0013-7227/08/$15.00/0
Printed in U.S.A.

Endocrinology 149(1):358–366

doi: 10.1210/en.2007-0467

Evodiamine Improves Diet-Induced Obesity in a Uncoupling Protein-1-Independent Manner: Involvement of Antiadipogenic Mechanism and Extracellularly Regulated Kinase/Mitogen-Activated Protein Kinase Signaling

Ting Wang,* Youxue Wang,* Yasuhide Kontani, Yoshinori Kobayashi, Yuzo Sato, Nozomu Mori, and Hitoshi Yamashita

Department of Biomedical Sciences (T.W., H.Y.), College of Life and Health Sciences, Chubu University, Kasugai 487-8501, Japan; Department of Surgery (Y.W.), University of Texas Southwestern Medical Center at Dallas, Dallas, Texas 75390; Department of Food Science for Health (Y.Kon.), Minami-Kyushu University, Miyazaki 880-0032, Japan; Laboratory of Pharmarognosy and Phytochemistry (Y.Kob.) School of Pharmaceutical Sciences, Kitasato University, Tokyo 108-8641, Japan; Department of Health Science (Y.S.), Faculty of Psychological and Physical Sciences, Aichi-Gakuin University, Nisshin 470-0195, Japan; and Department of Anatomy and Neurobiology (N.M.), Nagasaki University School of Medicine, Nagasaki 852-8523, Japan

Evodiamine is an alkaloidal compound with antiobesity effects that have been thought to be due to uncoupling protein-1 (UCP1) thermogenesis similar to the effects of capsaicin, but the underlying mechanisms are poorly understood. To clarify the mechanisms, we first examined whether the antiobesity effect of evodiamine could be attributed to the involvement of UCP1. When UCP1-knockout mice were fed a high-fat diet with 0.03% evodiamine (wt/wt) for 2 months, the increases in body weight, adiposity, and the serum levels of leptin and insulin were reduced in a manner indistinguishable from control mice fed a high-fat diet with evodiamine, suggesting that evodiamine triggered a UCP1-independent mechanism to prevent diet-induced obesity. By using preadipocyte cultures, we found that evodiamine, but not capsaicin, increased phosphorylation of ERK/MAPK, reduced the expression of transcription factors such as peroxisome proliferator-activated receptor-γ, and strongly inhibited adipocyte differentiation. Evodiamine treatment also reduced insulin-stimulated phosphorylation of Akt, a crucial regulator of adipocyte differentiation; and the reduction of phosphorylated-Akt and augmentation of phosphorylated ERK were reversed by blockade of the MAPK kinase/MAPK signaling pathway, restoring adipogenesis in the cultures. The changes in ERK and Akt phosphorylation levels were also observed in white adipose tissues of UCP1-knockout mice fed the evodiamine diet. These findings suggest that evodiamine has a potential to prevent the development of diet-induced obesity in part by inhibiting adipocyte differentiation through ERK activation and its negative cross talk with the insulin signaling pathway. (*Endocrinology* 149: 358–366, 2008)

OBESITY, THE STATE of excess fat deposition in the body, is a serious health problem in industrialized societies because it is associated profoundly with type 2 diabetes mellitus, coronary heart disease, atherosclerosis, and certain cancers (1, 2). Because obesity develops as the result of energy imbalance when energy intake exceeds energy expenditure, intervention to reduce caloric intake through hormonal regulation and/or to increase energy expenditure by thermogenic function such as uncoupling protein 1 (UCP1) would be reasonable ways for preventing or curing obesity (3–5). It has been recently reported that α-lipoic acid has these potentials because it causes weight loss in rodents by reducing food intake and enhancing energy expenditure through a hypothalamic AMP-activated protein kinase pathway (6).

The regulation of adipogenesis also appears to be a potential strategy for the treatment of obesity because the excessive growth of adipose tissue in obesity has been suggested to result from adipocyte hypertrophy and the recruitment of new adipocytes from precursor cells (4, 7). Adipogenesis is a complex process that is highly regulated by positive and negative stimuli, including molecules involved in the insulin signaling pathway and various transcription factors (8, 9). ERK/MAPK signaling is a pathway activated by insulin. To date, many studies including one showing the pivotal role of peroxisome proliferator-activated receptor (PPAR)-γ, 8) have brought about great advances in our understanding of the molecular mechanism of adipogenesis. Although contradictory results on the role of ERK1/2 (p44/42 MAPK) in adipogenesis have been obtained, recent results indicate that ERK activation is necessary to initiate the process of differentiation of preadipocytes into adipocytes and that, thereafter, this signal pathway needs to be shut off for adipoctye differentiation to proceed

First Published Online September 20, 2007
* T.W. and Y.W. contributed equally to this work.

Abbreviations: aP2, Adipocyte fatty acid-binding protein; AR, adrenergic receptor; BAT, brown adipose tissue; C/EBP, CCAAT/enhancer-binding protein; CS, calf serum; HF, high fat; IGF-IR, IGF-I receptor; IPGTT, ip glucose tolerance test; IR, insulin receptor; IRS, insulin receptor substrate; KO, knockout; PI3K, phosphoinositide 3-kinase; PPAR, peroxisome proliferator-activated receptor; UCP, uncoupling protein; WAT, white adipose tissue.

Endocrinology is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.

(10–13). Despite the high potential for antiobesity intervention, however, safe and effective agents inhibiting adipocyte differentiation, thereby preventing obesity, are not yet available.

Evodiamine, a major alkaloidal compound in the fruit of *Evodia fructus (Evodia rutaecarpa* Bentham, Rutaceae) was previously reported to exhibit capsaicin-like antiobesity effects (14). The major mechanism eliciting the effect was postulated to be enhancement of energy dissipation by UCP1 thermogenesis, probably through β3-adrenergic stimulation in brown adipose tissue (BAT). Capsaicin, the pungent main principle of red pepper, has also been reported to decrease body weight by reducing food intake in rats (15), although the molecular basis on this antiobesity effect of capsaicin is still obscure. If evodiamine has a high potential for preventing obesity, this compound may be suitable for dietary supplementation because it has no perceptible taste, unlike capsaicin. However, the mechanisms underlying the antiobesity effects of evodiamine are still not clear. In this report, we demonstrate that evodiamine inhibited adipocyte differentiation through stimulation of an ERK/MAPK pathway and that dietary supplementation with this nonpungent compound could ameliorate diet-induced obesity in animals lacking UCP1 thermogenesis. This work may lead to the development of drugs and therapeutic strategies for treatment of obesity in adult humans who are virtually UCP1 deficient.

Materials and Methods

Experimental animals

Ucp^{tm1} knockout (KO) mice on a C57BL/6J background (16) were kindly provided by Dr. Leslie Kozak (Pennington Biomedical Research Center, Baton Rouge, LA), and N13-N15 generations were used in the experiments. The mice were maintained according to our institutional guidelines for animal care under artificial lighting for 12 h/d and provided a standard chow (11.6% kcal from fat; Diet CE-2; CLEA Japan, Inc., Tokyo, Japan) and tap water *ad libitum* in our animal facility at 23 ± 1 C. In the experiments on the effects of evodiamine, UCP1-KO and the control (wild-type and hetero-type) littermates mice were fed the standard chow until they were 4 months old and then were fed a high-fat diet (HF: 41.9% kcal from fat, Diet B15040; CLEA Japan) (17) with or without 0.03% evodiamine (wt/wt; Kishida Chemical, Osaka, Japan) for 2 months. The 6-month-old mice were sampled to determine the effects of evodiamine on body weight, adiposity, blood biochemical parameters, histology of tissues, and/or gene expression.

Cell culture

3T3-L1 cells, which were provided kindly by Dr. Masayoshi Imagawa (Nagoya City University, Nagoya, Japan), were grown in DMEM (Invitrogen, Grand Island, NY) containing 10% calf serum (CS; ICN Biomedicals, Aurora, OH). The adipocyte differentiation was performed as described (18). Briefly, 2 d after confluence, the medium was changed to DMEM containing 10% fetal bovine serum (ICN Biomedicals), 10 μg/ml insulin, 1 μM dexamethasone, and 0.5 mM 3-isobutyl-1-methylxanthine. Dexamethasone and 3-isobutyl-1-methylxanthine were withdrawn after 2 d of exposure, and insulin was withdrawn after 4 d. To determine the effect of evodiamine, we induced 2-d postconfluent preadipocytes to differentiate in the presence of evodiamine or capsaicin (Wako Pure Chemical, Osaka, Japan) for 4 d and then in its absence for 6 d. After 10 d of differentiation, the cells were stained with oil Red O (Muto Pure Chemicals, Tokyo, Japan). Similarly, we determined the effects of evodiamine on the protein and mRNA expression and triglyceride content of the cultured cells.

For the preparation of primary cultures of adipocyte precursor cells, small pieces of epididymal white adipose tissue (WAT) of C57BL/6J mice were incubated at 37 C for 30 min in PBS containing 0.9 mM $CaCl_2$, 0.49 mM $MgCl_2$, 0.2% collagenase (Sigma, St. Louis, MO), 5 mM glucose, and 1.5% BSA (Sigma). The mixture was then passed through a 70-μm nylon filter (Falcon, Becton Dickinson Labware, Franklin Lake, NJ) and centrifuged at 130 × *g* for 3 min. After the upper (lipid) layer had been removed, the lower layer and pellets were suspended and passed through a 40-μm nylon filter (Falcon). After the filtrate had been mixed with an equal volume of DMEM supplemented with 10% CS, the mixture was centrifuged at 170 × *g* for 6 min, and the cell pellet including the stromal vascular fraction was recovered for use as the primary culture. The cells were inoculated into 6-well plates (7×10^5 cells/well) and cultured in 10% CS/DMEM. The conditions for adipocyte differentiation were the same as those in the experiment using 3T3-L1 cells.

Human hepatoma HepG2 cells were obtained from the European Collection of Cell Cultures (Wiltshire, UK). Cells were cultured in 10% fetal bovine serum/DMEM containing 5.5 mM D-glucose as described previously (19). After HepG2 cells reached approximately 70% confluence in 60-mm-diameter dishes, the cells were maintained in serum-free medium overnight and then incubated in DMEM containing 24.75 mM D-glucose for an additional 24 h. To determine the effect of evodiamine on ERK phosphorylation, the cells were treated with or without evodiamine.

Biochemical analysis

Blood samples were collected from a tail vein and used immediately to determine the glucose level by use of a glucometer (NovoAssist Plus, Novo Nordisk, Tokyo, Japan). The following other parameters were measured by using serum and commercial assay kits: insulin (ultrasensitive insulin ELISA; Mercodia, Winston-Salem NC, or immunoassay kit; Shibayagi, Gunma, Japan) and leptin (Enzyme Immunoassay kit, Cayman, Ann Arbor, MI). The concentrations of protein and triglyceride in the tissue lysate and cultured cells were measured by using a BCA protein assay (Pierce Biotechnology, Rockford, IL) and Triglyceride E test (Wako Pure Chemical), respectively. An ip glucose tolerance test (IPGTT) using 1.5 mg of glucose per gram body weight was performed after 17 h of starvation. The blood glucose level was measured by the glucometer before glucose injection (0 min) and at 30, 60, and 120 min after that. Hepatic lipids were extracted with CHCl3/MeOH (2:1) according to the method of Folch *et al.* (20) and were dissolved in 2-propanol. Triglyceride or cholesterol concentrations in the lipids were determined by using commercially available kits: triglyceride G-Test Wako (Wako Pure Chemical) or Determiner TC555 (KYOWA MEDEX Co., Ltd., Tokyo, Japan).

Northern blot analysis

Total RNA was prepared from tissues and cultured cells with TRIzol (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. Northern blot analysis was performed by using total RNA (WAT: 20 μg, 3T3-L1 cells: 10 μg), as described earlier (17). Blots were hybridized successively with probes (labeled with [^{32}P]dCTP) for the mRNAs of UCP2, β3-adrenergic receptor (AR), PPARγ, leptin, adipocyte fatty acid-binding protein (aP2), resistin, and 18S rRNA. In the analysis of β3-AR, three transcripts of 2.1, 2.8, and 3.6 kb were detected in WAT, as reported (21). Like probes for UCP2, β3AR, aP2, and leptin mRNAs and 18S rRNA (16, 17), probes for PPARγ and resistin mRNAs were produced by the RT-PCR technique. The sequences used were the following: PPARγ, positions 464-1945 of the mouse sequence (GenBank accession no. U01841), and resistin, positions 38–558 of the mouse sequence (GenBank accession no. AF323080). The PCR products were sequenced after subcloning into the pCRII vector (Invitrogen). Hybridization signals were quantified with Bioimage (FUJIFILM; Fuji, Tokyo, Japan).

