Atypical expression of mitochondrial 3-hydroxy-3-methyl-glutaryl-CoA synthase in subcutaneous adipose tissue of male rats

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Abstract **The mRNAs encoding mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase (mtHMG-CoA synthase), the rate limiting enzyme in ketone body production, are highly expressed in subcutaneous (SC) and, to a lesser extent, in peri-epididymal (PE) rat adipose tissues. This atypical mtHMG-CoA synthase gene expression is dependent on the age (from 9 weeks of age) and sex (higher in male than in female) of the rats. In contrast, the expression of mtHMG-CoA synthase in SC adipose deposit is independent of the nutritional state (fed versus starved) or of the thermic environment (24°C versus 4°C). The expression of mtHMG-CoA synthase is suppressed in SC fat pads of castrated male rats whereas treatment of castrated rats with testosterone restores a normal level of expression. Moreover, testosterone injection induces the expression mtHMG-CoA synthase in SC adipose tissue of age-matched females. The presence of the mtHMG-CoA synthase immunoreactive protein confers to mitochondria isolated from SC adipose deposits, the capacity to produce ketone bodies at a rate similar to that found in liver mitochondria (SC = 13.7 ± 0.7, liver = 16.4 ± 1.4 nmol/min/mg prot). mtHMG-CoA synthase is expressed in the stromal vascular fraction (SVF) whatever the adipose deposit considered. While acetyl-CoA carboxylase (ACC) is only expressed in mature adipocytes, the other lipogenic enzymes, fatty acid synthase (FAS) and citrate cleavage enzyme (CCE), are expressed both in SVF cells and mature adipocytes. The expression of lipogenic enzyme genes is markedly reduced in adipocytes but not in SVF cells isolated from 48-h starved male rats. When SVF is subfractionated, mtHMG-CoA synthase mRNAs are mainly recovered in two fractions containing poorly digested structures such as microcapillaries whereas the lowest expression is found in the pre-adipocyte fraction. Interestingly, FAS and CCE mRNAs co-segregate with mtHMG-CoA synthase mRNA. The possible physiological relevance of such atypical expression of mtHMG-CoA synthase is discussed.**—Thumelin, S., C. Kohl, J. Girard, and J-P. Pégorier. **Atypical expression of mitochondrial 3-hydroxy-3-methylglutaryl CoA synthase in subcutaneous adipose tissue of male rats.** ***J. Lipid Res.*** **1999.** 40: **1071–1077.**

Supplementary key words cultured explants of white adipose tissue • isolated mitochondria • ketone body production • lipogenic enzymes • stromal vascular fraction

Until recently, it was generally accepted that ketone body production was restricted to the liver of non-ruminant species. This was based on the fact that extrahepatic tissues were devoid of mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase (mtHMG-CoA synthase), the rate-limiting enzyme of ketogenesis (reviewed in ref. 1). However, mtHMG-CoA synthase gene expression was found in the intestinal mucosa and kidney cortex of suckling rats (2, 3) conferring to these tissues the capacity to produce ketone bodies (2, 4, 5). This ketogenic capacity disappeared after weaning as the result of the suppression of mtHMG-CoA synthase gene expression (2). These observations emphasized the crucial role of mtHMG-CoA synthase for net ketone body production. Nevertheless, the expression of mtHMG-CoA gene in tissues not usually considered as ketogenic organs raises the question of whether the mtHMG-CoA synthase pathway could have purposes other than the production of ketone bodies. For instance, it was suggested that mtHMG-CoA synthase could be involved in cholesterol synthesis in liver (6) or steroid synthesis in the testis and the ovary (7). However, the role of mtHMG-CoA synthase for this purpose remains uncertain as these tissues express the cytosolic isoform of HMG-CoA synthase, the regulatory enzyme of mevalonate synthesis. Similarly, the physiologic relevance for the presence of all ketogenic enzymes, including mtHMG-CoA synthase, in cortical astrocytes of suckling rats (8) remains unknown. Finally, the presence of ketogenic enzyme activities was reported in human white adipose tissue (9). However, it seems unlikely that human white adipose tissue

Abbreviations: mtHMG-CoA synthase; mitochondrial hydroxymethylglutaryl-CoA synthase; SC, subcutaneous adipose tissue; PE, peri-epididymal adipose tissue; BAT, brown adipose tissue; SVF, stromal vascular fraction; HSL, hormone-sensitive lipase; CPT I, carnitine palmitoyltransferase I; FAS, fatty acid synthase; ACC, acetyl-CoA carboxylase; CCE, citrate cleavage enzyme; MEM, minimum essential medium; DMEM, Dulbecco's modified Eagle's medium; BSA, bovine serum albumin.

