Expression and induction of cytochrome P450s in rat white adipose tissue

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Abbreviations: CYP, cytochrome P450; AHR, aryl hydrocarbon receptor; PB, phenobarbital; DEX, dexamethasone; CAR, constitutive androstane receptor; PXR, pregnane X receptor; ARNT, AHR nuclear translocator; PCB, polychlorinated biphenyl; DDT, 1,1,1-trichloro-2,2-bis-(p-chlorophenyl)ethane; DDE, 1,1-dichloro-2,2-bis-(p-chlorophenyl)ethylene; BNF, β-naphtoflavone (5,6-benzoflavone); OR, NADPH cytochrome P450 reductase; RT-PCR, reverse transcription-polymerase chain reaction; RPS9, ribosomal protein S9; EROD, ethoxyresorufin O-deethylase.

Recommended section: Toxicology
Abstract

Lipophilic environmental pollutants are often stored in adipose tissues after exposure. These compounds have been well studied in terms of their cell toxicity in organs such as liver and kidney, and their xenoestrogenic action on reproductive tissues as endocrine disruptors. However, the effects of these chemicals on the depot, adipose tissue, have not been studied, although adipose tissue is an important endocrine tissue secreting obesity/diabetes-related hormones and cytokines. In this study, we identified the expression of cytochrome P450s in rat white adipose tissues and investigated the effects of typical lipophilic cytochrome P450 inducers, namely phenobarbital, dexamethasone and β-naphthoflavone. The results showed that β-naphthoflavone was a strong CYP1A inducer in adipose tissue as well as in liver. It increased CYP1A1 mRNA, protein and its related activity, ethoxyresorufin O-deethylase. Phenobarbital and dexamethasone also induced both the mRNA and protein of CYP2Bs and CYP3As, respectively, in adipose tissue, although significant inter-individual differences were observed. Furthermore, we demonstrated that 48 hr fasting was as effective in adipose tissue as in the liver in the induction of CYP2E1 mRNA and protein. These results suggest that the mechanisms by which cytochrome P450 genes are regulated in the liver are also functional in rat adipose tissues. This has raised the possibility that lipophilic environmental contaminants accumulated in adipose tissue may dysregulate the gene expression profile.
Introduction

Cytochrome P450 (CYP), a heme-thiolate monooxigenase, is comprised of a gene superfamily, among which, members of the CYP1, CYP2 and CYP3 families show broad substrate specificities and play an important role in detoxification of xenochemicals such as pharmaceutical drugs and both man-made and naturally occurring chemicals in the environment (Gonzalez, 1989). CYP forms are expressed not only in the liver but also in the extrahepatic tissues including the intestine, kidney, lung, brain, adrenal gland, skin and placenta (Gonzalez, 1989; McKinnon and McManus, 1996; Ding and Kaminsky, 2003). On xenobiotic exposure, organisms can increase their metabolic activities to eliminate the chemicals effectively from the body, usually by activating the transcription of the CYP genes (Conney, 1967; Gonzalez, 1989). Whereas the liver is the main organ to metabolize the xenochemicals, the metabolizing enzymes in the exposed tissues such as lung, skin, nasal mucosa play important roles in reducing the toxicity of the chemicals before they enter the body circulation.

The mechanisms of the chemical-induced transcriptional activation of CYP genes have recently been revealed. Zinc finger-containing nuclear hormone receptors
and a helix-loop-helix type receptor, the aryl hydrocarbon receptor (AHR), play central roles in the phenomena (Whitlock JP Jr, 1999; Willson and Kliewer, 2002). Typical CYP inducer, phenobarbital (PB) and dexamethasone (DEX), activate nuclear receptors, the constitutive androstane receptor (CAR) and the pregnane X receptor (PXR), respectively. The activated receptor forms a heterodimer with retinoid X receptor in nucleus to bind to the PB-responsive enhancer module in the CYP2B genes or the CYP3A promoter sequences including the DR3, ER6 and xenobiotic-responsive enhancer module. Species differences in the induction of CYP2B and CYP3A forms are now explained by the differences in the binding affinities of nuclear receptors to inducing agents. For example, a mouse-specific CYP2B inducer, 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene, can only bind to mouse CAR, not to rat or human CAR (Poland et al., 1981; Tzameli et al., 2000). Rifampicin, which induces CYP3As in humans and rabbits, but not in mice and rats, prefers human and rabbit PXRs to mouse and rat PXRs, whereas DEX, which induces CYP3As in rodent cells, but not in human cells, binds to rodent PXRs more effectively than to human PXR (Jones et al., 2000). Other CYP inducers, such as dioxins and aryl hydrocarbons, bind to AHR in the cytoplasm. The ligand-bound AHR translocates into the nucleus and
forms a heterodimer with the AHR nuclear translocator (ARNT) to bind to a promoter sequence, the xenobiotic response element, found in aryl hydrocarbon-inducible genes such as CYP1A1. These xenobiotic receptors are expressed mainly in the liver, and a certain number are present in extrahepatic tissues, as are CYPs.

