THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 279, No. 38, Issue of September 17, pp. 39968–39974, 2004
Printed in U.S.A.

Cytosolic NADP$^+$-dependent Isocitrate Dehydrogenase Plays a Key Role in Lipid Metabolism*

Received for publication, March 1, 2004, and in revised form, June 14, 2004
Published, JBC Papers in Press, July 14, 2004, DOI 10.1074/jbc.M402260200

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NADPH is an essential cofactor for many enzymatic reactions including glutathione metabolism and fat and cholesterol biosynthesis. We have reported recently an important role for mitochondrial NADP$^+$-dependent isocitrate dehydrogenase in cellular defense against oxidative damage by providing NADPH needed for the regeneration of reduced glutathione. However, the role of cytosolic NADP$^+$-dependent isocitrate dehydrogenase (IDPc) is still unclear. We report here for the first time that IDPc plays a critical role in fat and cholesterol biosynthesis. During differentiation of 3T3-L1 adipocytes, both IDPc enzyme activity and its protein content were increased in parallel in a time-dependent manner. Increased expression of IDPc by stable transfection of IDPc cDNA positively correlated with adipogenesis of 3T3-L1 cells, whereas decreased IDPc expression by an antisense IDPc vector retarded adipogenesis. Furthermore, transgenic mice with overexpressed IDPc exhibited fatty liver, hyperlipidemia, and obesity. In the epididymal fat pads of the transgenic mice, the expressions of adipocyte-specific genes including peroxisome proliferator-activated receptor γ were markedly elevated. The hepatic and epididymal fat pad contents of acetyl-CoA and malonyl-CoA in the transgenic mice were significantly lower, whereas the total triglyceride and cholesterol contents were markedly higher in the liver and serum of transgenic mice compared with those measured in wild type mice, suggesting that the consumption rate of those lipogenic precursors needed for fat biosynthesis must be increased by elevated IDPc activity. Taken together, our findings strongly indicate that IDPc would be a major NADPH producer required for fat and cholesterol synthesis.

Abnormal lipid metabolism is frequently associated with obesity and hyperlipidemia. In fat and cholesterol biosynthesis, NADPH is an essential cofactor for numerous enzymes. For instance, 3-L-hydroxylacyl-coenzyme A dehydrogenase and enoyl-coenzyme A reductase in fatty acid synthesis and hydroxymethylglutaryl-coenzyme A reductase, the rate-limiting enzyme in cholesterol biosynthesis, require NADPH for their enzyme activities. It has been demonstrated that glucose-6-phosphate dehydrogenase (G6PDH),[1] 6-phosphogluconate dehydrogenase, and malic enzyme are considered as the major enzymes producing cytosolic NADPH (1). Nevertheless, the activities of these enzymes were markedly lower than that of cytosolic NADP$^+$-dependent isocitrate dehydrogenase (IDPc) in the rat liver (1, 2). Consistent with this observation, McLean and co-workers (3) reported that certain adaptive changes in the pentose phosphate pathway dehydrogenases did not take place in parallel with fat synthesis in adipose tissue and suggested that a major source of NADPH for fat synthesis could be IDPc. It is worthy of note that IDPc is expressed mainly in lipogenic tissues such as liver and adipocytes, whereas G6PDH and 6-phosphogluconate dehydrogenase are expressed ubiquitously (4, 5). These data indicate that NADPH-producing IDPc may have an important role in fat and cholesterol biosynthesis by supplying the required cofactor, NADPH.

In mammals, three classes of isocitrate dehydrogenase isoenzymes exist (6): mitochondrial NAD$^+$-dependent isocitrate dehydrogenase (EC 1.1.1.41), mitochondrial NADP$^+$-dependent isocitrate dehydrogenase (EC 1.1.1.42), and IDPc. Among these isoenzymes, mitochonrial NAD$^+$-dependent isocitrate dehydrogenase catalyzes oxidative decarboxylation of isocitrate in the tricarboxylic acid cycle (7). However, the exact role of mitochondrial NADP$^+$-dependent isocitrate dehydrogenase and IDPc, which produce α-ketoglutarate, CO_2, and NADPH from isocitrate in the mitochondria and cytosol, respectively, are not well defined. Recently, we have demonstrated that mitochondrial NADP$^+$-dependent isocitrate dehydrogenase and IDPc play important roles in providing NADPH for the regeneration of reduced glutathione in the mitochondria and the cytosol, respectively, in cellular defense against oxidative damage (8, 9). It is also possible that IDPc-mediated production of NADPH in the cytosol may be linked to elevated fat and cholesterol synthesis. However, the direct relationship between the role of IDPc and

* This work was supported by Grant PF0320902-00 from Plant Diversity Research Center of the 21st Century Frontier Research Program funded by the Ministry of Science and Technology of the Korean government and Grant R02-2003-000-10106-0 from the Basic Research Program of the Korea Science & Engineering Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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[1] The abbreviations used are: G6PDH, glucose-6-phosphate dehydrogenase; IDPc, cytosolic NADP$^+$-dependent isocitrate dehydrogenase; WT, wild type; PEPCK, phosphoenolpyruvate carboxykinase; MOPS, 4-morpholinepropanesulfonic acid; PPARγ, peroxisome proliferator-activated receptor-γ; IDPcS, sense strand of IDPc; IDPcAS, antisense strand of IDPc; FAS, fatty-acid synthase; LPL, lipoprotein lipase.

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cytosolic NAPDH production accompanied by increased lipid synthesis has not been established. Therefore, we investigated the potential role of IDPc in fat and cholesterol biosynthesis, which takes place in the cytosol and requires NADPH as a cofactor.