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performs a net ketone body synthesis as mtHMG-CoA synthase activity is very low in this tissue (9).

The present work reports that white adipose tissue of male rats is able to produce ketone bodies due to a high level of mtHMG-CoA synthase gene expression.

MATERIALS AND METHODS

Animals

Male (4-, 6-, 9-, and 12-week-old) and female Wistar rats (11- and 12-week-old), were housed in individual plastic cages at 24°C, with light from 15:00 to 03:00 h. They had free access to water and chow pellets (65% carbohydrate, 11% fat, 24% protein, % of energy). Subcutaneous (inguinal region), peri-epididymal, perioovarian, and retro-peritoneal fat pads, and interscapular brown adipose tissue, liver, heart, hindlimb muscle, brain, and intestine were sampled at 9.00, i.e., during the absorptive period, or after 48 h of starvation or cold exposure (4°C).

Surgery and injection of animals

Male rats were castrated at the age of 9 weeks under phenobarbital anesthesia. Two weeks later, they were injected daily (in the interscapular subcutaneous area) for 1 week with either commercial olive oil (controls) or testosterone propionate (10 mg/kg/day) diluted in olive oil. Age-matched females were injected using the same experimental design. After 3 weeks, castrated male rats expressed a reduction of seminal vesicle wet weight of more than 90% compared to aged-matched control rats, whereas testosterone treatment resulted in total recovery (non-castrated = 864 ± 68 mg; castrated = 82 ± 17 mg; castrated injected with testosterone = 905 ± 71 mg, n = 5).

Culture of white adipose tissue explants

Adipose tissue explants were prepared from subcutaneous and peri-epididymal fat pads of 48-h starved male rats and from subcutaneous adipose tissue of 48-h starved female rats as described by Foufelle et al. (10). Briefly, 300 mg of tissue was cut in small pieces and cultured in 75 cm^2 Petri dishes for 6, 24, or 48 h in a serum-free MEM medium supplemented with 1% BSA, 10 IU/ml penicillin and 50 μg/ml kanamycin. The cultures were maintained at 37°C in an incubator equilibrated with O_2/CO_2 (95/5%). The culture medium was collected and deproteinized with perchloric acid (4% final conc). Ketone bodies were determined in neutralized perchloric filtrates. Each experimental condition was performed in duplicate.

Isolation and incubation of mitochondria

Liver, heart, and subcutaneous fat pads from 12-week-old rats were finely minced and homogenized at 4°C in 0.25 M sucrose medium containing 10 mM Tris/HCl, 1 mM EGTA, 1 mM dithiothreitol (DTT), and 2% bovine serum albumin (BSA). After removal of lipids by filtration on gauze, mitochondria were isolated by a differential centrifugation technique as described previously (11). The subcutaneous fad pads from six female rats and four adult male rats were used to obtain sufficient amounts of mitochondria for determination of ketogenic capacity. Ketone body production was determined in isolated mitochondria (1 mg/ml) after a 15-min incubation period at 30°C in the presence of 0.5 mM l-carnitine and in the absence or in the presence of 0.1 mM oleate as described previously (12).

Isolation of adipocytes and non-adipocyte cells

Adipocytes were separated from the stromal vascular fraction (SVF) according to Rodbell (13), as modified by Parker, Lane, and Axelrod (14). Briefly, the fat pads (3–6 g) were rapidly excised, finely minced, and incubated (1 g/10 ml) for 60 min at 37°C in a low glucose (5 mM) Dulbecco's modified Eagle's medium (DMEM) supplemented with 2% BSA, and type C collagenase (1 mg/ml). Undigested tissue was then removed by filtering the suspension on a sterile nylon filter (250 μm). After decantation of adipocytes (10 min at 37°C), the infranatant (SVF) was collected. Isolated adipocytes from the supernatant were washed 3 times (2 min at 600 *g*) in a saline phosphate buffer (PBS: NaCl 130 mM; KCl 2.7 mM; Na_2HPO_4 22 mM; NaH_2PO_4 1.45 mM; pH 7.4) containing 2% BSA. This procedure was necessary to eliminate contaminating SVF cells from the adipocyte fraction (Ad). This fraction of SVF cells (subfraction Fa) was collected separately or pooled to other SVF subfractions (see below). In some experiments, SVF was filtered on a nylon filter (60–90 μm) to separate isolated cells (subfraction Fc) from tissular structure more resistant to collagenase digestion (subfraction Fb). Thus, SVF fraction represents a pool of subfractions Fa (from adipocyte washing), Fb (fraction resistant to collagenase digestion retained on the filter), and Fc (isolated cells). Cells from total SVF or from Fa, Fb, and Fc subfractions were washed once in PBS and sedimented by centrifugation for 10 min at 800 *g*. The cell pellets and adipocyte suspensions were immediately homogenized in 3 or 6 ml guanidinium thiocyanate denaturing solution, respectively, for total RNA extraction. Only samples arising from the same preparation were used for comparison.