Lipophilic environmental pollutants such as dioxins, polychlorinated biphenyls (PCBs), 1,1,1-trichloro-2,2-bis-(p-chlorophenyl)ethane (DDT) or 1,1-dichloro-2,2-bis-(p-chlorophenyl)ethylene (DDE) tend to escape from the organ’s detoxification system and are accumulated in the body, especially in the adipose tissue. In addition to the cell toxicity of those compounds, their effects on reproductive organs have been studied intensively and numerous compounds have been considered as endocrine disruptors (Birnbaum, 1995; Longnecker et al., 1997). In contrast, their effects on adipose tissue remain to be elucidated. Since these chemicals often induce CYPs in the liver through the activation of nuclear receptors or AHR (Blizard et al., 2001; Mimura and Fujii-Kuriyama, 2003; Wyde et al., 2003), they may also have similar activity in the adipose tissue. Adipose tissue is the main organ for the storage of triacylglycerol as an energy source and plays a crucial role in the development of obesity and diabetes since the organ secretes several hormones and cytokines (i.e.
adipocytekines) including tumor necrosis factor α and resistin, which induce insulin resistance, leptin and adiponectin, which improve insulin sensitivity (Ahima and Flier, 2000). Thus, if environmental pollutants accumulated in adipose tissues affect the gene expression profile of the tissue, there may be significant consequences for the endocrine system. In this study, we examined the effects of typical lipophilic CYP inducers on the expression of CYP genes in rat epididymal white adipose tissue. Moreover, the effect of fasting on the CYP2E1 expression in adipose tissue was studied.
Materials and Methods

Materials. Phenobarbital sodium salt and DEX were purchased from Wako Pure Chemical Industries (Osaka, Japan). β-naphtoflavone (BNF) and ethoxyresorufin were obtained from Sigma (St. Louis, MO). Resorufin was purchased from Molecular Probes (Eugene, OR). Anti-rat CYP1A2, anti-rat CYP2B1, anti-rat CYP2E1 and anti-rat NADPH cytochrome P450 reductase (OR) antibodies were obtained from Daiichi Pure Chemicals (Tokyo, Japan). The anti-CYP3A4 antibody was a generous gift from Dr. Yasushi Yamazoe (Tohoku University, Sendai, Japan). The anti-carleticulin antibody was purchased from Stressgen (Victoria, Canada). ECL Western blotting detection reagent and Hyperfilm MP were from Amersham Biosciences (Piscataway, NJ). Oligonucleotides were synthesized by Kurabo (Osaka, Japan) and their sequences are shown in Table 1.

Animal treatment. Six- to seven-week-old male Sprague-Dawley rats (Japan SLC, Hamamatsu, Japan) weighing 220-250 g were housed for at least a week before the experiments. The rats were housed under a 12 hr light/12 hr dark condition and were given water and standard chow diet *ad libitum*. They were given intraperitoneal
injections of PB sodium salt (80 mg/kg), DEX (40 mg/kg), BNF (40 mg/kg) or vehicle, saline for PB, and corn oil for DEX and BNF, for three consecutive days. Twenty-four hours after the final injection, the rats were killed and the liver and epididymal white adipose tissue were removed and weighed. For the fasting study, diet was removed 48 hr before killing.