Here we present evidence for the critical role of IDPc in modulating fat and cholesterol synthesis *in vitro* and *in vivo*. Increased expression of IDPc promoted adipogenesis of 3T3-L1 preadipocytes, whereas reduced IDPc expression inhibited adipogenesis. In addition, the transgenic mice overexpressing IDPc gained greater body weight accompanied with hyperlipidemia, fatty liver, and obesity than the wild type littermates. We also observed markedly elevated expression of certain genes involved in fat biosynthesis in the transgenic mice, possibly through up-regulation of PPARγ expression by increased production of its natural ligand. Increased fat and cholesterol contents most likely result from the IDPc-mediated NADPH production. Based on the current results, we conclude that IDPc is one of the major producers of the cytosolic NADPH required for fat and cholesterol biosynthesis in the liver and adipocytes.

EXPERIMENTAL PROCEDURES

Stable Cell Lines and Induction of Differentiation—IDPc cDNAs in pLNCX retroviral vector were permanently expressed in 3T3-L1 cells in sense (IDPcS) and antisense (IDPcAS) directions as described previously (9). Cultured cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal calf serum. When cells reached confluence in culture dishes, differentiation was induced in a differentiation-permissive medium (Dulbecco's modified Eagle's medium containing 10% fetal bovine serum, 1 μM dexamethasone, 0.5 mM methylisobutylxanthine, and 5 μg/ml insulin). After 2 days of differentiation induction, cells were further fed with Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 5 μg/ml insulin and were replaced with fresh medium at 2-day intervals. Cytoplasmic fat droplets were visualized by staining with Oil Red O dye (Sigma).

Transgenic Mice—To construct an IDPc mini-gene, a cytomegalovirus promoter in plasmid PCI-neo (Promega) was replaced with the 2.2 kb of rat phosphoenolpyruvate carboxykinase (PEPCK) promoter (10, 11). The 1.7-kb mouse IDPc cDNA (nucleotides 1–1714) (9) was inserted at the XhoI and SalI sites of the resulting recombinant plasmid PEPCK-neo to place IDPc cDNA downstream from the chimeric intron preexisting in plasmid PEPCK-neo. The resulting plasmid PEPCK-neoIDPc was linearized with NsiI, and then the 4.4-kb IDPc-mini gene including the SV40 late polyadenylation signal was isolated by the partial digestion of XbaI. Transgenic mice were produced by following the method described by McGrane *et al.* (11) using the fertilized eggs from the FVB mouse strain. The animals were housed in stainless steel cages in a room with controlled temperature (23 °C) and lighting (alternating 12-h periods of light and dark) and received standard laboratory chow and water *ad libitum*. All animals were maintained in conformity with National Institutes of Health standards for the care of laboratory animals.

RNA Preparation, Northern Blot, and Immunoblot Analyses—Total RNAs were prepared from cultured cells and mouse tissues using RN-Azol (Tel-Test Inc.) according to the manufacturer's protocol. RNAs from cultured cells (10 μg/lane) and mouse tissues (20 μg/lane) were separated by electrophoresis, transferred to GeneScreen membranes, and hybridized with ^{32}P-labeled cDNA probes. Plasmid constructs containing cDNA probes for ADD1/SREBP1, aP2, and PPARγ were obtained from B. M. Spiegelman (Harvard Medical School, Boston). The respective cDNA probes for mouse adipsin, C/EBPα, FAS, GLUT4, LPL, leptin, and resistin were amplified by reverse transcriptase-PCR by using poly(A)$^+$ RNA isolated from 3T3-L1 cells. The PCR primers used to obtain the selective DNA probes are as follows: adipsin, sense primer, 5′-GATGACGACTCTGTGCAGGTGCTCC-3′, and antisense primer, 5′-GATGACACTCGGGTATAGACGCCCG-3′; C/EBPα, sense primer, 5′-GAGCCGAGATAAAGCCAAACAACG-3′, and antisense primer, 5′-GG-GAAGCCCACTTCATTTCATTGG-3′; FAS, sense primer, 5′-AATCCA-ACATGGGACACCCT-3′, and antisense primer, 5′-TGCCCATCCCTG-AGCAGAT-3′; GLUT4, sense primer, 5′-AAACAAGATGCCGTCGGGT-TT-3′, and antisense primer, 5′-TGGCTCTCCCACCCTGTTTT-3′; LPL, sense primer, 5′-GAGGAATCTAATGGCCCATAGC-3′, and antisense primer, 5′-GGAACTCACTCTGTAAACCAGGC-3′; leptin, sense primer, 5′-AGCTGCAAGGTGCAAGAAG-3′, and antisense primer, 5′-GGGCT-AACATCCAACTGTTGA-3′; and resistin, sense primer, 5′-AACAAGA-AGGAGCTGTGGGA-3′, and antisense primer, 5′-TGGCTGTGCTGG-AAACCA-3′. The relative intensity of each mRNA band in WT and IDPc-Tg1 mice was determined by a densitometer and normalized to the relative intensity of β-actin mRNA band for each sample. To prepare IDPc polyclonal antibody, full-length mouse IDPc cDNA was introduced into the pGEX-KG bacterial expression vector (12). The recombinant mouse IDPc fusion protein from bacterial lysate was purified by elution through an affinity chromatography on glutathione-agarose gel. The purified protein was used to prepare polyclonal antibodies in rabbit.

