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Nur77 modulates hepatic lipid metabolism through suppression of SREBP1c activity

Thijs W.H. Pols [a], Roelof Ottenhoff [a], Mariska Vos [a], Johannes H.M. Levels [b], Paul H.A. Quax [d,e], Joost C.M. Meijers [b,c], Hans Pannekoek [a], Albert K. Groen [a], Carlie J.M. de Vries [a,*]

[a] *Medical Biochemistry, Academic Medical Center, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands*
[b] *Experimental Vascular Medicine, Academic Medical Center, Amsterdam, The Netherlands*
[c] *Vascular Medicine, Academic Medical Center, Amsterdam, The Netherlands*
[d] *TNO-Quality of Life, Gaubius Laboratory, Leiden, The Netherlands*
[e] *Department of Surgery, Leiden University Medical Center, Leiden, The Netherlands*

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Abstract

NR4A nuclear receptors are induced in the liver upon fasting and regulate hepatic gluconeogenesis. Here, we studied the role of nuclear receptor Nur77 (NR4A1) in hepatic lipid metabolism. We generated mice expressing hepatic Nur77 using adenoviral vectors, and demonstrate that these mice exhibit a modulation of the plasma lipid profile and a reduction in hepatic triglyceride. Expression analysis of >25 key genes involved in lipid metabolism revealed that Nur77 inhibits SREBP1c expression. This results in decreased SREBP1c activity as is illustrated by reduced expression of its target genes stearoyl-coA desaturase-1, mitochondrial glycerol-3-phosphate acyltransferase, fatty acid synthase and the LDL receptor, and provides a mechanism for the physiological changes observed in response to Nur77. Expression of LXR target genes *Abcg5* and *Abcg8* is reduced by Nur77, and may suggest involvement of LXR in the inhibitory action of Nur77 on SREBP1c expression. Taken together, our study demonstrates that Nur77 modulates hepatic lipid metabolism through suppression of SREBP1c activity.

Keywords: Lipid metabolism; NR4A nuclear receptors; Nur77; NR4A1; Sterol regulatory element binding protein-1; SREBP1c

Nuclear receptor Nur77 (NR4A1) belongs together with Nurr1 (NR4A2) and NOR-1 (NR4A3) to the NR4A subfamily of the nuclear receptor superfamily. NR4A nuclear receptors consist, like most other nuclear receptors, of an N-terminal activating-function-1 domain, a central DNA binding domain, and a C-terminal ligand-binding domain (LBD) [1]. It has been demonstrated that the ligand-binding pocket of Nurr1, located within the LBD domain, is filled with bulky side-chains of hydrophobic amino acids [2]. Although an induced fit of at present unknown ligands may not be excluded, it is very well possible that all three NR4A nuclear receptors are regulated independent of ligand binding to the LBD. Nur77 is a so-called 'immediate-early response gene' and is rapidly induced in response to various stimuli, among which growth factors, inflammatory stimuli and mechanical stimuli [3].

Nur77 initiates gene transcription by binding the so called NGFI-B consensus response element (NBRE; AAAGGTCA) [4]. Furthermore, Nur77 can form homo- or heterodimers with NR4A subfamily members or with Retinoid X Receptor (RXR), to bind the Nurr1 response element (NurRE; TGATATTTn_6AAATGCCA) or the DR5 consensus response element (GGTTCACCGAAAG GTCA), respectively [5,6]. In addition to directly

* Corresponding author. Fax: +31 20 6915519.
E-mail address: c.j.devries@amc.uva.nl (C.J.M. de Vries).

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modulating transcription, NR4A nuclear receptors have been described to trans-repress other transcription factors, such as E26 transformation specific sequence (ETS-1), nuclear factor κ beta (NFκB) and estrogen related receptor-1 (ERR1) [7–9].

Nur77 regulates distinct cellular processes in a tissue-specific manner. For example, we have previously demonstrated that Nur77 is expressed in atherosclerotic lesions and inhibits neointima formation [3]. More recently, Nur77 has been shown to regulate lipolysis, energy expenditure, and glucose metabolism in skeletal muscle, to enhance insulin sensitivity in 3T3-L1 cells, and to increase energy expenditure in murine brown adipocytes [10–13].

The liver exhibits a key regulatory function in metabolism as it controls the regulation of both glucose and lipid homeostasis. Nur77 is expressed in human adult liver [14], and has been shown to regulate gluconeogenesis [15]. In the current study we demonstrate, to our knowledge for the first time, that Nur77 modulates hepatic lipid metabolism by repressing SREBP1c gene expression.

Materials and methods

Recombinant adenoviruses. Full-length human Nur77 cDNA (GenBank D49728) was inserted into replication-defective adenoviruses expressing cDNAs under control of the cytomegalovirus promoter. The adenoviruses were purified by CsCl gradient centrifugation as described before [16].

