equilibrium. Because the prevalence of TT homozygosity in this cohort was only 0.3%, we analyzed it in combination with *CT* and labeled this group *CT/TT*. Logistic regression was performed to calculate the odds ratio (OR) for the *CT/TT* genotypes compared with the *CC* allele homozygote, and MetS was defined according to the IDF and modified NCEP criteria as described above—ie, it included hypertension, hypertriglyceridemia, low HDL cholesterol, elevated fasting glucose, and central obesity—or by specifying a number of components of MetS with the use of age as a covariate. A general linear model was applied to control for age. $P < 0.05$ was considered significant. The data were analyzed by using SAS software (version 8.2; SAS Inc, Cary, NC).

RESULTS

The genotype frequencies for GPX1 Pro198Leu polymorphism were 0.846, 0.151, and 0.003 for *CC*, *CT*, and *TT*, respectively, and the *T* allele frequency was 0.078 among this cohort (**Table 1**). These frequencies are consistent with those expected under Hardy-Weinberg equilibrium. There were no significant differences in the genotype distributions of GPX1 Pro198Leu polymorphism between men and women (Table 1).

The means (and SEs) of the anthropometric variables, lipid or glucose metabolic variables, or blood pressure tested in the *CC* and *CT/TT* genotypes are shown in **Table 2**. In men, there was a significant difference in WHR and a marginal difference in WC between genotypes (these variables were higher in the *CT/TT* genotypes), but no significant differences were observed in other anthropometric measurements. In women, higher body fat mass was detected in the *CT/TT* genotypes than in the *CC* genotype, but there were no significant differences in other anthropometric measurements. Although there were no significant differences in lipid metabolic variables between the genotypes in women, significant differences in triacylglycerol concentrations were detected in men, who had higher triacylglycerol concentrations in the *CT/TT* genotypes. No significant differences in fasting glucose and glycated hemoglobin concentrations were observed between the genotypes, and no difference was seen in the rate of participants of either sex who were previously diagnosed with diabetes mellitus (control group men: 86.1% and 13.9% in the *CC* and *CC/TT* genotypes, respectively; men with diabetes: 85.2% and 14.8% in the *CC* and *CT/TT* genotypes, respectively; $P = 0.7966$, chi-square test; control group women: 82.9% and 17.1% in the *CC* and *CC/TT* genotypes, respectively; women with diabetes: 88.3% and 11.8% in the *CC* and *CC/TT* genotypes, respectively; $P = 0.2763$). However, significantly higher IRI concentrations were observed in the *CT/TT* genotypes than in the *CC* genotype in both men and women. In addition, higher HOMA-IR and HOMA-β concentrations were observed in the *CT/TT* genotypes in both sexes, but the differences were significant only for HOMA-IR in women and for HOMA-β in men. There were significant differences in systolic and diastolic blood pressure between the genotypes only in men, with higher blood pressure in the *CT/TT* genotypes.

The prevalence of MetS and each of the MetS components in the study population is shown in **Table 3**. Compared with the wild type (*CC*), the variant GPX1 (*CT/TT*) was associated with a higher frequency of MetS according to both the IDF and modified NCEP definitions in men but not in women. These variants were also associated with central obesity and hypertriglyceridemia in men but not in women. There were no associations between the genotypes and low HDL-cholesterol concentration, elevated blood pressure or hypertension, or elevated fasting glucose concentrations or diabetes mellitus.

When logistic regression was performed to calculate the OR for the *CT/TT* genotypes as compared with the CC homozygote, with the prevalence of MetS defined according to the 2 different criteria of the IDF definition and the modified NCEP definition that uses age as a covariate, the *CT/TT* genotypes showed a significant association with a higher prevalence of MetS as defined by both criteria in men (OR: 2.02; 95% CI: 1.30, 3.15 for IDF; OR:1.49; 95% CI: 1.02, 2.18 for modified NCEP) but not in women (Table 3). When we examined whether this variant was associated with the prevalence of each component of MetS, no association between the *CT/TT* genotypes and the prevalence of low HDL cholesterol, elevated glucose concentrations or diabetes mellitus, or elevated blood pressure or hypertension was found in either sex. However, when the *CT/TT* genotypes were compared with the *CC* genotype, the OR for the prevalence of central obesity (WC > 90 cm and > 80 cm in men and women, respectively) was 1.93 (95% CI: 1.31, 2.85) for men. The OR for hypertriglyceridemia was 1.52 (95% CI: 1.08, 2.15) for men. However, no association was observed between the *CT/TT* genotypes and the prevalence of central obesity and hypertriglyceridemia among women.

The OR for the risk of showing different numbers of features of MetS for the *CT/TT* genotypes is shown in **Figure 1**. Significantly higher ORs for clusters of 2 to 4 risks of MetS (OR: 1.88; 95% CI: 1.07, 3.31 for 2 risks; OR: 1.97; 95% CI: 1.04, 3.74 for 3 risks; and OR: 2.66; 95% CI: 1.27, 5.56 for 4 risks) were observed in men with the *CT/TT* genotypes. In contrast, in women the only significant OR for this variant was observed at the 3-risk accumulation (OR: 2.05; 95% CI: 1.16, 3.64). In this cohort, only 60 participants (2.7%) showed a 5-risk accumulation.

DISCUSSION

In the present study, we observed that GPX1 Pro198Leu variant is associated with higher WHRs, triacylglycerol concentrations, IRI, HOMA-IR and HOMA-β, and blood pressure levels than is the *CC* genotype in men. In women, however, those with

TABLE 1
Genotype frequency of GPX1 Pro198Leu[1]

	CC	*CT*	*TT*	*CT/TT*
Total (n = 2183)	1846 (84.6)	330 (15.1)	7 (0.3)	337 (15.4)
Men (n = 1105)	948 (85.8)	154 (13.9)	3 (0.3)	157 (14.2)
Women (n = 1078)	898 (83.3)	176 (16.3)	4 (0.4)	180 (16.7)

[1] All values are n; percentage in parentheses. GPX1, glutathione peroxidase 1. *CC*, *CT*, and *TT* in men vs women: $\chi^2 = 2.63$, $P = 0.268$ (chi-square test); *CC* and *CT/TT* in men vs women: $\chi^2 = 2.59$, $P = 0.108$ (chi-square test).

TABLE 2
Anthropometric and metabolic variables according to *GPX1 Pro 198Leu* genotypes[1]

	Men				Women			
	Subject	*CC*	*CT/TT*	*P*	Subject	*CC*	*CT/TT*	*P*
	n				*n*			
Age (y)	1105	59.3 ± 0.4[2]	58.7 ± 0.9	0.566	1078	59.1 ± 0.4	59.6 ± 0.8	0.636
Waist (cm)	1115	82.2 ± 0.3	83.5 ± 0.7	0.072	1078	75.1 ± 0.3[3]	75.8 ± 0.7[3]	0.380
Waist-hip ratio	1105	0.901 ± 0.002	0.911 ± 0.005	0.045	1078	0.827 ± 0.002[3]	0.832 ± 0.005[3]	0.326
BMI (kg/m^2)	1105	22.9 ± 0.1	23.2 ± 0.2	0.165	1078	22.9 ± 0.1	23.1 ± 0.2	0.364
Weight (kg)	1105	62.0 ± 0.3	62.9 ± 0.7	0.293	1078	52.5 ± 0.3[3]	52.6 ± 0.6[3]	0.872
Height (cm)	1105	164.6 ± 0.2	164.5 ± 0.5	0.840	1078	151.4 ± 0.2[3]	150.7 ± 0.4[3]	0.188
Body fat mass (kg)	1103	21.3 ± 0.1	21.6 ± 0.4	0.406	1071	31.5 ± 0.2[3]	32.4 ± 0.4[3]	0.027
Total cholesterol (mmol/L)[4]	1025	5.47 ± 0.03	5.55 ± 0.07	0.290	963	5.86 ± 0.03[3]	5.80 ± 0.07[3]	0.460
LDL cholesterol (mmol/L)[4]	1017	3.40 ± 0.03	3.40 ± 0.07	0.978	948	3.59 ± 0.03[3]	3.56 ± 0.07	0.714
HDL cholesterol (mmol/L)[4]	1025	1.48 ± 0.01	1.51 ± 0.03	0.493	963	1.72 ± 0.01[3]	1.68 ± 0.03[3]	0.208
Triglyceride (mmol/L)[4]	1025	1.46 ± 0.03	1.68 ± 0.08	0.012	963	1.19 ± 0.02[3]	1.25 ± 0.05[3]	0.390
Fasting glucose (mmol/L)[5]	1036	5.73 ± 0.03	5.78 ± 0.07	0.544	1040	5.53 ± 0.03[3]	5.52 ± 0.06[3]	0.873
HbA_{1c} (%)[5]	1035	5.20 ± 0.02	5.25 ± 0.05	0.300	1040	5.14 ± 0.02[3]	5.13 ± 0.04[3]	0.735
IRI (μU/mL)[5]	1036	8.0 ± 0.2	9.2 ± 0.5	0.019	1040	8.1 ± 0.2	9.1 ± 0.4	0.010
HOMA-IR[5]	1036	2.11 ± 0.07	2.45 ± 0.17	0.065	1040	2.03 ± 0.06	2.40 ± 0.13	0.008
HOMA-β[5]	1036	76.6 ± 1.4	86.4 ± 3.5	0.011	1040	87.7 ± 1.6[3]	92.4 ± 3.6	0.231
Systolic blood pressure (mm Hg)[6]	846	120.1 ± 0.6	124.8 ± 1.5	0.004	844	119.4 ± 0.7	121.7 ± 1.6	0.176
Diastolic blood pressure (mm Hg)	846	74.8 ± 0.4	77.8 ± 0.9	0.003	844	72.4 ± 0.4[3]	74.0 ± 0.9[3]	0.112

[1] GPX1, glutathione peroxidase 1; HbA_{1c}, glycated hemoglobin; IRI, immunoneative insulin; HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-β, HOMA of β-cell function. Unpaired Student's *t* tests were used for all analyses.
[2] $\bar{x}$ ± SEM (all such values).
[3] Significantly different from men, $P < 0.05$.
[4] Subjects who received specific treatment for lipid abnormality were excluded.
[5] Subjects who were previously diagnosed with diabetes mellitus or who received hypoglycemia treatment were excluded.
[6] Subjects who received treatment for previously diagnosed hypertension were excluded.

the *CT/TT* genotypes had higher IRI, HOMA-IR, and body fat mass, but no differences were seen in triacylglycerol concentrations, WHRs, and blood pressure levels between genotypes. These results suggest that there is a sex difference in the association between the variant and these variables. In addition, higher body fat mass was observed in women in the *CT/TT* genotypes but not in men. The higher WHR and WC without any difference in body fat mass or BMI in men with the GPX1 Pro198Leu variant suggest that the *CT/TT* genotypes are associated with central obesity in men. On the basis of the role of GPX1 in the antioxidant defense system, it seems that subjects with the *T* allele have lower GPX1 activity, and thus those subjects have a weaker antioxidant defense system than do subjects with the *C* allele. The association between GPX1 198Leu variants and central obesity in men may suggest that a weaker antioxidant defense system or greater oxidative stress might be a causative factor for obesity. Further research will be required to elucidate the interactions between GPX1 and central obesity. It should be noted that the allelic distribution observed among Japanese persons in this study is different from those reported for other ethnicities. In fact, a greater frequency of the *T* allele was reported among other ethnicities—a *T* allele frequency of 0.326, 0.363, and 0.340 in African Americans (15), Finns (14), and Danes (29), respectively—than among Japanese, which indicates that there are ethnicity-specific variations at this position of GPX1.