Measurement of Lipids—The total cholesterol and triglyceride concentrations in serum were determined enzymatically using the commercial kits (Sigma) based on a modification of the cholesterol oxidase method and the lipase-glycerol phosphate oxidase method, respectively (13, 14). The hepatic lipids were extracted using Folch's method with a slight modification (15). The dried lipid residues were dissolved in 1 ml of ethanol for total cholesterol and triglyceride assays. Triton X-100 and sodium cholate solutions (in distilled H_2O) were added to 200 μl of the dissolved lipid solution to produce final concentrations of 0.5% and 3 mM, respectively. The hepatic total cholesterol and triglycerides were analyzed with the same enzymatic kit used in the serum.

Measurement of NADPH Level, Acetyl-CoA, Malonyl-CoA, and IDPc Activity—NADPH values were determined by the method described previously (9) and were expressed as the ratio of NADPH to the total NADP pool (NADPH/NADPt). Each male mouse of IDPc-Tg1 and WT littermates (n = 4–5 per group) was fed with standard laboratory chow from 8 p.m. to 10 p.m. daily. At 10 p.m., the mice were killed by cervical dislocation, and the livers and epididymal fat pads were rapidly removed and clamped between aluminum disks pre-chilled in liquid nitrogen. The time from cervical dislocation to frozen tissue was less than 8 s. Perchloric acid extractions of freeze-clamped tissues were prepared according to the method described by Veech *et al.* (1). The concentration of acetyl-CoA or malonyl-CoA in tissue extracts was determined by reverse-phase high pressure liquid chromatography as described by King *et al.* (16) with a slight modification, in which the C_{18} column (2.0 × 150 mm, 3.5 μM laureate series) from Methchem (Cleveland, OH) was used. IDPc activity in the cytosolic fraction of cultured cells and mouse tissues was measured at 25 °C in reaction mixture (50 mM MOPS, 5 mM *threo*-D_s-isocitrate, 35.5 mM triethanolamine, 2 mM $NADP^+$, 1 mM ADP, 2 mM $MgCl_2$, and 1 μg/ml rotenone, pH 7.2) by monitoring the production of NADPH at 340 nm. One unit of IDPc activity is defined as the amount of enzyme catalyzing the production of 1 μmol of NADPH/min.

Glucose Tolerance Test—Animals were fasted for 12 h, and blood was collected from the tail vein to determine fasting glucose levels. Mice then received 1 mg/g body weight of a sterile glucose solution by intraperitoneal injection. Blood samples were collected at 10, 20, 30, 70, 90, and 120 min following the injection, and circulating blood glucose concentration was measured using the Glucocard (Arkray, Kyoto, Japan).

RESULTS

Positive Correlation between the Levels of IDPc and Fat Accumulation in 3T3-L1 Adipocytes—To elucidate whether IDPc plays a role in adipogenesis, we scored the changes in the IDPc expression level and enzyme activity during adipocyte differentiation from 3T3-L1 preadipocytes. Both IDPc enzyme activity (Fig. 1*A*) and its protein content were increased in parallel in a time-dependent manner, whereas its mRNA level remained constant (Fig. 1*B*), suggesting a post-transcriptional activation of IDPc during the fat cell differentiation. The catalytic activity of G6PDH also increased in parallel with that of IDPc with a maximum activity observed at the 5th day of 3T3-L1 fat cell differentiation (data not shown). In contrast, the activity of malic enzyme gradually increased during 3T3-L1 adipocyte differentiation, but its activity at the 5th day of differentiation was ~30% of IDPc and G6PDH activities (data not shown). To determine whether the up-regulated IDPc activity is reflected as a cause or as a result of adipogenesis, we prepared 3T3-L1 preadipocytes stably transfected with the IDPc cDNA vector expressing the sense (IDPcS) or antisense (IDPcAS) strand, as reported previously (9). The IDPcS- and