Histological analysis

Tissues were fixed immediately in 10% formaldehyde in neutral buffer solution (Kishida Chemical) and embedded in paraffin. Tissue sections of 3 μm were cut and then stained with hematoxylin and eosin.

Immunoblotting and immunoprecipitation

Total cell lysates were prepared and analyzed as described previously (22). Briefly, cells in 100-mm dishes were washed with ice-cold PBS

containing 1 mM Na_3VO_4 and lysed with a lysis buffer (pH 7.2) consisting of 50 mM Tris-HCl, 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 1 mM EGTA, 25 mM NaF, 1 mM Na_3VO_4, and 0.25% protease inhibitor mixture solution (Sigma). The proteins of cell lysates were separated by 4–20% SDS-PAGE and electrotransferred onto a polyvinylidene difluoride membrane. Immunoblotting and immunoprecipitation were performed by using cell lysates (30 and 500 μg, respectively) and specific antibodies against CCAAT/enhancer-binding protein (C/EBP)-β, PPARγ, insulin receptor (IR)-β, IGF-I receptor β(IGF-IRβ), phosphatidylinositol 3-kinase (PI3K) p85 (Santa Cruz Biotechnology, Santa Cruz, CA), phospho-tyrosine (4G10; Upstate, Charlottesville, VA), insulin receptor substrate (IRS)-1, p44/42 MAPK, phospho-p44/42 MAPK, serine/threonine kinase Akt, and phospho-Akt (Cell Signaling Technology, Danvers, MA). The immunoreactive bands were visualized with an enhanced chemiluminescence reagent (Amersham Biosciences, Buckinghamshire, UK).

Statistical analysis

Data were expressed as the mean ± SE. Significant differences between groups were assessed by ANOVA or Student's *t* test.

Results

Effects of evodiamine on the development of diet-induced obesity in UCP1-KO mice

We provided UCP1-KO and the control mice with a HF diet with or without evodiamine for 2 months. Body weight gain and adiposity index were significantly lower in the mice with evodiamine (+Evo) than in the mice without it (−Evo) in both groups (Fig. 1, A and B), even though there was no significant difference in food intake between the +Evo and −Evo groups (0.43 ± 0.02 and 0.43 ± 0.02 kcal/d·g body weight in the control mice, 0.45 ± 0.01 and 0.48 ± 0.01 kcal/d·g body weight in the KO mice, respectively). The serum leptin levels in the +Evo group were reduced to 27 and 43% of the −Evo group in the control and KO mice, respectively (Fig. 1C). Although the nonfasting glucose level in the mice was not changed by the evodiamine diet (Fig. 1D), the insulin levels in mice treated with evodiamine were decreased to about one third of those without it in both groups (Fig. 1E). Moreover, the evodiamine diet improved the impaired glucose tolerance in UCP1-KO mice fed the HF diet, bringing it close to that in the mice fed the standard chow diet (Fig. 1F).

In addition to the data on adiposity (Fig. 1B), histological analysis supported the effectiveness of evodiamine in reducing fat accumulation in WAT and BAT, as well as fatty liver, in the mice fed the HF diet in both groups (Fig. 2). Liver mass in the −Evo group and +Evo group was 1.535 ± 0.113 and 1.286 ± 0.056 g in the control mice and 1.540 ± 0.119 and 1.391 ± 0.100 g in the KO mice, respectively. When the accumulation of hepatic lipids was evaluated, the triglyceride content tended to be reduced in the +Evo group, compared with that in the −Evo group (16.4 ± 2.9 and 57.5 ± 22.2

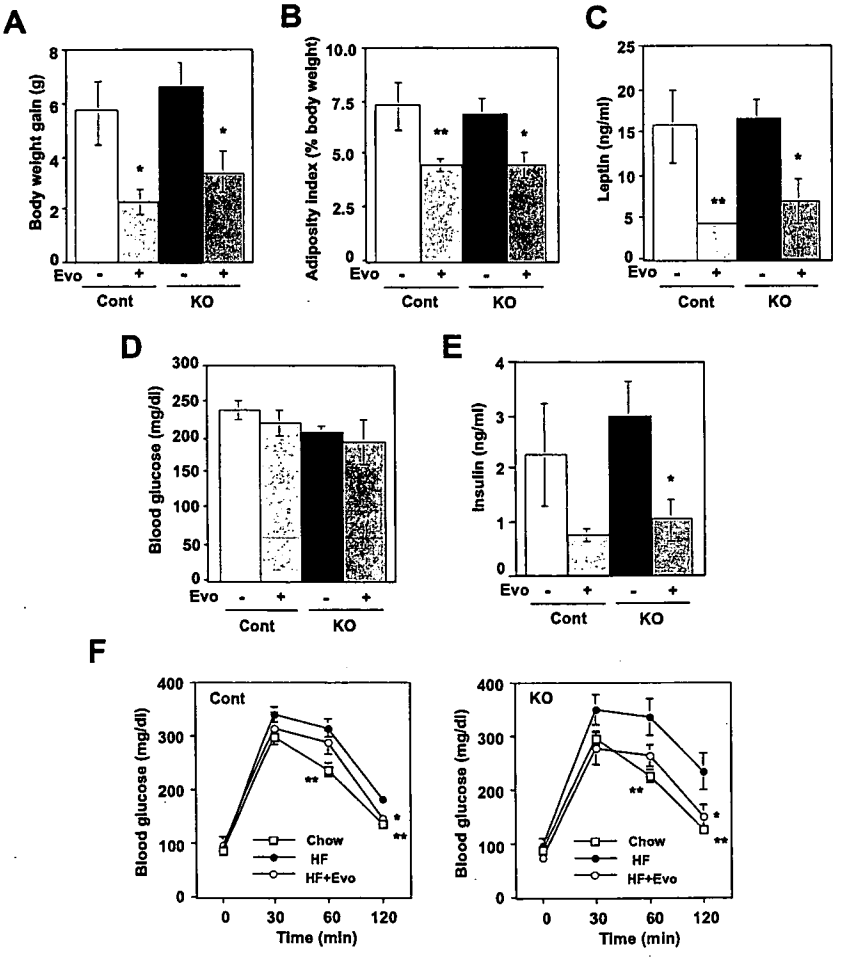


FIG. 1. Effects of evodiamine on body weight gain (A), adiposity (B), leptin (C), nonfasting levels of blood glucose (D) and insulin (E), and glucose tolerance (F) in the mice fed HF diet. Four-month-old control (Cont) and KO mice were fed the HF diet with or without evodiamine [Evo, 0.03% (wt/wt)] for 2 months. Data are expressed as the mean ± SE. The Cont/−Evo group contains four wild-type and one hetero-type mice. The Cont/+Evo group contains four wild-type and two hetero-type mice. The KO/−Evo and KO/+Evo groups contain six mice each. *, $P < 0.05$ and **, $P < 0.01$ *vs.* −Evo diet in the same group (ANOVA with Fisher's protected least significant difference test). F, IPGTT in Cont and KO mice after Evo feeding for 7 wk. The data in mice fed HF+Evo diet (*open circle*) were compared with those of age-matched mice fed the standard chow (*open square*) or HF diet (*closed circle*). Data are expressed as mean ± SE (chow: n = 9 and 8, HF: n = 8 and 9, HF+Evo: n = 6 and 6 for Cont and KO mice, respectively). *, $P < 0.05$ and **, $P < 0.01$ *vs.* HF group.

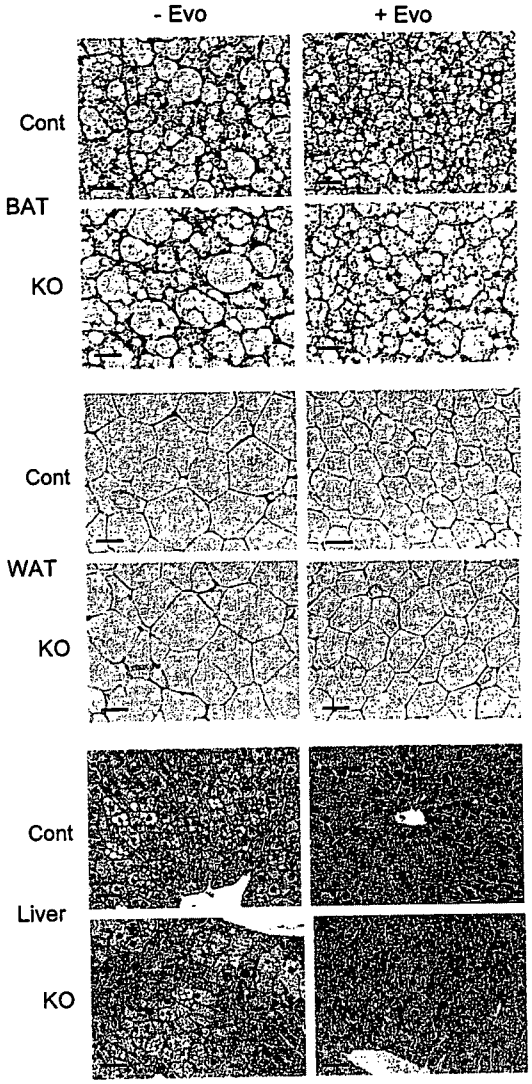


FIG. 2. Histological analysis of BAT, retroperitoneal WAT, and liver in the mice fed the HF diet with or without evodiamine (Evo). Tissue sections of the control (Cont) and KO mice were stained with hematoxylin and eosin. *Bars*, 25 μm for BAT and 50 μm for WAT and liver.

mg/g liver in the control mice, 25.1 ± 9.3 and 51.3 ± 10.8 mg/g liver in the KO mice, respectively). There was no difference in the total cholesterol content in the liver between the +Evo and −Evo groups (3.98 ± 0.37 and 3.42 ± 0.38 mg/g liver in the control mice, 3.75 ± 0.58 and 4.05 ± 0.47 mg/g liver in the KO mice, respectively).

In the analysis of gene expression in WAT, similar effects of evodiamine in the control and KO mice were observed (Fig. 3). Namely, reductions in the mRNA levels of leptin (control, 53%; KO, 26%), UCP 2 (control, 29%; KO, 22%), and PPARγ (control, 17%; KO, 26%) were found in the +Evo group, compared with those levels in the −Evo group, whereas the mRNA level of β3-AR increased in the +Evo group (WT: 201%, KO: 135%; Fig. 3), consistent with the effects of increased adiposity on the expression of these genes (17). There was no difference in the mRNA level of UCP1 in the BAT between the +Evo and −Evo groups in the control mice (data not shown). We also could not detect a significant

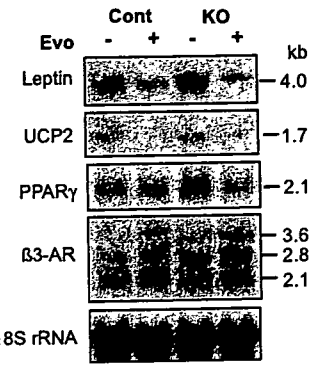


FIG. 3. Effects of evodiamine on the gene expression for leptin, UCP2, PPARγ, and β3-AR in WAT of the mice. Northern blot analyses were performed by using 20 μg of total RNA from WAT of the control and KO mice fed the HF diet with or without evodiamine (Evo). Three transcripts of 2.1, 2.8, and 3.6 kb were detected in the analysis for β3-AR. Hybridization signals were quantified and normalized by 18S rRNA levels. Representative images are shown.

difference in the mRNA levels of UCP2 and UCP3 in the BAT and muscles between the mice with or without evodiamine in either group (data not shown).

Evodiamine inhibits adipocyte differentiation in 3T3-L1 cells

The phenotypes of evodiamine *in vivo* could be mediated by changes in energy expenditure and lipolysis or through its effects on adipogenesis. Accordingly, we examined the effects of evodiamine on adipocyte differentiation *in vitro*. When the differentiation of 3T3-L1 preadipocytes was assessed quantitatively in terms of triglyceride contents, evodiamine dose-dependently inhibited the differentiation of the preadipocytes incubated in differentiation medium containing insulin, dexamethasone, and 1-isobutyl-3-methylxanthine (Fig. 4A). The addition of 1 μM evodiamine to the differentiation medium almost completely inhibited the adipocyte differentiation as evidenced by oil Red O staining of lipids. This inhibitory effect of evodiamine was reduced when it was added 2 d after the start of stimulation of differentiation, and the alkaloid did not suppress differentiation at all when it was added after d 4 (data not shown). Not only did evodiamine inhibit fat accumulation in adipocytes, but also the greatly reduced expression of aP2, leptin, and resistin indicated that adipogenesis in 3T3-L1 cells was strongly suppressed (Fig. 4B).

