Enhanced expression of hepatic lipogenic enzymes in an animal model of sedentariness

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Abstract The hindlimb-suspended rat was used as animal model to investigate the effects induced by immobilization of the skeletal muscle in the expression of the genes encoding hepatic lipogenic enzymes. Following a 14-day period of immobilization, rats were injected intraperitoneally with radioactive acetate, and the labeling of hepatic lipids and cholesterol was evaluated 15 min after the isotope injection. The incorporation of labeled acetate in lipids and cholesterol was almost three times higher in the liver of immobilized rats than in control animals as a consequence of the enhanced transcription of the genes encoding acetyl-CoA synthase, acetyl-CoA carboxylase, fatty acid synthase, and 3-hydroxy-3-methylglutaryl-CoA reductase. The high expression of the key enzymes for fatty acid and cholesterol synthesis induced by immobilization was not paralleled by an increase of the hepatic sterol-regulatory element binding protein (SREBP)-1 and SREBP-2 mRNA content. However, the expression of the mature form of SREBP-1 and SREBP-2 was higher in the nuclear fraction of immobilized rat liver than in controls due to a significant increase of the cleavage of the native proteins. Immobilization also affected the expression of proteins involved in lipid degradation. In fact, the hepatic content of peroxisome proliferator-activated receptor-α (PPARα) mRNA and of PPARα target genes encoding carnitine palmitoyl transferase-1 and acyl-CoA oxidase were significantly increased upon immobilization.—Vecchini, A., V. Ceccarelli, P. Orvietani, P. Caligiana, F. Susta, L. Binaglia, G. Nocentini, C. Riccardi, and P. Di Nardo. Enhanced expression of hepatic lipogenic enzymes in an animal model of sedentariness. J. Lipid Res. 2003. 44: 696–704.

Supplementary key words acetyl-CoA synthase • acetyl-CoA carboxylase • fatty acid synthase • 3-hydroxy-3-methylglutaryl-CoA reductase • gene expression • sterol-regulatory element binding protein • polyunsaturated fatty acid • sedentary lifestyle • immobilization

It is well known that a sedentary lifestyle provokes an increase in the plasmatic cholesterol and triglyceride levels (1, 2), whereas hepatic β-oxidation of fatty acid, ketone body production, and gluconeogenesis are highly active in experimental animals subjected to prolonged physical exercise (3–5). While evidence exists that exercise training reduces the expression of lipogenic enzymes (6–8), no information is available on the mechanisms involved in the modulation of hepatic lipid metabolism in sedentary animals.

A fundamental step in the advancement of knowledge of lipid metabolism regulation is represented by the discovery of integral proteins of the endoplasmic reticulum that play a pivotal role in the control of hepatic lipogenesis (9, 10). It is now clear that a feedback control of cholesterol and glycerolipid synthesis is operated in liver by a multiplicity of stimuli through a common mechanism involving the release of membrane-anchored transcription factors (10, 11). In particular, the expression of lipogenic enzymes is controlled by the regulated cleavage of endoplasmic reticulum integral proteins known as SREBPs (sterol-regulatory element binding protein factors). In sterol-depleted cells, SREBP is fragmented by two sequential proteolytic cleavages, thereby releasing in the cytoplasm its N-terminal domain, a member of the basic helix-loop-helix family of transcription factors (12). Cleavage of the native form of SREBP enables its mature form to enter the nucleus and act as a transcription factor for a series of lipogenic enzymes through interaction with sterol response elements and/or palindromic CATG sequences in the promoter region of SREBP target genes (13, 14). On the contrary, when cells are overloaded with cholesterol, SREBP cleavage is depressed and sterol-regulated gene transcription declines (15–16).