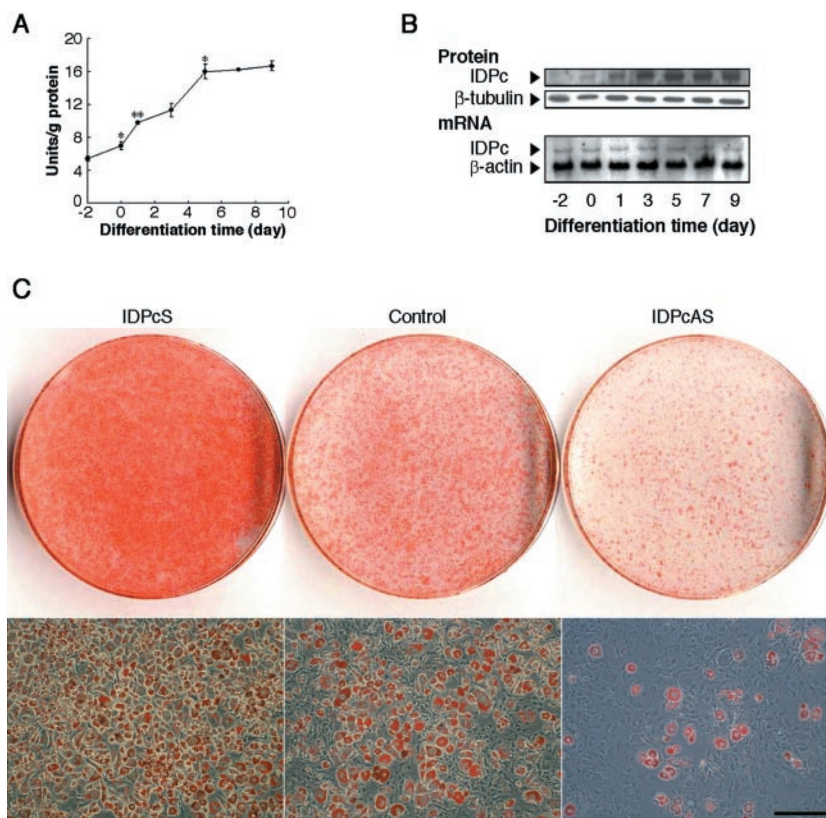

FIG. 1. **Post-transcriptional activation and the effect of transfected IDPc in 3T3-L1 adipocyte differentiation.** *A,* changes of IDPc enzyme activity during 3T3-L1 adipogenesis were analyzed. Values are means ± S.E. of at least three separate experiments. A significant difference of the mean from that of the preceding time point is indicated (*, $p < 0.05$; **, $p < 0.01$). *B,* the cytosolic homogenates (50 μg/lane) from cultured cells were subjected to 12% SDS-PAGE and followed by immunoblot analysis using antibodies against mouse IDPc and β-tubulin. For Northern blot analyses, total RNAs (10 μg/lane) from cultured cells were separated on 1% agarose gel and hybridized with cDNA probes for mouse IDPc and β-actin. *C,* pre-adipocyte 3T3-L1 cells stably transfected with sense strand of IDPc (*IDPcS*), viral vector alone (*Control*), or antisense strand of IDPc (*IDPcAS*) were allowed to differentiate into fat cells for 9 days and then stained with Oil Red O to visualize fat accumulation. *Bar,* 250 μm.

IDPcAS-infected cells exhibited 9.7 ± 0.5 and 3.4 ± 0.2 units of IDPc activity per g of protein, respectively. These values are 180% higher and 37% lower, respectively, than that of control cells infected with the empty vector alone. On the 9th day of differentiation, the amount of fat and the degree of adipocyte differentiation were markedly increased in IDPcS cells, whereas both were significantly reduced in IDPcAS cells, compared with those in the control cells (Fig. 1*C*). These results suggest that elevated IDPc activity positively correlated with the degree of adipogenesis of 3T3-L1 cells.

IDPc Transgenic Mice Displayed Hyperlipidemia, Fatty Liver, and Obesity—To confirm our *in vitro* results, we also studied the changes of fat accumulation in IDPc transgenic mice. In order to overproduce IDPc protein in the liver and adipose tissues, where IDPc is primarily expressed (Fig. 2*A*), we prepared a mini-IDPc gene construct using rat cytosolic PEPCK promoter. This promoter contained all the elements needed for the liver- and adipose tissue-specific expression (10, 11). Three independent transgenic founders gained significantly more body weight compared with WT littermates. These three male mice were mated with WT female mice, and two lines of heterozygous F1 male mice (IDPc-Tg1 and -Tg2) were obtained. Of the two, IDPc-Tg1 gained more weight than IDPc-Tg2 (Fig. 2, *B* and *C*). Therefore, we performed additional experiments with the offspring of the IDPc-Tg1 line. At the 10th week after birth, both male and female IDPc-Tg1 mice gained more weight compared with WT littermates (male, 32.8 ± 0.5 g in IDPc-Tg1 ($n = 12$) *versus* 26.8 ± 0.6 g in WT ($n = 9$), $p < 0.01$; female, 28.9 ± 1.4 g in IDPc-Tg1 ($n = 5$) *versus* 24.2 ± 0.4 g in WT ($n = 9$), $p < 0.05$) (Fig. 2*D*). At 26 weeks, the average body weight of IDPc-Tg1 male mice (39.8 ± 1.5 g, $n = 5$) was 35% higher than that of WT littermates (29.4 ± 0.2 g, $n = 6$) (Fig. 2*B*). Increased body size was much more evident in the 26-week-old IDPc-Tg1 mice (Fig. 2, *E* and *F*). The size of retroperitoneal (Fig. 2*G*) and epididymal fat pads (Fig. 2, *H* and *I*) in the abdominal cavities of the 26-week-old IDPc-Tg1 mice was markedly increased. The total weight of epididymal fat pads in IDPc-Tg1 mice (2.02 ± 0.14 g, $n = 5$) was 5.8-fold greater than that of WT littermates (0.35 ± 0.01 g, $n = 5$) (Fig. 2*C*). The size of adipocytes in epididymal fat pads in IDPc-Tg1 mice was 2.5-fold bigger than that of WT littermates (3,145 ± 62 μm^2 in IDPc-Tg1 *versus* 1,268 ± 33 μm^2 in WT, $n = 100$; $p < 0.001$), demonstrating adipocyte hypertrophy in IDPc transgenic mice (Fig. 2, *J–L*). The liver of the IDPc-Tg1 mice exhibited a typical sign of fatty liver with accumulation of fat droplets throughout the liver acini (Fig. 2, *M* and *N*). However, despite fatty liver in the transgenic mice, liver weights of the IDPc-Tg1 and WT mice were similar (1.70 ± 0.05 g in IDPc-Tg1 ($n = 5$) *versus* 1.61 ± 0.05 g in WT ($n = 6$)) (Fig. 2*O*).

We compared the IDPc activities in the liver and epididymal fat pads of IDPc-Tg1 mice and WT littermates (Table I). The IDPc activities in the liver and epididymal fat tissues of IDPc-Tg1 mice were elevated by 40 and 170%, respectively, compared with those of WT littermates (liver, 108.2 ± 1.7 units in IDPc-Tg1 ($n = 5$) *versus* 77.4 ± 1.1 units per g of protein in WT ($n = 6$); epididymal fat, 50.0 ± 0.4 units in IDPc-Tg1 ($n = 5$) *versus* 18.6 ± 0.6 units per g of protein in WT ($n = 6$)). The ratios of [NADPH]/[NADPt] in the liver and epididymal adipose tissues of the IDPc-Tg1 mice after 5 h of fasting were 0.38 ± 0.01 and 0.55 ± 0.02, respectively, whereas those of WT littermates were 0.34 ± 0.01 and 0.43 ± 0.01, respectively (Table I). The NADPH contents of the liver, although they were statistically different, did not correspond with the differences of IDPc activities in IDPc-Tg1 and WT littermates. This inconsistency might result from the different rates of NADPH consumption in those organs.