We then analyzed the induction of transcription factors regulating adipogenesis in the cells (Fig. 4C). In the absence of evodiamine, C/EBPβ, a crucial regulator in an early step of the transcriptional cascade in adipogenesis, and PPARγ were expressed in a time-dependent manner as the 3T3-L1 cells proceeded toward adipocyte differentiation, as previously described (23, 24). Treatment of postconfluent cultures with evodiamine severely suppressed the early induction of C/EBPβ and then blocked the induction of PPARγ (Fig. 4C), indicating that evodiamine acted to inhibit the early transcriptional steps of adipocyte differentiation. When the intracellular signaling molecules involved in adipocyte differentiation were examined, an increased level of phosphorylated ERK1/2 was found in

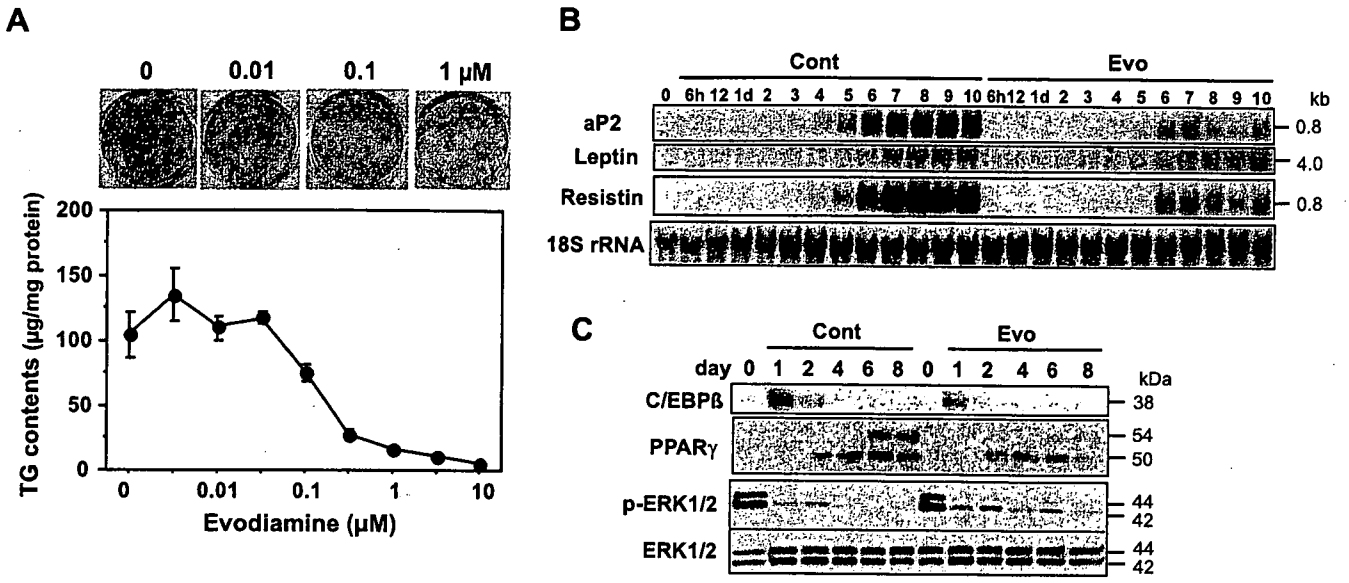


FIG. 4. Effects of evodiamine on adipocyte differentiation in 3T3-L1 cells. A, Dose-dependent inhibition of adipocyte differentiation by evodiamine. 3T3-L1 cells were cultured in the differentiation medium with the indicated concentration of evodiamine for 4 d and then without it for 6 d. On d 10, the lipid accumulation in the cells was evaluated by oil Red O staining (macroscopic images, *upper panel*) or determined in terms of triglyceride (TG) content (*lower panel*). Data are expressed as the mean ± SE (n = 4). B, Effects of evodiamine on the induction of adipocyte-characteristic genes. Northern blot analysis for aP2, leptin, resistin, and 18S rRNA was performed by using 10 μg of total RNA isolated from the cells. C, Effects of evodiamine on the activation of ERK and expression of transcription factors during adipogenesis. Western blot analysis for C/EBPβ, PPARγ, phospho- and total ERK was performed by using cell lysates. In B and C, 3T3-L1 cells were treated with 1 μM evodiamine for 4 d and then without it for 4–6 d (Evo) or without evodiamine through the differentiation culture (Cont). The cells were harvested at the indicated time point and used for Northern or Western blot analysis. Day 0, Time point before the stimulation of differentiation.

the cells treated with evodiamine, compared with that in the control cells (Fig. 4C). In the control cells, the very high level of ERK phosphorylation in growth medium before differentiation stimulation (d 0) was reduced greatly within the first 2 d, and it disappeared thereafter. On the other hand, a steady level of ERK phosphorylation was detected in Evo-treated cells in the first 2 d, and phosphorylated ERK was detectable by d 8. Evodiamine did not stimulate ERK phosphorylation in the 3T3-L1 mature adipocytes (data not shown).

Evodiamine inhibits adipocyte differentiation by the sustained activation of ERK in 3T3-L1 cells

To further assess the involvement of ERK signaling in the effects of evodiamine on adipocyte differentiation, we examined the acute effects of insulin and evodiamine on the stimulation of ERK phosphorylation. As previously reported (25), a transient increase in ERK phosphorylation that peaked after 5 min was observed in 3T3-L1 cells after insulin stimulation (Fig. 5A). Evodiamine addition led to a modest but considerable stimulation of ERK phosphorylation that lasted over a 1-h period (Fig. 5B), indicating the differences in mode of action between insulin and evodiamine. When the evodiamine effect was checked in a longer time course, ERK phosphorylation lasted 18 h after evodiamine stimulation (data not shown). Cotreatment of evodiamine with PD98059, a specific inhibitor of MAPK kinase (an upstream kinase for ERK), reduced ERK phosphorylation (Fig. 5B) and restored adipocyte differentiation (Figs. 5C). Interestingly, capsaicin had no effect on ERK phosphorylation (Fig. 5D) or adipocyte differentiation (Fig. 5E). In addition, the effect of evodiamine on ERK phosphorylation was detected in a nonadipogenic cell line, human hepatoma HepG2 cells. Similar to the results in 3T3-L1 preadipocytes, ERK phosphorylation greatly increased 1 h after evodiamine stimulation, and its increased level was detectable even after 24 h (Fig. 5F). As expected, the increase in ERK phosphorylation was blocked by cotreatment of evodiamine with PD98059.

Effects of evodiamine on insulin signaling pathway

We examined whether evodiamine would affect insulin signaling through the PI3K/Akt pathway during adipocyte differentiation because this pathway is important in transducing the proadipogenic effects of insulin through IR and/or IGF-IR (26, 27). As shown in Fig. 6A, evodiamine did not affect the tyrosine phosphorylation of either IR or IGF-IR in 3T3-L1 preadipocytes. Likewise, the tyrosine phosphorylation of IRS-1 and its binding with the PI3K p85 subunit in the preadipocytes were not changed by the evodiamine treatment (Fig. 6B). Evodiamine also did not affect PI3K activity (data not shown). However, we found that evodiamine strongly reduced the insulin-induced phosphorylation of Akt Ser473, a regulatory site of Akt activity, in the 3T3-L1 preadipocytes, which contrasted with the steady level of ERK phosphorylation (Fig. 7A). Reduced phosphorylation of Akt Ser303 was also detected in the evodiamine-treated cells (data not shown). When the cells were pretreated with PD98059, this inhibitory effect of evodiamine was strongly blocked, and Akt phosphorylation was restored by about 70% of level in the insulin treatment. The stimulation of ERK phosphorylation and inhibition of insulin-stimulated Akt phosphorylation were also observed in primary cultures of

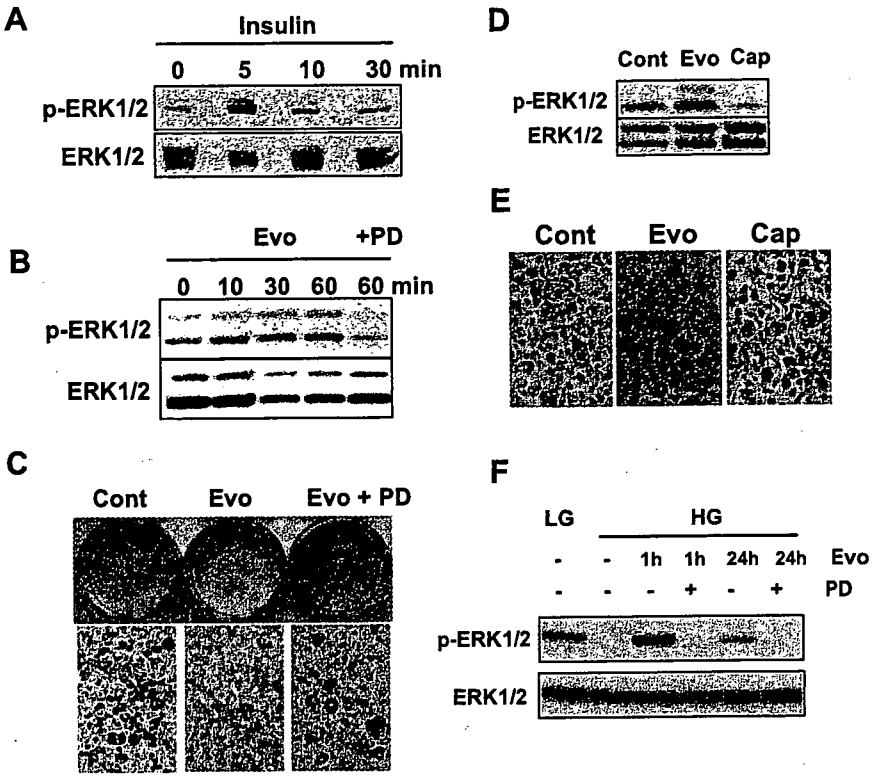


FIG. 5. Evodiamine inhibits adipocyte differentiation by stimulating the ERK pathway. A and B, Time course of ERK activation by insulin (A) and evodiamine (B). Two-day postconfluent 3T3-L1 cells were serum deprived for 4 h and then treated with 20 nM insulin or 10 μM evodiamine in the absence or presence of 10 μM PD98059 (+PD) for the indicated times, and the lysates were analyzed for activated ERK. C, PD98059 inhibits the evodiamine effect and restores adipogenesis. The cells were cultured in the differentiation medium with 1 μM evodiamine in the absence or presence of 10 μM PD by using the same protocol as in Fig. 4A. The lipid accumulation in the cells was evaluated by oil Red O staining. D and E, Capsaicin neither stimulates ERK phosphorylation (D) nor inhibits adipocyte differentiation (E). Cells were treated with 20 μM evodiamine (Evo), capsaicin (Cap), or an equal volume of dimethylsulfoxide control (Cont) for 30 min (D) or cultured in the differentiation medium with 1 μM evodiamine or capsaicin by using the same protocol as in Fig. 4A (E). F, Effect of evodiamine on ERK phosphorylation in HepG2 cells. Cells (~70% confluent) were quiesced in serum-free low-glucose (LG) medium (5.5 mM D-glucose) overnight and then stimulated in high-glucose (HG) medium (24.75 mM D-glucose) with or without evodiamine (Evo) for the indicated periods. One and 10 μM Evo were used for 24 h treatment and for 1 h treatment, respectively, in the absence or presence of 10 μM PD98059 (+PD), and the cell lysates were analyzed for activated ERK by Western blot analysis. Data shown are representatives of three independent experiments.

adipocyte precursor cells (Fig. 7B). Moreover, the effects of evodiamine on ERK and Akt signaling were determined in the WAT derived from the mice in the diet study (Fig. 8A). Compared with those for the −Evo group, the phosphorylation levels of ERK and Akt were significantly higher (2-fold) and lower (about half), respectively, in the WAT of the +Evo group in KO mice. In the control mice, the phosphorylation level of Akt in the +Evo group was reduced to 28% of the −Evo group, whereas the effect on ERK phosphorylation was not clear. The effects of evodiamine on ERK and Akt signaling were also confirmed in the WAT of mice administered with the compound. An injection of evodiamine significantly up-regulated ERK phosphorylation (1.7-fold) and down-regulated Akt phosphorylation (about a fourth) in the WAT of mice, compared with those for the control mice (Fig. 8B).