Western blot analysis

Mitochondrial proteins (100 μg to 300 μg) from liver, heart, and adipose deposits from male and female rats were subjected to a 8.5% SDS-PAGE under reducing conditions (100 mM DTT) as described previously (2). The blots were hybridized using the anti-ox liver mtHMG-CoA synthase antibody (1/500, v/v) according to Quant et al. (15).

Isolation of total RNA and Northern blot analysis

After homogenization of the freeze-clamped tissues and the cellular extracts in the guanidinium thiocyanate denaturing solution, homogenates were centrifuged at 3000 *g* for 5 min (4°C) to remove lipids. Total RNA was further extracted as described (16) and samples of 20 μg were size fractionated in 1% agarose gel containing 2.2 M formaldehyde. Then, they were transferred to hybond N membranes (Amersham) and hybridized at 42°C as previously reported (2). mRNA encoding mtHMG-CoA synthase and liver carnitine palmitoyltransferase I (L-CPT I) were hybridized with random priming radiolabeled probes prepared from a KpnI–KpnI fragment from the pMS1 plasmid (17), and an EcoR1–EcoR1 fragment from the p61a plasmid (18), respectively. The α_2-chain of type IV collagen (α_2ColIV/pOb24) was used as a pre-adipocyte marker; the hormone-sensitive lipase (HSL) and the glucose transporter, Glut-4, were used as markers of mature adipocytes. Radiolabeled probes for these markers were prepared, respectively, from an EcoR1–EcoR1 fragment of the C3-PU18-11 plasmid (19), a SmaI–EcoRV fragment of the pGEM-2 vector (20), and an EcoR1–EcoR1 fragment of the pBKSII(+) vector (21). The fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC), and citrate cleavage enzyme (CCE) where used as lipogenic markers. The specific mRNAs encoding these proteins were hybridized with probes prepared from an EcoR1–EcoR1 fragment of the pFAS plasmid (22), an EcoR1–EcoR1 fragment of the p181-6 plasmid (23), and an EcoR1–BamH1 fragment of the pBS 18 plasmid (24), respectively. Hybridization of the blots with an excess of [γ-^{32}P]ATP-labeled synthetic oligonucleotide specific for the 18S rRNA subunit (25) allowed us to correct for possible variations in the amount of RNA transferred onto the membranes.

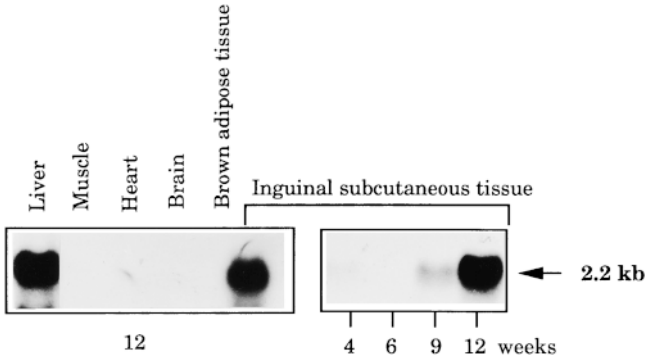

Fig. 1. Tissue specific expression of mtHMG-CoA synthase gene in starved adult rats. This northern blot is representative of four different experiments.

