells, the expression level of 11β-HSD1 is lower in preadipocytes but is dramatically increased during the course of differentiation into mature adipocytes (51). In fact, active glucocorticoids generated intracellularly by 11β-HSD1 are critical for normal adipose differentiation (33). On the other hand, TNF-α augments 11β-HSD1 expression in preadipocytes (Fig. 2). Of note, in proinflammatory milieu, TNF-α inhibits adipocyte differentiation by decreasing PPARγ expression (43, 46, 64). Depending on the

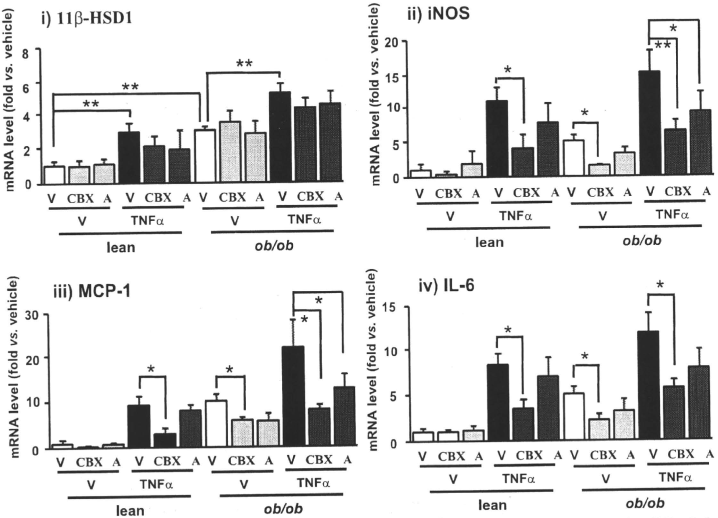

Fig. 7. Effects of pharmacological inhibition of 11β-HSD1 on iNOS, MCP-1, and IL-6 mRNA levels in SVF cells from *ob/ob* mice. SVF cells from *ob/ob* mice and lean control mice were treated with CBX (50 μM) or inhibitor A (10 μM), with or without TNF-α (10 ng/ml) for 24 h. 11β-HSD1 (*i*), iNOS (*ii*), MCP-1 (*iii*), and IL-6 mRNA (*iv*) levels were determined using real-time PCR. Values were normalized to that of 18S rRNA and expressed relative to lean control. Data are means ± SE of triplicate experiments. $*P < 0.05$, $**P < 0.01$.

hormonal milieu, it is therefore conceivable that 11β-HSD1 plays a role in both adipogenesis and inflammatory response in preadipoytes.

We assessed the expression of Pref-1 (a representative molecular marker for preadipocytes; Ref. 7) as well as aP2, PPARγ2, and GLUT4 (a set of representative markers for differentiated adipocytes; Refs. 32 and 59) in preadipocytes overexpressing 11β-HSD1. Consequently, forced augmentation of 11β-HSD1 did not affect the expression level of these genes (Suppl. Fig. S4), supporting that a line of our observation was not a facet of mature adipocytes but of preadipocytes.

Previous studies demonstrated that chronic inflammation is closely associated with insulin resistance in insulin-sensitive organs (24, 64). Glucocorticoids are widely used as anti-inflammatory agents in a clinical setting (49). On the other hand, this hormone simultaneously causes insulin resistance (4, 50). Regarding this apparent paradox, recent studies (34, 55) suggest that reactivated glucocorticoids within cells have the potential to enhance inflammatory or immune responses in a variety of cells. In the present study, replenished dexamethasone in the culture media at pharmacological doses did decrease the synthesis and secretion of proinflammatory molecules in preadipocytes in a dose-dependent manner (Fig. 3). On the other hand, in activated preadipocytes, 11β-HSD1 intensifies TNF-α-induced activation of NF-κB and the MAPK signaling cascade (Fig. 6). In this context, it is possible that intracellular activation of glucocorticoids within physiological range would likely cause proinflammatory responses in certain cell types. It should be noted that preadipocytes possess very few insulin receptors (51). Instead, preadipocytes express a large number of IGF-1 receptors (18). Insulin can bind to the IGF-1 receptor only at supraphysiological concentrations. However, it is likely that increased release of inflammatory cytokines from activated preadipocytes may aggravate insulin receptor signaling in adjacent mature adipocytes in obese adipose tissue. This notion is supported by a line of mouse experiments showing that pharmacological inhibition of 11β-HSD1 ameliorated diabetes, dyslipidemia, and even arteriosclerosis (1, 23).