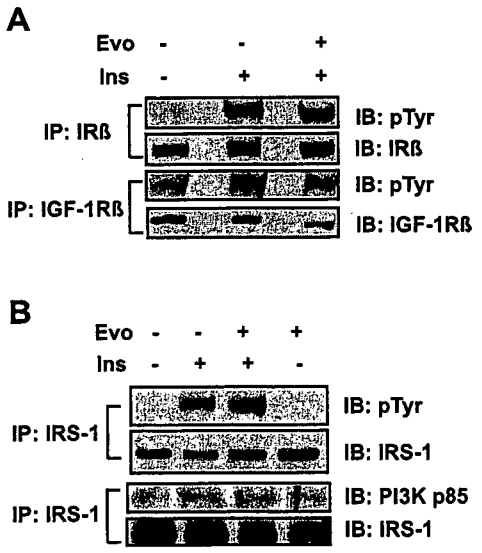


FIG. 6. Effects of evodiamine on insulin signaling pathway in 3T3-L1 cells. Two-day postconfluent cells were serum deprived for 4 h and then treated with 20 μM evodiamine (Evo) for 1 h and with 20 nM insulin (Ins) for the last 10 min. Tyrosine phosphorylation of IRβ or IGF-IRβ (A) and tyrosine phosphorylation of IRS-1 or its binding with PI3K (B) were determined by immunoprecipitation (IP) and immunoblot (IB) analysis. Immunoprecipitation experiments were performed by using the cell lysates and antibodies specific for each molecule. Data shown are representative of three independent experiments.

Discussion

It was previously reported that evodiamine showed an antiobesity effect, which was thought to depend on the enhancement of UCP1 thermogenesis through β3-adrenergic stimulation in BAT (14). To clarify whether the antiobesity effect of evodiamine depended on energy dissipation mediated by UCP1 thermogenesis, we first examined the effects of evodiamine on the development of diet-induced obesity in UCP1-KO mice in the present study. Interestingly, we found that evodiamine showed a potent effect of preventing the increases in body weight and adiposity even in the UCP1-KO mice fed the HF diet, which was indistinguishable from the effects of this compound in the control mice. The data on blood parameters suggested the improvement of leptin resistance and insulin sensitivity in the mice fed the evodiamine diet. In addition, IPGTT data supported the improvement of glucose metabolism in the mice treated with the evodiamine diet, in which the phenotypes in fed glucose and insulin levels were similar to those in PPARγ$^{+/-}$ mice (24). These results were unexpected because the diet-induced obe-

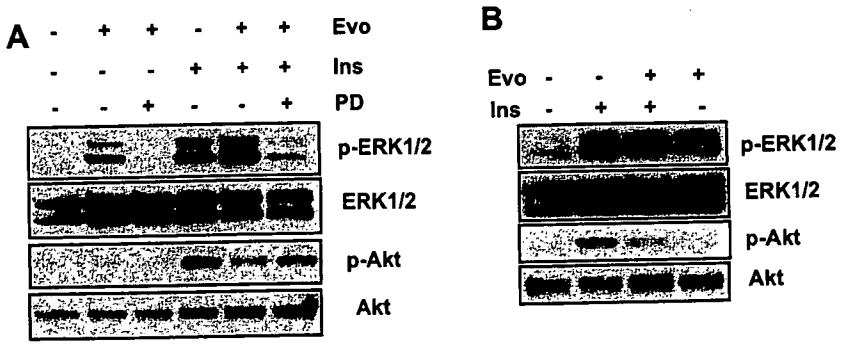


FIG. 7. Evodiamine negatively regulates insulin-stimulated Akt activation through an ERK pathway in preadipocytes. A, 3T3-L1 preadipocytes were serum deprived for 4 h and then treated with 20 μM evodiamine (Evo) for 1 h and with 20 nM insulin (Ins) for the last 10 min. PD98059 (PD; 20 μM) was added 1 h before the evodiamine treatment. Western blot analyses for ERK and Akt were performed by using cell lysates. B, Adipocyte precursor cells isolated from the WAT of mice were cultured as described in *Materials and Methods*. The cells were serum deprived for 4 h and then treated with 20 μM evodiamine for 1 h and with 20 nM insulin for the last 10 min. Representative images of three independent experiments are shown.

sity in UCP1-KO mice was not prevented by treatment with CL316,243, a strong β3-adrenergic agonist, whereas this compound effectively stimulated UCP1 thermogenesis and prevented the diet-induced obesity in wild-type mice (28). An injection of CL316,243 is known to increase oxygen consumption acutely in wild-type mice but not UCP1-KO mice (16, 28), whereas evodiamine did not appear to change oxygen consumption for the periods of 24 h after its injection in both of wild-type and UCP1-KO mice (our unpublished data). We also could not detect any change in UCP1 mRNA level in BAT of the control mice fed the evodiamine diet. These results suggest that energy dissipation mediated by UCP1 does not play a role in the antiobesity effect of evodiamine in mice, raising the possibility that this compound could be related more directly to the inhibition of fat deposition.

Therefore, we next investigated the direct effects of evodiamine on adipocyte differentiation. Our results suggest that reduced diet-induced obesity by evodiamine in the diet could be due to a reduction in the recruitment of new adipocytes from precursor cells. Such recruitment has been suggested to play a role in the expansion of adipose tissue in obesity (4, 7). In addition to the serial induction of transcriptional regulators, modulation of intracellular signaling molecules is essential for adipocyte differentiation. In the early steps of the differentiation, the regulation of MAPK activity is critical for initiating the entry of preadipocytes into the differentiation process (10). After its transient activation of ERK, its activity is down-regulated to enable the differentiation program to proceed because the sustained activation of the ERK signaling pathway inhibits adipocyte differentiation *in vitro* (13). Indeed, we found that evodiamine had a strong inhibitory effect for adipogenesis of the cells via sustained activation of the ERK/MAPK signaling pathway in 3T3-L1 and primary preadipocytes.

A similar ERK-activating effect was recently reported in 3T3-L1 preadipocytes treated with a quite high dose (500 μM) of α-lipoic acid (25). Because ERK stimulates the phosphorylation of PPARγ, causing a reduction in its transcriptional activity (29), the inactivation of PPARγ activity mediated by ERK together with reduced PPARγ mRNA levels could compound the inhibitory effect of evodiamine on adipogenesis. Because C/EBPβ is an important factor to initiate mitotic clonal expansion, which is a crucial step to enter the late stages of adipogenesis (23), the marked reduction of C/EBPβ in 3T3-L1 preadipocytes treated with evodiamine might affect the initiation of mitotic clonal expansion. The p38MAPK pathway has also been reported recently to play a role in

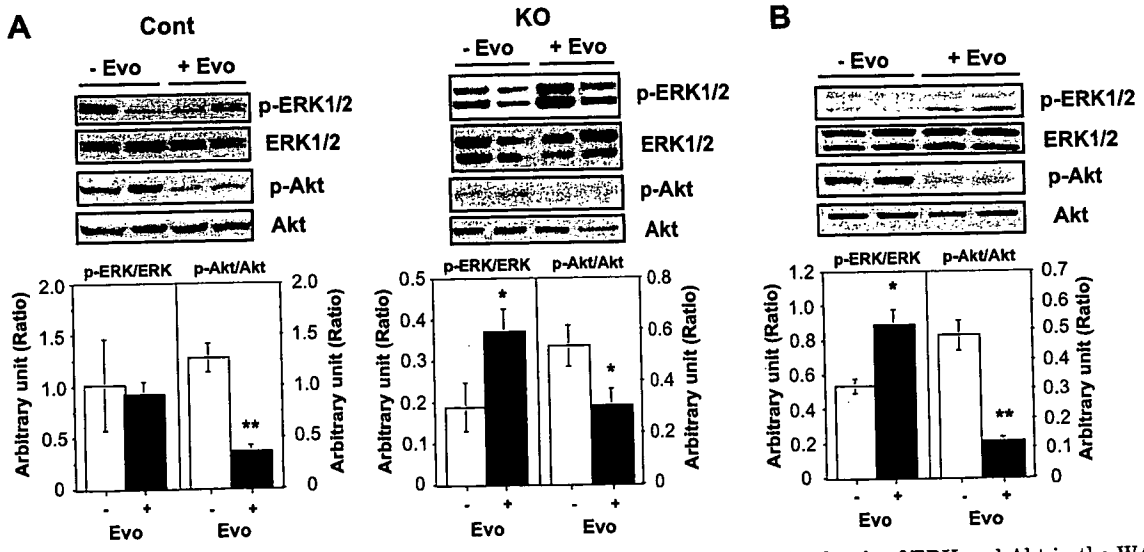


FIG. 8. Effects of evodiamine on the phosphorylation of ERK and Akt *in vivo*. Phosphorylation levels of ERK and Akt in the WAT of mice fed the HF diet with or without evodiamine (Evo) in the diet study (A) and in the WAT of wild-type mice treated with evodiamine (3 mg/kg, ip) or vehicle for 24 h (B). Western blot analyses for ERK and Akt were performed by using tissue lysates (50 μg protein) of epididymal WAT from the mice. Data are expressed as the mean ± SE (n = 4). *, $P < 0.05$ and **, $P < 0.01$ *vs.* −Evo group (Student's *t* test).

adipogenesis via regulation of C/EBPβ and PPARγ transcriptional activities (30). Of note, unlike evodiamine, capsaicin neither stimulated ERK phosphorylation nor inhibited adipogenesis, even though these compounds show similar actions *in vivo* on vasorelaxation and hypothermia (14, 31, 32). Considering the results on the signal analysis of mouse WAT, it is likely that evodiamine contributed to the suppression of diet-induced obesity in mice by inhibiting adipogenesis, although other mechanisms could be involved in the antiobesity effects of evodiamine.

We also found an effect of evodiamine on ERK phosphorylation in HepG2 cells. Similar to 3T3-L1 preadipocytes, evodiamine significantly stimulated ERK phosphorylation in the nonadipogenic cells. Kosone *et al.* recently suggested an involvement of ERK in a reducing effect of hepatocyte growth factor on lipid accumulation in HepG2 cells through induction of several genes related to lipid metabolism (33). Because the fatty liver observed in HF diet-induced obesity was improved considerably in the mice fed the evodiamine diet, it would be of interests to know the effect of evodiamine on lipid metabolism in hepatocytes.

In addition to ERK/MAPK signaling, PI3K/Akt is an important intracellular signal cascade in the regulation of many cellular activities including growth, glucose metabolism, and adipogenesis (34–36). Insulin stimulates tyrosine phosphorylation of the IR and/or IGF-IR, which promotes the activation of Akt via phosphorylation of PI3K. Differentiation of 3T3-L1 preadipocytes is stimulated strongly by the expression of a constitutively active form of Akt (37). On the other hand, adipogenesis is blocked in cultured cells or mice lacking Akt (26, 36). In the present study, the upstream signals within the insulin/IGF-I pathway were not affected by evodiamine in 3T3-L1 cells. However, we found that insulin-stimulated Akt phosphorylation was inhibited strongly in the preadipocytes treated with evodiamine in contrast to the stimulation of ERK phosphorylation. In addition, the Akt inhibition was restored by a MAPK kinase inhibitor, which profoundly blocked ERK phosphorylation in the cells, suggesting a connection between PI3K/Akt and ERK/MAPK pathways in the preadipocytes. Taken together, evodiamine may inhibit adipogenesis by suppressing insulin-stimulated Akt phosphorylation through the activation of ERK signaling. Similar effects of evodiamine on Akt and ERK phosphorylation were detected in the WAT from the UCP1-KO mice in the diet study. Although we could not detect a significant effect of evodiamine on ERK phosphorylation in the WAT from the control mice, the effects of evodiamine on ERK and Akt signaling *in vivo* were supported from the evidence that an injection of evodiamine to wild-type mice considerably stimulated ERK phosphorylation and reduced Akt phosphorylation in the WAT. Because Akt has important roles in growth (34), the decrease in Akt phosphorylation by evodiamine might inhibit mitotic clonal expansion in preadipocytes. Takada *et al.* (38) recently reported that evodiamine inhibits Akt activation in tumor cells.

We presently do not know how ERK regulates Akt phosphorylation in preadipocytes. Because the Akt activity is regulated negatively by several phosphatases such as the phosphatidylinositol 3′ lipid phosphatase (39) or protein phosphatase type 2A (40), we examined the effect of evodiamine on the phosphorylation level of these phosphatases. However, evodiamine did not affect the phosphorylation levels of phosphatidylinositol 3′ lipid phosphatase and protein phosphatase type 2A in the presence of insulin (Wang, T., unpublished data). Therefore, the contribution of these phosphatases to Akt inactivation in 3T3-L1 preadipocytes stimulated with evodiamine appears to be low, so other molecules may be involved in the negative cross talk of ERK signaling for the regulation of Akt activity in adipocyte differentiation.