Abbreviations: ACC, acetyl-CoA carboxylase; ACL, ATP citrate lyase; ACO, acyl-CoA oxidase; ACS, acetyl-CoA synthase; CPT-1, carnitine palmitoyltransferase-1; FAS, fatty acid synthase; GPAT, mitochondrial glycerol-3-phosphate acyltransferase; PPARα, peroxisome proliferator-activated receptor-α; SCD-1, stearoyl-CoA Δ9-desaturase-1; SREBP, sterol-regulatory element binding protein.

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Both insulin and dietary carbohydrates are involved in the control of hepatic lipogenesis by enhancing the expression of the nuclear mature form of SREBP-1c (16, 17). On the contrary, dietary PUFAs depress the hepatic lipogenesis by lowering both SREBP-1 mRNA level and nuclear content of the SREBP-1c protein (13, 18).

PUFAs are also involved in hepatic lipid metabolism regulation by activating the peroxisome proliferator-activated receptor-α (PPARα), a transcription factor that up-regulates the expression of enzymes involved in the oxidation of fatty acids (18, 19).

In the present work, the role played by the above-mentioned transcription factors in the modulation of hepatic lipid metabolism has been examined in an animal model of sedentary lifestyle. The hindlimb-suspended rat, widely used for studying the physiological and biochemical changes of immobilized skeletal muscle, was employed as an experimental model of sedentariness. Indeed, besides the metabolic effects induced on muscle, immobilization affects also the metabolism of extra-muscular districts. Among these changes, it is noteworthy to mention the onset of an insulin-resistance syndrome (20), the increase of norepinephrine turnover in the brain (21), and the reduction of sympathetic nervous system activity (22).

In this context, the increase of circulating insulin level induced by immobilization (20) was considered of particular interest due to the involvement of this hormone in the transcriptional control of hepatic lipogenesis (17, 23). Therefore, hepatic lipogenic enzyme expression and activity were examined in immobilized rats by evaluating the acetate utilization for glycerolipid and cholesterol synthesis, the hepatic content of the mRNAs encoding lipogenic enzymes, and related transcription factors.

**EXPERIMENTAL PROCEDURES**

**Materials**

- [2-14C]acetate (specific activity, 60 Ci/mol) and 3-hydroxy-3-methyl-[3-14C]glutaryl-CoA (specific activity, 19 Ci/mol) were purchased from NEN (Boston, MA). Sodium [3H]bicarbonate (specific activity 0.1 Ci/g) was obtained from ICN Biomedicals, Inc. (Milano, Italy). [α-32P]UTP (specific activity, 800 Ci/mmole) was obtained from Amersham Biosciences Europe (Milano, Italy). Rabbit polyclonal antibodies against the N-terminal region of SREBP-1 (H-160: sc-8984) and of SREBP-2 (N-19: sc-8151) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA).

Hors eradish peroxidase-conjugated anti-rabbit and anti-goat immunoglobulin secondary antibodies were obtained from Sigma-Aldrich Co. (Milano, Italy). All other chemicals were purchased from Sigma-Aldrich.

**Animal treatments**

Sixteen male Wistar rats [140 ± 5 g body weight] were randomly divided into two groups. Eight rats were immobilized by hindlimb suspension, as previously described (20). Control Wistar rats [140 ± 5 g body weight] were caged in standard conditions. Both groups were placed in light-cycling rooms with alternating 12 h periods of light and darkness. All animals had free access to food and water for the entire period preceding blood withdrawal and sacrifice.

The daily food intake was not significantly different in immobilized rats (8.6 ± 1.3 g/100 g body weight) and in control rats (8.5 ± 0.7 g/100 g body weight).

Blood samples were withdrawn from the tail vein of animals at 9 AM and collected in sodium heparinate-coated plastic tubes immediately before the sacrifice. Plasma was prepared from each collected blood sample to quantify insulin, corticosterone, glucose, cholesterol, and triglycerides.

Glucose plasma level was determined according to a standard laboratory technique (24). Plasma insulin and corticosterone were assayed using the Rat Insulin RIA Kit from Linco Research, Inc. (St. Charles, MO) and the RIA Kit from Immuno-Biological Laboratories (Hamburg, Germany), respectively, following the manufacturers’ instructions.

