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# Developmental regulation of hormone-sensitive lipase mRNA in the rat: changes in steroidogenic tissues

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**Abstract** The hydrolysis of triglycerides and cholesteryl esters stored within cells is mediated by the enzyme, hormone-sensitive lipase. In adipose tissue and heart, hormone-sensitive lipase primarily hydrolyzes stored triglycerides to free fatty acids, while in steroidogenic tissues, it principally converts cholesteryl esters to free cholesterol for steroid hormone production. To determine whether hormone-sensitive lipase is under tissue-specific, developmental regulation, the steady state levels of hormone-sensitive lipase mRNA were determined in normal rats from late fetal life through 2 years of age. Hormone-sensitive lipase mRNA levels did not appear to vary in adipose tissue from epididymal fat pads obtained from animals between 3 weeks and 2 years of age. In heart, hormone-sensitive lipase mRNA levels were lowest in the fetus increased rapidly within the first day postnatally, and then gradually increased to stable adult levels by 2 months that were 3-fold higher than observed in fetal rats. Steady state mRNA levels of hormone-sensitive lipase in the adrenals were lowest in fetal rats, increased 4-fold during the first day and peaked at levels that were 9-fold higher by the end of the first week. Thereafter, levels fell and remained 3- to 4-fold higher than at birth throughout adult life. Hormone-sensitive lipase mRNA was undetectable in testes before 4 weeks of age and increased 25-fold to stable adult levels between 4 and 12 weeks. Thus, hormone-sensitive lipase is differentially expressed and regulated in a tissue-specific fashion during development and aging.—**Kraemer, F. B., K. Tavangar, and A. R. Hoffman.** Developmental regulation of hormone-sensitive lipase mRNA in the rat: changes in steroidogenic tissues. *J. Lipid Res.* 1991. **32:** 1303–1310.

**Supplementary key words** hormone-sensitive lipase • development • adipose tissue • heart • adrenal • testis

Free fatty acids, which are derived from the breakdown of stored triacylglycerols in adipose tissue, are a major source of energy for most tissues (1, 2). The major enzyme responsible for the mobilization of free fatty acids from adipose tissue is hormone-sensitive lipase (HSL), whose name was coined to reflect the ability of hormones such as catecholamines, ACTH, and glucagon to stimulate the activity of this intracellular neutral lipase (3). Unlike other neutral lipases, HSL purified from adipose tissue exhibits both triacylglycerol and cholesterol ester hydrolase activities (4). HSL is expressed in a variety of tissues, including adipose tissue, adrenals, ovaries, testes, placenta, macrophages, heart, and skeletal and smooth muscles (5–7). The enzyme's triacylglycerol lipase activity is predominant in adipose tissue, and, perhaps, in heart (5, 8). The neutral cholesterol ester hydrolase activity found in adrenals, ovaries, and testes has been identified as HSL (9–11), and, thus, the ability of HSL to hydrolyze stored cholesteryl esters to free cholesterol for use in steroid hormone production appears to be paramount in these steroidogenic tissues (9–14). This dual activity allows HSL to play an important role in two independent and apparently unrelated processes: lipolysis and steroidogenesis (5, 15). Although the exact functional role of HSL in tissues other than adipose and steroid-producing tissues is not completely understood, it would appear that in those other tissues, HSL participates in intracellular cholesterol homeostasis by mobilizing stored cholesterol for use by the cell or for excretion.

Tissue-specific HSL activity, therefore, may reflect the organism's ability to utilize stored fuels, as well as its capacity to synthesize adrenal and gonadal steroids under both basal and stress conditions. Since an animal's ability to respond to food deprivation and other stresses varies throughout development (16), it would be important to determine whether HSL also undergoes age-specific changes. Little is known about the tissue-specific ontogenic regulation of the expression of the HSL gene. Traditional in vitro and in vivo enzymatic assays are difficult to interpret because of a combination of problems stemming from the varying and confounding effects of lipolytic

Abbreviations: HSL, hormone-sensitive lipase.