PPARγ agonists potently suppress the activity of 11β-HSD1 exclusively in adipose tissue (6). The present finding that amplified glucocorticoids within activated preadipocytes may enhance inflammatory responses does not contradict the notion that PPARγ agonists exert potent anti-inflammatory effects in a variety of cell types (37).

Recent studies showed that phosphoinositide 3-kinase (PI3K)-Akt pathways, IL-1 receptor-associated kinase-M (IRAK-M), and suppressors of cytokine signaling-1 (SOCS-1) are negative regulators of NF-κB and MAPK signaling (21). Under inflammatory stimuli, a physiological dose of glucocorticoids positively regulates the expression of SHIP1, a phosphatase that negatively regulates PI3K signaling, resulting in the activation of NF-κB and MAPK in activated macrophages (67). Considering the close biological similarities between activated preadipocytes and activated macrophages (11, 13), we explored whether PI3K-Akt pathways, SHIP1, or other phosphatases could be involved in the 11β-HSD1-induced NF-κB and MAPK activation. Western blot analyses indicated that phosphorylation of Akt or protein levels of SHIP1, PP2A, or MKP-1 did not change significantly with inhibition or overexpression of 11β-HSD1 (Suppl. Fig. S5). Further studies are warranted to unravel the entire mechanism.

In summary, the present study provides novel evidence that inflammatory stimuli-induced 11β-HSD1 reinforces NF-κB and MAPK signaling pathways and results in further induction of proinflammatory molecules in activated preadipocytes. Our findings highlight an unexpected, inflammatory role of reactivated glucocorticoids within preadipocytes in obese adipose tissue.

ACKNOWLEDGMENTS

We thank A. Ryu, S. Maki, M. Nagamoto, T. Fukui, Y. Kobayashi, S. Yamauchi, and K. Takahashi for assistance.

GRANTS

This work was supported in part by a Grant-in-Aid for Scientific Research (B2:19390248), the Takeda Medical Research Foundation, and the Lilly Research Foundation.

DISCLOSURES

No conflicts of interest are declared by the author(s).