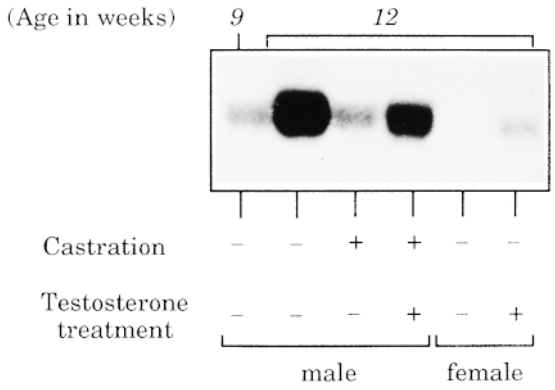

Fig. 3. Effects of castration and testosterone treatment on mtHMG-CoA synthase gene expression in subcutaneous adipose tissue. Male rats were castrated at 9 weeks of age and total RNAs were extracted from subcutaneous adipose deposits at 12 weeks of age in castrated male rats which had been treated for 1 week with either testosterone (10 mg/kg/day) or the same volume of vehicle (olive oil). Age-matched females were treated under same conditions. This Northern blot is representative of four different experiments.

Analytical methods

Acetoacetate and 3-hydroxybutyrate were determined using enzymatic methods (26). Proteins were determined by the method of Lowry et al. (27) with bovine serum albumin as standard.

Statistical analysis

Results are expressed as means ± SEM. Statistical analyses were performed using an analysis of variance for unpaired variables.

Chemicals

Culture medium was obtained from Gibco (Cergy-Pontoise, France). All substrates and enzymes were obtained from Boehringer-Mannheim (Meylan, France). Testosterone propionate, fatty acid-free albumin, Type C collagenase for adipocytes isolation, l-carnitine, oleate, antibiotics, and reagents for Northern and Western blot analysis were purchased from Sigma (St. Louis, MO).

RESULTS

Tissue specific expression and regulation of mtHMG-CoA synthase gene in adult rats

As shown in **Fig. 1**, the mtHMG-CoA synthase mRNAs are undetectable in hindlimb muscle, heart, brain, and brown adipose tissue of 48-h starved adult rats. In contrast, the transcript is present in the subcutaneous adipose tissue at a level similar to the one found in the liver of the starved adult rat (Fig. 1). This atypical expression of mtHMG-CoA synthase is age-dependent as its transcript is not detected in pre-pubescent rats (Fig. 1). mtHMG-CoA synthase is highly expressed in subcutaneous fat pads of both fed and starved adult male rats (**Fig. 2**). In contrast, the mtHMG-CoA synthase mRNA levels are undetectable in subcutaneous fat pads of fed age-matched females (Fig. 2) and are very low in subcutaneous fat pads of 48-h starved female (Fig. 2). They are virtually absent from other adipose deposits in both starved male and female adult rats (Fig. 2). Moreover, mtHMG-CoA synthase mRNA levels in subcutaneous deposits of male rats are not affected by thermic environment (Fig. 2) and are undetectable in brown adipose tissue, even after cold exposure.

Castration of 9 week-old male rats results, 3 weeks later, in a complete disappearance of mtHMG-CoA synthase mRNAs in subcutaneous adipose deposit (**Fig. 3**). Treatment of castrated rats with testosterone allows partial restoration of mtHMG-CoA synthase mRNA at a level similar to the one found in 9-week-old rats (Fig. 3). Interestingly, when age-matched females are injected with the same amount of testosterone, mtHMG-CoA synthase is induced in the subcutaneous adipose tissue of fed female rats (Fig. 3).

As shown for the transcript, the level of mtHMG-CoA synthase immunoreactive protein is not affected by the nutritional status of the male rats and is similar in mitochondria isolated from subcutaneous adipose tissue or from liver of 48-h starved rats (**Fig. 4**). In contrast, the amount of mtHMG-CoA synthase protein is low in mitochondria isolated from peri-epididymal fat pads of starved

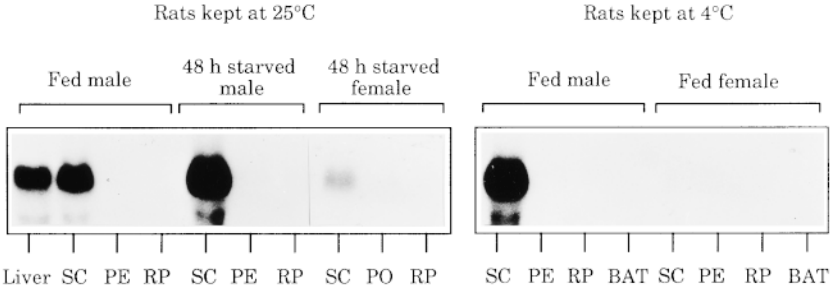

Fig. 2. Effect of sex and/or nutritional or thermic environments on mtHMG-CoA synthase gene expression in subcutaneous adipose tissue. Total RNAs were extracted from subcutaneous (SC), peri-epididymal (PE), retro-peritoneal (RP), and peri-ovarian (PO) white fat pads and brown adipose tissue (BAT). Twelve-week-old female or male rats were either starved for 48 h at 24°C or fed and exposed for 48 h to a cold environment (4°C). This Northern blot is representative of four different experiments.