We also showed that *CT/TT* genotypes were associated with the higher prevalence of MetS as defined by both the IDF and modified NCEP criteria in men but not in women. It should be noted that higher ORs were observed in MetS according to the IDF definition than in MetS according to the modified NCEP definition in men. It is obvious that this difference is attributable to the inclusion of central obesity (large waist) as an essential component of MetS in the IDF criteria. In fact, there is an association between *CT/TT* genotypes and central obesity in men. In addition, these variants are more likely to be associated with a higher prevalence of hypertriglyceridemia in men but not in women. In contrast, there was no association between the *CT/TT* genotypes and a higher prevalence of low HDL cholesterol, elevated blood pressure or hypertension, or elevated fasting glucose concentrations or diabetes mellitus in either sex. The possible explanation for the lack of the association with other components of MetS except for central obesity and hypertriglyceridemia may be a lack of statistical power or the low frequency of the *T* allele in this population. The men with *CT/TT* genotypes were associated with a greater number of MetS components, but no apparent association was observed in women. These results indicate that the GPX1 Pro198Leu variant is associated with the prevalence of MetS and the cluster of risks of MetS in men but not in women, which suggests that GPX1 genetic susceptibility to MetS is dependent on sex.

As described above, we showed that the *CT/TT* genotypes had higher HOMA-IR, as well as higher IRI and HOMA-β, although there was no significant difference in HOMA- IR in men and HOMA-β in women between the *CC* and *CT/TT* genotypes. We also observed no difference in the prevalence of diabetes mellitus between the *CC* and *CT/TT* genotypes and no association between the *CT/TT* genotypes and a greater prevalence of elevated fasting glucose concentrations or diabetes mellitus in either sex.

TABLE 3
The odds ratio (OR) for the metabolic syndrome (MetS) and each of the MetS components[1]

	OR[2] (95% CI)
MetS by IDF	
Men	
Control (n = 970)	
Case (n = 135)	2.02 (1.30, 3.15)
Women	
Control (n = 901)	
Case (n = 177)	1.14 (0.75, 1.75)
MetS by modified NCEP	
Men	
Control (n = 970)	
Case (n = 135)	1.49 (1.02, 2.18)
Women	
Control (n = 901)	
Case (n = 177)	1.40 (0.97, 2.03)
Central obesity	
Men	
Control (n = 900)	
Case (n = 205)	1.93 (1.31, 2.85)
Women	
Control (n = 790)	
Case (n = 288)	1.20 (0.84, 1.72)
High triglyceride concentration	
Men	
Control (n = 746)	
Case (n = 359)	1.52 (1.08, 2.15)
Women	
Control (n = 806)	
Case (n = 270)	1.11 (0.76, 1.62)
Low HDL cholesterol	
Men	
Control (n = 942)	
Case (n = 162)	1.01 (0.63, 1.63)
Women	
Control (n = 842)	
Case (n = 234)	1.22 (0.82, 1.80)
Elevated blood pressure or hypertension	
Men	
Control (n = 612)	
Case (n = 493)	1.25 (0.88, 1.77)
Women	
Control (n = 608)	
Case (n = 469)	1.22 (0.87, 1.73)
Elevated glucose or diabetes mellitus	
Men	
Control (n = 519)	
Case (n = 586)	0.99 (0.70, 1.39)
Women	
Control (n = 667)	
Case (n = 411)	1.17 (0.83, 1.65)

[1] IDF, International Diabetes Federation; NCEP, National Cholesterol Education Program. Logistic regression analysis among men and women was adjusted for age.
[2] The OR for the *CT/TT* genotypes compared to the *CC* homozygote.

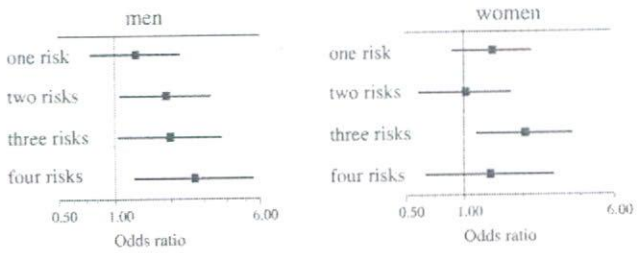

FIGURE 1. Odds ratio (and 95% CIs) of the GPX1 Pro198Leu polymorphism for the cluster of risks of the metabolic syndrome (MetS). The y-axis represents the odds ratio on a log scale for the risks associated with the various numbers of features of MetS for *CT/TT* genotypes (reference group: *CC* genotype). ■, Point estimates from a logistic regression model adjusted for age; error bars represent 95% CIs. Men: 1 risk (n = 353), 2 risks (n = 283), 3 risks (n = 161), and 4 risks (n = 76); women: 1 risk (n = 261), 2 risks (n = 203), 3 risks (n = 154), and 4 risks (n = 84).

The insulin resistance observed in the *CT/TT* genotypes may be compensated for by the elevated β-cell function (insulin secretion). Therefore, there appear to be no apparent differences in the prevalence of diabetes mellitus or of elevated glucose in this cohort.

The reasons for these sex differences are not known. However, the result is consistence with a number of prior reports (30–33). Among coronary heart disease patients, the polymorphism in the LDL receptor–related protein gene, *LRPAP1*, is associated with MetS in women but not in men (30). Regulator of G-protein signaling-2 polymorphism is associated with MetS in men but not in women in European populations (31). The beta2-adrenergic receptor gene (Arg16Gly, Gln27Glu) is associated with MetS only in men in the World Health Organization–Monitoring Trends in Cardiovascular Disease population survey (33). These observations and those of the present study indicate that genetic risk factors for MetS may differ between men and women. Although the biological basis of gene-sex interactions in the etiology of MetS remains to be elucidated, the clustering of the traits making up MetS may be due to pleiotropy, when the same gene or genes influence several traits, or to common environmental determinants. It has been shown that erythrocyte GPX activity was significantly higher in premenopausal than in postmenopausal women and higher in premenopausal women than in age-matched men (34). Estrogen seems to be responsible for the sex-related differences in GPX activity. This sexual dimorphism potentially may be conditioned by the activity of sex hormones, differences in lifestyle, or exposure to various environmental factors. Another possibility is that there may be sex differences in free radical homeostasis (35).

The present study has various strengths and limitations. It was conducted in a representative sample of the population, and therefore a possible bias due to the selection of participants was avoided. These findings may not be generalizable to other populations, given that differences in racial and ethnic attitudes toward lifestyle may influence these results. Limitations include the lack of the lifestyle and dietary data for the participants in the analysis, which may affect the prevalence of MetS.

In the present study, we observed that GPX1 Pro198Leu variants are associated with the prevalence of MetS in Japanese men but not in Japanese women. This result may support the hypothesis that oxidative stress is involved in the pathogenesis of MetS. However, further research is needed to establish whether the GPX1 Pro198Leu polymorphism is also associated with MetS in other populations and ethnicities and whether the functional variants of the potential antioxidative enzymes besides GPX1 may be associated with the prevalence of MetS.

The authors' responsibilities were as follows—MK: contributed to the study concept and design and manuscript writing and editing; AF: contributed to data collection and statistical analysis; AI: contributed to the study concept and design; and HS: helped obtain funding and contributed to the study concept and design, data analysis, and statistical support. None of the authors had a personal or financial conflict of interest.

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Impaired Basal Thermal Homeostasis in Rats Lacking Capsaicin-sensitive Peripheral Small Sensory Neurons

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We studied the effects of selective loss of capsaicin-sensitive primary sensory neurons on thermosensation and thermoregulation in rats. Neonatal capsaicin treatment in rats caused a remarkable decrease in the number of small-diameter neurons in the dorsal root ganglion (DRG) compared with their number in the control rats. Gene expression analysis for various thermo-sensitive transient receptor potential (TRP) channels indicated marked reductions in the mRNA levels of TRPV1 (70%), TRPM8 (46%) and TRPA1 (64%), but not of TRPV2, in the DRG of capsaicin-treated rats compared with those in the control rats. In addition to the heat and cold insensitivity, capsaicin-treated rats showed lower rectal core temperature, higher skin temperature and decreased sensitivity to ambient temperature alteration under normal housing at room temperature, suggesting impaired thermosensation and change in thermoregulation in the rats. Uncoupling protein 1 (UCP1) expression and the thermogenic ability in brown adipose tissues were attenuated in the capsaicin-treated rats. These results indicate a critical role of capsaicin-sensitive sensory neurons in both heat and cool sensation and hence in basal thermal homeostasis, which is balanced by heat release and production including UCP1 thermogenesis, following sensation of the ambient temperature.

Key words: dorsal root ganglion, sensory neuron, thermoregulation, transient receptor potential channel, uncoupling protein 1.

Abbreviations: BAT, brown adipose tissue; DRG, dorsal root ganglia; LE, living environment; TE, test environment; Trectal, rectal temperature; TRP, transient receptor potential; Tskin, tail skin temperature; UCP, uncoupling protein.

Body temperature in homeothermic animals is controlled at a constant level by the balance between heat release and production. Animals sense the ambient temperature at all times, presumably through thermoreceptors belonging to the transient receptor potential (TRP) family of ion channels (temperature-sensitive channels) in primary sensory neurons of the periphery; and, based on the thermosensation, the central nervous system (CNS) regulates the responses to maintain body temperature (*1–3*). Several physiological mechanisms such as cutaneous vasoreaction or insulation are involved in the control of heat loss in animals. In particular, cutaneous vasodilation is the major mechanism for heat loss from the skin surface (*4–6*). Effective heat loss is achieved through various body parts, *e.g.* the hand in humans and the tail in rats, both of which have a high surface-to-volume ratio, absence of hair or fur and a high density of arteriovenous anastomoses. In rats, ~25% of the basic metabolic heat can be dissipated at the tail (*7*).

Likewise, heat production by thermogenic organs, such as muscle and brown adipose tissue (BAT), controlled *via* CNS, is indispensable (*8*). To date, BAT-specific uncoupling protein (UCP1) is known to be the most potent thermogenic protein (*9*, *10*); and the critical role of UCP1 in adaptive non-shivering thermogenesis in a cold environment has been verified by studies using UCP1-deficient mice (*11*, *12*). UCP2 and UCP3, homologues of UCP1, were discovered in 1997, but their roles in thermogenesis seem to be low (*8–10*). With respect to the connection between thermosensation and thermoregulation, Jancso-Gabor *et al.* (*13*) were the first to show that capsaicin treatment of adult rodents causes desensitization of assumed warmth detectors and impaired regulation of rectal core temperature, suggesting the involvement of capsaicin-sensitive neurons in thermoregulation. However, the roles of these sensory neurons

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and their thermoreceptors in the mechanism of thermal homeostasis still remain to be understood.