**Reverse transcription-polymerase chain reaction.** Total RNA was prepared from the rat livers and adipose tissues using the acid guanidine/phenol/chloroform method with TRIzol reagent (Invitrogen, Carlsbad, CA). First strand cDNA was synthesized using the SuperScript II first strand synthesis system (Invitrogen). Conventional PCR was performed with rTaq polymerase (Takara Bio, Ohtsu, Japan). PCR products were subcloned into the pCR2.1 plasmid using a TOPO TA cloning kit (Invitrogen) and their sequences were confirmed by dideoxy sequencing. Real-time reverse transcription-polymerase chain reaction (RT-PCR) experiments were carried out using QuantiTect SYBR Green PCR kit (Qiagen, Valencia, CA) with GeneAmp 5700 (Applied Biosystems, Foster City, CA). cDNA synthesized from 10 ng of total RNA and the plasmid DNAs subcloned above were used as the template in the reaction. After the
reaction, dissociation curve analyses were carried out to confirm the amplification of a single PCR product. Relative mRNA levels were calculated using the standard curve determined with the plasmid DNA. For normalization, ribosomal protein S9 (RPS9) mRNA levels were used.

**Western blotting.** Microsomal fractions were prepared from the rat livers and adipose tissues as described previously (Yamazoe et al., 1986). To remove the lipid layer effectively, the adipose homogenate in 1.15% KCl was first centrifuged at 2,000 x g for 10 min and the resultant middle layer (other than the upper lipid layer and pellet) was subjected to 9,000 x g centrifugation. The protein concentration of the microsome was determined with BCA kit (Pierce, Rockford, IL) with bovine serum albumin as the standard. A portion of the microsome was separated by 9% SDS-polyacrylamide gel electrophoresis and transferred onto a polyvinylidene fluoride membrane Immobilon-P (Millipore, Billerica, MA). The membrane was incubated with the primary antibodies and appropriate horseradish peroxidase-labeled secondary antibodies, and signals were detected with ECL reagent. For reprobing, the membrane was treated with an SDS-containing solution as described in the user manual for ECL reagent.
**Ethoxyresorufin O-deethylase activity.** Microsomal activities of ethoxyresorufin O-deethylation were measured according to the method by Burke *et al.* (1985) with 20 µg (liver) or 40 µg (adipose tissue) of microsomal protein.
Results

Detection of CYP mRNAs in rat white adipose tissue

Real-time RT-PCR was carried out to examine whether CYP forms were expressed in rat white adipose tissues. The relative mRNA levels of several drug-metabolizing CYPs and OR in the adipose and liver of untreated male rats are shown in Table 2. A housekeeping gene, \( RPS9 \) encoding ribosomal protein S9, was used for normalization. Among the CYPs examined, adipose CYP1A1, CYP1B1 and CYP2B1 mRNA levels, which show little expression in normal livers, were comparable to or higher than those in the liver. Interestingly, the mRNA level of OR in adipose tissue was also as high as that in the liver. These data suggest that several CYP forms are present such as CYP1A1, CYP1B1 and CYP2B1, and may function in rat white adipose tissue.

Increase in adipose CYP mRNA levels by CYP inducers

It was then investigated whether the CYP expression in the adipose tissue was induced by treatment with typical CYP inducers, PB, DEX and BNF, which induce mainly CYP2B, CYP3A and CYP1A/1B1, respectively. These chemicals are relatively lipophilic and were given for three consecutive days to increase their concentration in
the peripheral tissues. Control animals received saline (for PB) or corn oil (for DEX and BNF). While PB or BNF treatment did not alter the mass of epididymal white adipose tissue significantly, DEX treatment reduced it by 18%. Since this reduction was accompanied with a reduction in body weight (17%), the adipose/body weight ratio was not different from the other groups. The mRNAs of CYPs and OR in adipose tissues and livers were amplified individually by RT-PCR (Figs. 1A, 1B, 1C). RPS9 mRNA was also amplified as a control (Fig. 1D). In the liver, PB, DEX and BNF increased the mRNA levels of CYP2B1/2B2, CYP3A1 and CYP1A1/1A2/1B1, respectively. PB treatment also increased the OR mRNA level in the liver. In adipose tissue, the inducers also induced CYP mRNAs as in the liver, although significant inter-individual differences were observed in the case of CYP2B and CYP3A induction. In addition, BNF was not a good CYP1B1 inducer in adipose tissue.

**Induction of CYP proteins in adipose tissue**

To examine whether CYP protein levels were also increased by chemical treatment, microsomal fractions were prepared from the liver and adipose tissue, and Western blotting was carried out. As expected, the treatment of rats with PB but not with DEX or
BNF increased the total amount of microsomal protein in the liver. In contrast, DEX and BNF treatment, but not PB, increased the total adipose microsomal protein 2- and 7-fold, respectively, suggesting an increase in CYP protein. Because of inability of the anti-CYP3A4 antibody used in this study to distinguish CYP3A1, CYP3A2 and CYP3A9, we measured the sum of these forms and expressed it hereafter as CYP3As protein.