In summary, our results indicate that evodiamine has the previously unrecognized action of inhibiting adipogenesis by a mechanism in which the stimulation of ERK/MAPK signaling down-regulates the expression of adipocyte transcription factors and insulin-induced Akt signaling. Because evodiamine clearly showed an antiobesity effect in UCP1-deficient mice, this compound may offer a new approach to circumvent the development of diet-induced obesity, especially in animals lacking UCP1 thermogenesis including adult humans; however, further details of the inhibitory mechanism and the effects on insulin sensitivity remain to be clarified.

Acknowledgments

We thank Dr. L. P. Kozak for the UCP1-deficient mice and critical review of the manuscript; Dr. M. Imagawa for 3T3-L1 cells; Ms. Y. Kadokawa, Ms. Z. Wang, and Dr. J. Yao for technical assistance; Dr. K. Takaba for histological analysis; and Dr. I. Shimomura for valuable suggestions. Early phase of this study was performed at the Departments of Molecular Genetics and Aging Intervention in National Institute for Longevity Sciences.

Received April 11, 2007. Accepted September 10, 2007.

Address all correspondence and requests for reprints to: Hitoshi Yamashita, Department of Biomedical Sciences, College of Life and Health Sciences, Chubu University, Kasugai 487-8501, Japan. E-mail: hyamashi@isc.chubu.ac.jp.

This work was supported by Research Grant 15C-8 for Longevity Sciences from the Ministry of Health, Labor, and Welfare (to H.Y.).

Disclosure Statement: All authors have nothing to declare.

References

1. **Kopelman PG** 2000 Obesity as a medical problem. Nature 404:635–643
2. **Flier JS** 2004 Obesity wars: molecular progress confronts an expanding epidemic. Cell 116:337–350
3. **Bray GA, Tartaglia LA** 2000 Medicinal strategies in the treatment of obesity. Nature 404:672–677
4. **Crowley VE, Yeo GS, O'Rahilly S** 2002 Obesity therapy: altering the energy intake-and-expenditure balance sheet. Nat Rev Drug Discov 1:276–286
5. **Kopecky J, Clarke G, Enerback S, Spiegelman B, Kozak LP** 1995 Expression of the mitochondrial uncoupling protein gene from the aP2 gene promoter prevents genetic obesity. J Clin Invest 96:2914–2923
6. **Kim MS, Park JY, Namkoong C, Jang PG, Ryu JW, Song HS, Yun JY, Namgoong IS, Ha J, Park IS, Lee IK, Viollet B, Youn JH, Lee HK, Lee KU** 2004 Anti-obesity effects of α-lipoic acid mediated by suppression of hypothalamic AMP-activated protein kinase. Nat Med 10:727–733
7. **Kolonin MG, Saha PK, Chan L, Pasqualini R, Arap W** 2004 Reversal of obesity by targeted ablation of adipose tissue. Nat Med 10:625–632
8. **Rosen ED, Spiegelman BM** 2000 Molecular regulation of adipogenesis. Annu Rev Cell Dev Biol 16:145–171
9. **Koutnikova H, Auwerx J** 2001 Regulation of adipocyte differentiation. Ann Med 33:556–561
10. **Bost F, Aouadi M, Caron L, Binetruy B** 2005 The role of MAPKs in adipocyte differentiation and obesity. Biochimie (Paris) 87:51–56
11. **Kim SW, Muise AM, Lyons PJ, Ro HS** 2001 Regulation of adipogenesis by a transcriptional repressor that modulates MAPK activation. J Biol Chem 276: 10199–10206
12. **Prusty D, Park BH, Davis KE, Farmer SR** 2002 Activation of MEK/ERK signaling promotes adipogenesis by enhancing peroxisome proliferator-acti-

vated receptor γ (PPARγ) and C/EBPα gene expression during the differentiation of 3T3-L1 preadipocytes. J Biol Chem 277:46226–46232
13. **Sakaue H, Ogawa W, Nakamura T, Mori T, Nakamura K, Kasuga M** 2004 Role of MAPK phosphatase-1 (MKP-1) in adipocyte differentiation. J Biol Chem 279:39951–39957
14. **Kobayashi Y, Nakano Y, Kizaki M, Hoshikuma K, Yokoo Y, Kamiya T** 2001 Capsaicin-like anti-obese activities of evodiamine from fruits of *Evodia rutaecarpa*, a vanilloid receptor agonist. Planta Med 67:628–633
15. **Cui J, Himms-Hagen J** 1992 Long-term decrease in body fat and in brown adipose tissue in capsaicin-desensitized rats. Am J Physiol 262:R568–R573
16. **Enerback S, Jacobsson A, Simpson EM, Guerra C, Yamashita H, Harper ME, Kozak LP** 1997 Mice lacking mitochondrial uncoupling protein are cold-sensitive but not obese. Nature 387:90–94
17. **Kontani Y, Wang Y, Kimura K, Inokuma KI, Saito M, Suzuki-Miura T, Wang Z, Sato Y, Mori N, Yamashita H** 2005 UCP1 deficiency increases susceptibility to diet-induced obesity with age. Aging Cell 4:147–155
18. **Hemati N, Ross SE, Erickson RL, Groblewski GE, MacDougald OA** 1997 Signaling pathways through which insulin regulates CCAAT/enhancer binding protein α (C/EBPα) phosphorylation and gene expression in 3T3-L1 adipocytes. Correlation with GLUT4 gene expression. J Biol Chem 272:25913–25919
19. **Zang M, Zuccollo A, Hou X, Nagata D, Walsh K, Herscovitz H, Brecher P, Ruderman NB, Cohen RA** 2004 AMP-activated protein kinase is required for the lipid-lowering effect of metformin in insulin-resistant human HepG2 cells. J Biol Chem 279:47898–47905
20. **Folch J, Lees M, Sloane-Stanley GH** 1957 A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497–509
21. **Revelli J-P, Preitner F, Samec S, Muniesa P, Kuehne F, Boss O, Vassalli J-D, Dulloo A, Seydoux J, Giacobino J-P, Huarte J, Ody C** 1997 Targeted gene disruption reveals a leptin-independent role for the mouse β3-adrenoceptor in the regulation of body composition. J Clin Invest 100:1098–1106
22. **Summers SA, Lipfert L, Birnbaum MJ** 1998 Polyoma middle T antigen activates the Ser/Thr kinase Akt in a PI3-kinase-dependent manner. Biochem Biophys Res Commun 246:76–81
23. **Tang QQ, Otto TC, Lane MD** 2003 CCAAT/enhancer-binding protein β is required for mitotic clonal expansion during adipogenesis. Proc Natl Acad Sci USA 100:850–855
24. **Kubota N, Terauchi Y, Miki H, Tamemoto H, Yamauchi T, Komeda K, Satoh S, Nakano R, Ishii C, Sugiyama T, Eto K, Tsubamoto Y, Okuno A, Murakami K, Sekihara H, Hasegawa G, Naito M, Toyoshima Y, Tanaka S, Shiota K, Kitamura T, Fujita T, Ezaki O, Aizawa S, Kadowaki T, et al** 1999 PPAR γ mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance. Mol Cell 4:597–609
25. **Cho KJ, Moon HE, Moini H, Packer L, Yoon DY, Chung AS** 2003 α-Lipoic acid inhibits adipocyte differentiation by regulating pro-adipogenic transcription factors via mitogen-activated protein kinase pathways. J Biol Chem 278:34823–348233
26. **Xu J, Liao K** 2004 Protein kinase B/AKT 1 plays a pivotal role in insulin-like growth factor-1 receptor signaling induced 3T3-L1 adipocyte differentiation. J Biol Chem 279:35914–35922
27. **Zhang B, Berger J, Zhou G, Elbrecht A, Biswas S, White-Carrington S, Szalkowski D, Moller DE** 1996 Insulin- and mitogen-activated protein kinase-mediated phosphorylation and activation of peroxisome proliferator-activated receptor γ. J Biol Chem 271:31771–31774
28. **Inokuma K, Okamatsu-Ogura Y, Omachi A, Matsushita Y, Kimura K, Yamashita H, Saito M** 2006 Indispensable role of mitochondrial UCP1 for antiobesity effect of β3-adrenergic stimulation. Am J Physiol Endocrinol Metab 290:E1014–E1021
29. **Hu E, Kim JB, Sarraf P, Spiegelman BM** 1996 Inhibition of adipogenesis through MAP kinase-mediated phosphorylation of PPARγ. Science 274:2100–2103
30. **Aouadi M, Laurent K, Prot M, Le Marchand-Brustel Y, Binetruy B, Bost F** 2006 Inhibition of p38MAPK increases adipogenesis from embryonic to adult stages. Diabetes 55:281–289
31. **Chiou WF, Chou CJ, Shum AY, Chen CF** 1992 The vasorelaxant effect of evodiamine in rat isolated mesenteric arteries: mode of action. Eur J Pharmacol 215:277–283
32. **Wang Y, Kimura K, Inokuma K, Saito M, Kontani Y, Kobayashi Y, Mori N, Yamashita H** 2006 Potential contribution of vasoconstriction to suppression of heat loss and homeothermic regulation in UCP1-deficient mice. Pflugers Arch 452:363–369
33. **Kosone T, Takagi H, Horiguchi N, Ariyama Y, Otsuka T, Sohara N, Kakizaki S, Sato K, Mori M** 2007 HGF ameliorates a high-fat diet-induced fatty liver. Am J Physiol 293:G204–G210
34. **Cho H, Thorvaldsen JL, Chu Q, Feng F, Birnbaum MJ** 2001 Akt/PKBα is required for normal growth but dispensable for maintenance of glucose homeostasis in mice. J Biol Chem 276:38349–38352
35. **Gagnon A, Chen CS, Sorisky A** 1999 Activation of protein kinase B and induction of adipogenesis by insulin in 3T3-L1 preadipocytes: contribution of phosphoinositide-3,4,5-trisphosphate versus phosphoinositide-3,4-bisphosphate. Diabetes 48:691–698
36. **Peng XD, Xu PZ, Chen ML, Hahn-Windgassen A, Skeen J, Jacobs J, Sundararajan D, Chen WS, Crawford SE, Coleman KG, Hay N** 2003 Dwarfism, impaired skin development, skeletal muscle atrophy, delayed bone development, and impeded adipogenesis in mice lacking Akt1 and Akt2. Genes Dev 17:1352–1365
37. **Kohn AD, Summers SA, Birnbaum MJ, Roth RA** 1996 Expression of a constitutively active Akt Ser/Thr kinase in 3T3-L1 adipocytes stimulates glucose uptake and glucose transporter 4 translocation. J Biol Chem 271:31372–31378
38. **Takada Y, Kobayashi Y, Aggarwal BB** 2005 Evodiamine abolishes constitutive and inducible NF-κB activation by inhibiting IκBα kinase activation, thereby suppressing NF-κB-regulated antiapoptotic and metastatic gene expression, up-regulating apoptosis, and inhibiting invasion. J Biol Chem 280:17203–17212
39. **Marino M, Acconcia F, Trentalance A** 2003 Biphasic estradiol-induced AKT phosphorylation is modulated by PTEN via MAP kinase in HepG2 cells. Mol Biol Cell 14:2583–2591
40. **Ugi S, Imamura T, Maegawa H, Egawa K, Yoshizaki T, Shi K, Obata T, Ebina Y, Kashiwagi A, Olefsky JM** 2004 Protein phosphatase 2A negatively regulates insulin's metabolic signaling pathway by inhibiting Akt (protein kinase B) activity in 3T3-L1 adipocytes. Mol Cell Biol 24:8778–8789

Endocrinology **is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

Erratum

In the article "Dihydrotestosterone Differentially Modulates the Mitogen-Activated Protein Kinase and the Phosphoinositide 3-Kinase/Akt Pathways through the Nuclear and Novel Membrane Androgen Receptor in C6 Cells" by Joshua W. Gatson, Paramjit Kaur, and Meharvan Singh (*Endocrinology* **147**: 2028-2034), the authors note the following error in their paper. In the legend to Fig. 10, the sentence "The fluorescence histogram, depicting increasing fluorescence intensity on the x-axis and cell number on the y-axis, shows significant labeling of cells with DHT-BSA-FITC" should read "The fluorescence histogram, depicting increasing fluorescence intensity on the x-axis and cell number on the y-axis, shows significant labeling of cells with testosterone-BSA-FITC". Similarly, the description of the compound within the *Methods* and *Results* sections, both pertaining to Fig. 10, should read 'testosterone-BSA-FITC' rather than 'DHT-BSA-FITC'. This change does not alter the interpretation of data shown in Fig. 10. *The authors apologize for the error.*