Recent advances towards understanding the molecular mechanism of thermosensation including that in the noxious range have been made since the cloning of the capsaicin receptor (vanillloid receptor 1, VR1/TRPV1) by Julius' group in 1997 (*14*). TRPV1 is located in small-diameter neurons with unmyelinated C-fibres and thinly myelinated Aδ-fibres in sensory ganglia such as the dorsal root and trigeminal ganglia. After the discovery of TRPV1, a series of other thermoreceptors sensing different ranges of ambient temperature were identified by using the techniques of expression cloning and electrophysiology *in vitro* (*15–23*). Thermoreceptors are gated by noxious heat (>43°C, TRPV1; >52°C, VRL1/TRPV2), warmth to hot (about 25–40°C, TRPV3 and TRPV4), cold to cool (about 15–25°C, TRPM8) and cold (<18°C, ANKTM1/TRPA1). In addition, recent studies using gene knockout mice have clarified the physiological and pathological roles of these receptors. For example, mice lacking TRPV1 show a deficiency in response to noxious heat and in the development of heat hyperalgesia (*24*), but the mutant mice maintain a normal resting body temperature (*25*). TRPV4-knockout mice exhibit an impaired pressure sensation (*26*), whereas circadian body temperature and thermoregulation in the mutant mice were normal at warm environmental temperatures of 25–35°C (*27*). Two studies using TRPA1-deficient mice demonstrated that TRPA1 is essential for transduction of chemical stimuli in nociceptor sensory neurons, although the role of TRPA1 in sensing noxious cold is controversial (*28, 29*). Most recently, three groups have reported a predominant role of TRPM8 in cold sensation by using mice lacking this receptor (*30–32*). Nevertheless, the contribution of these receptors to the regulation of thermal homeostasis *in vivo* remains to be fully understood. To extend our understanding about the roles of primary sensory neurons in thermosensation and basal thermal homeostasis and to assess the involvement of thermoreceptors in the mechanisms, we investigated the thermal responses and heat production in conscious animals with selective loss of capsaicin-sensitive primary afferent neurons in the present study.

MATERIALS AND METHODS

Animals—F344/N pregnant rats were obtained from Japan SLC. On Day 1 after birth, the neonatal rats were injected or not intraperitoneally with capsaicin (Cap, 50 mg/kg of BW; Wako, Japan) dissolved in a solvent of 10% ethanol, 10% Tween-80, and 80% saline, as described earlier (*33*). The newborn rats in both groups were weaned at the age of 4 weeks and reared until they were 9–10 weeks old, during which time they were given a regular chow diet (Labo MR stock, Nihon Nousan Co., Japan) and tap water *ad libitum* under a 12-h:12-h light–dark cycle at 23±1°C. In the present study, five independent experiments were conducted; and a total of 35 (19 females and 16 males) and 33 (13 females and 20 males) animals were used in control and Cap groups, respectively. The physiological analyses were conducted after maturation of the rats (after 8 weeks of age) under the conscious condition, because anaesthetization greatly affects the regulation of body temperature (*34*). After a series of analyses, the rats were killed by decapitation; and tissues including BAT and skeletal muscles were then dissected for RNA and/or protein analyses. The lumbar dorsal root ganglia (DRG, level 1–5) were removed and frozen on dry ice. The average body weights of 9-week-old rats in the control and capsaicin groups were 124.4±2.0 and 119.1±3.3 g, respectively, for females and 187.4±3.9 and 186.1±4.1 g, respectively for males. All experiments were carried out according to the institutional guidelines for animal care and the principles in the Helsinki Declaration.

Physiological Analysis—The hot-plate test was performed in an aluminium cage (D165 mm × W250 mm × H100 mm), the floor temperature of which was controlled at 52±1°C by a hot plate (MODEL PC-420, Corning) beneath it. The floor temperature of the test cage was monitored with a thermal sensor (TD-300, Shibaura Electronics, Tokyo). The latency until rats showed the first signs of discomfort (paw-lifting, -licking or -shaking) was recorded with a cutoff time of 60 s. The cold-plate test was designated to assess any difference in thermal sensation to cold temperature. For this purpose, we used an experimental setup similar to that for the hot-plate test, in which the aluminium cage, which had been placed in a polystyrene box, was surrounded by small ice cubes. The floor temperature of the test cage, monitored as in the hot-plate test, was ~1°C. Rats showed similar signs of discomfort to those in the hot-plate test, although the responses to the cold stress took a longer time compared with those to the hot stress. The shortest and longest latency times in the cold-plate test were 34.6 s in the control group and 216.7 s in the capsaicin group, respectively. In the cold tolerance test, rats were maintained in a cold room (5°C) individually for 5 h. Rectal temperature (Trectal) of the animals was measured every hour with an electronic thermistor equipped with a rectal probe (Mon-a-therm 4070TM, Mallinckrodt Medical Inc., St. Louis, MO). Changes in the skin-surface temperature (Tskin) of conscious rats were recorded at 10-s intervals by use of an infrared thermographic device, Thermotracer (TH5100, NEC San-ei, Tokyo Ltd., Tokyo) or Thermo-Viewer (JTG-5200, JEOL Ltd., Tokyo), as described (*6*), which is a good tool to evaluate heat release in conscious, unrestrained animals non-invasively and successively. We determined the Tskin in the area of the tail, because it has no fur, thus facilitating accurate analysis, and is a crucial site for regulation of heat release in rodents (*4, 35*). After the Tskin of rats in their regular cage (living environment, LE: 23.5–24.3°C) had been recorded for several minutes, the rats were transferred singly to a new cage without wooden chips (test environment, TE: 21.5–22.4°C). This transfer gave the rats a drop of ~1–2°C in the ambient temperature around them. Two cages were set in the field of the device, and the recording for two rats from each group was done at the same time for 5 min after transferring the rats to the TE. The highest skin temperature in a fixed area of the tail was measured by using image analysing software (TH51-701, NEC San-ei, or TG-5000CNTA, JEOL Datum., Tokyo).

Histological Analysis—Horizontal sections (15-μm thickness) of the lumbar DRGs were cut on a cryostat and thaw-mounted on Superfrost slides. The slides were stained with cresyl violet and thionine and observed with OptiPhoto2 (Nikon). The microscopic images were scanned by a digital camera (HC-2000, Fujix). The contours of thionine-stained neurons in the images were traced, and the cell diameter was measured by using a digital caliper (Mitutoyo, Japan).

RNA Analysis—For northern blot analysis, total RNA (10 or 20 μg), isolated from the DRG, BAT or gastrocnemius muscles by use of TRIzol reagent (GIBCO BRL), was electrophoresed on 1.25% formaldehyde–agarose gels. The separated RNA was transferred onto a GeneScreen membrane (NEN™ Life Science Products Inc.; Boston, MA) in 10 × saline sodium citrate (SSC) by capillary blotting and was immobilized by exposure to ultraviolet light (0.35 J). Blots were hybridized with probes (labelled with [^{32}P] dCTP) for the mRNAs of TRPV1, TRPV2, TRPM8, UCP1 and 18S rRNA, as previously described (*36*). The cDNA probes for TRPV1, TRPV2 and TRPM8 mRNAs were produced from positions 81 to 580 of the rat VR1 sequence (GenBank accession No. AF029310), from positions 208 to 676 of the rat VRL1 sequence (GenBank accession No. AF129113), and from positions 446 to 3765 of the rat TRPM8 sequence (GenBank accession No. AY072788), respectively, by using rat DRG total RNA and the reverse transcription PCR technique. The PCR products were sequenced after subcloning into the pCRII or pCR2.1 vector (Invitogen, CA, USA). The blots were hybridized sequentially with the probes after having stripped away the previous probe. Each probe was confirmed to react with the specific mRNA. Hybridization signals were quantified with a Fuji Bioimage Analyzer. Gene expression of TRPA1 was evaluated by the technique of real-time quantitative PCR. After first-strand cDNAs for TRPA1 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNAs had been synthesized with 1 μg total RNA and SuperScript III Reverse Transcriptase (Invitrogen, Carlsbad, CA), the cDNAs were analysed by using Light Cycler FastStrat DNA Master^{PLUS} SYBR Green I and Light Cycler 2.0 system (Roche Diagnostics, Mannheim, Germany). The gene expression of TRPA1 was normalized to the level of that of GAPDH. The sequences of primers used were the following: TRPA1, GAAAACTAAGCAAGTACGAG (forward) and CTCCCAC TGAAATTAGGTAG (reverse); GAPDH, forward primer, ACCACAGTCCATGCCATCAC (forward) and TCCACCA CCCTGTTGCTGTA (reverse).

Biochemical Analysis—Immunodetection of UCP1 and cytochrome oxidase subunit IV (COX IV) was performed by using the mitochondrial fraction isolated from BAT, as described previously (*11*). The protein concentration of the fraction was measured with a BCA protein assay kit (Pierce, Rockford, IL, USA). Equal amounts of mitochondrial protein (2 μg) were separated on 12.5% gels (Daiichi Pure Chemicals; Tokyo, Japan) and transferred onto Immobilon polyvinylidene difluoride membranes (Millipore Corporation; Bedford, MA, USA). The membranes were incubated with affinity-purified rabbit polyclonal antibodies specific for UCP1 (STRATA GENE, USA) or monoclonal antibody specific for COX IV (Molecular Probes, Inc., USA). After the secondary antibody reaction for 1 h at room temperature, the specific signals were detected by using an ECL kit (Amersham Pharmacia Biotech). The resulting images were quantified with NIH Image (version 1.63). Thermogenic activity was evaluated by using the mitochondrial fraction isolated from BAT, as previously described (*37*). Briefly, [^{3}H] GDP-binding was measured by incubation of 1 mg/ml mitochondria with 1 μM [8, 5′-^{3}H] GDP (specific radioactivity 32.5 Ci/mmol, PerkinElmer Life Sciences, Boston, MA, USA) in the presence or absence of unlabelled 1 mM GDP at 37°C for 15 min in an assay buffer of 100 mM sucrose, 0.1 mg/ml fatty acid-free bovine serum albumin, 2 μM rotenone and 10 mM sodium Na–Tris (hydroxymethyl) methyl-2-aminoethane sulphonic acid (pH 7.2). After the reaction mixture had been centrifuged at 8,500*g* for 5 min, the pellets were recovered, dissolved in 20 μl of 5% SDS, and then transferred into vials for scintillation counting. The mitochondrial samples pooled from six rats were used for Scatchard plot analysis.

Statistical Analysis—Data were expressed as means ± SE. The statistical significance of the data was assessed by using the unpaired Student's *t*-test or repeated measure analysis of variance (ANOVA).

RESULTS

Reduction in Small-diameter Neurons and Expression of TRPV1, TRPM8 and TRPA1 in DRG of Capsaicin-treated Rats—Capsaicin treatment of neonatal rats resulted in a marked reduction in the small-diameter DRG neurons (Fig. 1). The number of neurons with the diameter <20 μm in Cap rats reduced to 23% of the control rats. The number of small-diameter neurons (20–<30 μm) in Cap rats was about a half compared to that in the control rats. These results indicated a marked loss of small-diameter neurons in the DRG of Cap rats. On the other hand, there was no significant difference in the number of medium-diameter neurons (30–<40 μm) between the control and Cap groups. The numbers of medium- to large-diameter neurons (40–<50 and 50–<60 μm) were rather greater in Cap rats than in the control rats. When the effect of the capsaicin treatment on the expression of TRP channels in the DRG was examined by northern blot analysis (Fig. 2A), an ~70% decrease in the TRPV1 mRNA level in the DRG was found in Cap rats. The results also indicated a significant reduction (about 46%) in the TRPM8 mRNA level in the DRG of Cap rats, compared with that of the control rats; however, there was no difference in the TRPV2 mRNA level between the two groups. Moreover, a marked reduction (about 64%) in the TRPA1 mRNA level was detected in Cap rats (Fig. 2B).