Plasma levels of cholesterol and neutral lipids were quantified photodensitometrically (25) after chromatographic separation by TLC on silica plates using petroleum ether-diethyl ether-acetic acid (95:5:1, v/v/v) as developing mixture.

**Analysis of liver lipids**

After blood withdrawal, livers were excised from immobilized and control rats by rapid dissection, cut into small pieces, frozen in liquid nitrogen, and stored at −80°C.

Frozen liver slices (about 1 g, fresh weight) from immobilized and control rats were homogenized in 10 vol of 0.25 M sucrose. Proteins were quantified in aliquots of the homogenates according to Bradford (26). Lipids were extracted according to Folch et al. (27). Neutral lipids were isolated by TLC on silica plates using diethyl ether-diethyl ether-acetic acid (20:80:1, v/v/v) as developing mixture, and quantified photodensitometrically as described above.

Free fatty acids were eluted from the silica scrapes with chloroform-methanol (2:1, v/v). After solvent removal under vacuum, free fatty acids were converted into the respective methyl esters by reaction with diazomethane. Gas chromatography of fatty acid methyl esters was performed using a Carlo Erba Instruments (Milano, Italy) model HRGC 5300 gas chromatograph equipped with a SP-2330 capillary column (30 m × 0.25 mm, Supelco Inc. Bellefonte, PA) and a flame ionization detector.

Polar lipids were separated by two-dimensional TLC on silica plates according to Horrocks (28) and quantified as above.

**HPLC analysis of acetyl-CoA and malonyl-CoA**

Frozen liver slices (about 0.5 g, fresh weight) from immobilized and control rats were homogenized in ice-cold 5% sulfosalicylic acid in 50 mM dithioerythritol to obtain 10% w/v homogenates. The cellular levels of acetyl-CoA and malonyl-CoA were quantified by RP-HPLC on Supelcosil LC-18 columns (Sigma-Aldrich), as previously described (29).

**RNase protection assay**

Total RNA was extracted from frozen liver slices (about 0.5, fresh weight) by the cesium chloride purification procedure (30). For RNA probes, fragments of mRNA encoding the proteins listed in Table 1 (31–42) were amplified by reverse transcriptase polymerase chain reaction from rat liver total RNA using the primers listed in the same table. The amplified products were subcloned into pCR 2.1 TOPO vector (Invitrogen S.r.l., Milano, Italy) and verified by nucleotide sequencing. After plasmid linearization, antisense RNAs were transcribed with [α-32P]UTP (800 Ci/mmole) using T7 RNA polymerase (Promega Italia S.r.l., Milano, Italy).

RNA samples were subjected to RNase protection assay using an RPA III™ kit (Ambion Inc. Austin, TX), 10 µg RNA from rat liver, 100,000 cpm of the specific antisense probe, and 20,000–2,000 cpm of antisense β-actin (Ambion) with a 5- to 50-fold excess.
lower specific activity to give a β-actin signal comparable to the test mRNAs. After digestion with RNase A/T1, the protected fragments were separated on 8 M urea/6.0% polyacrylamide gels. Gels were dried and subjected to quantitative analysis using an Instant Imager autoradiography system (Packard BioScience).

**Immunoelectrophoretic analysis of SREBPs**

Frozen rat liver slices (about 200 mg, fresh weight) were homogenized in 7% SDS. Aliquots of the homogenates containing 30 μg protein were added with 0.5 vol of 3X PAGE sample buffer. The resulting suspensions were kept for 1 min at 100°C and centrifuged for 10 min at 3,000 g. The supernatants were loaded into the wells of 10% polyacrylamide gels (1.5 mm thick). After the electrophoretic run (30 mA/gel), proteins were electrophoretically transferred onto nitrocellulose membranes and stained with Ponceau. After destaining with water, membranes were rinsed with PBS and submitted to the reaction with antibody against SREBP-1. The bound antibody was revealed by peroxidase-conjugated, affinity-purified anti-rabbit IgG antibody using the SuperSignal CLHRP substrate (Pierce, Rockford, IL) according to the manufacturer’s instructions. Blots were exposed to Kodak X-Omat film at room temperature and quantified photodensitometrically. The same procedure was used to determine the native-mature SREBP-2 ratio by using anti-goat IgG antibody. SDS-PAGE gels were calibrated with molecular weight markers (Bio-Rad, CA).