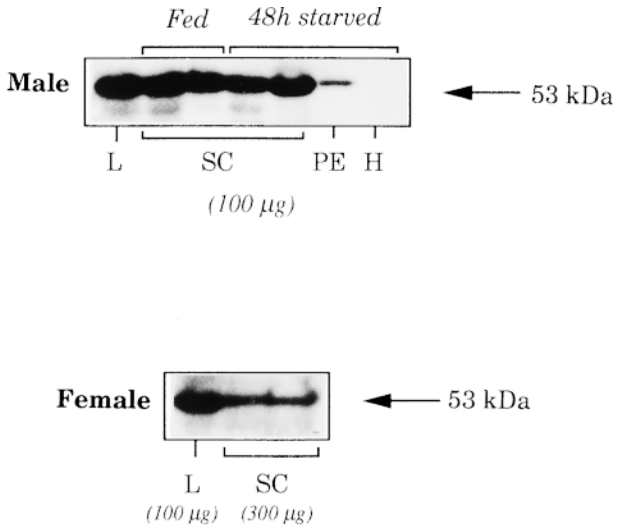

Fig. 4. Determination of mtHMG-CoA synthase immunoreactive protein in liver and subcutaneous adipose tissue. Mitochondria were isolated from liver (L), heart (H), subcutaneous (SC), or peri-epididymal (PE) adipose deposits of fed or 48-h starved male rats (upper panel) and from liver and subcutaneous fat pads of 48-h starved female rats (lower panel). Mitochondrial proteins (100 or 300 μg as indicated) were size fractionated in 8.5% acrylamide gel and blotted with an anti-ox liver HMG-CoA synthase antibody as described in Material and Methods. These Western blots are representative of three to five different experiments.

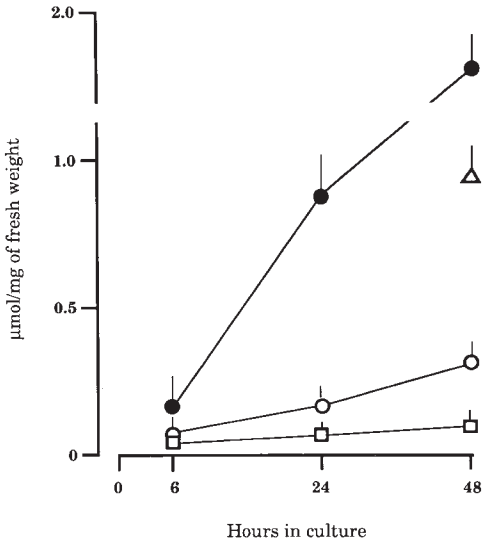

Fig. 5. Ketone body production in cultured explants from male and female adipose tissue. Explants were prepared either from subcutaneous or peri-epididymal fat pads and cultured as described in Materials and Methods. Subcutaneous adipose deposits were sampled from fed adult male rats (△) or from 48-h starved male (●) or female (○) rats. Peri-epididymal fat pads were sampled in 48-h starved male rats (□). Results are expressed as μmol of total ketones (acetoacetate + β-hydroxybutyrate) produced per mg of fresh tissue. The tissue ketone body concentration at the time of plating was similar whatever the origin of the deposit and was subtracted for the rate of ketone body production in each experimental condition. Results are means ± SEM of five different cultures performed in duplicate. The respective concentration of β-hydroxybutyrate (B) and acetoacetate (A) in various cultured explants are (in nmol/mg): subcutaneous pads from fed males: B = 574 ± 122; A = 323 ± 95; subcutaneous pads from starved males: 6 h in culture B = 93 ± 28; A = 89 ± 18; 24 h in culture B = 546 ± 108; A = 294 ± 96; 48 h in culture B = 1180 ± 212 A = 441 ± 87. Subcutaneous pads from starved females: 6 h in culture B = 43 ± 9; A = 36 ± 12; 24 h in culture B = 93 ± 23; A = 68 ± 12; 48 h in culture B = 190 ± 52; A = 121 ± 32. Peri-epididymal pads from starved males: 6 h in culture B = 13 ± 2; A = 17 ± 6; 24 h in culture B = 34 ± 8; A = 37 ± 5; 48 h in culture B = 60 ± 12; A = 41 ± 6.

male rats (Fig. 4) or from subcutaneous adipose tissue of female rats (Fig. 4).