Distinct Thermosensation and Thermoregulation in Rats Lacking Capsaicin-sensitive Neurons—To ascertain the functional defect expected for the rats lacking capsaicin-sensitive small sensory neurons in their DRGs, we first examined the response of rats to noxious heat. In the hot-plate test, Cap rats exhibited a significantly longer response latency than the control

rats (Fig. 3A). We then performed the cold-plate test to examine whether the reduction in small sensory neurons affected the sensation of cold temperature as well (Fig. 3B). When the rats were put on a cold plate (1°C), they showed several signs of discomfort such as paw-lifting, -licking and -shaking, which were quite similar to those in the hot-plate test. There was a tendency for a longer latency in the Cap group than in the control group ($P=0.0895$), although the latency time was not significantly different between the two groups.

We also performed a cold tolerance test to examine whether the reduction in small sensory neurons affected the regulation of body temperature (Trectal) in the cold (Fig. 3C). The core temperature was slightly but significantly lower in Cap rats (37.3 ± 0.1°C) than in the control rats (37.6 ± 0.1°C) at the room temperature of 23°C (Time 0). When the rats were exposed to the cold at 5°C, Cap rats retained tolerance against the cold just like the control rats did; whereas the decrease in the Trectal of rats in the first 1 h seemed to be slow in Cap group (Δ 0.2°C) compared with that in the control group (Δ 0.6°C). The Trectal of rats was reduced 0.8 and 0.6°C by 5 h of cold exposure in the control and Cap group, respectively. We then measured the Tskin, an index of heat release, by using an infrared thermographic device. In contrast to the Trectal (Fig. 3C), the Tskin in the regular cage was slightly higher in Cap group than in the control group (27.1 ± 0.2 and 26.7 ± 0.1°C, respectively, $P<0.01$; Fig. 3D). To further test the sensitivity to a small change in ambient temperature, the rats were transferred from their LE (LE, 23.5–24.3°C) to the TE (TE, 21.5–22.4°C), a drop of ~1–2°C. The Tskin of rats immediately decreased just after the change in ambient temperature in both groups (at 0 time), though the initial fall in Tskin was considerably smaller (~1.7°C) in Cap rats than in the control rats (~2.7°C). After that, the Tskin in the control rats was kept at a constant level, while the Tskin in Cap rats further decreased gradually and became close to the level in the control rats by 5 min (Fig. 3D).

Decrease in UCP1 Thermogenic Ability in the BAT of Capsaicin-treated Rats—To determine whether the lack of capsaicin-sensitive neurons affected the thermogenic ability, we examined the expression of UCPs in major thermogenic tissues; *i.e.* BAT and skeletal muscles. As shown in Fig. 4A, we detected a decrease in the steady-state level of UCP1 mRNA in the BAT of Cap group (about 80% of the control). There were no

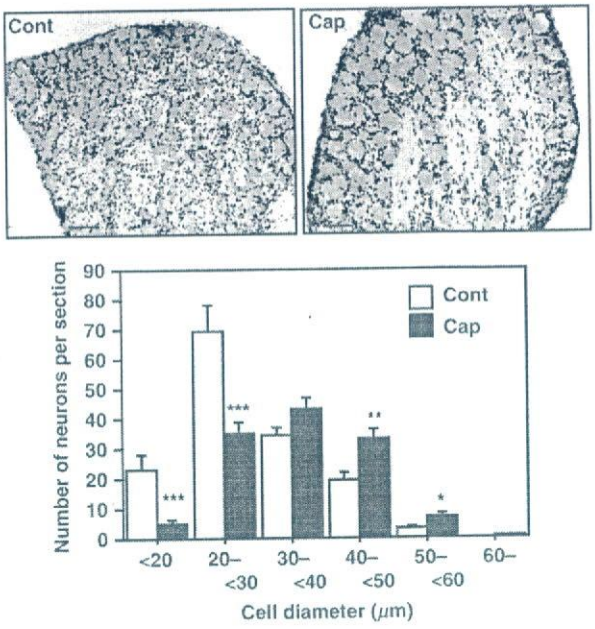

Fig. 1. **Histological analysis of the DRG in rats with neonatal treatment of capsaicin.** Tissue sections of the lumbar DRGs in the control (Cont) and capsaicin-treated (Cap) rats were stained with cresyl violet and thionine. Representative images are shown (scale bar, 100 μm). Cell soma diameter of the DRG neurons was measured and the data are expressed as mean ± SE. The numbers of sections analysed were 14 for the control group and 18 for capsaicin group. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ versus control group.

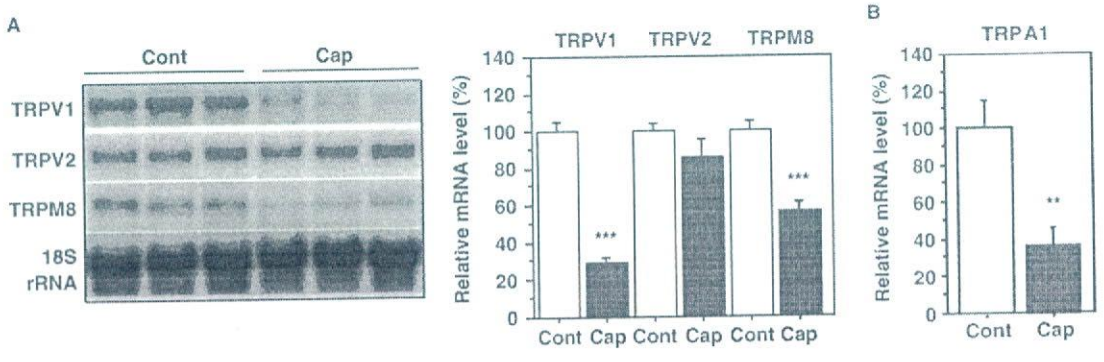

Fig. 2. **mRNA levels of TRPs in the DRG of rats.** (A) mRNA of TRPV1, TRPV2 and TRPM8 was analysed by northern blots using total RNA (10 μg) from DRGs of the control (Cont) and capsaicin-treated (Cap) rats, as described in MATERIALS AND METHODS section. The relative levels of mRNA for TRPs are expressed as means ± SE after normalization by 18S rRNA levels. The numbers of rats were 28 and 32 (for TRPV1 and TRPM8), and 12 and 11 (for TRPV2), in the control and capsaicin groups, respectively. $^{***}P<0.001$ versus control group. (B) mRNA of TRPA1 was measured by real-time PCR and expressed as relative to GAPDH mRNA. There was no significant difference in the GAPDH mRNA levels between the groups. Data are expressed as means ± SE ($n=8$ for control group, $n=9$ for capsaicin group). $^{**}P<0.01$ versus control group.

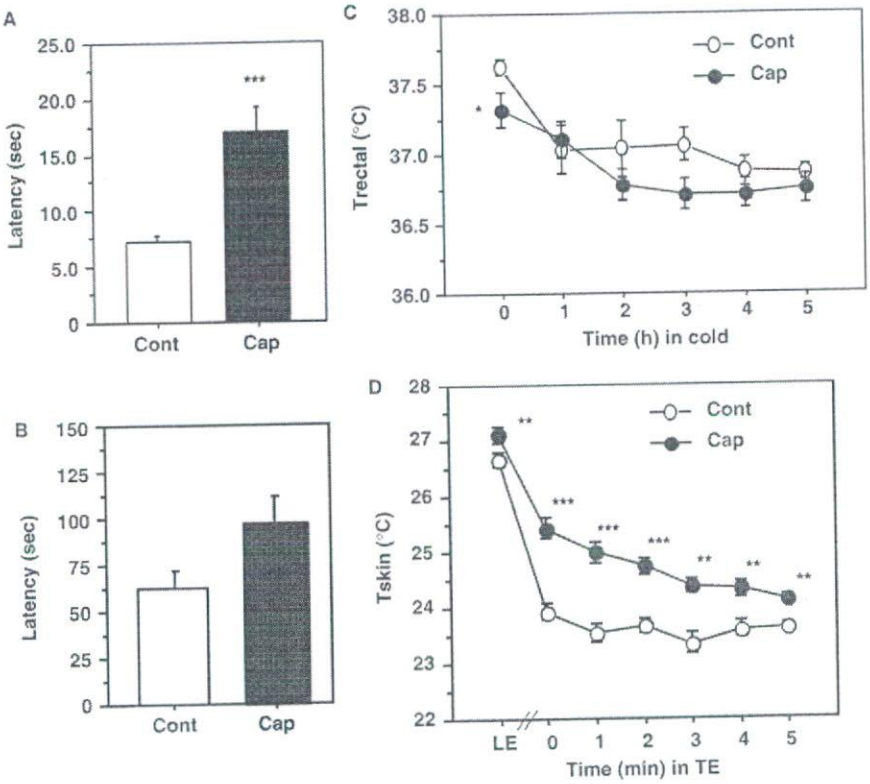

Fig. 3. **Thermal responses to various stimuli in the control (Cont) and capsaicin-treated (Cap) rats.** (A) Hot-plate test at 52 ± 1°C ($n = 24$ for the control group, $n = 22$ for capsaicin group). (B) Cold-plate test at 1°C ($n = 8$ for the control group, $n = 10$ for capsaicin group). (C) Cold tolerance test. Rectal temperature (Trectal) of the rats exposed to a cold temperature of 5°C was measured as described in MATERIALS AND METHODS section. The data for time 0 are those obtained at 23°C ($n = 13$ for the control group, $n = 16$ for capsaicin group). (D) Regulation of heat release in response to a small change in ambient temperature. Tail skin temperature (Tskin, an index of heat release) of the unrestrained rat ($n = 6$ for each groups) was recorded for 5 min by an infrared thermographic device before and after the rats had been transferred from their living environment (LE: 23.5–24.3°C) to the test environment (TE: 21.5–22.4°C). Data are expressed as means ± SE. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ versus control group.

significant differences in UCP2 and UCP3 levels in the BAT or skeletal muscles between the two groups (data not shown). The decreased expression of UCP1 in the BAT of Cap rats was confirmed by the decrease in protein level, which was 67% of the control (Fig. 4B). In contrast, the COX IV level in Cap group was 30% higher than that in the control group. The GDP-binding activity, a marker of thermogenic activity, was significantly lower in the BAT of Cap rats than in that of the control ones (Fig. 4C). In addition, Scatchard plot analysis showed a considerable decrease in total GDP-binding sites (Bmax), but not in binding affinity (Kd; Fig. 4D), in Cap rats compared with those in the control rats.