The nuclear abundance of the truncated form of SREBP-1 and SREBP-2 was determined according to the same protocol. Nuclear proteins were isolated from 0.2 g liver aliquots using the Pierce NE-PER kit (Pierce) according to the manufacturer’s instructions.

**De novo lipid biosynthesis**

Ten male Wistar rats (140 ± 4 g, body weight) were randomly divided into two groups. Five rats were submitted to hindlimb suspension in separate cages, whereas the others were separately caged in normal conditions. All animals had free access to food and water for the entire period preceding the intraperitoneal isotope injection.

After 14 day conditioning, immobilized and control rats were injected intraperitoneally with 0.5 ml of a sterile PBS physiological solution containing [1,2-14C]acetate (30 μCi, specific activity, 60 Ci/mol). Fifteen minutes after the isotope injection, rats were sacrificed by decapitation. Livers were excised, washed with ice-cold saline, cut into small pieces, weighed, and frozen in liquid nitrogen for future analyses.

Lipids were extracted according to Folch et al. (27). Incorporation of the 14C-labeled precursor in whole lipids was measured in aliquots of the extracts by liquid scintillation counting. The labeling of individual lipids, isolated by TLC on silicic acid as described above, was also quantified.

To evaluate the incorporation of the labeled acetate into individual fatty acids, aliquots of the lipid extracts were submitted to saponification. Fatty acids were extracted, converted into the respective methyl esters, and separated by RP-HPLC (23). The radioactivity of the labeled fractions separated chromatographically was determined by liquid scintillation counting.

In order to quantify the isotopic labeling of hepatic acetylCoA and malonyl-CoA, 5 g aliquots of tissue were submitted to the acidic extraction-concentration procedure described by Hosokawa et al. (43) before HPLC analysis. The isotopic labeling of the chromatographic fractions (0.3 ml/fraction) containing acetylCoA and malonyl-CoA was determined by liquid scintillation counting.
In vitro enzymatic assays

The activity of selected key enzymes of fatty acid and cholesterol synthesis was assayed in vitro. Three rats (145 ± 7 g, body weight) were immobilized for 14 days as described previously, while three control rats (146 ± 4 g, body weight) were caged for the same time in the standard conditions. Immediately after sacrifice, the liver was excised and 2 g aliquots were homogenized in 9 vol of 0.3 M sucrose containing 50 mM Tris HCl (pH 7.4), 10 mM EDTA, and 10 mM dithiothreitol. The homogenates were centrifuged 15 min at 20,000 g. The resulting supernatants were centrifuged 1 h at 100,000 g. The microsomal pellets were recovered and suspended in 5 ml of 20 mM imidazole buffer (pH 7.4). 3-Hydroxy-3-methylglutaryl-CoA reductase activity was quantified according to Angelin et al. (44) in aliquots of the microsomal preparations containing 100 µg protein in the absence of fluoride and without alkaline phosphatase pretreatment. Acetyl-CoA synthetase, acetyl-CoA carboxylase (ACC), and fatty acid synthetase activities were assayed in the post-microsomal supernatant according to Knudsen et al. (45), Thampy et al. (46), and Halestrap and Denton (47), respectively.