In the liver, PB, DEX and BNF treatment significantly increased the amount of CYP2B1/CYP2B2, CYP3As and CYP1A1/CYP1A2 proteins, respectively, as expected (Figs. 2A, 2B and 2C). The OR protein level was slightly increased by PB treatment (Fig. 2D). In adipose tissue without chemical treatment, only CYP2B1 but not CYP2B2 was detected with the anti-CYP2B1 antibody (Fig. 2A), which contrasted with the expression profile of the normal liver. These tissue-specific differences in CYP2B1/2B2 protein levels were consistent with the mRNA levels. After PB treatment, the adipose CYP2B1 protein was increased (Fig. 2A). With the anti-CYP3A4 antibody, a trace of CYP3A protein was detected in corn oil-treated rats, and DEX treatment increased the intensity of the band (Fig. 2B). As noted in the CYP2B and CYP3A mRNA levels (Fig. 1), significant inter-individual differences were observed in the CYP2B and CYP3A
protein levels. When the membrane was reprobed with anti-calreticulin, which is exclusively localized in the endoplasmic reticulum (Smith and Koch, 1989), no significant inter-individual differences were observed (data not shown), suggesting that differences in the CYP2B and CYP3A levels resulted from differences in the magnitude of induction rather than differences in the quality or quantity of the microsomal fractions. The reason for these inter-individual differences remains unknown at present. Unknown factor(s) may affect the degree of CYP induction at both mRNA and protein levels.

With the anti-CYP1A2 antibody, neither CYP1A1 nor CYP1A2 was detected in vehicle-treated rat adipose tissues (Fig. 2C). Instead, an immunoreactive band, showing slightly higher electrophoresis mobility than that of CYP1A2, was detected. BNF treatment strongly induced both CYP1A1 and CYP1A2 proteins except in one individual (Fig. 2C). Anti-calreticulin immunoblotting demonstrated that the individual showing low CYP1A induction had a small amount of calreticulin protein (data not shown), suggesting that the low intensity of CYP1A proteins in this individual may be due to the poor quality or partial degradation of the microsomal protein rather than a low degree of induction. This was supported by data showing that CYP1A1/1A2
mRNAs were equally induced by BNF in every individual (Fig. 1).

In contrast to the CYP forms, the OR protein level in adipose was comparable to that in the control rat livers, and PB treatment did not alter its level (Fig. 2D). These results agreed with the mRNA data (Table 2 and Fig. 1).

Among the compounds tested, BNF most effectively increased the total adipose microsomal protein and it also increased CYP1A1 apoprotein. Thus, we next measured the hepatic and adipose microsomal ethoxyresorufin O-deethylase (EROD) activity, which is mainly catalyzed by CYP1A1 (Table 3) to examine whether CYP1A1 holoprotein was induced in adipose tissue. BNF treatment increased the activity in adipose tissue as well as in the liver, although the magnitude of induction (4-fold) was lower than that expected from the significant increase in the amount of total microsomal protein and CYP1A1. This may have been due to the contribution of CYP forms other than CYP1A1 to EROD.

**Expression of nuclear receptors and AHR**

Nuclear receptors, CAR and PXR, and a helix-loop-helix transcription factor AHR play important roles in xenobiotic-responsive transcriptional activation of CYP genes. Thus,
we investigated whether rat adipose tissue expressed those receptors. To this end, RT-PCR analyses were carried out for CAR, PXR, RXRα, AHR and ARNT mRNAs in untreated rat livers and adipose tissues. In adipose tissue as well as in livers, all mRNAs were detected, although the adipose mRNA levels of CAR, PXR, RXRα and AHR were lower than those in livers (Fig. 3). Among them, adipose/liver mRNA ratio was highest for AHR. Interestingly, the ARNT mRNA level in adipose tissue was comparable to that in the liver (Fig. 3).

mRNA levels of phase 2 enzymes and transporters

In addition to CYPs, phase 2 conjugating enzymes such as UDP-glucuronosyltransferase and sulfotransferases and drug transporters such as ATP-binding cassette transporters and organic anion transporters also play important roles in the excretion of xenobiotics. Therefore, we performed RT-PCR analyses for those mRNAs including UGT1A1, SULT1A1, MDR1a (ABCB1a), MRP2 (ABCC2) and Slc21a10 (also called rat liver-specific organic anion transporter-1 or Oatp4) to investigate the expression of those mRNAs in rat adipose tissue (Fig. 4). All mRNAs were detected in both adipose tissues and livers, although those levels were much
lower in adipose tissue than in the liver except SULT1A1. The adipose SULT1A1 mRNA level was comparable to that in the liver. In addition, inter-individual differences were observed in the adipose mRNA levels of UGT1A1, MDR1a, MRP2 and Slc21a10.