DISCUSSION

To understand the mechanism of thermal homeostasis, many studies have been performed previously. Jancso-Gabor *et al.* (*13*) for the first time reported the effect of capsaicin treatment of adult rodents on thermoregulation. After this, capsaicin has been used as a tool to study the mechanism of thermoregulation as well as that of nociception, leading to the discovery of the capsaicin receptor, TRPV1 (*14*). Later Osaka *et al.* (*38*, *39*) demonstrated the role of capsaicin-sensitive nerve fibres in the regulation of BAT thermogenesis and thermal homeostasis by using anaesthetized rats. However, the role of capsaicin-sensitive neurons in the sensation of ambient temperature in conscious animals remained unknown. Likewise, the degree of desensitization by capsaicin treatment of mature rodents in previous studies is unclear. In the present study, therefore, we used neonatal capsaicin treatment, which is different from the desensitized rat model, as it is well established that a single capsaicin administration selectively destroys small sensory neurons in the peripheral ganglia in newborn rats, but not in mature rats (*33*, *40*).

Indeed, Mezey *et al.* (*41*) showed that the capsaicin treatment of neonatal rats significantly decreased the number of TRPV1-positive cells in their DRG, whereas the treatment did not affect the expression and/or distribution of the mRNA in the CNS except in the spinal trigeminal nucleus. Our results of the histological and RNA analyses of DRG also suggested a marked

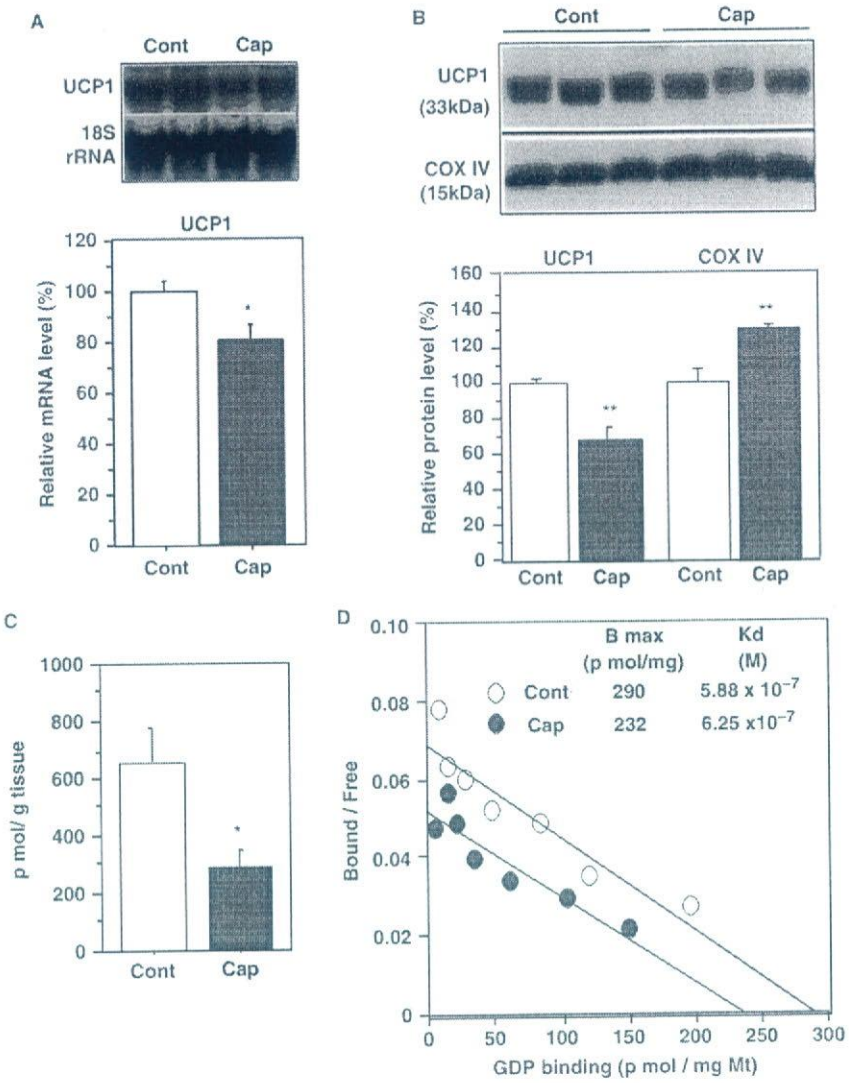

Fig. 4. **Reduction in UCP1 expression and thermogenic ability in the BAT of capsaicin-treated rats.** (A) Northern blots using total RNA (20 μg) of BAT from the control (Cont) and capsaicin-treated (Cap) rats were hybridized with the probes for UCP1 or 18S rRNA as described in MATERIALS AND METHODS section. The numbers of rats were 23 and 26 in the control and capsaicin groups, respectively. (B) Immunodetection of UCP1 and COX IV. (C) GDP-binding activity. (D) Scatchard plot analysis. Western blot analysis and GDP-binding assay using mitochondrial proteins isolated from the BAT of rats were performed as described in MATERIALS AND METHODS section. Data are expressed as means ± SE ($n = 6$ for each group). $^{*}P < 0.05$ and $^{**}P < 0.01$ versus control group.

reduction in the number of small sensory neurons expressing TRPV1 in the DRG of Cap rats. On the other hand, an increase in the numbers of medium- to large-diameter neurons was detected in the DRG of Cap rats. We presently do not know the reason; however, there may be a compensatory mechanism for the loss of small-diameter neurons in Cap rats. In addition, we found that the loss of capsaicin-sensitive small-diameter neurons was associated with a marked decrease in the mRNA level of TRPA1. This result may be reasonable, because TRPA1 is co-expressed with TRPV1 in a subset of nociceptive sensory neurons (*42*). We also found a significant reduction in the mRNA level of TRPM8 in the DRG of Cap rats. The present data suggest that TRPM8 was expressed in a subpopulation of TRPV1-expressing neurons in the rat DRG, which is consistent with the report of McKemy *et al.* (*18*), although the co-expression of these receptors in same subpopulation was controversial (*19*, *42*). Story *et al.* (*22*) have demonstrated that NGF treatment of DRG neurons isolated from adult rats elicited co-expression of TRPM8 and TRPV1 in culture. One may consider another possibility that the death of capsaicin-sensitive neurons caused a decrease in TRPM8-expressing neurons by some

unknown mechanism. Although the expression of TRPV3 in DRGs of monkeys and humans was reported previously (*21*, *23*), we could not detect a clear signal for TRPV3 in the rat DRG, in agreement with Peier *et al.* (*20*). These observations led us to the experiments to examine the effects of loss of the small sensory neurons expressing TRPV1, TRPA1 and/or TRPM8 on thermoregulation in rats.

To assess the responsiveness of rats to the ambient temperature, we used four different methods, *i.e.* the hot-plate test for the sensation of high temperature (~52°C), cold-plate test for the sensation of low temperature (~1°C), cold tolerance test for the regulation of body temperature in the cold (~5°C) and infrared thermography for the sensation of mild temperature (~23°C). As a result of these methods, rats lacking capsaicin-sensitive small sensory neurons displayed not only the heat-insensitivity but also a tendency to be insensitive to noxious cold, suggesting that heat and cool sensors are in the same subset of small sensory neurons. A reduction in the neurons expressing TRPM8 and/or TRPA1 might cause this insensitivity of cold sensation in rats, as suggested by knockout studies (*29–32*). Moreover, we found novel phenotypes indicating changes in thermal homeostasis in Cap rats. Particularly, the thermographic analysis revealed greater heat release in Cap rats than in the control ones. This finding may indicate an adaptive response to dissipate excess heat for the control of body temperature, which was significantly lower in Cap rats than in the control rats; because heat release from the body surface to the environment is huge and profoundly affects the regulation of body temperature (*6*, *39*, *43*). In addition, Cap rats were significantly insensitive to a small change in ambient temperature around 23°C as well as to noxious temperature, suggesting that capsaicin-sensitive small sensory neurons in DRG is responsible to sense the mild temperature. This may be related to a considerable decrease in the mRNA level of TRPM8, which functional range covers the ambient temperature in a normal animal facility (*18*, *19*). In the cold tolerance test, the change in Trectal after 1 h of cold exposure was smaller in Cap rats than in the control rats. Since TRPA1 senses the lowest temperature (<18°C) among the members of the thermosensitive TRP channel family (*22*), the blunt response of Trectal to acute cold exposure in Cap rats could be associated with the decrease in the number of TRPA1 and an impaired sensation of cold temperature.

Adaptive thermogenesis is an important response in mammals to environmental alterations such as cold and excessive food intake in order to maintain body temperature or energy homeostasis (*8–10*). BAT and skeletal muscle are the principal thermogenic organs and dominantly express UCP1 and UCP3, respectively. The critical role of UCP1 in adaptive thermogenesis in the cold was verified by studies using UCP1-knockout mice (*11*, *12*). With respect to the effect of capsaicin on the regulation of body fat, Cui and Himms-Hagen (*44*) reported an atrophy of and decrease in the UCP1 level in the BAT of the capsaicin-desensitized rats; however, the functional change in thermosensation was not determined. Our results showed a significant decrease in BAT theromogenic ability in Cap rats, which displayed a lowered core temperature and an increased heat release under normal housing conditions. Interestingly, these phenotypes were contrast to those in UCP1-knockout mice, in which thermosensation seems to be normal but the core temperature tended to be higher than that in wild-type mice (*45*). Several groups including our group have demonstrated that induction of UCP1-independent thermogenesis and strong vasoconstrictor response contributed to the regulation of body temperature in mice lacking UCP1 under the thermal conditions of ~20–25°C in the normal animal facilities (*6*, *12*, *45*). The difference in thermoregulation between Cap rats and UCP1-knockout mice may be related to that of the target tissue in these animal models. Namely, capsaicin-sensitive afferent neurons were destroyed and hence several types of thermo-sensitive channels were markedly reduced in Cap rats, which caused abnormal integration of thermosensation and affected the mechanisms to maintain body temperature such as thermogenesis in BAT (an effector of thermoregulation). In UCP1-knockout mice, however, BAT thermogenesis was eliminated by the target manipulation of *Ucp1* gene but thermosensation was normal, in which UCP1-independent thermogenesis was induced and heat conservation was strengthened for homeothermic regulation. Taken together, the results in the two animal models suggest that normal recognition of ambient temperature *via* thermosensory neurons is a crucial factor to elicit proper thermal responses in homeothermic regulation. Furthermore, the changes in thermoregulation in Cap rats could be related to a decline in sympathetic nervous activity, because norepinephrine stimulates UCP1 expression and BAT thermogenesis and simultaneously has a vasoconstriction effect, which decreases peripheral blood flow and suppresses heat loss from the body (*12*, *43*). Scatchard plot analysis indicated a decrease in BAT thermogenic capacity and a reduced demand for UCP1 thermogenesis in Cap rats. The present data also suggest a role of UCP1 in the regulation of basal thermal homeostasis. If there is an intimate interplay between thermosensation and thermoregulation, how is the thermoregulation in mice lacking TRP channels such as TRPM8 and TRPA1? Unfortunately, the studies using knockout mice did not look into the consequences of loss of cold sensitivity on thermoregulation and core temperature, as pointed out by Nilius and Voets (*46*). Because body temperature is controlled by the balance between heat loss and production, the changes in thermosensation by gene knockout of TRP channels may affect thermal responses such as thermogenesis through regulation of UCP1 expression.