RESULTS

Effect of immobilization on plasma levels of hormones, glucose, and lipids

After 14 days of conditioning, the body weight increase was lower in the immobilized rats than in controls (Table 2). Hindlimb-suspended rats exhibited high plasmatic levels of insulin compared with rats caged in normal conditions. In contrast, both glucose and corticosterone plasmatic levels were not significantly different in hindlimb-suspended and control rats. Immobilization induced an increase of the plasmatic cholesterol level while plasma triglyceride concentration was not significantly different in hindlimb-suspended and control rats (Table 2).

Effect of immobilization on liver lipid composition

The hepatic lipid composition of control and hindlimb-suspended rats is shown in Table 3. Triglycerides and free fatty acids were almost three times more abundant in the liver of immobilized rats than in controls. The main free fatty acids accumulated in the liver of immobilized rats were palmitic acid (7.8 ± 0.28 nmol/g liver), oleic acid (3.36 ± 0.44 nmol/g liver), and linoleic acid (3.11 ± 0.14 nmol/g liver). The content of the same fatty acids in the liver of control rats was, respectively, 2.97 ± 0.09, 0.43 ± 0.10, and 0.38 ± 0.07 nmol/g liver. Also, phosphatidylcholine and phosphatidylethanolamine content was higher in the liver of hindlimb-suspended rats than in the control liver, whereas the hepatic cholesterol content was not significantly affected by immobilization.

Incorporation of labeled acetate into hepatic lipids

After intraperitoneal injection of control rats with labeled acetate, triglycerides, phosphatidylcholine, phosphatidylethanolamine, and cholesterol were the most heavily labeled liver lipids (Table 4). The incorporation of the labeled precursor into glycerolipid and cholesterol was about 2.5 times and 2.9 times higher in immobilized rats than in controls.

The label distribution among individual fatty acids separated by RP-HPLC is shown in Fig. 1. The efficiency of the de novo synthesis of fatty acids in liver, evaluated by in-
Fig. 1. Incorporation of intraperitoneally injected [1,2-14C]acetate in the fatty acids of whole-liver lipids. Control and immobilized rats were injected intraperitoneally with 30 μCi [1,2-14C]acetate and sacrificed after 15 min. Fatty acid methyl esters, obtained by transesterification of whole lipids extracted from 1 g of liver, were separated by RP-HPLC. The label incorporation into fatty acids was quantified by liquid scintillation counting of the isolated peaks. The data represent the means ± SD from five separate experiments. Statistical significance of the difference between immobilized and control rats was evaluated by Student’s t-test. * P < 0.001; b P < 0.05; c P < 0.01.

Fig. 2. Effect of immobilization on the level of mRNAs encoding proteins of lipid metabolism. Total RNA was isolated from the liver of control and immobilized rats. Ten microgram aliquots of total RNA were hybridized to 32P-labeled cRNA probe specific for each protein using β-actin cRNA as internal standard. After RNA digestion, the protected fragments were separated by gel electrophoresis, and the radioactivity associated with each band was quantified using a Phosphor Imager autoradiography system. For each probe, one representative out of five or six autoradiographic images is reported. The fold change in each mRNA relative to the control was calculated after correction for loading differences with β-actin. The values are shown below each blot. C, control rats; I, immobilized rats. ACC, acetyl-CoA carboxylase; ACL, ATP citrate lyase; ACO, acyl-CoA oxidase; ACS, acetyl-CoA synthase; CCT, choline cytidylyltransferase; CPT-1, carnitine palmitoyltransferase-1; DGAT, diglyceride acyltransferase; FAS, fatty acid synthase; HMG-CoA reductase; ME, malic enzyme; PPARα, peroxisome proliferator-activated receptor-α; SCD-1, stearoyl-CoA Δ9-desaturase-1; SREBP, sterol-regulatory element binding protein.

Immunoelectrophoretic analysis of native and mature SREBPs

The relative content of membrane-bound and nuclear forms of SREBP-1 and SREBP-2 was evaluated after electrophoretic separation by immunoenzymatic-chemiluminescence detection of the proteins electroblotted on nitrocellulose membranes.