Significantly high levels of triglycerides and cholesterol are usually detected in the sera of obese mice (17). To determine whether this phenomenon could be also applied to the IDPc-transgenic mice, we measured the lipid contents in the serum of IDPc-Tg1 and WT littermates. Triglycerides and cholesterol contents in the serum of IDPc-Tg1 mice were elevated by 1.7-fold

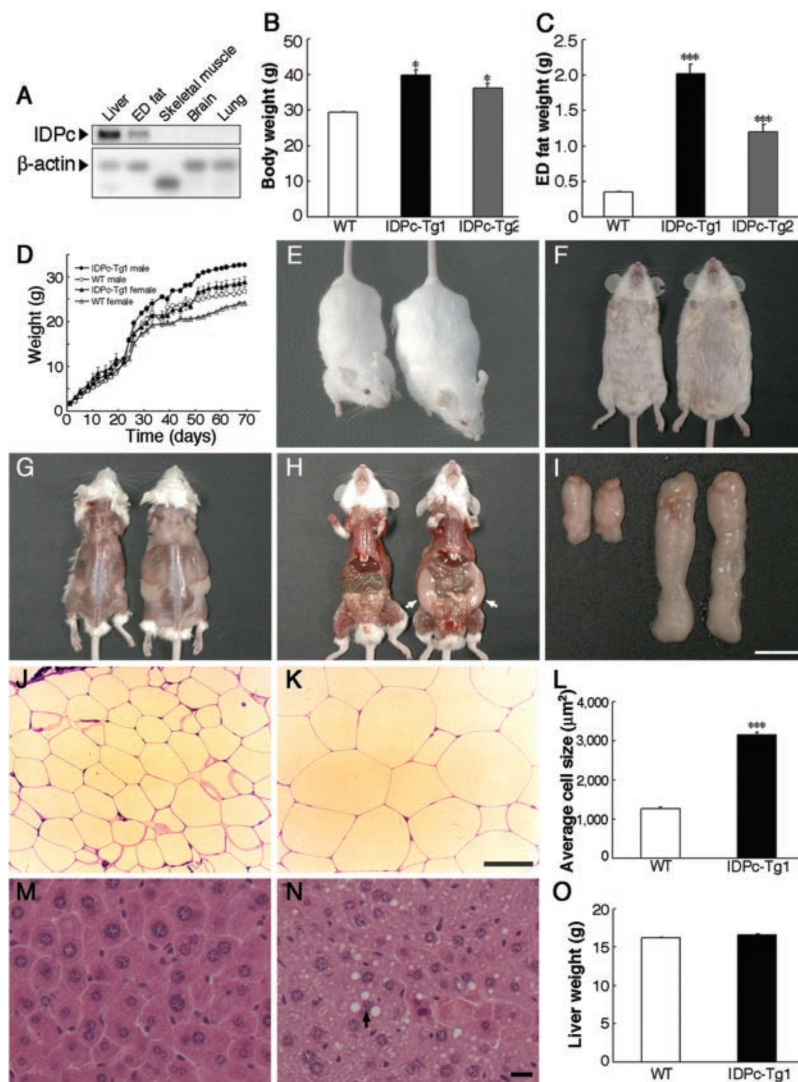

FIG. 2. **Phenotypic changes in transgenic mice overexpressing IDPc gene.** *A*, expression of IDPc mRNA transcript in various tissues of adult mouse. *ED fat* represents epididymal fat. *B* and *C*, comparison of weight of body (*B*) and epididymal fat (*C*) of 26-week-old male mice for wild type littermates (*WT*) (n = 5, 6) and IDPc-Tg1 (n = 5) and Tg2 mice (n = 5) are depicted in the *histogram*. *D*, change in body weights of male and female mice for wild type littermates (*WT*) (n = 9) and IDPc-Tg1 mice (male, n = 12; female, n = 5). Body weight of every mouse in each group was measured three times per week. The data are presented as means ± S.E. *E–H*, gross views of the 26-week-old WT (*left*) and IDPc-Tg1 mouse (*right*). *E,* dorsal view; *F*, ventral view; *G*, exposed dorsal view; *H*, ventral view of opened abdomen. The enlarged retroperitoneal and epididymal fat pads in IDPc-Tg1 are indicated by *arrows. I*, epididymal fat pads removed from WT (*left*) and IDPc-Tg1 mouse (*right*). *Bar*, 1 cm. *J* and *K*, histological analysis of epididymal fat tissues from WT (*J*) and IDPc-Tg1 mouse (*K*). *Bar*, 100 μm. *L*, the average size of fat was measured on image generated by microscope. *M* and *N*, histological analysis of liver from WT (*M*) and IDPc-Tg1 mice (*N*). An *arrow* indicates fat droplet accumulated in the liver of IDPc-Tg1 mouse. *Bar*, 250 μm. *O*, liver weight between WT and IDPc-Tg1 was compared. Each value represents the mean ± S.E. *, $p < 0.05$; ***, $p < 0.001$ compared with WT mice.