Thus, the present study indicated a critical role of capsaicin-sensitive small sensory neurons in thermosensation not only of noxious heat but also of mild room temperature. The change in thermosensation by loss of the capsaicin-sensitive neurons profoundly affected basal thermal homeostasis, which is balanced by heat release and production. It is plausible that the decreased sensitivity of Cap rats to a change in the ambient temperature in the LE was originated, at least in part, from the marked reduction in the numbers of TRPM8- and/or TRPA1-expressing neurons, coincident with the reduction in TRPV1-expressing small-diameter neurons

relating to another phenotype of heat insensitivity. In addition, UCP1 thermogenesis appears to be involved in basal thermal homeostasis in rats. However, further studies such as those using double-knockout animals are required to understand the precise mechanisms of the cross-talk between thermosensation and thermoregulation and their contribution to basal thermal homeostasis *in vivo*.

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Induction of fatty acid-binding protein 3 in brown adipose tissue correlates with increased demand for adaptive thermogenesis in rodents

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ABSTRACT

We investigated the contribution of fatty acid-binding protein 3 (FABP3) to adaptive thermogenesis in brown adipose tissue (BAT) in rodents. The expression of FABP3 mRNA in BAT was regulated discriminatively in response to alteration of the ambient temperature, which regulation was similar and reciprocal to the regulation of uncoupling protein 1 (UCP1) and leptin, respectively. FABP3 expression in the BAT was significantly higher in the UCP1-knockout (KO) mice than in the wild-type ones, and these KO mice showed a higher clearance rate of free fatty acid from the plasma. In addition, FABP3 expression in the BAT was increased greatly with the development of diet-induced obesity in mice. These results indicate that the induction of FABP3 in BAT correlates with an increased demand for adaptive thermogenesis in rodents. FABP3 appears to be essential for accelerating fatty acid flux and its oxidation through UCP1 activity for non-shivering thermogenesis in BAT.

Brown adipose tissue (BAT) is the most important organ for adaptive thermogenesis, which is a response to environmental alterations such as cold (cold-induced thermogenesis) and excessive food intake (diet-induced thermogenesis) in order to maintain body temperature and energy homeostasis, respectively, in mammals [1–3]. Uncoupling protein 1 (UCP1), which is located exclusively in the inner mitochondrial membrane of BAT, is a unique thermogenic protein and its expression level is increased by cold exposure in rodents [4]. UCP1 functions to dissipate chemical energy produced by oxidative phosphorylation of energy fuels such as fatty acid (FA) and glucose, resulting in heat generation. The important roles of UCP1 in body temperature regulation in cold and in energy metabolism have been demonstrated in previous studies using UCP1-knockout (KO) mice [5–8].

Fatty acid-binding proteins (FABPs) are small cytosolic proteins that are members of the superfamily of lipid-binding proteins [9,10]. FABPs are expressed in a variety of cells, and nine molecules with different tissue distribution patterns are presently known. FABP4/aP2 is expressed mainly in adipocytes while FABP3, also called H-FABP, is most abundant in heart and skeletal muscles [11,12]. FABPs have been postulated to be translocators of long-chain FAs to intracellular organelles such as mitochondria and nucleus [9]. It has been suggested that FABP3 is specifically involved in FA oxidation in energy metabolism in heart and skeletal muscles [12,15–17]. Actually, genetic lack of FABP3 severely impairs FA uptake and oxidation [15]. We have reported that a 12.5-kD protein was markedly induced in BAT of cold-acclimated rats [13]; and this cold-inducible protein was clarified later to be FABP3 [14]. However, the contribution of FABP3 expressed in BAT to adaptive thermogenesis still remains to be understood.

In the present study, we examined the regulation of FABP3 expression in BAT under three different thermal conditions in rats. In addition to the contribution of FABP3 to FA utilization, we also investigated the induction of FABP3 in the BAT of UCP1-KO mice, which showed cold sensitivity and increased susceptibility to diet-induced obesity with age.

Materials and methods

Animals. Experiment-1: Male Wistar strain rats were obtained from Japan SLC at 7 weeks of age. The rats were housed individually under barrier conditions and reared at 23 °C under artificial lighting for 12 h and provided standard chow (CR-1, CLEA JAPAN, INC.) and tap water *ad libitum*. At 8 weeks of age, the rats (243 ± 7 g, $n = 15$) were divided into three groups and maintained either at 5, 23 or 32 °C for 2 weeks. After the rats had been killed by decapitation, their interscapular BAT was dissected and used for RNA

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preparation. Experiment-2: UCP1-KO (C57BL/6J background, N12-N15 generations) and wild-type (WT) mice were bred and reared at 23 °C under artificial lighting for 12 h per day, and provided a different standard chow (CE-2, Clea Japan, Inc.) and tap water *ad libitum*. A number of mice were fed a high-fat diet (HF: B15040, Clea Japan, Inc.) from the age of 3 months, as described previously [7]. The KO and WT mice were sampled at various ages. The experiments in the present study were performed according to our institutional guidelines for animal care.

Preparation of FABP3-expressing cells and culture. Rat FABP3 cDNA containing the open reading frame was generated as described previously [18] and inserted into a pIRES2 vector (Clontech, CA). Cos7 cells were grown in Dulbecco's modified Eagle's medium (DMEM; Nacalai Tesque, Kyoto, Japan) containing 10% fetal bovine serum (FBS; JRH Biosciences, Lenexa, IA, USA) at 37 °C in 5% CO_2. The cells were transfected with the pIRES2-FABP3 vector by using Lipofectamine (Invitrogen) and were used to determine free fatty acid (FFA) uptake after 48 h of transfection.

RNA analysis. Total RNA (15 μg), prepared from the BAT with TRIzol (Invitrogen, Carlsbad, CA), was analyzed by Northern blotting as described previously [7]. Blots were hybridized successively with probes (labeled with [^{32}P]dCTP) for the mRNAs of UCP1, leptin, FABP3, FABP4, β-actin, and/or 18S rRNA. These probes were produced by the reverse transcription PCR technique as previously described [7,19]. Hybridization signals were quantified with Fuji Bioimage.

Protein analysis. Western blot analyses for FABP3 and β-tubulin were performed by using the cytosolic fraction or whole cell lysates recovered from BAT or cultured cells and specific antibodies, as described previously [18]. The protein concentration in the samples was measured by conducting BCA protein assays (PIERCE, Rockford, IL). Equal amounts of protein (20 μg) were separated on 12.5% gels (Daiichi Pure Chemicals; Tokyo, Japan) and transferred onto immobilon polyvinylidene difluoride membranes (Millipore, Bedford, MA). The membranes were incubated with the specific antibodies for rat FABP3 [18] or β-tubulin (Santa Cruz, CA). After the secondary antibody reaction for 1 h at room temperature, the specific signals were detected by using an ECL kit (Amersham Pharmacia Biotech, Piscataway, NJ). The resulting images were quantified with NIH Image (version 1.63).

Fat-loading and FA uptake tests. For the fat-loading test, 6-month-old mice fasted for 17 h were injected with 30 ml of 20% Intralipid (Research Biochemicals International) through a tail vein to generate a sudden rise in their plasma FFA [20]. Blood samples were collected from the tail vein before and 3, 15, 30, and 60 min after the Intralipid injection. FFA levels in the serum were measured by using a commercial assay kit (NEFA C-test, Wako Pure Chemical, Osaka, Japan). FA uptake in cells was determined according to the method of Darimont et al. with minor modification [21]. Cos7 cells transfected with the pIRES2-FABP3 vector were washed with warm PBS and subsequently incubated with a serum-free DMEM supplemented with 0.1% BSA (fatty acid-free, Sigma) for 24 h. [^{14}C]-Palmitate/sodium taurocholate micelles were prepared by mixing 50 μM palmitate and 5 μM [^{14}C]-palmitate (55 Ci/mol; PerkinElmer Life Sciences, Boston, MA) with serum-free DMEM supplemented with 8 mM sodium taurocholate (Fluck). This solution was equilibrated for 30 min at 37 °C and added to the culture. After incubation for 30 or 120 min, the medium was removed; and the cells were then washed twice with 0.5 ml of ice-cold PBS. Thereafter they were lysed in 250 μl of 1% sodium dodecyl sulfate. The lysate (100 μl) was diluted in 2 ml of scintillation fluid, and its radioactivity was quantified. The final amount of uptake was expressed as pmol per 10^5 cells.

Statistical analysis. Data were expressed as means ± SE. Significant differences among groups were assessed by analysis of variance (ANOVA) with Fisher's PLSD test.

Results and discussion

Ambient temperature profoundly affects energy metabolism and body temperature regulation. In rodents, BAT is the most important organ to produce heat through the activity of UCP1 for homeothermic regulation [5,6,8]. UCP1 also plays a crucial role in diet-induced thermogenesis, in which excess caloric energy is dissipated as heat [1,2]. To confirm the role of BAT in thermoregulation, we maintained rats in a room at three different ambient temperatures, i.e., 5 °C (cold condition), 23 °C (standard condition), and 32 °C (thermoneutral condition), in the present study. Especially, the thermoneutral condition is essential to clearly determine the effects of ambient temperature in thermal physiology. After 2 weeks, the body weight increased in the cold and standard groups (28 ± 2 and 59 ± 3 g, respectively), whereas it was reduced in the thermoneutral group (−9 ± 7 g). Average food intake during the exposure was 33.1 ± 1.3, 28.4 ± 0.8, and 14.8 ± 1.0 g/day/rat in the cold, standard, and thermoneutral groups, respectively. We first examined the gene expression of UCP1 and leptin mRNAs in the BAT of rats (Fig. 1). As expected, the level of UCP1 mRNA significantly increased (290%) in the cold group and decreased (60%) in the thermoneutral group compared with that in the standard group. On the contrary, that of leptin mRNA was dramatically reduced and almost disappeared (~2% of the standard group) in the cold group, but it was increased (154%) in the thermoneutral group compared with that in the standard group, being in agreement with the levels of food intake in each group. This reciprocal regulation between UCP1 and leptin genes in BAT is consistent with the previous observation of Cancello et al. [22]. Our data also support the functions of these genes under the thermal conditions. We then measured the level of FABP3 mRNA in the BAT of rats. Similar to the response of the UCP1 gene, this level discriminatively increased (810%) in the cold group and decreased (60%) in the thermoneutral group compared with that in the standard group, suggesting that FABP3 expression correlates with UCP1 function and the degree of heat requirement. Moreover, our data clearly illuminated the opposite regulation of gene expression between FABP3 and leptin in BAT. The mRNA level of FABP4, which is the major type of FABP in adipocytes, was also significantly higher (206%) in the BAT of the cold group, but a steady-state level was maintained in the thermoneutral group, compared with that in the standard group, suggesting a basal role of FABP4 in FFA utilization in the adipose tissue. A similar observation on the cold-induced expression of FABP3 mRNA in BAT was previously reported by Daikoku et al. [14]. Considering the primary function of FABP3, its marked increase in BAT would supply a large amount of long-chain FA for the mitochondria, where UCP1 efficiently "burns" the substrate to make heat in the cold condition.