**Effect of fasting on adipose CYP2E1**

Physiological changes are known to alter the expression of CYPs in the liver (Cheng and Morgan, 2001). In fact, CYP2E1 mRNA and protein levels are significantly induced by fasting or diabetes (Song et al., 1987; Yamazoe et al., 1989; Johansson et al., 1990; Liu et al., 1993), although the mechanisms have not been completely understood. In such conditions, the weight and size of adipose tissues also change. While fasting or insulin-dependent diabetes decreases the weight and size of white adipose tissue, obesity/non insulin-dependent diabetes increases them. In this study, we investigated the effects of fasting on CYP2E1 mRNA and protein levels in adipose tissues (Fig. 5). Since 48 hr fasting decreased the size of epididymal white adipose tissue, making it difficult to prepare microsomes from individual animals, pooled tissues from four rats were used. In the liver, CYP2E1 mRNA and protein levels were increased by 48 hr fasting. Similarly, RT-PCR analysis revealed that fasting dramatically increased in the
level of CYP2E1 mRNA in adipose tissue (Fig. 5A). Fold of induction was higher in adipose tissue than in the liver. In agreement with the mRNA data, Western blotting analysis demonstrated that CYP2E1 protein levels in the fasted rat adipose tissue were higher than those in the control animals (Fig. 5B). These data suggest that the induction mechanism for CYP2E1 mRNA and protein observed in the liver is also conserved in adipose tissue.
Discussion

Lipophilic environmental pollutants, such as dioxins, PCBs and DDT-related compounds, are accumulated in adipose tissues after exposure and have relatively long half-lives for disappearing from the tissue or the body. In this study, we found that the drug-metabolizing forms of CYPs were expressed in rat white adipose tissue, although the mRNA levels were much lower than in the liver, with the exception of CYP1A1, CYP1B1 and CYP2B1. Interestingly, the OR mRNA and protein levels in adipose tissue were comparable to those in the liver. When rats were treated with typical inducers, the CYP levels in the adipose tissue were increased at both the mRNA and protein levels. Moreover, BNF treatment increased the total amount of microsomal proteins in the adipose tissue, and the induction of CYP1A1 protein by BNF was accompanied with increased activity (i.e. EROD). These data indicate the presence of active CYPs as well as the conserved molecular mechanisms for the transcriptional activation of CYP genes in the adipose tissue.

Nuclear hormone receptors and AHR play vital roles in the transcriptional activation of CYP genes. (Whitlock JP Jr, 1999; Willson and Kliewer, 2002). In this study, we chose three typical CYP inducers that use different receptor systems: CAR...
for PB, PXR for DEX and AHR for BNF. Among them, BNF seemed to be the most effective inducer in adipose tissue in terms of enrichment in total amount of microsomal proteins in addition to CYP1A1 mRNA and holoprotein increase. While the expressions of CAR and PXR are limited to the liver and small intestine (Baes et al., 1994; Choi et al., 1997; Kliwer et al., 1998; Lehmann et al., 1998), AHR expression is ubiquitous. Its mRNA was detected in many extrahepatic tissues as well as in the liver (Ema et al., 1992; Carver et al., 1994). In this study, we found that the adipose/liver ratio of mRNA levels was highest for AHR, then for PXR and that the ratio was correlated with the extent of increase in microsomal protein contents. Thus, the expression level of these receptors may be one of the determinants for the degree of induction.