III. 脂肪組織の代謝

ミトコンドリア：UCP1，PGC1ファミリーなど

山下　均
(中部大学生命健康科学部生命医科学科)

はじめに

脂肪組織におけるミトコンドリアの機能は白色脂肪細胞と褐色脂肪細胞で大きく異なる．この違いは，内分泌細胞としての脂肪細胞の働きとは別に，二つの脂肪細胞の基本的な役割が白色脂肪細胞ではエネルギー貯蔵 fat storage であり，褐色脂肪細胞では熱産生 thermogenesis にあることに基づく．本項では，2種類の脂肪細胞の代謝特性の違いを担う主要な分子として，ミトコンドリア脱共役蛋白質1型(uncoupling protein-1：UCP1)やペルオキシソーム増殖剤応答性受容体γコアクチベーター1(PPARγ coactivator-1：PGC1)などの役割について，最近の知見を踏まえて概説する．

I. UCP1ファミリー

褐色脂肪細胞のみに特異的に発現する分子量32,000の蛋白質として最初に発見されたUCP1の研究は，そのcDNAがクローニングされた1985年以降急速に進んできた[1]．UCP1はミトコンドリア内膜に存在する6回膜貫通型蛋白質で，2量体として作用していると考えられている．基本的なミトコンドリアの役割は，エネルギー基質の酸化的リン酸化によりATPを産生し，さまざまな生体反応を支えることにある．図1に示すように，摂取した食物由来の脂質や糖質などのエネルギー基質は，ミトコンドリアの呼吸鎖で酸化され，生じたプロトン(H^+)はATP合成酵素F_0F_1-ATPaseの作用によりATP合成に共役される．しかし，この共役反応は不完全であり，プロトンの一部はUCP1の働きによりATP合成と共役することなく熱として消費される．実際に，UCP1による脱共役反応はATP合成を阻害した実験条件下において，ミトコンドリア膜ポテンシャルの低下(プロトンリーク)という形で観察される．この脱共役反応は脂肪酸や活性酸素により亢進し，GDPやADPなどプリンヌクレオチドにより阻害されるが，そのメカニズムについては今なお不明な点が多い．現在までに，UCP1以外に四つのUCPファミリー分子(UCP2-UCP5)が同定され，各UCPは*in vitro*の実験系においてUCP1と同様の脱共役機能を有することが確認されている．各UCPは300前後のアミノ酸からなり，ヒトホモログでは一次構造の比較からUCP2とUCP3はそれぞれ59％と57％，UCP4とUCP5は30％程度UCP1との相同性を示す．ここでは，肥満や糖尿病と関連して研究の進んでいるUCP1～UCP3について研究の現状を以下に述べる．

A. UCP1

UCP1の生理的役割については，トランスジェニックマウスを用いた研究などから寒冷環境下における体温維持のための熱産生(寒冷誘導性熱産生 cold-induced thermogenesis)と過食時における余剰摂取カロリーの熱変換による消費(食事誘導性熱産生 diet-induced thermogenesis)と考えられてきた．これらの役割をより明確に検証するためにUCP1遺伝子を欠損する(UCP1-KO)マウスが作製されて研究が進められ，現在までに多くのことが明らかとなってきた．まず，最初の報告において，UCP1-KOマウスは急激な寒冷曝露(5°C)に対して著しい体温低下を示したことから，UCP1の寒冷誘導性熱産生における重要性が証明された[2]．また，UCP1は室温環境下(～23°C)での恒温性維持における熱産生においても重要であ

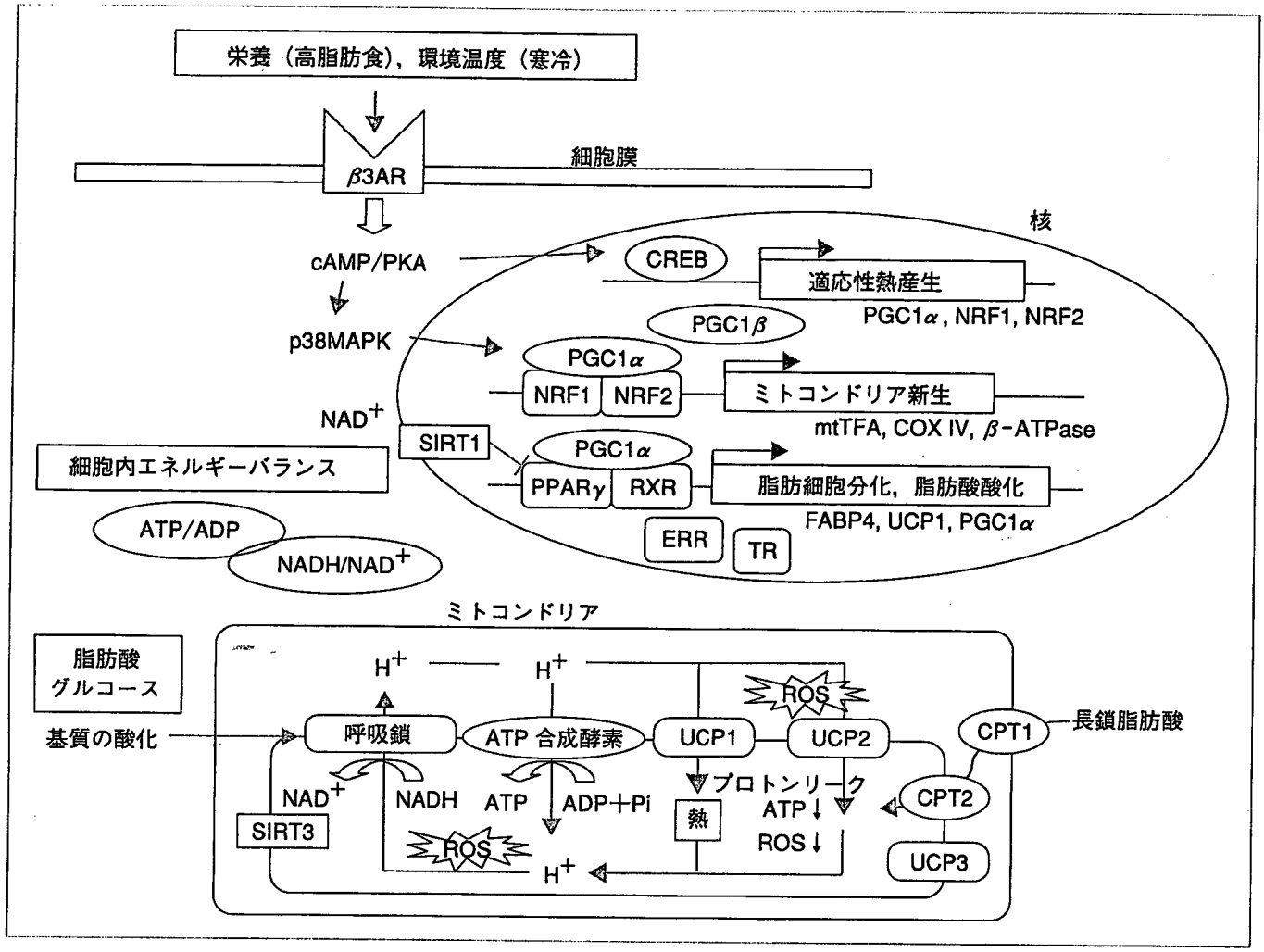


図1 脂肪細胞におけるミトコンドリア機能とエネルギー代謝の調節 褐色脂肪細胞のミトコンドリアでは，細胞機能を維持するためのATP合成と細胞の役割としての熱産生が行われる．熱産生はUCP1の作用に大きく依存し，種々の外的刺激に応じて，その機能が強化される(適応性熱産生)．白色脂肪細胞のミトコンドリアには，この機能は付与されていない．PGC1は脂肪細胞の分化と適応性熱産生にきわめて重要であり，さまざまな転写因子と協調して両脂肪細胞の機能を遺伝子発現の面から支える．CPT1やSIRT3は，長鎖脂肪酸やグルコースの利用を促進し，褐色脂肪細胞における爆発的な代謝回転に寄与する．また，UCP2やUCP3は活性酸素種(ROS)などの産生を抑制する．これらの反応は互いに関連し，細胞内のエネルギーバランス(ATP/ADP比やNADH/NAD^+比)の変化に応じて巧みに調節されると考えられる．
FABP4：fatty acid binding protein 4，COX IV：cytochrome-c-oxidase subunit IV，TR：thyroid hormone receptor

ることが明らかとなっている．しかし予想に反して，UCP1-KOマウスの体重は標準食ばかりでなく高脂肪食摂取においても野生型マウスの体重と差がないことから，UCP1の食事誘導性熱産生における役割に疑問がもたれると同時に，UCP1熱産生以外の熱産生機構がUCP1欠損を代償した可能性が考えられた．現在そのメカニズムとして，甲状腺ホルモンT3やCa^{2+}サイクリングの関与が示唆されている[3]．一方Kontaniらは，UCP1-KOマウスは若齢期には肥満にならないが，高脂肪食の摂取により加齢とともにインスリン抵抗性を伴う肥満を呈することを報告した[4]．UCP1-KOマウスの褐色脂肪組織では，β3アドレナリン受容体(β3AR)やPGC1のmRNAレベルの上昇とUCP3蛋白量の増加が観察され，これらの発現上昇が加齢と高脂肪食という環境要因により減弱したことがUCP1-KOマウスにおける肥満進展の理由と考えられた．

図1に示すように，UCP1の発現と脂肪分解は主としてβ3ARを介するcAMP/PKA経路の活性化により強く促進されることが知られている．実際に，マウスへのβ3ARアゴニストの投与に

より褐色脂肪細胞と UCP1 の発現が誘導され，食事誘導性の肥満とインスリン抵抗性が軽減することが報告されていた．最近 Inokuma らは，β3AR アゴニストが UCP1-KO マウスの食事誘導性肥満を改善しないことを示し，β3AR アゴニストの抗肥満効果が UCP1 作用に依存することを証明した[5]．一方，褐色脂肪細胞では β3AR の刺激により UCP1 の活性化と同時にインスリン非依存性のグルコース利用が高まるが，そのメカニズムとして細胞内 AMP レベルの上昇による AMP キナーゼの活性化がグルコースの細胞内への取り込みを促進することによることが明らかとなっている[6]．以上の結果は，UCP1 熱産生の促進が脂質代謝のみならず，糖代謝をも改善する可能性を強く示唆する．興味深いことに，腹部内の白色脂肪組織における低レベルの UCP1 発現誘導が全身のインスリンならびにレプチン感受性を高めることが最近報告された[7]．このメカニズムの詳細については明らかではないが，末梢の脂肪組織から中枢へ求心性の神経経路を介して何らかの情報が送られている可能性があり，その解明が待たれる．

B．UCP2

1997 年にクローニングされた UCP2 は，脂肪組織や免疫系，神経系を含めて全身の組織で広く発現が認められる．UCP2 の生理的役割について，当初は UCP1 と同様の熱産生作用が予想されたが，UCP2-KO マウスの解析が進むにつれて主な役割が UCP1 とは異なることが明らかとなってきた．エネルギー産生の場であるミトコンドリアでは，代謝回転の副産物として活性酸素種 (reactive oxygen species：ROS) が生成する．すなわち，ATP 合成に共役しない過剰なプロトンの滞留は活性酸素生成の引き金となる (図 1)．この ROS は，DNA，蛋白質，脂質を酸化しさまざまな細胞障害の原因となるが，ミトコンドリアにはスーパーオキシドディスムターゼなど，ROS の毒性を消去する系が存在する．UCPs はプロトンリークにより酸化ストレスを低減する可能性が以前から示唆されていたが，UCP2 にはこのプロトンの滞留を解消して ROS 生成を抑える作用があること，その活性は活性酸素と脂肪酸により上昇することがいくつものグループから報告された．病態との関連では，肥満や 2 型糖尿病では脂肪組織における UCP2mRNA レベルの上昇など，酸化ストレスに関連する種々のマーカーが上昇することが知られている．最近 Houstis らは，UCP2 の関与については検討していないが，脂肪細胞における酸化ストレスの上昇がインスリン抵抗性の一つの要因となることを実験的に示した[8]．

一方，UCP2 の活性化は酸化ストレスの抑制と同時に ATP 合成の低下を意味し，ATP レベルの低下はさまざまな生体反応に影響する可能性がある．例えば，膵臓の β 細胞では血糖レベルの上昇を感知して取り込まれたグルコースからの ATP 産生が増加すると，この ATP レベル (ATP/ADP 比) の上昇により K_{ATP} チャネルが閉鎖して細胞膜の脱分極を誘導し，最終的にインスリン分泌が促進される機構が知られている．したがって，UCP2 活性の上昇は ATP/ADP 比の低下を招きインスリン分泌を抑制するように働き，肥満におけるインスリン抵抗性の増大や 2 型糖尿病の進行に関与していることが考えられた．実際に，UCP2 によるインスリン分泌の負の制御機構は，UCP2-KO マウスを用いた解析により証明され，ob/ob マウスにおける UCP2 欠損は糖代謝を改善することが報告されている[9]．