Hepatic content of mRNA encoding enzymes of lipid and cholesterol metabolism

The high labeling of saturated and monoenoic fatty acids in the liver of immobilized rats paralleled the high content of the mRNA encoding fatty acid synthase (FAS) (Fig. 2), while stearoyl-CoA Δ9-desaturase-1 (SCD-1) mRNA, encoding liver SCD, was not significantly different in immobilized and control rats.

Immobilization also affected the mRNA content for ancillary enzymes of fatty acid synthesis in liver (Fig. 2). Indeed, the mRNA levels of acetyl-CoA synthase (ACS) and ACC were higher in the liver of immobilized rats than in controls, whereas immobilization induced a decrease of the hepatic mRNA content for ATP citrate lyase (ACL) and did not affect the mRNA content for malic enzyme.

Also, the hepatic level of the mRNAs encoding enzymes involved in glyceroipid synthesis was influenced by immobilization. Indeed, the mRNA content for choline cytidylyltransferase, the enzyme catalyzing the rate-limiting step of phosphatidylcholine synthesis, was higher in immobilized rats than in controls, whereas the mRNA for liver mitochondrial glycerol-3-phosphate acyltransferase (GPAT) was less abundant in immobilized rats than in controls. On the contrary, the levels of the mRNA encoding diglyceride acyltransferase were comparable in immobilized and control rats.

Im mobilization also induced a relevant increase of the mRNA for HMG-CoA reductase, the key enzyme of cholesterol biosynthesis (Fig. 2).

The high transcription level of genes encoding enzymes of fatty acids and cholesterol biosynthesis was paralleled by that of selected enzymes involved in the degradative pathway of fatty acids. Indeed, carnitine palmitoyltransferase-1 (CPT-1) and acyl-CoA oxidase (ACO) mRNA levels were increased upon immobilization.

The mRNA levels of PPARα, SREBP-1, and SREBP-2, the transcription factors involved in the expression of the above-mentioned enzymes, were measured in control and sedentary rat livers. The PPARα mRNA content was about 30% higher in the liver of immobilized rats than in controls, whereas SREBP-1 and SREBP-2 mRNA content was not significantly affected by immobilization.

Fig. 2. Incorporation of intraperitoneally injected [1,2-14C]acetate in the fatty acids of whole-liver lipids. Control and immobilized rats were injected intraperitoneally with 30 μCi [1,2-14C]acetate and sacrificed after 15 min. Fatty acid methyl esters, obtained by transesterification of whole lipids extracted from 1 g of liver, were separated by RP-HPLC. The label incorporation into fatty acids was quantified by liquid scintillation counting of the isolated peaks. The data represent the means ± SD from five separate experiments. Statistical significance of the difference between immobilized and control rats was evaluated by Student’s t-test. * P < 0.001; b P < 0.05; c P < 0.01.

Fig. 2. Effect of immobilization on the level of mRNAs encoding proteins of lipid metabolism. Total RNA was isolated from the liver of control and immobilized rats. Ten microgram aliquots of total RNA were hybridized to 32P-labeled cRNA probe specific for each protein using β-actin cRNA as internal standard. After RNA digestion, the protected fragments were separated by gel electrophoresis, and the radioactivity associated with each band was quantified using a Phosphor Imager autoradiography system. For each probe, one representative out of five or six autoradiographic images is reported. The fold change in each mRNA relative to the control was calculated after correction for loading differences with β-actin. The values are shown below each blot. C, control rats; I, immobilized rats. ACC, acetyl-CoA carboxylase; ACL, ATP citrate lyase; ACO, acyl-CoA oxidase; ACS, acetyl-CoA synthase; CCT, choline cytidylyltransferase; CPT-1, carnitine palmitoyltransferase-1; DGAT, diglyceride acyltransferase; FAS, fatty acid synthase; HMG-CoA reductase; ME, malic enzyme; PPARα, peroxisome proliferator-activated receptor-α; SCD-1, stearoyl-CoA Δ9-desaturase-1; SREBP, sterol-regulatory element binding protein.