In this study, we also observed the induction of both CYP2E1 mRNA and protein in adipose tissue as well as in the liver by fasting. The induction of CYP2E1 is regulated at many stages and the whole mechanism is not fully understood. Fasting or type I diabetes induced by streptozotocin increases CYP2E1 mRNA levels (Song et al., 1987; Yamazoe et al., 1989; Johansson et al., 1990; Liu et al., 1993). It has been reported that this increase is, at least in part, achieved by mRNA stabilization and that hormonal changes may be involved (Yamazoe et al., 1989). A couple of papers have
demonstrated that the addition of insulin to cultured hepatocytes or hepatomas decreases the half-life of CYP2E1 mRNA and that inhibition of the insulin signal pathway restores the insulin-mediated decrease in CYP2E1 mRNA levels (Woodcroft and Novak, 1997; Woodcroft et al., 2002). Since adipocytes are one of the main targets of insulin action and the mRNA level of the insulin receptor in white adipose tissue was as high as in the liver (Leal et al., 1994; Yoshinari et al., unpublished data), it is reasonable to assume that the mechanism reported in the liver or hepatocytes is also functional in adipose tissue. In addition to transcriptional and posttranscriptional regulation, CYP2E1 induction is also regulated at the posttranslational level. Typical CYP2E1-inducing agents including ethanol, acetone and imidazole, are known to increase CYP2E1 protein by inhibiting its rapid turnover (Song et al., 1989; Eliasson et al., 1992; Roberts et al., 1995). Ketone bodies whose serum levels elevate during fasting or diabetes are also considered to work in a similar manner (Barnett et al., 1992). Thus, the 48 hr fasting in this study may have increased the serum ketone bodies enough to stabilize adipose CYP2E1 apoprotein. This suggests that common regulatory mechanisms, at least in fasting, are involved in CYP2E1 induction in the liver and adipose tissue, although we cannot rule out the possibility that there is a
adipose-specific mechanism for CYP2E1 induction since the magnitude of induction of CYP2E1 mRNA and protein was different between the liver and adipose tissues.

When rats were treated with PB or DEX, large inter-individual differences were observed in CYP mRNA and protein levels. At present, the reason of this remains unclear. One possible reason is that since the blood flow in adipose tissue is very small, a little difference in uptake of inducers or in physiological conditions of animals may cause a large difference in drug concentration in the tissue, leading to a large inter-individual difference in the magnitude of CYP induction. In addition to drugs, the CYP expression in adipose tissue seems to be regulated by hormones and/or physiological factors as well since 48 hr fasting drastically increased CYP2E1 mRNAs and proteins in the tissue. Thus, if adipose tissue was more sensitive to changes in animals' physiological conditions than the liver regarding the CYP expression, inter-individual differences could be observed more strongly in adipose tissue than in the liver. Another possibility is that there may be unequal distribution of drugs within adipose tissue because of the limited vascular system in the tissue. Moreover, epididymal adipose tissue is directly exposed to chemicals after intraperitoneal injections, which may result in higher drug concentrations in surrounding adipocytes.
than in inside cells since lipophilic compounds are able to enter cells by passive
diffusion. When preparing total RNAs or microsomal fractions in this study, we used a
portion of the tissue from each animal. Since each rat has a different shape of
epididymal adipose tissue, we may have collected a different part of the tissue with
different drug concentrations. Although future studies are necessary to evaluate these
possibilities, yet this could also happen to the human population, which may cause
inter-individual differences in the sensitivity to toxic lipophilic compounds such as
environmental pollutants.

Historically, it was believed that highly lipophilic compounds tend to be stored
in adipose tissues at a high rate. However, Bickel summarized a tremendous amount
of data related to the distribution and storage of drugs and found that basic drugs such
as chlorpromazine and imipramine do not accumulate in adipose tissue in spite of their
high lipophilicity (Bickel, 1984). Moreover, it was reported that there were large
differences in the pharmacokinetics of lipophilic model compounds. For example,
although thiopental, DDE and 2,4,5,2',4',5'-hexachlorobiphenyl have similar
octanol/water partition coefficients and show comparable maximum adipose
concentration, thiopental enters and disappears from the fat (or body) very rapidly: the
half lives of adipose accumulation and disappearance are 0.4 hr and 2 hr, respectively (Muhlebach et al., 1985). In contrast, it take quite a long period (more than a month) for 2,4,5,2',4',5'-hexachlorobiphenyl to reach its maximum concentration in the adipose and it barely disappears from the fat or body (Muhlebach et al., 1985). DDE has a medium rate. The reason for these differences remains unclear. In this study, we identified a functional CYP system and detected mRNAs for phase 2 enzymes and transporters in the adipose tissue. These data have raised the possibility that drug-metabolizing enzymes and/or drug transporters may affect the half-lives of lipophilic compounds in adipose tissue as they do in other tissue such as the liver and intestine. Further studies in the future will confirm whether this hypothesis is accurate.