Animals. Animal care and experimental procedures were approved by the Animal Experimental Committee at our institute. Male 10–12 weeks old C57Bl/6 mice ($n = 6$ per group; Charles River Laboratories, Wilmington, MA) were fed a standard chow diet (Special diet services, Witham, Essex, UK). Hepatic overexpression of Nur77 in mouse liver was achieved by intravenous injection of recombinant adenovirus as described previously [17]. Mice were sacrificed 2 days after adenoviral treatment, after fasting for 4 h. The liver was dissected and part of the liver was fixed in formalin for immunohistochemistry, a part was used for lipid extraction, and a part was used for RNA extraction. Triglycerides and cholesterol were extracted from mouse liver as described earlier [18], and analyzed with a colorimetric assay (Wako Chemicals, Neuss, Germany). Plasma cholesterol and triglyceride concentrations in the main lipoprotein classes were determined using high performance gel filtration chromatography (HPGC) as described before [19].

RNA extraction, cDNA synthesis, and RT-PCR analysis. RNA was extracted from mouse liver using Trizol (GIBCO–Invitrogen Life Technology, Breda, The Netherlands), after which cDNA was synthesized from 1 μg total RNA (iScript; Bio-Rad, Veenendaal, The Netherlands). Semi-quantitative real-time RT-PCR was performed using iQ SYBR-Green Super-Mix in the MyiQ RT-PCR system (Bio-Rad) using gene-specific primers. Primer sequences (see Supplementary Tables 1 and 2) were obtained from literature, designed (Beacon designer 3, Premier Biosoft International, Palo Alto, CA), or obtained from the Harvard Primer Bank (pga.mgh.harvard.edu/primerbank). All expression levels were corrected for expression of the housekeeping gene cyclophillin A. The heat map was generated using Spotfire software (Spotfire Inc., MA, USA).

Immunohistochemistry. Immunohistochemistry was performed using the M210 Antibody against Nur77 (Santa Cruz, Biotechnology, Santa Cruz, CA), a biotin-labeled goat-anti-rabbit IgG secondary antibody incubation (DAKO, Glostrup, Denmark), followed by streptavidin-HRP (DAKO) and AEC (Sigma, Zwijndrecht, The Netherlands) detection.

Statistical analysis. All data are shown as means ± standard error (SD). The nonparametric Mann–Whitney U test was used to calculate statistical significance. P values less than 0.05 were considered statistically significant.

Results

Adenoviral expression of Nur77 in C57Bl/6 mice

To achieve hepatic overexpression of Nur77 in mouse liver, we injected chow-fed C57Bl/6 mice via the tail vein with control adenovirus (Ad.Mock) or with adenovirus encoding human Nur77 (Ad.Nur77). Starting plasma cholesterol and triglyceride levels, as well as liver weight (Wt), body Wt, and food intake did not reveal significant changes between the two groups (Table 1). Mice were sacrificed 2 days after adenoviral injection, after which livers were dissected and used for immunohistochemistry, lipid analysis, and RNA expression analysis. We detected hepatic mRNA expression of the Nur77 transgene in all mice injected with Ad.Nur77 virus (Fig. 1A). Immunohistochemistry demonstrated enhanced expression of Nur77 protein, predominantly in the nuclei of liver cells, in mice treated with Ad.Nur77 virus as compared to Ad.Mock-treated animals (Fig. 1B). In addition, expression of enolase 3 (*Eno3*) and fructose-1,6-bisphosphatase 2 (*Fbp2*), two direct target genes of Nur77 in mouse liver [15], was increased in livers of mice with enhanced expression of Nur77 (7.3 and 18.0 fold induction, respectively; Fig. 1C), which confirmed expression of functional Nur77 protein.

Nur77 modulates the plasma lipid profile

The plasma lipid profile analyzed 2 days after adenoviral treatment by HPGC revealed that Nur77 induced a redistribution of plasma cholesterol and trigylceride. Plasma HDL-cholesterol showed a moderate 12% reduction in response to Nur77 (2.15 ± 0.18 mmol/L vs. 2.52 ± 0.25 mmol/L in controls; Table 2). Concomitantly, plasma LDL-cholesterol was 98% increased in animals with hepatic expression of Nur77 as compared to control animals (0.79 ± 0.15 vs. 0.40 ± 0.03 mmol/L, respectively; Table 2). In addition, plasma LDL-triglyceride was 67% increased in mice overexpressing hepatic Nur77 as compared to control animals (0.55 ± 0.11 mmol/L vs. 0.33 ± 0.03 mmol/L, respectively; Table 2). Total plasma triglyceride and cholesterol was found similar between the two groups.

Nur77 reduces hepatic triglyceride levels

We next analyzed cholesterol and triglyceride content of the liver. Hepatic cholesterol level did not change in response to hepatic expression of Nur77 (Fig. 2A). Hepatic triglyceride levels, however, were reduced in response to