REFERENCES

1. **Alberts P, Nilsson C, Selen G, Engblom LO, Edling NH, Norling S, Klingstrom G, Larsson C, Forsgren M, Ashkzari M, Nilsson CE, Fiedler M, Bergqvist E, Ohman B, Bjorkstrand E, Abrahmsen LB.** Selective inhibition of 11 beta-hydroxysteroid dehydrogenase type 1 improves hepatic insulin sensitivity in hyperglycemic mice strains. *Endocrinology* 144: 4755–4762, 2003.
2. **Andrew R, Phillips DI, Walker BR.** Obesity and gender influence cortisol secretion and metabolism in man. *J Clin Endocrinol Metab* 83: 1806–1809. 1998.
3. **Andrews RC, Rooyackers O, Walker BR.** Effects of the 11 beta-hydroxysteroid dehydrogenase inhibitor carbenoxolone on insulin sensitivity in men with type 2 diabetes. *J Clin Endocrinol Metab* 88: 285–291, 2003.
4. **Asensio C, Muzzin P, Rohner-Jeanrenaud F.** Role of glucocorticoids in the physiopathology of excessive fat deposition and insulin resistance. *Int J Obes Relat Metab Disord* 28 *Suppl* 4: S45–52, 2004.
5. **Balachandran A, Guan H, Sellan M, van Uum S, Yang K.** Insulin and dexamethasone dynamically regulate adipocyte 11beta-hydroxysteroid dehydrogenase type 1. *Endocrinology* 149: 4069–4079, 2008.
6. **Berger J, Tanen M, Elbrecht A, Hermanowski-Vosatka A, Moller DE, Wright SD, Thieringer R.** Peroxisome proliferator-activated receptor-gamma ligands inhibit adipocyte 11beta-hydroxysteroid dehydrogenase type 1 expression and activity. *J Biol Chem* 276: 12629–12635, 2001.
7. **Boney CM, Fiedorek FT Jr, Paul SR, Gruppuso PA.** Regulation of preadipocyte factor-1 gene expression during 3T3–L1 cell differentiation. *Endocrinology* 137: 2923–2928, 1996
8. **Bujalska IJ, Kumar S, Stewart PM.** Does central obesity reflect "Cushing's disease of the omentum"? *Lancet* 349: 1210–1213, 1997.
9. **Cancello R, Henegar C, Viguerie N, Taleb S, Poitou C, Rouault C, Coupaye M, Pelloux V, Hugol D, Bouillot JL, Bouloumie A, Barbatelli G, Cinti S, Svensson PA, Barsh GS, Zucker JD, Basdevant A, Langin D, Clement K.** Reduction of macrophage infiltration and chemoattractant gene expression changes in white adipose tissue of morbidly obese subjects after surgery-induced weight loss. *Diabetes* 54: 2277–2286, 2005.
10. **Chapman KE, Coutinho AE, Gray M, Gilmour JS, Savill JS, Seckl JR.** The role and regulation of 11beta-hydroxysteroid dehydrogenase type 1 in the inflammatory response. *Mol Cell Endocrinol* 301: 123–131, 2009.
11. **Charriere G, Cousin B, Arnaud E, Andre M, Bacou F, Penicaud L, Casteilla L.** Preadipocyte conversion to macrophage. Evidence of plasticity. *J Biol Chem* 278: 9850–9855, 2003.
12. **Chung S, Lapoint K, Martinez K, Kennedy A, Boysen Sandberg M, McIntosh MK.** Preadipocytes mediate lipopolysaccharide-induced inflammation and insulin resistance in primary cultures of newly differentiated human adipocytes. *Endocrinology* 147: 5340–5351, 2006.
13. **Cousin B, Munoz O, Andre M, Fontanilles AM, Dani C, Cousin JL, Laharrague P, Casteilla L, Penicaud L.** A role for preadipocytes as macrophage-like cells. *FASEB J* 13: 305–312, 1999.
14. **De Sousa Peixoto RA, Turban S, Battle JH, Chapman KE, Seckl JR, Morton NM.** Preadipocyte 11beta-hydroxysteroid dehydrogenase type 1 is a keto-reductase and contributes to diet-induced visceral obesity in vivo. *Endocrinology* 149: 1861–1868, 2008.
16. **Dembinska-Kiec A, Pallapies D, Simmet T, Peskar BM, Peskar BA.** Effect of carbenoxolone on the biological activity of nitric oxide: relation to gastroprotection. *Br J Pharmacol* 104: 811–816, 1991.