(181.5 ± 22.2 mg/dl in IDPc-Tg1 (n = 5) *versus* 106.7 ± 2.5 mg/dl in WT (n = 6)) and by 2.4-fold (165.0 ± 20.2 mg/dl in IDPc-Tg1 (n = 5) *versus* 68.3 ± 1.9 mg/dl in WT (n = 6)), respectively (Table I). Similarly, triglycerides and total cholesterol contents in the liver of IDPc-Tg1 mice were also 3.4 times higher (42.3 ± 1.8 mg/g wet tissue in IDPc-Tg1 (n = 4) *versus* 12.5 ± 1.0 mg/g wet tissue in WT (n = 6)) and 2.8 times higher (9.7 ± 0.8 mg/g wet tissue in IDPc-Tg1 (n = 5) *versus* 3.5 ± 0.2 mg/g wet tissue in WT (n = 6)), compared with those of WT littermates (Table I). These results indicate that elevated IDPc activity is positively related to hypertriglyceridemia and hypercholesterolemia.

We also measured the concentration of acetyl-CoA, a common precursor for fatty acid and cholesterol synthesis, in freeze-clamped liver and epididymal fat pads (Table I). Acetyl-CoA concentrations in the liver and epididymal fat tissues of IDPc-Tg1 male mice were lower than those of WT male mice by 56 and 26% (liver, 79.6 ± 5.9 nmol/g frozen tissue in IDPc-Tg1 (n = 5) *versus* 182.3 ± 3.9 nmol/g frozen tissue in WT (n = 4); epididymal fat pad, 9.1 ± 0.3 nmol/g frozen tissue in IDPc-Tg1 (n = 5) *versus* 12.4 ± 0.6 nmol/g frozen tissue in WT (n = 4)). Similarly, the concentration of the malonyl-CoA in the liver, the immediate precursor for fatty acid synthesis, was reduced by 24% in IDPc-Tg1 mice (11.3 ± 0.4 nmol/g frozen tissue of IDPc-Tg1 (n = 5) *versus* 14.9 ± 0.4 nmol/g frozen tissue of WT (n = 4)). These data suggest that the elevated NADPH level caused by the high IDPc activity was likely to increase fat and cholesterol synthesis, which consumes the precursor molecules at a high rate, leading to reduced levels of both acetyl-CoA and malonyl-CoA.

It is well established that food intake and weight gain are positively correlated with each other. Most interestingly, our IDPc-Tg1 mice did not seem to obey this relationship, because no significant difference in food intake was observed between the two groups (male, 4.30 ± 0.4 g in IDPc-Tg1 (n = 6) *versus* 4.4 ± 0.5 g in WT (n = 3); female, 4.6 ± 0.12 g in IDPc-Tg1 (n = 4) *versus* 4.7 ± 0.70 g in WT (n = 4)) (Table I). Thus, obesity, fatty liver, and hyperlipidemia observed in IDPc-Tg1 mice did not appear to be caused by the increased food intake. This conflict could be explained by the different metabolic flux rates in IDPc-Tg1 mice.

Overexpression of IDPc Was Linked to the Activation of PPARγ-mediated Adipogenesis—It is well established that activation of PPARγ is an indispensable factor in adipogenesis (18). To evaluate whether the enhanced adipogenesis observed in IDPc-Tg1 mice is mediated through PPARγ activation, we determined the mRNA levels of PPARγ and other genes known to be associated with adipogenesis. In the epididymal fat tissues of IDPc-Tg1 mice, expression of IDPc mRNA was increased by 70% compared with that of littermates. PPARγ expression in this tissue of IDPc-Tg1 was much higher than that of the littermates (4.5-fold; Fig. 3). Other important genes with significantly elevated mRNA expression in the fat cells of IDPc-Tg1 mice over those of WT

TABLE I
Biochemical comparisons of wild type and IDPc-Tg1 mice

Each value represents the mean ± S.E. of the indicated number of 26-week-old mice. All mice were fed normal chow diet. Metabolic parameters of the 26-week-old male WT and IDPc-Tg1 transgenic mice were measured after fasting for 5 h. Concentrations of acetyl-CoA and malonyl-CoA in the liver and epididymal (ED) fat of 26-week-old WT and IDPc-Tg1 mice (n = 4–5 per group) were determined as indicated (see "Experimental Procedures").

Genotype	Wild type	IDPc-Tg1
Liver IDPc activity (units/g protein)	77.4 ± 1.1	108.2 ± 1.7[a]
ED fat IDPc activity (units/g protein)	18.6 ± 0.6	50.0 ± 0.4[a]
The ratio of [NADPH/NADPt] in liver	0.34 ± 0.01	0.38 ± 0.01[b]
The ratio of [NADPH/NADPt] in ED fat	0.43 ± 0.01	0.55 ± 0.02[c]
Serum triglycerides (mg/dl)	106.7 ± 2.5	181.5 ± 22.2[b]
Serum cholesterol (mg/dl)	68.3 ± 1.9	165.0 ± 20.2[c]
Liver triglycerides (mg/g)	12.5 ± 1.0	42.3 ± 1.8[a]
Liver cholesterol (mg/g)	3.5 ± 0.2	9.7 ± 0.8[a]
Liver acetyl-CoA (nmol/g)	182.3 ± 3.9	79.6 ± 5.9[a]
ED fat acetyl-CoA (nmol/g)	12.4 ± 0.6	9.1 ± 0.3[c]
Liver malonyl-CoA (nmol/g)	14.9 ± 0.4	11.3 ± 0.4[a]
Male food intake (g/day)	4.4 ± 0.5	4.3 ± 0.4
Female food intake (g/day)	4.7 ± 0.7	4.6 ± 0.1

[a] $p < 0.001$, significantly different when compared with the values for wild type littermates.
[b] $p < 0.05$.
[c] $p < 0.01$.