Ketone body production by cultured explants and isolated mitochondria from various adipose deposits

The rate of ketone body production from endogenous substrates is time-dependent whatever the origin of adipose tissue explant (**Fig. 5**). After 48 h of culture, the rate of ketone body production is 40% lower in subcutaneous adipose tissue of fed male rats than in 48-h starved rats (Fig. 5). In contrast, it is 15-fold higher in subcutaneous than in peri-epididymal fat pads of starved rats (Fig. 5). Moreover, the rate of ketone body production is 5-fold higher in subcutaneous explants from male than from age-matched female rats (Fig. 5). Similar differences are observed when the ketogenic capacity is measured in isolated mitochondria incubated in the presence of saturating concentrations of oleate. While heart mitochondria (used as negative controls) are unable to synthesize ketone bodies from oleate (**Table 1**), mitochondria isolated from subcutaneous fat pads of male rats have a ketogenic rate similar (80%) to the one found in liver mitochondria used as positive controls (Table 1). The ketogenic capacity of mitochondria isolated from female subcutaneous deposits is much lower than in the homologous adipose pads of male rats (Table 1).

Distribution of mtHMG-CoA synthase mRNA inside the adipose tissue

On the basis of pre-adipocyte (α_2ColIV/pOb24) and adipocyte (HSL and Glut-4) markers, mtHMG-CoA synthase is mainly expressed in the total SVF fraction (SVFt) whatever the origin of the adipose pad (**Fig. 6**). This localization is unaffected by the nutritional state of the male rats (Fig. 6). As recently reported (28), liver-type carnitine palmitoyltransferase I (L-CPT I) is expressed in pre-adipocytes. When we look at the distribution of lipogenic enzyme gene expression, it is interesting to emphasize that fatty acid synthase (FAS) and, to a lesser extent, citrate cleavage enzyme (CCE) are expressed not only in mature adipocytes but also in SVF of subcutaneous fat pads of adult male rats (**Fig. 7**). In contrast, acetyl-CoA carboxylase is found exclusively in mature adipocytes (Fig. 7). In mature adipocytes isolated from subcutaneous fat pads of male rats, the expression of lipogenic enzyme genes is markedly decreased after starvation (Fig. 7) whereas FAS and CCE mRNA concentrations are still elevated in SVF (Fig. 7). When the SVF is subfractionated, the highest mtHMG-CoA synthase gene expression is associated with Fa and Fb fractions (Fig. 7), two fractions containing poorly digested

TABLE 1. Ketone body production in incubated mitochondria isolated from liver, subcutaneous adipose tissue, and heart

Mitochondrial Source	Fed Male	Starved Male	Starved Female
Liver	16.4 ± 1.4 (7)	20.9 ± 1.2 (7)	18.2 ± 1.4 (5)
Subcutaneous fat pads	13.7 ± 0.7 (7)	17.0 ± 0.6 (7)	2.9 ± 0.5 (5)[a]
Heart	1.5 ± 0.4 (6)	0.9 ± 0.2 (3)	0.7 ± 0.2 (5)

Isolated mitochondria (1 mg/ml) were incubated for 15 min in the absence or in the presence of oleate bound to 2% fatty acid-free albumin. Endogenous ketogenesis (absence of oleate) was subtracted from the rate of ketone body production (acetoacetate + β-hydroxybutyrate) determined from 0.1 mm oleate. Results are expressed as nmol/min/mg of protein. Values are means ± SEM for the number of experiments shown in parentheses.

[a] $P < 0.01$ when compared to mitochondria isolated from subcutaneous adipose tissue of male rats whatever the nutritional status.

structures such as microcapillaries and capillaries (14, 29, 30). In contrast, mtHMG-CoA synthase mRNA concentration is lower in the Fc fraction (Fig. 7) a pre-adipocyte enriched fraction as suggested by the increase in the α2ColIV/pOb24 mRNA concentration (Fig. 7). Interestingly, FAS and, to a lesser extent, CCE mRNA co-segregate with mtHMG-CoA synthase mRNA (Fig. 7).