以上のように，UCP2 の生理機能は活性酸素と ATP レベルの制御が中心と考えられる．これらの機能は，UCP2 の脱共役作用により同時に進行するものであることから，その機能を別々に制御する他の因子が存在しない限り，二つの機能を切り離すことはむずかしい．UCP2 の働きは，おそらく組織や細胞固有の特性と関連して制御されているのであろう．

C．UCP3

UCP2 に続いてクローニングされた UCP3 は，褐色脂肪細胞と骨格筋や心臓などの筋組織において高い発現レベルが認められる．また，UCP3 の発現は PGC1s や PPARs による転写制御を受け，脂肪酸代謝の高まる高脂肪食摂取や絶食，寒冷曝

露の際に上昇することが報告されている．遺伝子改変マウスを用いた解析から，UCP3が熱産生により全身のエネルギー代謝の制御にかかわる可能性が示唆されたが，熱産生における役割については今なお明確な答えが出ていない．むしろ現在は，UCP2と同様に，ミトコンドリアにおける酸化ストレスの低減やミトコンドリアマトリクスからの脂肪酸の排出に関連する可能性が指摘されている[10]．すなわち，脂質の代謝が進む過程で脂肪酸がミトコンドリアに蓄積すると脂肪毒性が生じミトコンドリア機能が低下する．この際，UCP3はミトコンドリア内に蓄積する脂肪酸をマトリクスの外側へ排出する．排出された脂肪酸は脂肪酸サイクリングと呼ばれるメカニズムなどにより，マトリクス内に再移行することにより脂肪酸の代謝回転が進むという．ヒトでは，褐色脂肪細胞が非常に少ないことからUCP3の作用は主に骨格筋や心臓に限定されるものと考えられる．

II. PGC1ファミリー

PGC1ファミリー分子としては，PGC1α，PGC1β，PRC(PGC1-related coactivator)の3種類が知られ，褐色脂肪組織，骨格筋，心臓，肝臓など脂肪酸の酸化能力の高い組織を中心に分布する．分子構造としては，ホモログ間で相同性の高いN末領域の転写活性化ドメインとC末領域のRNA結合ドメインを特徴とし，N末領域に存在する結合モチーフ(LxxLL)を介してPPARs，レチノイドX受容体(RXR)，あるいはエストロゲン受容体関連受容体(ERR)などの転写因子と結合し，その活性を制御すると考えられる[11]．現在までに，PGC1αの生理的役割を中心に精力的な研究が進められ，エネルギー代謝全般においてきわめて重要な役割を果たすことが明らかとなってきた．PRCの機能についてはいまだ十分理解されていない．

A. PGC1α

最初にクローニングされたPGC1αは，脂肪細胞分化のマスターレギュレーターとして働く核内受容体PPARγと相互作用する分子として見出された．PGC1αは寒冷刺激による交感神経系の活性化により褐色脂肪細胞において誘導され，UCP1遺伝子の発現とミトコンドリアの新生を促進する(図1)．そのメカニズムとしては，β3ARを介するcAMP/PKA系の活性化がCREB(cAMP response element-binding protein)をリン酸化してPGC1α遺伝子の転写を促進することに始まる．PGC1αはUCP1遺伝子のプロモーター領域に結合したPPARγとRXRのヘテロダイマーと複合体を形成することにより，UCP1遺伝子の転写を促進する．PGC1α遺伝子もPPARγとRXRによる転写制御を受け，自らの転写を増幅する[12]．同時にp38MAPキナーゼによりリン酸化されたPGC1αはNRF1(nuclear respiratory factor-1)とNRF2の複合体に結合することにより，呼吸鎖を構成する種々の蛋白質やミトコンドリアDNAの複製とコード遺伝子の転写の主要な制御因子であるmtTFA (mitochondrial transcription factor A) の転写を高め，急速なミトコンドリアの新生と爆発的な脂肪酸酸化の亢進に中心的役割を果たすと考えられる．

白色脂肪細胞におけるPGC1α遺伝子の発現レベルは低く，PGC1αを白色脂肪細胞に導入することにより，UCP1の発現誘導を含めて褐色脂肪細胞の特徴をもつ細胞に転換することができる．一方，PGC1α遺伝子欠損マウスでは，褐色脂肪組織の機能低下に加えて肝臓，心臓，脳などにも異常が認められることは，代謝の活発な組織でのPGC1αの重要性を示唆するものと思われる．

B. PGC1β

PGC1βの遺伝子発現は，PGC1αと同様に，白色脂肪組織に比べて褐色脂肪組織で高い発現レベルが認められるが，寒冷誘導性ではなく，絶食により強く誘導される．また，PGC1βは脂肪細胞の分化とともに発現レベルが上昇し，脂肪酸酸化にかかわる遺伝子の転写を促進する．PGC1βを過剰発現するトランスジェニックマウスでは，エネルギー消費量が高く，肥満に対して抵抗性を示すことが報告され，PGC1βがエネルギー代謝に深くかかわることが示された[13]．最近Uldryらは，褐色脂肪細胞におけるPGC1αとPGC1βの役割について検討し，それぞれ白色脂肪細胞と

しての分化には必ずしも必須ではないが，UCP1やミトコンドリア新生など褐色脂肪細胞の特性にかかわる遺伝子群の発現には少なくともどちらか一方のPGC1の発現が必要であることを報告した[14]．しかし，PGC1βはPGC1αと異なりcAMP/PKA系を介する転写制御を受けないので，生体内では褐色脂肪細胞特有のミトコンドリア機能の発現・維持に対して互いに相補的に働いている可能性が高いと考えられる．また，肝臓や骨格筋での糖新生や脂肪酸β酸化などにおける役割の違いを含めてPGC1αとは異なる生理的条件下におけるPGC1βの役割が予想される．

III．SIRT1 ファミリー

NAD^+(nicotinamide adenine dinucleotide)依存性の蛋白質脱アセチル化酵素であるSIRT1(sirtuin 1)ファミリーは7種類のホモログからなり，エネルギー代謝と関連して重要な役割を果たすことが明らかとなってきた[15]．現在，SIRT1ファミリー分子の役割の一つとしては細胞内の$NADH/NAD^+$比の変化に応じてエネルギー貯蔵を制御するメカニズムにおいて働いていると考えられる．特に，最初に見出されたSIRT1はカロリー制限下において強く活性化され，個体の栄養状態を感知して代謝の活発な組織を中心にエネルギー代謝関連遺伝子の転写制御などに関与するらしい．ファミリー分子の多くは，発現レベルに違いはあるものの白色脂肪組織と褐色脂肪組織で発現が認められる．このうち，SIRT1は脂肪組織では転写抑制因子とともにPPARγと複合体を形成することにより前駆脂肪細胞の脂肪細胞への分化を阻害する[16]．したがって，カロリーが制限される状況下では，SIRT1は脂肪細胞の分化を抑制することによりエネルギー貯蔵を制限するように働いていると思われる．さらに，SIRT1は肝臓ではPGC1αと結合して糖代謝を調節するほか，膵臓のβ細胞ではUCP2の発現を負に制御してインスリン分泌に関与することが明らかとなっている．

SIRT3は褐色脂肪細胞のミトコンドリア内膜に局在し，寒冷曝露によりUCP1とともに発現レベルが上昇する[17]．また，培養細胞においてSIRT3の発現上昇はCREBの活性化を促進するとともに，PGC1α，UCP1，あるいは他のミトコンドリア関連遺伝子の発現レベルを高めるという．SIRT3がどのようにしてこれらの遺伝子の発現調節にかかわるかを含めて，他のSIRT1ファミリー分子の脂肪細胞での役割の解明に興味が持たれる．

IV．CPT1 ファミリー

CPT1(carnitine palmitoyl-transferase-1)はミトコンドリアにおける脂肪酸酸化において長鎖脂肪酸のミトコンドリアへの取り込みにかかわる律速酵素として重要である[18](図1)．肝臓，筋，脳組織特異型CPT1が報告されているが，脂肪組織では褐色脂肪組織において筋型CPT1の発現が認められる．細胞質でアシル化された長鎖脂肪酸はミトコンドリア外膜に存在するCPT1の作用によりカルニチンと結合した形でミトコンドリアマトリックスに運ばれた後，内膜に存在するCPT2の作用によりカルニチンから切り離され酸化されることになる．脂肪酸のβ酸化により生成したNADHは呼吸鎖で酸化され，生じたH^+はATP合成，あるいはUCP1による熱産生に利用されるであろう．また，CPT1の活性は脂肪酸合成経路のマロニルCoAにより阻害されるが，UCP1熱産生とともに活性化されたAMPキナーゼはアセチルCoAカルボキシラーゼを不活性化してマロニルCoA濃度を低下させる．このような状況下では，CPT1の活性が維持され，脂肪酸の代謝回転とグルコースの代謝が並行して進むと考えられる．

おわりに

ミトコンドリアにおけるエネルギー代謝には，上記の他にもATPやグリセロール3リン酸の輸送，電子供与体間のH^+転移にかかわる分子などが多数存在し，栄養状態の変化などに対して協調的に応答しているものと考えられる．それらの分子の機能低下や異常は，組織や個体レベルでのエネルギーバランスの乱れとなり，肥満や糖尿病につながることが動物実験により明らかとなっている．しかしながら，UCPをはじめミトコンドリ

ア蛋白質の多くについて，その活性制御のメカニズムについては十分理解されておらず，今後の研究のさらなる進展が期待される．

文 献

1) Kraus S et al : The mitochondrial uncoupling-protein homologues. Nature Rev Mol Cell Biol 2005, 6 : 248-261
2) Enerback S et al : Mice lacking mitochondrial uncoupling protein are cold-sensitive but not obese. Nature 1997, 387 : 90-94
3) Ukropec J et al : Leptin is required for uncoupling protein-1-independent thermogenesis during cold stress. Endcrinol 2006, 147 : 2468-2480
4) Kontani Y et al : UCP1 deficiency increases susceptibility to diet-induced obesity with age. Aging Cell 2005, 4 : 147-155
5) Inokuma K et al : Indispensable role of mitochondrial uncoupling protein 1 (UCP1) for anti-obesity effect of β3-adrenergic stimulation. Am J Physiol 2006, 290 : E1014-E1021
6) Inokuma K et al : Uncoupling protein 1 is necessary for norepinephrine-induced glucose utilization in brown adipose tissue. Diabetes 2005, 54 : 1385-1391
7) Yamada T et al : Signals from intra-abdominal fat modulate insulin and leptin sensitivity through different mechanisms : Neuronal involvement in food-intake regulation. Cell Metab 2006, 3 : 223-229
8) Houstis N et al : Reactive oxygen species have a causal role in multiple forms of insulin resistance. Nature 2006, 440 : 944-948
9) Zhang C-Y et al : Uncoupling protein-2 negatively regulates insulin secretion and is a major link between obesity, B cell dysfunction, and Type 2 diabetes. Cell 2001, 105 : 745-755
10) Schrauwen P et al : Oxidative capacity, lipotoxicity, and mitochondrial damage in type 2 diabetes. Diabetes 2004, 53 : 1412-1417
11) Finck B et al : PGC-1 coactivators : inducible regulators of energy metabolism in health and disease. J Clin Invest 2006, 116 : 615-622
12) Hondares E et al : Thiazolidinediones and rexinoids induces peroxisome proliferators-activated receptor-coactivator (PGC)-1 α gene transcription : an autoregulatory loop controls PGC-1α expression in adipocytes via peroxisome proliferator-activated receptor-γ coactivator. Endocrinol 2006, 147 : 2829-2838
13) Kamei Y et al : PPARγ coactivator 1 β/ERR ligand 1 is an ERR protein ligand, whose expression induces a high-energy expenditure and antagonizes obesity. Proc Natl Acad Sci USA 2003, 100 : 12378-12383
14) Uldry M et al : Complementary action of the PGC-1 coactivators in mitochondrial biogenesis and brown fat differentiation. Cell Metab 2006, 3 : 333-341
15) Guarente L et al : Calorie Restriction—the *SIR2* connection. Cell 2005, 120 : 473-482
16) Picard F et al : Sirt 1 promotes fat mobilization in white adipocytes by repressing PPAR-γ. Nature 2004, 429 : 771-776
17) Shi T et al : SIRT3, a mitochondrial sirtuin deacetylase, regulates mitochondrial function and thermogenesis in brown adipocytes. J Biol Chem 2005, 280 : 13560-13567
18) Jensen MD : Fatty acid oxidation in human skeletal muscle. J Clin Invest 2002, 110 : 1607-1609