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and antilipolytic factors that are released locally in these systems. Moreover, there are methodological problems in some of the assays of HSL in which neutral lipase activity has been measured under conditions where HSL activity is not clearly differentiated from other lipases such as lipoprotein lipase (17, 18). The recent cloning of the HSL gene (19, 20) has allowed a more direct approach to address the question of whether HSL expression is differentially regulated in various tissues. As an initial attempt to explore this issue, we examined changes in steady state HSL mRNA levels in normal rats of various ages, from late fetal life through 2 years of age.

## METHODS

### Animals

Male Sprague-Dawley rats (Simenson, Gilroy, CA) were maintained according to Stanford University guidelines on ad lib rat chow and tap water with a 12 h light/dark cycle. All animals were killed between 0800–1000 h by decapitation. The tissues were immediately removed, frozen in liquid nitrogen, and stored at $-80°C$ prior to RNA isolation. Fetal tissues were obtained from timed-pregnant rats (Simenson). Near term, the rats were checked hourly and the start and end of delivery was noted; delivery lasted approximately 1 h and "zero-hour" was set at the end of delivery. Rats were kept with their dams until used or until weaned at 21 days. Aged animals were obtained from the vendor as 9-month-old retired breeders. They then lived for 3–15 months in the Stanford University vivarium in single cages before being used.

### RNA isolation

RNA was isolated from tissues pooled from 3–12 rats and results represent the average of two or three RNA preparations at each time point. Total cellular RNA was isolated from frozen tissue as previously described (21, 22). Briefly, tissues were homogenized with a Polytron (heart, testis, adrenal) or with a brief sonication (fat) in the presence of 4 M guanidine thiocyanate (Fluka Chemical Corp., New York) and 0.1 M 2-mercaptoethanol. RNA from heart, fat, and adrenals was purified via a series of ethanol precipitations as described by Chirgwin et al. (23). For testis, RNA was purified by adding CsCl to a final concentration of 1.5 M, and layering the solution over a 5.7 M CsCl cushion containing 6 mM 2-mercaptoethanol. The RNA was pelleted by ultracentrifugation at 35,000 rpm for 20 h. RNA pellets were dissolved in sterile water, quantitated by standard UV absorbance, and analyzed for RNA integrity by agarose gel electrophoresis (24).

### Synthesis of probes

The HSL cDNA probe (a kind gift from Dr. Michael Schotz, UCLA) was the 1.9-kb Eco R1 fragment representing nucleotides 592 to 2486 of rat HSL (19, 20). A β-actin plasmid was a kind gift from Dr. Lawrence Kedes (University of Southern California). Probes were nick translated with [$^{32}$P]dCTP and [$^{32}$P]dATP to a specific activity $\sim 10^8$ cpm/μg using standard techniques (25).

### RNA quantification

Total HSL mRNA was quantitated by slot blot hybridization as previously described (21). Briefly, total RNA was serially diluted (1.0–0.25 μg/sample) in a 400 μl solution containing 6.15 M formaldehyde and 10 × SSC (0.15 M NaCl, 0.017 M sodium citrate, pH 7.0). The RNA was denatured at 65°C for 20 min and then immobilized on 0.1 μm nitrocellulose filters (Schleicher & Schuell, Keene, NH) by slow vacuum filtration. The wells were rinsed with 400 μl of 10 × SSC and the RNA was fixed to the filters by baking at 80°C in vacuo for 2 h. Prehybridization and hybridization procedures were performed at 42°C in 50% formamide, 3 × SSC, 10 × Denhardt's, 20 mM Tris (pH 7.6), 10 mM EDTA (pH 8.0), 200 μg/ml sheared salmon sperm DNA, and 0.2% SDS (sodium dodecyl sulfate) with the hybridization buffer containing 2.5–3.0 × $10^6$ cpm of [$^{32}$P]cDNA probe. After hybridization, the membranes were rinsed with 2 × SSC (24°C) followed by sequential 42°C washes in 2 × SSC, 0.2% SDS, and 0.2 × SSC, 0.2% SDS. Duplicate filters were hybridized with actin (42°C in a modified hybridization solution containing 5 × SSC). The filters were washed at 50°C. In addition, control experiments comparing actin hybridized to duplicate filters versus stripped re-used filters revealed the same pattern of actin mRNA expression. Autoradiographs were obtained by exposure to Kodak XAR-5 film with an intensifying screen at $-80°C$ for 12–96 h. The autoradiographs were scanned and analyzed with a Hoeffer Scientific Instruments (San Francisco, CA) GS-300 scanning densitometer and HSI GS370 Apple Macintosh program to quantify the relative amount of hybridized probe. Control experiments confirmed quantitative binding of the RNA to the filters. Multiple exposure times were obtained to ensure that the autoradiograph was within the linear range for quantitative scanning.