17. **Elsen FP, Shields EJ, Roe MT, Vandam RJ, Kelty JD.** Carbenoxolone induced depression of rhythmogenesis in the pre-Botzinger complex. *BMC Neurosci* 9: 46, 2008.
18. **Entingh-Pearsall A, Kahn, CR.** Differential roles of the insulin and insulin-like growth factor-i (igf-i) receptors in response to insulin and IGF-I. *J Biol Chem* 279: 38016–38024, 2004.
19. **Fernandez-Real JM, Vayreda M, Richart C, Gutierrez C, Broch M, Vendrell J, Ricart W.** Circulating interleukin 6 levels, blood pressure, and insulin sensitivity in apparently healthy men and women. *J Clin Endocrinol Metab* 86: 1154–1159, 2001.
20. **Fried SK, Bunkin DA, Greenberg AS.** Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: depot difference and regulation by glucocorticoid. *J Clin Endocrinol Metab* 83: 847–850, 1998.
21. **Fukao T, Koyasu S.** PI3K and negative regulation of TLR signaling. *Trends Immunol* 24: 358–363, 2003.
22. **Hauner H.** Secretory factors from human adipose tissue and their functional role. *Proc Nutr Soc* 64: 163–169, 2005.
23. **Hermanowski-Vosatka A, Balkovec JM, Cheng K, Chen HY, Hernandez M, Koo GC, Le Grand CB, Li Z, Metzger JM, Mundt SS, Noonan H, Nunes CN, Olson SH, Pikounis B, Ren N, Robertson N, Schaeffer JM, Shah K, Springer MS, Strack AM, Strowski M, Wu K, Wu T, Xiao J, Zhang BB, Wright SD, Thieringer R.** 11beta-HSD1 inhibition ameliorates metabolic syndrome and prevents progression of atherosclerosis in mice. *J Exp Med* 202: 517–527, 2005.
24. **Hotamisligil GS.** Inflammation and metabolic disorders. *Nature* 444: 860–867, 2006.
25. **Hult M, Shafqat N, Elleby B, Mitschke D, Svensson S, Forsgren M, Barf T, Vallgarda J, Abrahmsen L, Oppermann U.** Active site variability of type 1 11beta-hydroxysteroid dehydrogenase revealed by selective inhibitors and cross-species comparisons. *Mol Cell Endocrinol* 248: 26–33, 2006.
26. **Ishii T, Masuzaki H, Tanaka T, Arai N, Yasue S, Kobayashi N, Tomita T, Noguchi M, Fujikura J, Ebihara K, Hosoda K, Nakao K.** Augmentation of 11beta-hydroxysteroid dehydrogenase type 1 in LPS-activated J774.1 macrophages–role of 11beta-HSD1 in pro-inflammatory properties in macrophages. *FEBS Lett* 581: 349–354, 2007.
27. **Jamieson PM, Chapman KE, Edwards CR, Seckl JR.** 11 beta-hydroxysteroid dehydrogenase is an exclusive 11 beta- reductase in primary cultures of rat hepatocytes: effect of physicochemical and hormonal manipulations. *Endocrinology* 136: 4754–4761, 1995.
28. **Julien P, Despres JP, Angel A.** Scanning electron microscopy of very small fat cells and mature fat cells in human obesity. *J Lipid Res* 30: 293–299, 1989.
29. **Kanda H, Tateya S, Tamori Y, Kotani K, Hiasa K, Kitazawa R, Kitazawa S, Miyachi H, Maeda S, Egashira K, Kasuga M.** MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. *J Clin Invest* 116: 1494–1505, 2006.
30. **Kershaw EE, Morton NM, Dhillon H, Ramage L, Seckl JR, Flier JS.** Adipocyte-specific glucocorticoid inactivation protects against diet-induced obesity. *Diabetes* 54: 1023–1031, 2005.
31. **Kotelevtsev Y, Holmes MC, Burchell A, Houston PM, Schmoll D, Jamieson P, Best R, Brown R, Edwards CR, Seckl JR, Mullins JJ.** 11beta-hydroxysteroid dehydrogenase type 1 knockout mice show attenuated glucocorticoid-inducible responses and resist hyperglycemia on obesity or stress. *Proc Natl Acad Sci USA* 94: 14924–14929, 1997.
32. **Lane MD, Tang QQ, Jiang MS.** Role of the CCAAT enhancer binding proteins (C/EBPs) in adipocyte differentiation. *Biochem Biophys Res Commun* 266: 677–683, 1999.