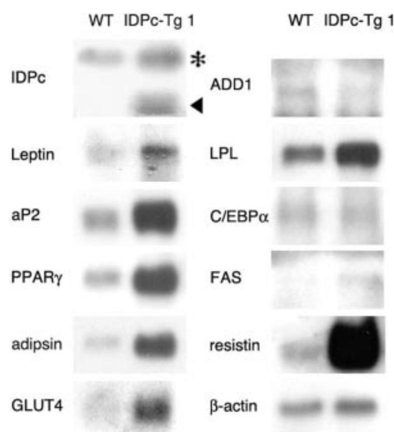

FIG. 3. **Differential expression of various mRNA transcripts in epididymal fat tissues of WT and IDPc-Tg1 mice.** Total RNA (20 μg/lane) isolated from the epididymal fat pads of WT or IDPc-Tg1 (male, n = 5–6 per group) mice were pooled, separated on agarose gels, and hybridized with the respective ^{32}P-labeled cDNA probe, as indicated. An equal amount of total RNA loaded in each lane was verified by hybridization with β-actin cDNA probe. The *asterisk* and *arrowhead* (*top, left panel*) represent the intrinsic IDPc transcript (2.2 kb) and PEPCK-neoIDPc transgene (1.7 kb), respectively.

littermates are as follows: *adipsin* (10.8-fold), adipocyte P2 (*aP2*; 3.4-fold), lipoprotein lipase (LPL; 2.3-fold), *resistin* (16.6-fold), fatty-acid synthase (FAS; 3.1-fold), and glucose transporter 4 (*GLUT4*; 4.4-fold), whereas the leptin level was moderately elevated (1.8-fold) in Northern blots (Fig. 3). Most interestingly, little change in the expression of *C/EBPα* was observed, whereas slightly decreased expression of *ADD1/SREBP1* was detected in the IDPc-Tg1 mice. Although *C/EBPα* and *ADD1/SREBP1* are considered the landmarks en route to adipocyte differentiation in cooperation with *PPARγ*, the two genes did not apparently contribute to the enhanced adipogenicity in our IDPc-stimulated obese model. Taken together, these observations suggest that the increased IDPc activity in the IDPc-Tg1 transgenic mice may

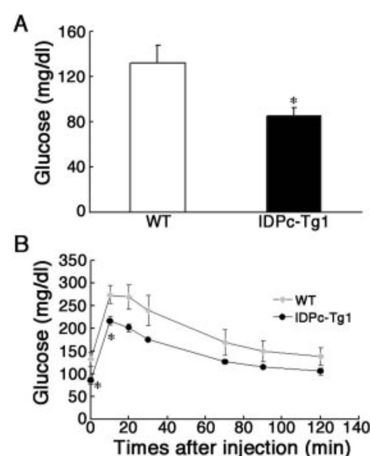

FIG. 4. **Blood glucose level and glucose tolerance test in IDPc-Tg1 mice.** *A*, blood glucose content of 26-week-old mice was measured after 12 h of fasting. *B*, glucose tolerance tests were carried out after glucose injection to the 26-week-old mice. Values are expressed as mean ± S.E. *, $p < 0.05$ significantly different when compared with the values for WT mice.

lead to the activation of PPARγ-mediated adipogenesis program through increased production of various lipids, which could serve as ligands/inducers of PPARγ gene expression.

In Northern blot analysis, *GLUT4* expression in IDPc-Tg1 was much higher than that of WT littermates (Fig. 3). The higher ratio of [NADPH]/[NADPt] in the liver and adipocytes of IDPc-Tg1 mice (Table I) appears to accelerate the consuming rate of the precursors with enhanced PPARγ-mediated fat synthesis (Fig. 3). Based on these results, we initially hypothesized that IDPc-Tg1 mice may accumulate fats through their efficient conversion of glucose to lipids without extra food intake. This hypothesis was supported by two observations. One is that the blood glucose level in IDPc-Tg1 mice after 12 h of fasting was slightly but significantly lower than that of the WT littermates (Fig. 4*A*). Additionally, in glucose tolerance test, IDPc-Tg1 mice showed improvement of glucose sensitivity compared with WT littermates (Fig. 4*B*).

In accordance with the results shown in Figs. 1–4, a positive correlation between the plasma IDPc activity and the body mass index (r = 0.69, $p < 0.01$) was found among 98 randomly selected human volunteers undergoing routine medical exams. In addition, their total cholesterol contents in plasma also correlated with the plasma IDPc activity (r = 0.58, $p < 0.01$) (data not shown). Similarly, hepatic IDPc activity in *ob/ob* mice was 50% higher than that in C57/BL, a parental strain of *ob/ob* mice (data not shown).

DISCUSSION

In fat and cholesterol biosynthesis, acetyl-CoA and NADPH are absolutely required as the common precursor and the essential cofactor, respectively. Therefore, it would be reasonable that a key NADPH producer may contribute to the regulation of body fat and cholesterol contents. Among the enzymes producing NADPH, G6PDH and malic enzyme were considered to be major NADPH producers for lipid synthesis, and treatment of dehydroepiandrosterone, an inhibitor for G6PDH, to 3T3-L1 fat cells decreased fat accumulation (19). However, our current results demonstrate time-dependent increases in IDPc and G6PDH with much less activation of malic enzyme during 3T3-L1 fat cell differentiation. These data suggest the possibility that IDPc and G6PDH may be the major contributors for NADPH production required for lipid synthesis during 3T3-L1 fat cell differentiation.