### Northern blot hybridization

Total RNA was denatured with 1 M glyoxal, 50% dimethyl sulfoxide and electrophoresed on a horizontal 0.9% agarose gel (24). The RNA was transferred and fixed to Hybond-N nylon membrane (Amersham Corp.). The filter was then prehybridized and hybridized with the HSL cDNA probe as described above.



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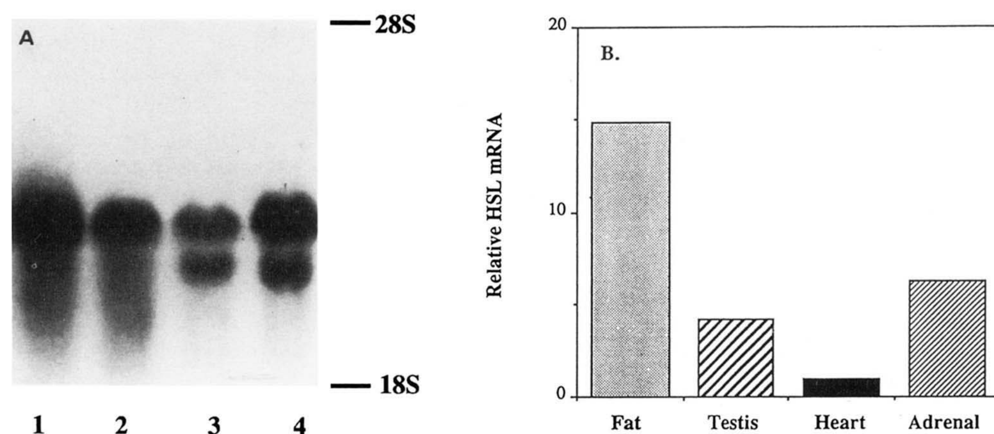


**Fig. 1.** Northern blot of total RNA isolated from fat (lane 1), testis (lane 2), heart (lane 3), and adrenal (lane 4) from adult rats probed with HSL cDNA. (B) Relative distribution of HSL mRNA in tissues. (A) Total RNA (10 $\mu g$) was isolated, separated by agarose electrophoresis, and transferred onto nitrocellulose filters as described in the Methods. Filters were hybridized with a $^{32}P$-labeled cDNA probe for HSL and autoradiographs developed after 24 h exposure. (B) Densitometric scan of Fig. 1A. Results are expressed relative to the amount of HSL mRNA in heart which was set as 1.

## RESULTS

In order to determine whether steady state HSL mRNA levels change during development and aging, tissues were obtained from well-fed rats at various ages. **Fig. 1** displays a Northern blot of RNA obtained from fat, testis, heart, and adrenal of an adult rat. The HSL cDNA hybridized to an RNA of 3.3–3.5 kb in each tissue. HSL mRNA was most abundant in fat, with levels approximately 15-fold greater than in heart (Fig. 1B). Testis and adrenal contained approximately 28 and 42%, respectively, of the level of HSL mRNA found in fat. Since HSL activity is highest and its function most clearly defined in adipose tissue, epididymal fat was examined in rats varying in age from 3 weeks to 2 years; there was insufficient epididymal fat in animals less than 3 weeks of age for analysis. Fat was not examined from other depot sites. As shown in **Fig. 2A**, no apparent differences appeared to occur in steady state HSL mRNA levels in epididymal fat across this large age range. Thus, while these rats grew from approximately 75 g to >1000 g, no major changes in HSL expression were observed. Likewise, no significant changes were noted in the steady state expression of actin mRNA with age (Fig. 2B).