33. **Liu Y, Sun Y, Zhu T, Xie Y, Yu J, Sun WL, Ding GX, Hu G.** 11β HSD1 promotes differentiation of 3T3-L1 preadipocyte. *Acta Pharmacol Sin* 28: 1198–204, 2007.
34. **McEwen BS, Biron CA, Brunson KW, Bulloch K, Chambers WH, Dhabhar FS, Goldfarb RH, Kitson RP, Miller AH, Spencer RL, Weiss JM.** The role of adrenocorticoids as modulators of immune function in health and disease: neural, endocrine and immune interactions. *Brain Res Brain Res Rev* 23: 79–133, 1997.
35. **McLaughlin T, Sherman A, Tsao P, Gonzalez O, Yee G, Lamendola C, Reaven GM, Cushman SW.** Enhanced proportion of small adipose cells in insulin-resistant vs insulin-sensitive obese individuals implicates impaired adipogenesis. *Diabetologia* 50: 1707–1715, 2007.
36. **Mohamed-Ali V, Goodrick S, Rawesh A, Katz DR, Miles JM, Yudkin JS, Klein S, Coppack SW.** Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. *J Clin Endocrinol Metab* 82: 4196–4200, 1997.
37. **Moller DE, Berger JP.** Role of PPARs in the regulation of obesity-related insulin sensitivity and inflammation. *Int J Obes Relat Metab Disord* 27 *Suppl* 3: S17–21, 2003.
38. **Montague CT, O'Rahilly S.** The perils of portliness: causes and consequences of visceral adiposity. *Diabetes* 49: 883–888, 2000.
39. **Morton NM, Paterson JM, Masuzaki H, Holmes MC, Staels B, Fievet C, Walker BR, Flier JS, Mullins JJ, Seckl JR.** Novel adipose tissue-mediated resistance to diet-induced visceral obesity in 11β-hydroxysteroid dehydrogenase type 1-deficient mice. *Diabetes* 53: 931–938, 2004.
40. **Muller S, Martin S, Koenig W, Hanifi-Moghaddam P, Rathmann W, Haastert B, Giani G, Illig T, Thorand B, Kolb H.** Impaired glucose tolerance is associated with increased serum concentrations of interleukin 6 and co-regulated acute-phase proteins but not TNF-alpha or its receptors. *Diabetologia* 45: 805–812, 2002.
41. **Napolitano A, Voice MW, Edwards CR, Seckl JR, Chapman KE.** 11beta-hydroxysteroid dehydrogenase 1 in adipocytes: expression is differentiation-dependent and hormonally regulated. *J Steroid Biochem Mol Biol* 64: 251–260, 1998.
42. **Ohara Y, Peterson TE, Harrison DG.** Hypercholesterolemia increases endothelial superoxide anion production. *J Clin Invest* 91: 2546–2551, 1993.
43. **Pape ME, Kim KH.** Effect of tumor necrosis factor on acetyl-coenzyme A carboxylase gene expression and preadipocyte differentiation. *Mol Endocrinol* 2: 395–403, 1988.
44. **Paulmyer-Lacroix O, Boullu S, Oliver C, Alessi MC, Grino M.** Expression of the mRNA coding for 11beta-hydroxysteroid dehydrogenase type 1 in adipose tissue from obese patients: an in situ hybridization study. *J Clin Endocrinol Metab* 87: 2701–2705, 2002.
45. **Perreault M, Marette A.** Targeted disruption of inducible nitric oxide synthase protects against obesity-linked insulin resistance in muscle. *Nat Med* 7: 1138–1143, 2001.
46. **Petruschke T, Hauner H.** Tumor necrosis factor-alpha prevents the differentiation of human adipocyte precursor cells and causes delipidation of newly developed fat cells. *J Clin Endocrinol Metab* 76: 742–747, 1993.
47. **Pickup JC, Mattock MB, Chusney GD, Burt D.** NIDDM as a disease of the innate immune system: association of acute-phase reactants and interleukin-6 with metabolic syndrome X. *Diabetologia* 40: 1286–1292, 1997.
48. **Poulain-Godefroy O, Froguel P.** Preadipocyte response and impairment of differentiation in an inflammatory environment. *Biochem Biophys Res Commun* 356: 662–667, 2007.
49. **Rhen T, Cidlowski JA.** Antiinflammatory action of glucocorticoids–new mechanisms for old drugs. *N Engl J Med* 353: 1711–1723, 2005.
50. **Roberge C, Carpentier AC, Langlois MF, Baillargeon JP, Ardilouze JL, Maheux P, Gallo-Payet N.** Adrenocortical dysregulation as a major player in insulin resistance and onset of obesity. *Am J Physiol Endocrinol Metab* 293: E1465–E1478, 2007.