In conclusion, we have identified the expression of drug metabolism-related genes in rat white adipose tissues and demonstrated that lipophilic CYP inducers increased CYP mRNA and protein levels in the tissue probably through the same mechanism as in the liver. Taken together with facts that adipose tissue plays an important role in energy homeostasis as an endocrine organ (Ahima and Flier, 2000) and that environmental pollutants, such as dioxins, PCBs and DDT-related compounds, activate CAR, PXR or AHR (Blizard et al., 2001; Mimura and Fujii-Kuriyama, 2003;
Wyde et al., 2003), lipophilic compounds accumulated in adipose tissue may unexpectedly affect the expression profile of physiologically important genes.
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Footnotes

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Legends

Figure 1. RT-PCR analysis of CYP mRNAs in rat livers and white adipose tissues.

RT-PCR was carried out with total RNA prepared from rat livers (left panels) and white adipose tissues (right panels) as described in Materials and Methods. Amplicons were electrophoresed on agarose gel, stained with ethidium bromide and detected under a UV illuminator. mRNA levels in each individual were analyzed after PB (A, D), BNF (B, D) or DEX (C, D) treatment. The numbers in parentheses indicate the numbers of PCR amplification cycles. Lane M contains 100 bp ladder DNA markers.

Figure 2. Western blot analysis of microsomal proteins prepared from rat livers and white adipose tissues.

Microsomal proteins prepared from pooled livers (left panels; n = 4 or 5; 1μg/lane) or white adipose tissues from the same animals used in the Figure 1 (right panels: A and D, 12.5 μg/lane; B, 25 μg/lane; C, 50 μg/lane) were subjected to Western blotting with anti-CYP2B1 (A), anti-CYP3A4 (B), anti-CYP1A2 (C) and anti-OR (D)
antibodies as described in Materials and Methods. The order of individuals for the adipose samples was same as Figure 1. Molecular weight markers (kDa) are shown on the right. S, saline; CO, corn oil.

**Figure 3. mRNA levels of transcription factors involved in the CYP induction.**

RT-PCR was carried out with total RNA prepared from livers and adipose tissues from four individual untreated rats as described in Materials and Methods. Amplicons were electrophoresed on agarose gel, stained with ethidium bromide and detected under a UV illuminator. The numbers in parentheses indicate the numbers of PCR amplification cycles. Lane M contains 100 bp ladder DNA markers.

**Figure 4. mRNA levels of conjugating enzymes and transporters in rat livers and adipose tissues.**

RT-PCR was carried out as described in Materials and Methods and the legend to Figure 3. The numbers in parentheses indicate the numbers of PCR amplification cycles. Lane M contains 100 bp ladder DNA markers.
Figure 5. Changes in the CYP2E1 mRNA and protein levels in rat livers and adipose tissues after fasting.

Livers and white adipose tissues from normal and 48 hr-fasted rats were subjected to RT-PCR (A) and Western blot (B) analyses as described in Materials and Methods. A. CYP2E1 and RPS9 mRNA levels were examined in four individual rats per group. Amplification was carried out for 22 (liver CYP2E1), 28 (adipose CYP2E1) or 24 (RPS9) cycles. Lane M contains a 100 bp DNA ladder. B. Microsomal proteins were prepared from pooled livers and adipose tissues (n = 4), and 1 µg (liver) or 50 µg (adipose tissue) of proteins were used for Western blotting with an anti-CYP2E1 antibody. The arrowhead and asterisks indicate CYP2E1 and nonspecific immunoreacted bands, respectively. Molecular weight markers are shown on the left.
Table 1. Sequences of oligonucleotide primers used for the RT-PCR analysis

All sequences are shown in 5’ to 3’ direction.