Obvious developmental changes in HSL gene expression were observed, however, in heart, where HSL presumably hydrolyzes small stores of triglycerides for use as fuel. HSL mRNA levels were lowest in late fetal development, increased approximately 50% within the first 24 h after birth, and then gradually rose over the next 2 months to reach stable adult levels that were threefold higher than those seen in the fetus (**Fig. 3A**). In contrast to HSL, the steady state levels of $\beta$-actin mRNA remained constant from the late fetal period throughout the 2-year life span of the rat (Fig. 3B).

In contrast to adipose tissue and heart, where HSL regulates triglyceride hydrolysis, the developmental pat-

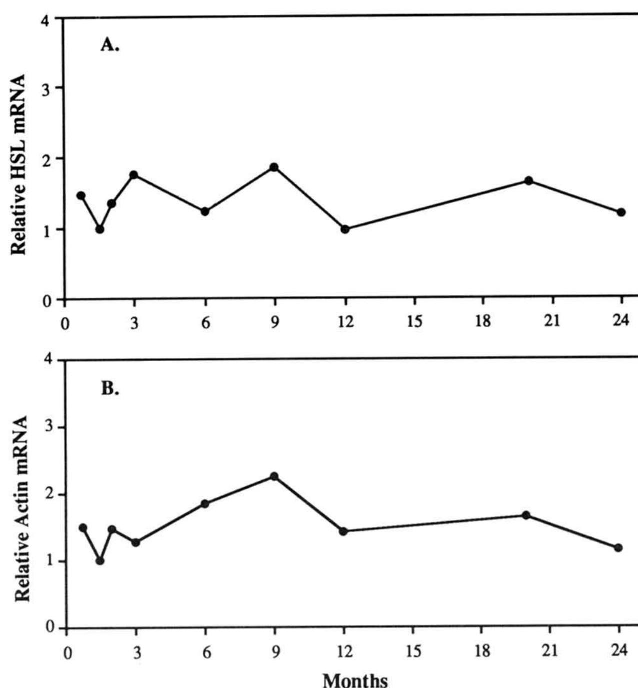


**Fig. 2.** Developmental expression of HSL mRNA (A) and $\beta$-actin mRNA (B) in rat epididymal fat. Total RNA was isolated and serial dilutions of RNA were slotted in duplicate onto nitrocellulose filters as described in Methods. Filters were hybridized with $^{32}P$-labeled cDNA probes for HSL (A) and $\beta$-actin (B). Autoradiographs of the washed filters were quantitated by scanning densitometry. The results are expressed relative to the lowest value of HSL mRNA (A) and $\beta$-actin mRNA (B) in epididymal fat, respectively.