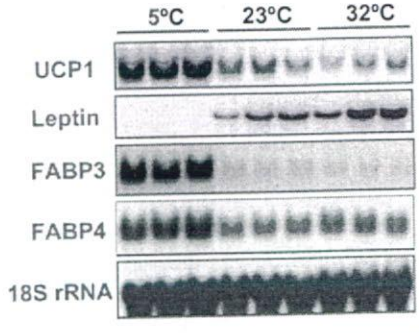

Fig. 1. Effect of ambient temperature on the expression of UCP1, leptin, FABP3 and FABP4 mRNAs in the BAT of rats. Northern blot analysis was conducted by using total RNA from the BAT of rats maintained at 5, 23, or 32 °C for 2 weeks. Hybridization signals were quantified and normalized by 18S rRNA level.

UCP1-KO mice are sensitive to cold and therefore heat demand to maintain body temperature appears to be high even under the standard thermal condition of ~23 °C in animal facilities, as suggested by our previous studies [7,8]. In order to make sure the involvement of FABP3 in adaptive thermogenesis, we analyzed the expression level in BAT of UCP1-KO mice. As shown in Fig. 2A, at either 3 or 11 months of age and fed the standard chow diet, the UCP1-KO mice showed a significantly higher level (250–300%) of FABP3 mRNA in their BAT than the WT mice. The mRNA level of FABP4 in BAT was also significantly higher (218%) in the KO mice than in the WT ones at 3 months of age, whereas no difference between genotypes was detected at 11 months of age (data not shown), as described in previous study [7]. The increase in the steady-state level of FABP3 mRNA in the BAT of UCP1-KO mice may reflect their requirement for thermogenesis, because the FABP3 mRNA level in their BAT was further increased after cold exposure (data not shown). Although the expression level was relatively low, an increased level of FABP3 mRNA was also detected in inguinal WAT of UCP1-KO mice compared with that of the WT mice (data not shown). The increase in FABP3 expression in the BAT of UCP1-KO mice was confirmed at the protein level. In mice fed the standard chow diet, significant increases in the FABP3 protein level (1.8-fold and 5.3-fold in 3-month-old and 11-month-old mice, respectively) were detected in BAT of UCP1-KO groups compared with those of WT groups (Fig. 2B). In addition, the protein level in the BAT was considerably higher in the older mice than in the younger ones in the KO group. Since UCP1-KO mice show increased susceptibility to diet-induced obesity with age [7], we also examined the effect of a HF diet on FABP3 expression in the BAT of mice. The FABP3 protein level in the BAT of WT mice fed the HF diet were increased markedly (4.4-fold) compared with that of control mice fed the standard chow at 11 months of age, and this higher level was retained in the BAT of older WT mice on the HF diet. In KO mice fed the HF diet, FABP3 protein level in their BAT was further increased compared with that of KO mice fed the standard

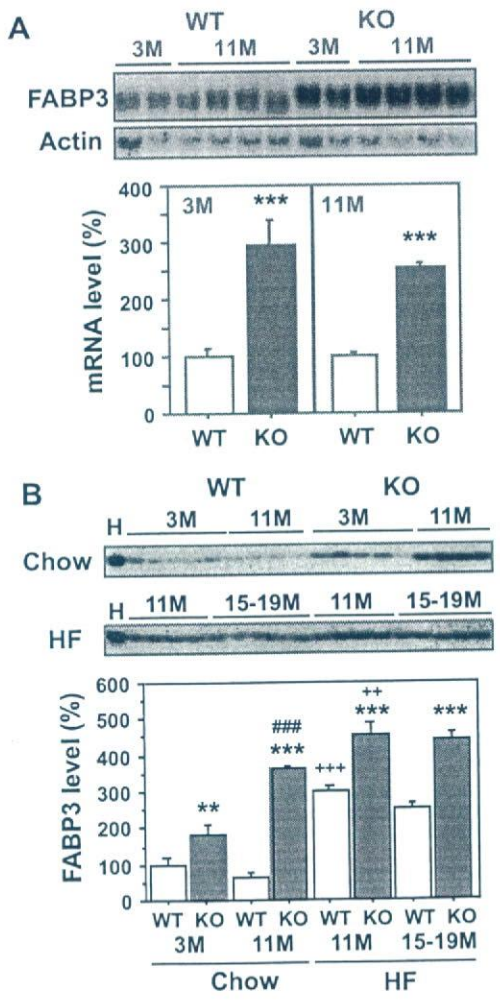

Fig. 2. Effects of age and diet on FABP3 expression in the BAT in WT and UCP1-KO mice. (A) Northern blot analysis was carried out by using total RNA from the BAT of 3- and 11-month-old mice fed a standard chow diet. Hybridization signals were quantified and normalized by β-actin mRNA level. Representative images are shown. The data (means ± SE) are presented as a percentage of the levels in WT mice at each age. The numbers of mice were 10 (5 each for female and male) for each genotype of 3-month-old mice and 4 females for each genotype of 11-month-old mice. $^{***}P<0.001$ *vs.* WT mice. (B) Western blot analysis for FABP3 was performed by using the cytosolic fraction (20 μg) from the BAT of mice fed the chow or HF diet. A fraction from heart (H, 1 μg) was used as a positive control. The data (means ± SE) are presented as a percentage of the level in 3-month-old WT mice. The numbers of mice were 4–5 for each group. $^{**}P<0.01$ and $^{***}P<0.001$ *vs.* WT mice in the same age and diet groups. $^{###}P<0.001$ *vs.* younger mice in the same genotype and diet groups. $^{++}P<0.01$ and $^{+++}P<0.001$ *vs.* the mice with the chow diet in the same age and genotype groups.

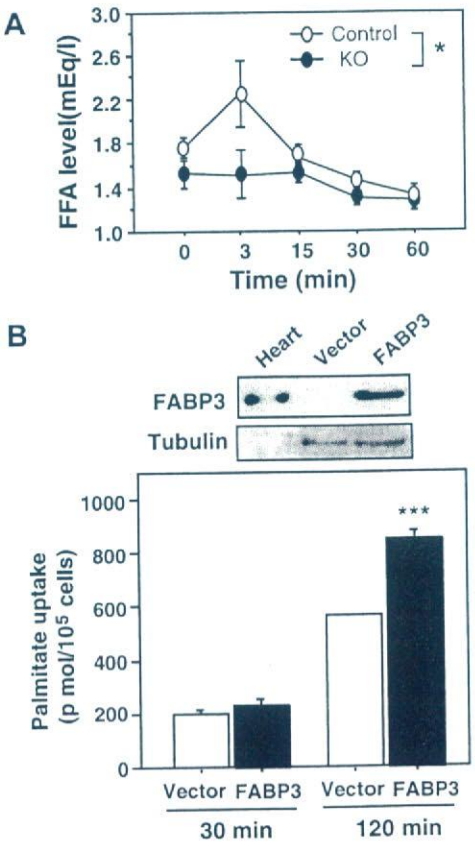

Fig. 3. FFA uptake in UCP1-KO mice and in cultured cells expressing recombinant FABP3. (A) Fat-loading test. Two groups of mice were injected with Intralipid via a tail vein after a 17-h fast. FFA levels in the serum at the indicated time points were measured. Data are expressed as means ± SE ($n=6$ for each group). $^{*}P<0.05$ (repeated measure ANOVA). (B) Cos7 cells were transfected with the pIRES2 vector bearing FABP3 cDNA (FABP3) or with the vector alone (Vector) and were cultured in a medium containing [^{14}C]-palmitate. Upper: Cell lysates (30 μg) were analyzed by Western blotting for FABP3 and β-tubulin. The cytosolic fraction of heart (1 μg) was used as a positive control. Lower: The radioactivity in the cells was determined after 30 and 120 min and expressed as pmol per 10^5 cells. Data are expressed as means ± SE ($n=3$ for each group). $^{***}P<0.001$ *vs.* the corresponding vector control.

chow at 11 months of age; and this higher level was retained in the BAT of older KO mice. We also found a positive correlation between the level of FABP3 mRNA in the BAT and BW in the mice in a regression analysis, whereas there was no relation between the level of FABP4 mRNA in the BAT and BW (data not shown). These results strongly suggest that FABP3 expression in the BAT increases with the development of diet-induced obesity in mice.

Since the steady-state levels of FABP3 protein were elevated considerably in the BAT of UCP1-KO mice compared with those of the WT mice, we analyzed the ability for FFA uptake in these mice. In the fat-loading test, the serum FFA level increased in 3 min after the lipid injection and then decreased with time in the control group (Fig. 3A). On the other hand, the increase in the FFA level observed at 3 min in the control mice was blunted in the UCP1-KO mice, indicating accelerated FFA clearance from the plasma in the mutant mice. In an *in vitro* analysis to see the effect of FABP3 induction on FFA uptake, we transfected Cos7 cells with a FABP3 expression vector and incubated them in medium containing [^{14}C]-palmitate. There was no difference in palmitate uptake at 30 min between the vector and FABP3 groups, whereas a significant increase in the FFA uptake was detected at 120 min in Cos7 cells expressing FABP3 compared with the cells transfected with the vector alone (Fig. 3B), indicating the potential of this protein for stimulating FFA uptake. Taken together, our data indicate that it is likely that the augmented FABP3 level in BAT (and probably WAT in a lesser extent) increases FFA flux into the tissue from the bloodstream and stimulates UCP1 thermogenesis, which contributes to maintain body temperature in the cold condition or to dissipate excess energy in the HF diet condition in the WT mice. In UCP1-KO mice, however, FFA will not be utilized effectively without UCP1 in spite of FFA uptake strengthened by FABP3 induction, and the unmetabolized FFA is stored eventually as triacylglycerol in the tissue. Indeed, this scenario is supported by the fact that BAT hypertrophy is a typical characteristic of UCP1-KO mice [5,7]. The exhaustion seen after swimming training in FABP3-KO mice could be related partly to intolerance against the cold caused by evaporative heat loss [15], suggesting that the function of FABP3 is important for UCP1 thermogenesis in BAT.

Thus, our data clearly show that the induction of FABP3 in BAT correlates with an increased demand for adaptive thermogenesis in rodents. FABP3 appears to be essential for accelerating FFA flux and its oxidation through UCP1 activity for non-shivering thermogenesis in BAT. Further study is needed to understand the precise transcriptional regulation of FABP3 and the differences in the function between FABP3 and other FABPs such as FABP4.