Immunoelectrophoretic analysis of native and mature SREBPs

The relative content of membrane-bound and nuclear forms of SREBP-1 and SREBP-2 was evaluated after electrophoretic separation by immunoenzymatic-chemiluminescence detection of the proteins electroblotted on nitrocellulose membranes.

When whole-liver proteins were submitted to SDS-PAGE...
and immunoblotting, two electrophoretic bands, with approximate molecular weights of 125 and 65 kDa, respectively, immunoreacted with the antibody against the N-terminal domain of SREBP-1 (Fig. 3A). The 125 kDa band was taken as the membrane-bound native form of SREBP-1. The low-molecular-weight band, corresponding to the unique immunoreactive polypeptide revealed when proteins of isolated nuclei were submitted to electrophoresis and immunostaining (Fig. 3B), represents the mature form of SREBP-1.

The nuclear to membrane-bound SREBP-1 signal ratio was higher in the liver of immobilized rats than in controls.

The relative abundance of native and mature SREBP-2 was evaluated by using the same Western blot procedure (Fig. 3). Also, in this case the mature-native SREBP ratio was higher in the liver of immobilized rats than in controls.

Water-soluble intermediates of cholesterol and fatty acid synthesis

The liver of immobilized and control rats contained comparable amounts of acetyl-CoA (30.8 ± 5.3 and 28.9 ± 5.1 nmol/g wet tissue, respectively).

The malonyl-CoA content in the liver of control rats (3.2 ± 0.6 nmol/g wet tissue) was not significantly different from that of immobilized animals (2.8 ± 0.5 nmol/g wet tissue).

The isotopic labeling of both acyl-CoA thioesters was very low, and therefore specific activity data were not quantifiable in the experimental conditions used.

In vitro enzymatic assays

Hepatic ACC activity was higher in immobilized rats (6.1 ± 0.8 μmol·min⁻¹·mg protein⁻¹) than in controls (4.2 ± 0.4 μmol·min⁻¹·mg protein⁻¹). The activity of ACS was 16.5 ± 2.2 nmol·min⁻¹·mg protein⁻¹ in the liver cytosol of immobilized rats and 10.6 ± 3.0 nmol·min⁻¹·mg protein⁻¹ in control liver cytosol. Immobilization also induced a significant increase of hepatic FAS activity (62.1 ± 3.2 nmol·min⁻¹·mg protein⁻¹ in control rats and 81.4 ± 2.4 nmol·min⁻¹·mg protein⁻¹ in immobilized rats).

HMG-CoA reductase activity was about 50% higher in the liver of immobilized rats (712 ± 98 pmol·min⁻¹·mg protein⁻¹), as compared with controls (484 ± 85 pmol·min⁻¹·mg protein⁻¹).

DISCUSSION

In the present work, the changes in lipid metabolism occurring in the rat liver upon immobilization of skeletal muscles was investigated. As expected from previous evidence, cholesterol and triglyceride metabolism was significantly affected by long-term immobilization. Among the multiple factors involved in such changes, expression and activity of selected hepatic enzymes were evaluated.

After 14 day immobilization conditioning, both activity and mRNA levels of ACS, ACC, FAS, and HMG-CoA reductase were higher in the liver of immobilized rats than in controls. As the transcription of these genes is known to be modulated through a mechanism involving SREBPs, the hepatic mRNA contents for SREBP-1 and SREBP-2 were quantified in control and sedentary rats. The high level of plasmatic insulin in immobilized rats was expected to increase the transcription of the gene encoding SREBP-1c in liver (48). Conversely, immobilization did not affect the SREBP-1 mRNA content but only the maturation of the encoded native protein to the nuclear form. In this respect, it is relevant to notice that the liver of immobilized rats exhibited high levels of nonesterified linoleate, as compared with controls. According to previous observations, accumulation of free linoleate in the liver of sedentary rats might be responsible both for the lacking effect of insulin on SREBP-1c mRNA level (13, 49, 50) and for the low SCD-1 mRNA and ACL mRNA levels (51, 52).