DISCUSSION

The present data demonstrate that unidentified cells from SVF of white adipose tissue are able to produce ketone bodies due to a mitochondrial HMG-CoA synthase pathway whose activity is quite similar to that found in liver mitochondria, at least in term of maximal capacity. However, it must be emphasized that the proportion of mitochondria is much lower in adipose tissue that in the liver, suggesting that the contribution and/or the purpose of ketogenesis in these tissues is not the same. Indeed, if we take into account the mass of subcutaneous fat pads of male rats (around 6 g) this allows us to estimate, from cultured explant experiments, that daily ketone body production is 90% lower than that estimated from liver explants cultured under the same conditions (data not shown). Moreover, the present work suggests that the regulation of adipose tissue mtHMG-CoA synthase differs from that of hepatic enzyme as its expression is not stimulated by starvation, unlike in the liver (31). The fact that ketone body production is 40% higher in subcutaneous explants from starved rats than from fed ones could result *1*) from a higher proportion of SVF cells due to a smaller size of

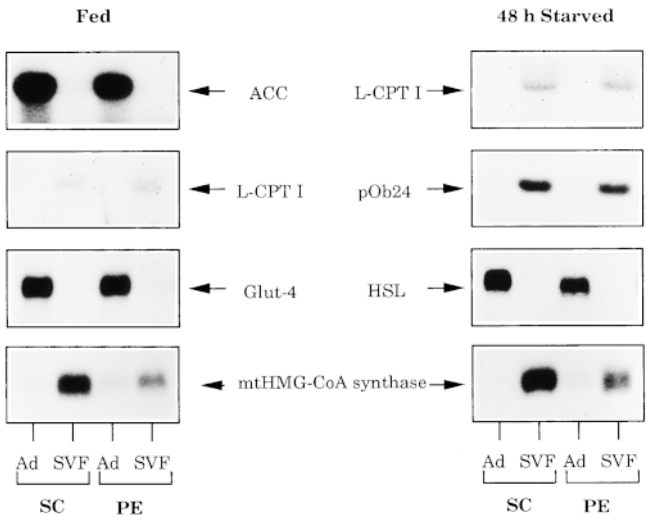

Fig. 6. Localization of mtHMG-CoA synthase mRNA in adipocyte or stromal vascular fraction of various fat pads. Total RNAs were extracted from adipocytes (Ad) and stromal vascular fraction (SVF) cells isolated from subcutaneous (SC) or peri-epididymal (PE) fad pads of 12-week-old fed (left panel) or starved (right panel) adult male rats. Fractions were characterized according to the expression of marker gene specific for mature adipocytes (glucose transporter Glut-4; hormone sensitive lipase, HSL, and acetyl-CoA carboxylase, ACC), or non-adipocyte cells (α2 chain of collagen IV, α2ColIV/pOb24; liver type carnitine palmitoyltransferase I, L-CPT I). These Northern blots are representative of four different experiments.