51. **Sakaue H, Ogawa W, Matsumoto M, Kuroda S, Takata M, Sugimoto T, Spiegelman BM, Kasuga M.** Posttranscriptional control of adipocyte differentiation through activation of phosphoinositide 3-kinase. *J Biol Chem* 273: 28945–28952, 1998.
52. **Sandeep TC, Andrew R, Homer NZ, Andrews RC, Smith K, Walker BR.** Increased in vivo regeneration of cortisol in adipose tissue in human obesity and effects of the 11beta- hydroxysteroid dehydrogenase type 1 inhibitor carbenoxolone. *Diabetes* 54: 872–879, 2005.
53. **Schaffler A, Scholmerich J, Buchler C.** Mechanisms of disease: adipocytokines and visceral adipose tissue–emerging role in intestinal and mesenteric diseases. *Nat Clin Pract Gastroenterol Hepatol* 2: 103–111, 2005.
54. **Seckl JR, Walker BR.** Minireview: 11beta-hydroxysteroid dehydrogenase type 1- a tissue-specific amplifier of glucocorticoid action. *Endocrinology* 142: 1371–1376, 2001.
55. **Smoak KA, Cidlowski JA.** Mechanisms of glucocorticoid receptor signaling during inflammation. *Mech Ageing Dev* 125: 697–706, 2004.
56. **Straub RH, Hense HW, Andus T, Scholmerich J, Riegger GA, Schunkert H.** Hormone replacement therapy and interrelation between serum interleukin-6 and body mass index in postmenopausal women: a population-based study. *J Clin Endocrinol Metab* 85: 1340–1344, 2000.
57. **Tchkonia T, Giorgadze N, Pirtskhalava T, Thomou T, DePonte M, Koo A, Forse RA, Chinnappan D, Martin-Ruiz C, von Zglinicki T, Kirkland JL.** Fat depot-specific characteristics are retained in strains derived from single human preadipocytes. *Diabetes* 55: 2571–2578, 2006.
58. **Tilg H, Moschen AR.** Adipocytokines: mediators linking adipose tissue, inflammation and immunity. *Nat Rev Immunol* 6: 772–783, 2006.
59. **Tontonoz P, Hu E, Graves RA, Budavari AI, Spiegelman BM.** mPPAR gamma 2: tissue-specific regulator of an adipocyte enhancer. *Genes Dev* 8: 1224–1234, 1994.
60. **Ulick S, Tedde R, Mantero F.** Pathogenesis of the type 2 variant of the syndrome of apparent mineralocorticoid excess. *J Clin Endocrinol Metab* 70: 200–206, 1990.
61. **Wake DJ, Rask E, Livingstone DE, Soderberg S, Olsson T, Walker BR.** Local and systemic impact of transcriptional up-regulation of 11beta-hydroxysteroid dehydrogenase type 1 in adipose tissue in human obesity. *J Clin Endocrinol Metab* 88: 3983–3988, 2003.
62. **Wamil M, Andrew R, Chapman KE, Street J, Morton NM, Seckl JR.** 7-oxysterols modulate glucocorticoid activity in adipocytes through competition for 11beta-hydroxysteroid dehydrogenase type. *Endocrinology* 149: 5909–5918, 2008.
63. **Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr.** Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 112: 1796–1808, 2003.
64. **Xing H, Northrop JP, Grove JR, Kilpatrick KE, Su JL, Ringold GM.** TNF alpha-mediated inhibition and reversal of adipocyte differentiation is accompanied by suppressed expression of PPARg without effects on Pref-1 expression. *Endocrinology* 138: 2776–2783, 1997.
65. **Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, Chen H.** Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest* 112: 1821–1830, 2003.
66. **Yeager MP, Guyre PM, Munck AU.** Glucocorticoid regulation of the inflammatory response to injury. *Acta Anaesthesiol Scand* 48: 799–813, 2004.
67. **Zhang TY, Daynes RA.** Glucocorticoid conditioning of myeloid progenitors enhances TLR4 signaling via negative regulation of the phosphatidylinositol 3-kinase-Akt pathway. *J Immunol* 178: 2517–2526, 2007.

厚生労働科学研究費補助金（医療技術実用化総合研究事業）
脂肪細胞由来ホルモン、レプチンのトランスレーショナルリサーチ
平成２０年度～平成２２年度　総合研究報告書

発行者　厚生労働科学研究費補助金（医療技術実用化総合研究事業）
脂肪細胞由来ホルモン、レプチンのトランスレーショナルリサーチ
研究代表者　　中尾　一和

連絡先：〒606-8507　京都市左京区聖護院川原町
京都大学大学院医学研究科　内分泌代謝内科
TEL：075-751-3168