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<thead>
<tr>
<th>Gene</th>
<th>Sense primer</th>
<th>Anti-sense primer</th>
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<td>CYP1A1</td>
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<td>CYP2E1</td>
<td>AGACCACCAAGCACAATCC</td>
<td>AGGGCTGAGGTCGATATC</td>
</tr>
<tr>
<td>CYP3A1</td>
<td>GGAAATTCCGATGTGGAGTGCC</td>
<td>AGGTTTTCCTTTCTCTTGCC</td>
</tr>
<tr>
<td>CYP3A2</td>
<td>ACTGCAGGAGGAGATCGATG</td>
<td>GTCAGGCTCATTATGACTG</td>
</tr>
<tr>
<td>OR</td>
<td>GAAGAGCTACGAGAACCAG</td>
<td>TCAGGTACAGCTCCTTGCC</td>
</tr>
<tr>
<td>CAR</td>
<td>GAAGAGCTACGAGAACCAG</td>
<td>TCAGGTACAGCTCCTTGCC</td>
</tr>
<tr>
<td>PXR</td>
<td>CAGATCTCCTGCTGAAG</td>
<td>GAGAAGAGGGAGATGGCC</td>
</tr>
<tr>
<td>RXRα</td>
<td>CCCAGCTCAACAAATGAC</td>
<td>GATCAGCAGCTCGTTCCA</td>
</tr>
<tr>
<td>AHR</td>
<td>ATAGCTACTCCACTTCAGGCC</td>
<td>TCATGCCACTTTCTCCAGTC</td>
</tr>
<tr>
<td>ARNT</td>
<td>GTGCACCCAGATGTGGTG</td>
<td>CATTCTGCATCTGTTTCC</td>
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<tr>
<td>UGT1A1</td>
<td>CCTACGTGCCCCAACAGATTG</td>
<td>ATCTCTGAGACCATTGGATCC</td>
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<tr>
<td>SULT1A1</td>
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<td>TGATCCAGCAGACTCTGAGG</td>
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<tr>
<td>MDR1a</td>
<td>TGGCCATCAGCCTGTTC</td>
<td>AGAAAAGCTGCAACCATG</td>
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<td>MRP2</td>
<td>TGAGGTGAAATCTCGACC</td>
<td>TGCTACTGTGCTCATGATG</td>
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<tr>
<td>Slc21a10</td>
<td>TCTCTGAGGAGCATCCTAC</td>
<td>TCCACTGGGTTCATCCATC</td>
</tr>
<tr>
<td>RPS9</td>
<td>TCCGGAACAAACGTGAGG</td>
<td>TCCAGCTTCATCTTGCCC</td>
</tr>
</tbody>
</table>
Table 2. Relative mRNA levels of CYPs and POR in rat livers and adipose tissues

Relative mRNA levels in arbitrary units were calculated by dividing CYP or POR mRNA levels by RPS9 mRNA levels determined by real-time RT-PCR as described in Materials and Methods. Data are means ± S.E.M. (n = 3). WAT, white adipose tissue.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Relative mRNA level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td>CYP1A1</td>
<td>0.068 ± 0.007</td>
</tr>
<tr>
<td>CYP1A2</td>
<td>198 ± 23</td>
</tr>
<tr>
<td>CYP1B1</td>
<td>0.016 ± 0.002</td>
</tr>
<tr>
<td>CYP2B1</td>
<td>2.6 ± 0.8</td>
</tr>
<tr>
<td>CYP2B2</td>
<td>78 ± 13</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>150 ± 16</td>
</tr>
<tr>
<td>CYP3A1</td>
<td>2280 ± 140</td>
</tr>
<tr>
<td>CYP3A2</td>
<td>1320 ± 330</td>
</tr>
<tr>
<td>OR</td>
<td>58 ± 4</td>
</tr>
</tbody>
</table>
Table 3. Microsomal EROD activity in rat livers and adipose tissues

EROD activity was determined as described in Materials and Methods. Twenty microgram (liver) or 40 µg (adipose tissue) of the pooled microsomal protein prepared from the rats treated with corn oil (n = 5) or BNF (n = 4) were used. Data are means ± S.E.M. of triplicate determinants. WAT, white adipose tissue.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Treatment</th>
<th>Activity (pmol/mg/min)</th>
<th>Ratio&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>Corn oil</td>
<td>121 ± 1.4</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>BNF</td>
<td>4080 ± 110</td>
<td>34</td>
</tr>
<tr>
<td>WAT</td>
<td>Corn oil</td>
<td>1.6 ± 0.9</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td>BNF</td>
<td>6.6 ± 0.3</td>
<td>0.055 (4.1)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Ratio represents the relative activity to that in the corn oil-treated rat liver. The number in a parenthesis is the ratio to the activity in the corn oil-treated rat WAT.
A

Liver

CYP2E1
RPS9

M Control Fasted

White adipose tissue

CYP2E1
RPS9

M Control Fasted

B

Liver White adipose tissue

62 kDa

47.5 kDa

Control Fasted Control Fasted

*