The increased level of mature SREBP-1 in immobilized rat liver accounted for the high levels of the hepatic mRNAs for SREBP-1 target genes encoding ACC, FAS, and ACS (16, 17, 53). Similarly, the high HMG-CoA reductase mRNA level in the liver of immobilized rats was in good agreement with the increased cleavage of SREBP-2, the transcription factor specifically involved in modulating its expression (54).

Besides increasing lipogenic enzyme expression, immobilization also induced an increase of lipid catabolic processes. Rough evidence for this effect is provided by the observation that the body mass of immobilized rats is
lower than that of age-matched controls, assuming the same amount of food. Direct evidence for an active lipid catabolism was obtained by the observation that the expression of PPARα and of PPARα target genes is higher in immobilized rats than in controls.

Although plasma corticosterone level was not significantly different in immobilized and control rats, the high PPARα mRNA content in the liver of sedentary rats is in agreement with previous observations by Lemberger et al. (55), who demonstrated that immobilization stress is a potent and rapid stimulator of PPARα expression in liver. Both high plasma insulin and fatty acids accumulated in the liver of immobilized rats could be responsible for the induction of PPARα transcription and for the increased expression of PPARα target genes encoding CPT-1 and ACO. In fact, it has been demonstrated (56, 57) that insulin enhances the transcriptional activity of PPARα, probably by modulating its insulin-mediated phosphorylation, and that fatty acids increase both transcription and activity of the same receptor.

Mitochondrial GPAT has been claimed to play an auxiliary role in lipogenesis, as it converts fatty acyl-CoAs to lysophosphatidic acid, thereby preventing fatty acyl-CoA from undergoing β-oxidation in mitochondria (58). In this respect, the low mRNA level of mitochondrial GPAT in the liver of immobilized rats might be functional to the enhancement of fatty acid oxidative pathways.

The results obtained from the isotope-labeling experiments are consistent with the changes induced by immobilization on the expression of lipogenic enzymes. In fact, the incorporation of [1,2-14C]acetate in fatty acids and cholesterol was significantly higher in immobilized rats than in controls. In this connection, considering the short incubation time and the relative abundance of ACL mRNA and ACS mRNA in the liver of immobilized and control rats, it appears that labeled acetate was converted to acetyl-CoA mainly by the ACS-catalyzed reaction.

The low level of acetyl-CoA and malonyl-CoA labeling did not allow us to accurately quantify their specific radioactivity. However, since the hepatic pool sizes of acetyl-CoA and malonyl-CoA were comparable in control and immobilized rats, the high fatty acid and cholesterol labeling in the liver of immobilized rats implies that the activity of the enzymes involved in their biosynthesis from [1,2-14C]acetate was higher than in controls. Both the increased level of hepatic mRNAs for key enzymes of cholesterol and fatty acid synthesis in immobilized rats and the high specific activity of ACS, ACC, FAS, and HMG-CoA reductase confirm this interpretation of the results obtained from the in vivo experiments.

The high rate of fatty acid and cholesterol synthesis in the liver of immobilized rats induced compositional changes of both liver and plasma. Indeed, after 14 days of conditioning, the plasmatic cholesterol concentration was significantly higher in immobilized rats than in controls, whereas livers from control and sedentary rats exhibited the same cholesterol content. On the contrary, the liver of sedentary rats accumulated triglycerides, whose plasmatic concentration was not affected by long-term immobilization.

In light of previous observations (59, 60), this finding raises the hypothesis that the enhanced maturation of hepatic SREBPs in immobilized rats might also affect the mechanisms involved in production and secretion of lipoproteins.∞

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