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脂肪細胞の役割からみる肥満
－新しい肥満予防法開発の試み－

Roles of adipocytes in the development of obesity

山　下　　均

Hitoshi YAMASHITA

要　旨

肥満は古くは富や健康（美）のシンボルであったが、現在では肥満状態が続くと健康に良くないことがわかってきた。最近問題となっているメタボリックシンドロームは、様々な生活習慣病につながることから全国的な予防への取り組みが本格的に始まりつつあるが、その第一が肥満の予防である。従来から、食べる量を制限する、あるいはエネルギーの消費を高めることにより肥満を予防する薬剤などの開発が行なわれてきたが未だ十分な効果を挙げるに至っていない。我々は、ヒトにおける肥満の成り立ちとその中心となる脂肪細胞の役割を理解し、真に効果のある肥満予防法の開発を試みてきた。本稿では、脂肪細胞の分化を阻害する植物成分を用いる新しい肥満予防法についての研究成果を含め、肥満における脂肪細胞の役割について概説する。

キーワード：メタボリックシンドローム、肥満、白色脂肪細胞、褐色脂肪細胞、UCP1、エボジアミン、シグナル伝達分子

1．メタボリックシンドロームと肥満

2008年4月から生活習慣病を予防するための国家プロジェクトとしてメタボリックシンドローム特定健康診査（通称、メタボ検診）がスタートする。メタボ検診では40-74歳の約5600万人が対象となり腹囲測定と血液検査を実施するもので、メタボリックシンドロームの増加がいかに深刻な社会問題となっているかがうかがわれる。内臓脂肪症候群とも呼ばれるメタボリックシンドロームは、食べ過ぎや運動不足によって内臓脂肪が蓄積し、高脂血症、高血圧、高血糖を合併した病態を指す[1]（診断基準を表1に示す）。この病態が続くと動脈硬化が進行して心筋梗塞や脳卒中などの重篤な心血管系疾患をまねく原因となる。従って、心血管系疾患が発症する前にメタボリックシンドロームの主因となる肥満の予防が重要となる。

肥満の程度は、BMI（Body mass index：肥満度＝体重kgを身長mの二乗で割った値）とい

表1　メタボリックシンドローム診断基準

内臓脂肪蓄積
＊腹囲　　男性≧85cm，　女性≧90cm
（内臓脂肪面積：男女とも≧100cm^{2}に相当）
上記に加えて以下の2項目以上
＊血中の中性脂肪含量　≧150mg/dl
又は　HDLコレステロール含量　<40mg/dl
＊収縮期血圧　≧130mmHg
又は　拡張期血圧　≧85mmHg
＊空腹時血糖　≧110mg/dl

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う簡単な指標をもとに定義することができ、BMIが25を超えると太り過ぎで肥満であると判断される。運動やダイエットにより比較的短期間に脂肪を減らすことが可能であることから、肥満を病気だと思っている人は少ないが、肥満状態が続くとメタボリックシンドロームとなり様々な生活習慣病を発症する確率が大きく上昇することになる。この肥満における陰の主役が「脂肪細胞」であり、脂肪細胞の集まりが脂肪組織である。

2．白色脂肪細胞と褐色脂肪細胞

脂肪は大切な栄養素の一つであり、完全に燃焼すると糖や蛋白質の2倍以上のエネルギーを放出する。多くの動物は進化の歴史の中で飢餓や寒さに備えるために中性脂肪を貯蔵する白色脂肪細胞（一般的にいう脂肪細胞）と中性脂肪をエネルギー源として熱をつくり体温を維持する褐色脂肪細胞を発達させてきた。つまり、白色脂肪細胞は燃料タンク、褐色脂肪細胞はヒーターという役割である。太古の昔には、十分な食べ物を常に確保することは難しかったので、厳しい自然の中で生き延びるためには獲得した食物エネルギーを中性脂肪のかたちで白色脂肪細胞に蓄えることが必要であった。同時に、ヒトを含む恒温動物は極寒の中でも体温を常に一定に保たなければ生きていけないので、貴重な貯蔵エネルギーである中性脂肪を効率良く燃やし熱を産み出す褐色脂肪細胞が必要であったと考えられる。

これら二種類の脂肪細胞の働きは脳－交感神経系により調節される。例えば、寒冷環境下では皮膚に存在する感覚神経を介して“寒い！”という情報が脳に伝えられると、交感神経が刺激されてその神経末端からノルアドレナリンが分泌される（図1）。続いて、ノルアドレナリンは白色脂肪細胞と褐色脂肪細胞の細胞膜上に存在するβ3アドレナリン受容体（β3-AR）を活性化し、白色脂肪細胞においては貯蔵されている中性脂肪を分解して脂肪酸がつくられる。一方、褐色脂肪細胞ではアンカップリングプロテイン1（UCP1）という特殊な熱産生タンパク質が活性化されて脂肪酸を燃料として熱をつくり体温の低下が抑えられる。食事の際にも交感神経が活性化され脂肪分解と熱産生が起こる。最終的には、食事で摂取するエネルギー量が身体活動で消費するエネルギー量を上回ると余ったエネルギーが中性脂肪として白色脂肪細胞に蓄積される（図2）。しかし、一つの白色脂肪細胞が貯め込む中性脂肪量には限界があるので、余剰のエネルギーをさらに貯め込むために脂肪前駆細胞（未分化の脂肪細胞）が分化して白色脂肪細胞の数が増える。すなわち、肥満は中性脂肪を蓄積した白色脂肪細胞が増加して白色脂肪組織量が大きくなった状態である。

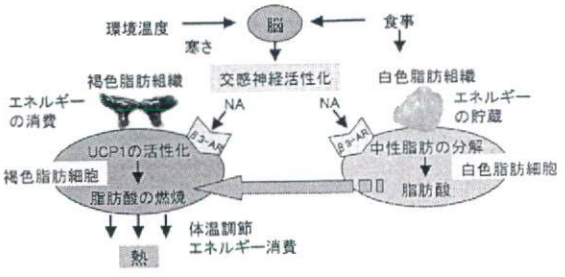

図1 脂肪細胞の役割。脂肪細胞には白色脂肪細胞と褐色脂肪細胞の2種類がある。白色脂肪細胞は摂取したエネルギーを中性脂肪のかたちで貯蔵するが、中性脂肪の過剰な蓄積は肥満の原因となる。寒さや食事などにより交感神経が活性化されて分泌されたノルアドレナリン（NA）は、脂肪細胞上のβ3アドレナリン受容体（β3-AR）に作用し白色脂肪細胞における中性脂肪の脂肪酸への分解を促進する。同時に、褐色脂肪細胞のアンカップリングプロテイン1（UCP1）が活性化されて脂肪酸を燃焼し熱を産み出す。

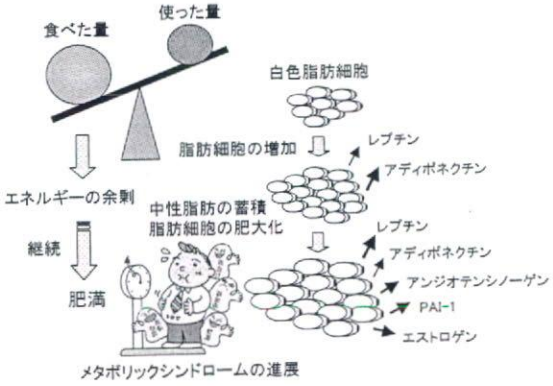

図2 肥満と脂肪細胞の関係。肥満は食べた量が使った量を上回ることによるエネルギーバランスの乱れが継続することにより進行する。一つの白色脂肪細胞が貯蔵できる中性脂肪量には限界があるので、余剰のエネルギーを蓄積していくために新しい脂肪細胞が生まれ細胞数が増加する。一方、脂肪細胞はレプチン、アディポネクチン、アンジオテンシノーゲン、プラスミノーゲンアクチベーターインヒビター1（PAI-1）、エストロゲンなど、様々なホルモンや生理活性分子をつくる。これらの血中レベルは肥満と共に変化し、メタボリックシンドロームの病態発症に関与する。

3．肥満と脂肪細胞

上記の基本的役割に加えて、脂肪細胞の新たな機能が近年明らかとなってきた。すなわち、脂肪細胞は単なる燃料タンクやヒーターではなく、種々のホルモンや生理活性分子をつくる内分泌細胞であることがわかってきた[2]（図２）。脂肪細胞が分泌するホルモンとして最初に見つかったレプチンは、食欲を抑制するホルモンで肥満の程度と比例して血中レベルが上昇する。また、レプチンは交感神経を活性化して褐色脂肪細胞における熱産生を促進してエネルギー消費を高める作用がある。その他にも、糖および脂質の代謝調節に係わるアディポネクチン、血液の凝固に係わるプラスミノーゲンアクチベーターインヒビター1（PAI-1）、血圧調節に働くアンジオテンシノーゲン、あるいは女性ホルモンであるエストロゲンなど、脂肪細胞は驚くほど多様な分子をつくる。

最近の研究から、内臓脂肪が蓄積すると脂肪細胞の働きが悪くなりアディポネクチンの分泌量が低下して、脂肪細胞だけではなく肝臓や筋肉などにも中性脂肪が蓄積しインスリン抵抗性や高脂血症の原因となることがわかってきた。一方、脂肪細胞によるPAI-1合成が上昇し血栓の形成が促進されて動脈硬化が進行する。加えて、アンジオテンシノーゲン合成の上昇による血圧上昇など、内臓肥満をベースとしてメタボリックシンドロームが進むことになる。

4．従来の肥満予防法

食べる量を減らし、適度な運動を行なうことが肥満にならないために重要であることは誰しも分かっていることである。しかし、肥満者の数が増え続ける現状をみると話はそれほど簡単ではないようである。従って、エネルギー摂取を減らす、あるいはエネルギー消費を高めることにより肥満を予防または治療する薬剤の開発が行われてきた。その一つは、前述のβ3-ARの活性化による褐色脂肪細胞の熱産生を介してエネルギー消費を促進し肥満を予防するβ3-AR活性化剤の開発である（図１）。しかし、褐色脂肪細胞はリスやマウスなど小型のげっ歯類では今なお必須の熱産生器官であるが、断熱と保温の効いた衣と住の中でくらす現代人では一般的に褐色脂肪細胞は生後急速に消退し、成人以降UCP1を検出することが難しい。また、β3-ARには遺伝子多型が存在し、褐色脂肪細胞の熱産生が活性化されにくい日本人の割合は３－４割に達するとみられることから、β3-AR活性化剤の効果には個人差があり有効性にも限界があると予想される。実際に、マウスなどの実験動物で有効なβ3-AR活性化剤がヒトでは効果が十分認められず今尚利用されるに至っていない。最近我々は、UCP1を欠損するマウス（UCP1-KOマウス）が高脂肪食の摂取により肥満となること[3]、β3-AR活性化剤はUCP1-KOマウスの肥満を予防することができないこと[4]を明らかにしている。

一方、植物など天然素材に含まれる種々の化合物に肥満を予防する作用が見出されている。α-リポ酸は摂食抑制作用とUCP1熱産生活性化作用により抗肥満効果を示す生体分子であり、健康補助剤として注目を集めている。食品機能を利用した肥満予防としては、古くから食材として使われてきた唐辛子に含まれるカプサイシンがその代表として挙げられる。カプサイシンは強力な血管拡張作用により末梢からの熱放散を促進し、交感神経及び褐色脂肪細胞を活性化してUCP1熱産生を亢進することにより肥満予防効果を発揮すると考えられる。最近、辛みのないカプサイシン類縁体が見出され市販されはじめている。同様に、薬用植物として利用されてきたゴシュユの主要成分であるエボジアミンがカプサイシンと類似の肥満予防効果を有することが報告された[5]。しかしながら、UCP1熱産生機能をほとんど喪失したヒトにおいて、これらの機能性化合物が抗肥満作用を本当に発揮することができるのか疑問がもたれた。

5．エボジアミンの新しい抗肥満作用

我々は、カプサイシンと異なり辛みがないエボジアミンを0.03%の濃度で配合した高脂肪食を調製し、正常マウスとUCP1-KOマウスに２ヶ月間食べさせてエボジアミンの肥満予防効果を検討した。その結果、エボジアミンは高脂肪食によるUCP1-KOマウスの肥満の進展を正常マウスと同様に抑制することが明らかとなった[6]。すなわち、エボジアミンを添加した高脂肪食を摂取したマウスでは、高脂肪食のみを摂取したマウスに比べて、摂食量に差はみられなかったが、体重や肥満度