During our attempt to identify key enzymes and their chemical modulators, we initially hypothesized that IDPc is the critical

enzyme as the major producer of NADPH needed for body fat and lipid synthesis based on the following thoughts and observations. First, we reported recently (9) that IDPc is a major cytosolic NADPH producer required for the regeneration of reduced glutathione. In this case (9), neither increased nor decreased IDPc expression altered G6PDH or other NADPH-producing enzymes in the cultured cells, strongly suggesting that the changes observed in the *in vitro* model of adipogenesis and glutathione biosynthesis are mainly driven by the increased or decreased IDPc activity. Second, earlier reports (1, 2) indicated that IDPc activity in rat liver was 13- and 24-fold higher than that of G6PDH and malic enzyme, respectively. In rat epididymal adipose tissue, IDPc activity was almost comparable with the G6PDH activity (20), whereas IDPc activity was much higher than that of G6PDH and malic enzyme in the lactating sheep mammary gland (21), suggesting an important role for IDPc in certain tissues. Third, modulation of G6PDH, expressed ubiquitously in various tissues (4), would bring potentially negative consequences because this enzyme is coupled to the pentose phosphate pathway, which is critical for the nucleic acid synthesis. In contrast, modulation of IDPc, mainly expressed in the lipogenic tissues including liver and white adipose tissue, would be achievable without major adverse consequences. Finally, differential metabolic regulation of IDPc *versus* G6PDH also provides the evidence for the important role of IDPc as the major NADPH producer in fat and cholesterol synthesis. In lipogenic tissues, a very high level of NADPH/NADPt is maintained (22). Therefore G6PDH, which could be inhibited by NADPH (23), may not be catalytically active. In contrast, our unpublished *in vitro* results[2] revealed that high concentrations of NADPH did not significantly inhibit the catalytically active IDPc purified from *Escherichia coli* expressing the recombinant IDPc DNA. In one study, less than 20% of IDPc activity was inhibited by the presence of 20 mM NADPH (NADPH/NADPt = 0.9), when determined by measuring α-ketoglutarate production in high pressure liquid chromatography analyses (data not shown). These observations together with the data in earlier reports (3, 24) support the possible role of IDPc as the major source of the cytosolic NADPH during fat synthesis. Consistent with this conclusion, overexpressed IDPc stimulated fat accumulation during adipogenesis of 3T3-L1 cells, whereas reduced IDPc activity inhibited fat accumulation, suggesting that relatively high IDPc activities could serve as a cause, rather than a result, of adipogenesis.

In this study, we also observed that the IDPc transgenic mice displayed obese phenotypes accompanied by marked elevation of both triglyceride and cholesterol contents in the serum, liver, and adipose tissues in parallel to the increased IDPc activity and NADPH amounts. Furthermore, hepatic acetyl-CoA and malonyl-CoA concentrations were reduced in the IDPc-Tg1 mice, most likely through their utilization in fat and/or cholesterol synthesis. The decrease in acetyl- and malonyl-CoA concentrations may also reflect the expression of FAS (3.1-fold), slightly lower blood glucose levels, and improvement of glucose sensitivity observed in IDPc-Tg1 mice. The data from our *in vitro* model for increased fat synthesis after IDPc overexpression and *in vivo* model of IDPc-Tg mice are in agreement with the results in humans for their plasma IDPc activity, the body mass index, and cholesterol contents as well as the data from *ob/ob* mice. These results demonstrate that IDPc activity is positively correlated with the degree of fat and cholesterol synthesis in mice and human, and taken together our results further suggest a causative role of IDPc in increased contents of body fat and cholesterol. However, knock-out experiments of the *IDPc* gene as well as treatment of obese rodents with the IDPc inhibitors remain to be undertaken to reveal the detailed function of IDPc in body fat and lipid synthesis.

It is well established that certain fatty acids and their derivatives can activate PPARγ. These include natural polyunsaturated fatty acids and their derivatives such as linoleic acid, linolenic acid, and 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 (25–27). Kim *et al.* (28) also showed that increased production of PPARγ ligands in 3T3-L1 cells led to simultaneous stimulation of PPARγ expression and its transcriptional activity in the presence of transfected ADD1/SREBP1 gene, which activates several genes involved in fatty acid synthesis. Recently, Wu *et al.* (29) reported a strong cross-regulation between PPARγ and C/EBPα during adipogenesis. However, in our 26-week-old IDPc-Tg1 mice, the strong cross-regulation between the two transcription factors was not observed, as has been reported by other investigators (28, 30, 31). Nonetheless, our data strongly suggest that elevated NADPH in IDPc-Tg1 mice was likely to promote synthesis of various fatty acids, some of which may subsequently activate PPARγ, but not C/EBPα and ADD1/SREBP1.

In our IDPc-Tg1 mice, the expression of *resistin*, which is highly expressed in white adipose tissues and known to serve as a hormonal link between obesity and insulin resistance in a mouse model (32), was markedly increased in epididymal fat pads. However, the blood glucose level in 26-week-old IDPc-Tg1 mice after 12 h of fasting was in the normal range although it was slightly lower than that of WT littermates, and IDPc-Tg1 showed improvement of glucose sensitivity compared with WT littermates. This inference is also supported by the 4.4-fold up-regulation of *GLUT4* expression in the epididymal fat pads of IDPc-Tg1 mice. These findings suggest that the obesity induced in IDPc-Tg1 mice is different from that observed in other rodent models (33) and may not be associated with diabetes or insulin resistance.

The concurrent induction of fatty liver, hyperlipidemia, and obesity by alteration of a single *IDPc* gene in liver and adipose tissues without an increase in caloric intake or diet composition is unprecedented to the best of our knowledge. Therefore, IDPc may become a potential therapeutic target for abnormal fat synthesis.

Acknowledgments—We thank B. M. Spiegelman for providing the cDNA clones for aP2 and PPARγ and R. W. Hanson for providing the PEPCK promoter. We also thank M. T. King for technical support and I. J. Lee and R. S. Goody for critical reading of this manuscript.

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