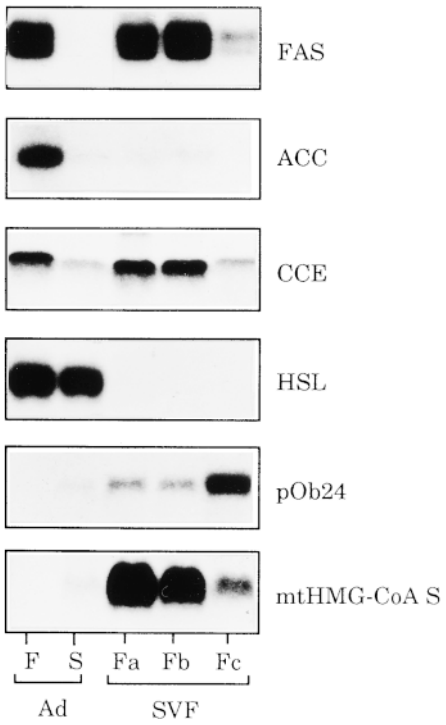

Fig. 7. Distribution of mRNA encoding lipogenic enzymes between adipocytes and subfractions of SVF isolated from subcutaneous fat pads of adult male rats. Adipocytes (Ad) were isolated from subcutaneous deposits of 12-week-old fed (F) or 48-h starved (S) male rats. SVF isolated from subcutaneous adipose tissue of 48-h starved male rats were subfractionated in three fractions as described in Materials and Methods. After collagenase digestion of the tissue and decantation, two phases were clearly identified. 1) the upper phase containing adipocytes contaminated with some SVF cells. This fraction was washed and centrifuged, the pellet being the Fa subfraction of SVF; 2) the infranatant containing SVF cells. This fraction was filtered on 60-μm nylon fibers and two fractions were obtained: Fb which represents the fiber-retained fraction and Fc the filtrate fraction. Each fraction was characterized by adipocyte (hormone-sensitive lipase, HSL) and SVF (a2C olIV/pOb24) markers. Northern blots were hydridized with probes encoding mtHMG-CoA synthase, lipogenic enzymes (acetyl-CoA carboxylase ACC, fatty acid synthase FAS, citrate cleavage enzyme CCE). These blots are representative of three to four different experiments.

adipocytes in subcutaneous fat pads of starved rats; and *2*) from a lower inhibition of CPT I by malonyl-CoA, whose concentration is probably low as suggested by the very low level of mRNA encoding acetyl-CoA carboxylase. In keeping with this, the rates of ketone body production are similar in mitochondria (a malonyl-CoA free system) isolated from fed or starved male rats.

This work also shows that the expression of mtHMG-CoA synthase is strongly dependent upon the site of adipose deposit and the sex of the animal. Such site and sex differences in white adipose tissue metabolism were previously reported for glucose metabolism (32, 33), insulin sensitivity (34–36), lipolytic activity (37), and *ob* gene expression (reviewed in ref. 38). This differences between male and female are also observed for liver metabolism (39) where is was shown that estrogens decrease the rate of hepatic fatty acid oxidation (40) as the result of an increased sensitivity of CPT I to malonyl-CoA inhibition (41). The mechanisms by which testosterone controls mtHMG-CoA synthase in subcutaneous adipose tissue remain unknown. However, it is noteworthy that changes in mtHMG-CoA synthase mRNA concentration in response to testosterone in the SVF are similar to those observed in total adipose deposits (data not shown). This suggests that the regulation of mtHMG-CoA synthase by testosterone is specific and does not result from variations in the adipocytes to SVF ratio inside the subcutaneous adipose tissue. The fact that preadipocytes are sensitive to physiological concentrations of testosterone in vitro (42) suggests that testosterone could directly control the expression of mtHMG-CoA synthase in subcutaneous adipose deposits. Moreover, sex hormones could be involved in the regionalization of mtHMG-CoA synthase gene expression as previously described for the control of lipolytic activity in white adipose tissue (reviewed in ref. 37).

mtHMG-CoA synthase mRNA is not detected in skin, salivary or preputial glands of adult male rats, suggesting that the expression of this gene in subcutaneous fat pads is not due to a contamination by these tissues. In contrast, preliminary histochemical experiments suggest that this high expression could be related to changes in the morphology of male rat subcutaneous adipose tissue as the result of an increase in uncharacterized glandular structures (sebaceous, sweat, or undifferentiated mammary glands?). Sebaceous glands are characterized by high rates of lipogenesis, cholesterol and triglyceride synthesis (43, 44). It was proposed some 25 years ago that acetoacetate production in subcutaneous adipose tissue would represent a system that allows the transfer of acetyl-CoA units from the mitochondria to the cytosol (9, 45) to sustain an active lipogenesis in a tissue characterized by low CCE activity (32). However, it seems unlikely that mtHMG-CoA synthase would play this role in "uncharacterized SVF cells" of subcutaneous fat pads as ACC, the key lipogenic enzyme, is not expressed in the same fraction as mtHMG-CoA synthase. Nevertheless, despite the absence of ACC in SVF cells expressing FAS and mtHMG-CoA synthase genes, the synthesis of short- or medium-chain fatty acyl-CoA cannot be excluded as acetoacetyl-CoA is a more efficient precursor for FAS than acetyl-CoA (46). In addition to a putative role for mtHMG-CoA synthase in fatty acid and triglyceride synthesis, it could play a role in sustaining cholesterol synthesis as previously reported in testis and ovary (7) or in liver (6). This role could be essential if cholesterogenesis takes place, at least in part, in mitochondria as shown in Leydig cells from rat testis (47).

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