日常診療に役立つ
高齢者の周術期管理

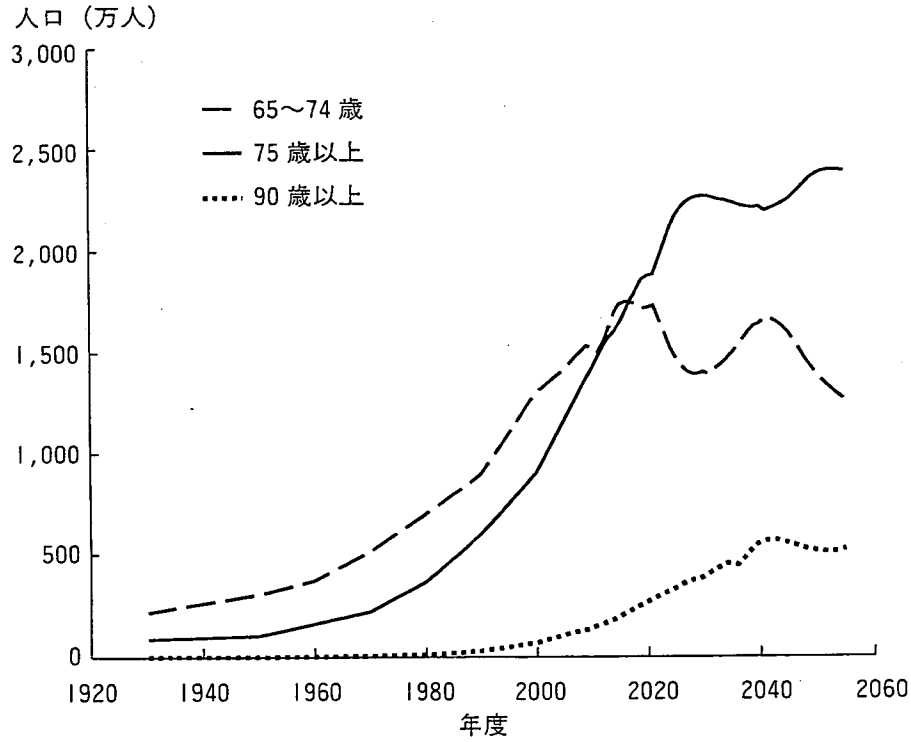
監修　並木昭義
岩手医科大学医学部麻酔科教授
編集　山蔭道明
札幌医科大学医学部麻酔科講師

真興交易㈱医書出版部

9 高齢者の臨床検査

1 急増する高齢者と高齢者医療

平均寿命の延長に伴って，高齢者人口は急速に増加している．2006年度の「高齢社会白書」によると2005年10月1日時点での65歳以上の高齢者人口は2,560万人で，総人口に占める割合は20.04%と日本の歴史上，初めて20%を超えた[1]．今後もこの増加は続くが，2015年には，日本の全人口の4人に1人が65歳以上の高齢者となる．高齢者のうちでも，とくに75歳以上の後期高齢者の人口が増えて，2020年以降には65～74歳までの前期高齢者の数よりも多くなると推定されている（図❶）．



図❶ 前期高齢者，後期高齢者，超高齢者の将来推計人口

国立社会保障・人口問題研究所・日本の将来推計人口・平成18年12月推計による．

90歳以上の超高齢者の数も年々増加している．超高齢者の人口は108万に達し，100万人の大台を超えている．1996年には47万人であった90歳以上の人口は8年で

Coffee Break (1) 長寿世界記録

日本の女性は世界一長生きであるが，長寿の世界記録保持者は残念ながら日本人ではない．「アルルの女」と言われている，南仏アルルのジャンヌ・カルマンという女性である．1875年2月21日に画材屋の娘として生まれた．店に画材を買いに来たゴッホにも会ったことがあるという．1997年8月4日，122歳でこの世を去っている．

倍増し，2010年には144万人に，2040年には555万人に増加するものと推定されている．

厚生労働省では毎年，100歳以上の高齢者名簿を発表してきた．2006年9月末の100歳以上の高齢者は2万8,000人に達した．高齢者名簿が初めて公表された1963年には100歳以上の百寿者は日本全体で153人しかいなかったことを考えると，驚くほどの増加である．百寿者は今後はさらに増加を続け，2025年に19万人，50年には68万人と予測されている．

このような高齢者，超高齢者の急激な増加は，医療の現場においても小児科や産科を除く科で，患者のほとんどを高齢者が占める状況となって，高齢者医療の重要性がますます高まっている．

2 高齢者医療と臨床検査

高齢者医療には過剰診断，過剰治療が多く，このため薬物副作用の頻度の増大や高齢者医療費の高騰を招く結果につながっているとの批判がある．高齢者では一般成人に比べて高齢者では代謝機能や排泄能などの予備力が低下しており，とくに重大な疾患をもたない場合でも異常値を示すことがある．一般成人での基準値を医療の現場でそのまま使用することは過剰診断，過剰治療につながってしまっている可能性がある．しかし，一方では高齢者では疾患による症状が出にくく症状も非定型的で，このため症状からの診断が難しく検査値が診断の決め手となることがある．臨床検査を行う場合には，高齢者では一般成人と区別して検査基準値を設け，判断を行う必要がある．さらに高齢者では同時に多くの慢性疾患が合併していることが多く，それぞれの疾患による異常が互いに影響しあい，検査値の変動が複雑となる．このように高齢者では検査基準値の設定と評価に多くの解決すべき問題点がある[2]．

3 正常値，基準値，診断値とは

検査値の判定には以前から「正常値」が使われてきた．しかし「正常」を医学的に定義し判定することはとくに高齢者ではきわめて難しい．このため「正常値」という言葉は曖昧なままに使用されている．特定の検査値をもとにして臨床的判断を下す場合には，何らかの基準の設定が必要であり，「正常」であるか「異常」であるかを区

別するために用いられる検査値の「正常範囲」を正常値とすべきである．「正常値」は，個人の健康，生命予後や疾患に関する判断を下すための値であり，本来，個人個人によって正常値が異なっているはずである．とくに高齢者では個人差が大きく，集団としての「正常値」を設定することは困難である．

こうした問題をもつ「正常値」に対して，最近では「基準値」という用語が使われることが多い．健康な基準個体を集めて基準母集団を設定し，その中から基準標本群を無作為に抽出し，抽出された集団の検査測定値を基準値とするものである．基準値の分布をもとにして，その95%内に含まれる値が基準範囲とするものである．これはNCCLS（アメリカ臨床検査標準協議会）が定めた検査基準値の設定方法であり，測定値の基準となる値としての検査基準値が求められる[3]．この基準値は健康者集団における平均的な検査値の範囲を示すものであり，基本的に健康状態の判定や疾患の有無の判断を行うように求められたものでないことに注意する必要がある．

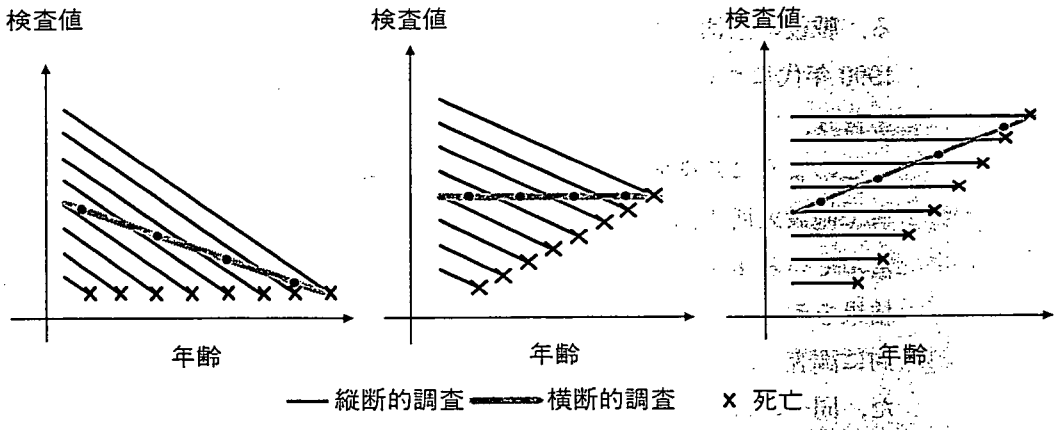
特定の疾患が存在するかどうかの目的に使われる検査値の基準を診断値と言う．判別値とかカットオフ値とも言われる．疾患をもつ患者の検査値の95%上限もしくは下限値，疾患群および健康群の検査値分布の交差点，検査値による疾患識別の感度と特異度から求めた疾患の最良の判定値などが使われる．疾患ごとの患者の検査測定値に加えて健康群での検査測定値が必要であり，一般的な臨床検査では診断値がはっきりと示されていることはほとんどなされていない．

4　高齢者における基準値設定の問題点

高齢者では基準値設定が重要であるにもかかわらず，その具体的な設定には多くの問題がある．通常行われる高齢者における基準値の設定では，「正常な」高齢者をまず定義する必要がある．一般的には日常生活活動に介助を要しておらず，貧血，糖尿病，腎不全，肺機能障害，認知症，高血圧，高脂血症，脳卒中，虚血性心疾患などを有していないなどの基準が設けられるが，このような基準に当てはまる高齢者はきわめて少数の特別な人たちである．ほとんどの高齢者では何らかの慢性疾患をもっている．このため基準となる「正常な」母集団の設定が困難であり，またこうした特別な母集団での検査基準値の設定が臨床の場でどのような意義をもつのかの評価も難しい．また，高齢者は健康状態の良し悪しにかかわらず，最近ではビタミン剤などのサプリメントを含めて何らかの薬物を服用している場合が多い．薬物は検査値に影響を与えることがあり，基準値を設定する際に問題となる．

高齢者では疾患があっても症状や所見が非定型的であり，検査の基準値からは異常として捉えられないことがある．逆に日常生活を送る上で支障となるような障害がなく，また特定の愁訴もない「健常な」高齢者でも検査所見で異常が認められることが多い．たとえば自己抗体の出現は検査データとしては異常であるが，高齢者ではそれ自体が健康の障害を意味するわけではなく，必ずしも異常と捉えられるわけではな

い．このような個人差としての検査値の分布幅は，小児期から青年，中年，初老期と加齢と共に増大していき，高齢者では一般成人よりも大きな分布幅を示すようになる．また，高齢者では予備力が低下していることが多い．機能的予備力の低下の影響は，検査値としての変化は出にくいが負荷時の変動が問題となり，高齢者では一般検査所見よりも負荷検査で大きな加齢変化が認められる．



図❷　検査値の横断的調査と縦断的調査による加齢変化（文献4より引用改変）

検査値が悪いと死亡リスクが高くなる場合には検査結果が良い者だけが生き残っていく選択効果のため，横断的調査では検査値の加齢変化を過小評価する結果になる．

さまざまな年齢を含む対象者に同時に検査を行って年齢群ごとの検査値の差を加齢変化として捉える調査方法を横断的調査と言う．横断的調査での検査値を縦軸，年齢を横軸にしてプロットしてみると，超高齢者では検査値には加齢変化がみられなかったり，むしろ高齢になるほど検査値が良くなったりしている．これを選択効果と言う[2]．加齢と共に検査値が低下していくが，一定の値まで悪くなると死んでしまう場合を考えてみよう．検査値が一定のレベルまで下がってしまった人は死んでしまうため，横断的調査では，生き残った人での検査値の加齢変化は見かけ上，個人を実際に追跡した縦断的変化よりも小さくなってしまう（図❷左）．加齢と共に検査値が低下するが，若い人では低い検査値でも生き残れるが，高齢になるほど低い検査値では生き残れない場合では，横断的調査による見かけ上の検査値では加齢変化はなくなってしまう（図❷中）．さらに個人個人では加齢で検査値は変化しないけれども，検査値が悪い人では長くは生きられないという場合を考えてみる．検査値が悪い人は早く死んでしまうため，生き残った人たちだけでの横断的調査では，年齢が高くなるほど検査値は良くなっていってしまう結果になる（図❷右）．高齢まで生き残っている人たちは，数々の致命的な疾患にかからずに生き延びてきた，健康に優れたエリートであり，このエリートからさらに健康に優れた少数を選んで設定された基準値は，実際の臨床的に使用できるかどうかに疑問がある．

高齢者では生まれた時代の生活環境が現在と大きく異なり，このことが出生年代に