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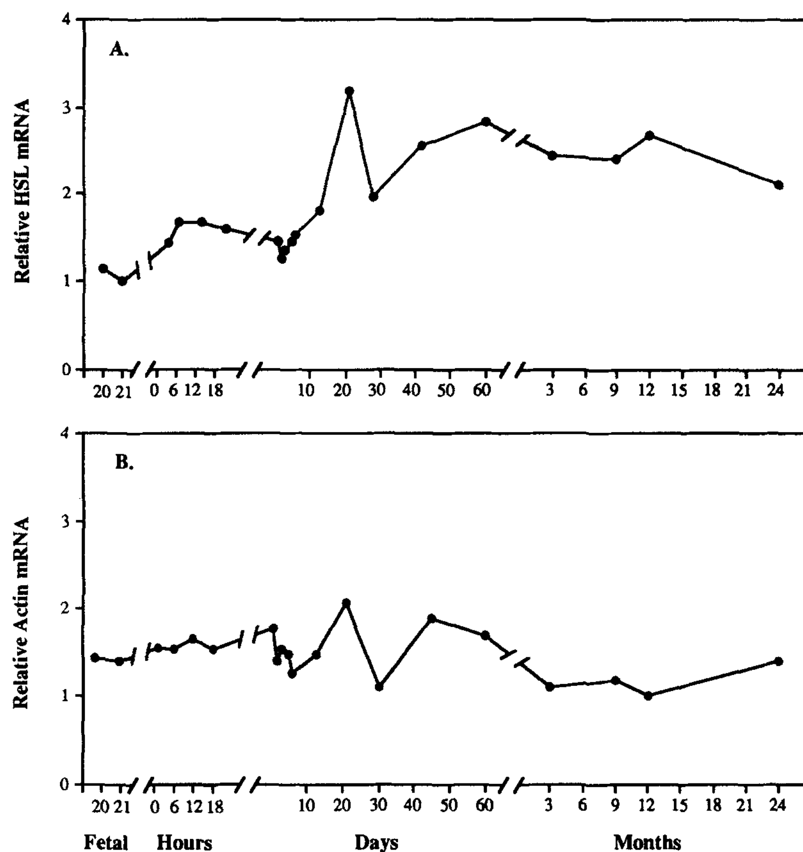


**Fig. 3.** Developmental expression of HSL mRNA (A) and $\beta$-actin mRNA (B) in rat heart. Total RNA was isolated and specific mRNA quantitated as described in Fig. 2 and in Methods. The results are expressed relative to the lowest value of HSL mRNA (A) and $\beta$-actin mRNA (B) in heart, respectively.

terns of HSL gene expression were quite different in steroidogenic tissues where HSL directs cholesteryl ester hydrolysis and plays a role in steroid hormone production. When steady state HSL mRNA levels were examined in the adrenal, levels increased 4-fold within the first 24 h of life (**Fig. 4A**), and continued to rise to levels that were 9-fold greater than fetal concentrations by the end of the first week of life. Thereafter, HSL mRNA abundance declined precipitously, stabilizing by age 2 months at adult values which were 3- to 4-fold higher than those found at birth. Thus, starting at birth and continuing for the first 7 days of life, there is a dramatic increase in HSL mRNA levels in the adrenals that coincides with the stress of birth and early suckling. Steady state levels of $\beta$-actin mRNA in the adrenals were not constant throughout life, but were highest during the first week of life, declining approximately 2-fold by day 10 and remaining constant thereafter (Fig. 4B).

In the testes, HSL mRNA levels were undetectable (days 10 and 21) or extremely low (day 28) prior to sexual maturation. Between weeks 4 and 12, the period when male rats complete their sexual development, steady state HSL mRNA levels increased approximately 25-fold and subsequently remained at this relatively high abundance throughout life (**Fig. 5A**). As observed in the adrenals, steady state levels of $\beta$-actin mRNA in the testes were not constant throughout life, but increased approximately 2-fold from 3 to 6 weeks of life, remaining constant from that point onward (Fig. 5B).

## DISCUSSION

HSL is an enzyme that hydrolyzes intracellular stores of triglycerides and cholesteryl esters, which places it in a physiologically important role in the regulation of two independent processes: lipolysis and cholesterol homeostasis. Many investigations have shown that HSL activity is rapidly modulated by lipolytic and antilipolytic hormones via phosphorylation-dephosphorylation reactions, yet very little information exists on the regulation of the expression of the HSL gene. By examining HSL mRNA levels in various tissues of rats from late fetal life through 2 years of age, a period during which remarkable metabolic changes occur in all tissues, we were able to demonstrate that the HSL gene is differentially expressed and regulated in tissues during growth and development. These findings suggest that HSL activity may be partially governed by alterations in the degree of gene expression.

Previous workers detected a single species of HSL mRNA in adipose tissue and adrenals that was 3.3 kb in



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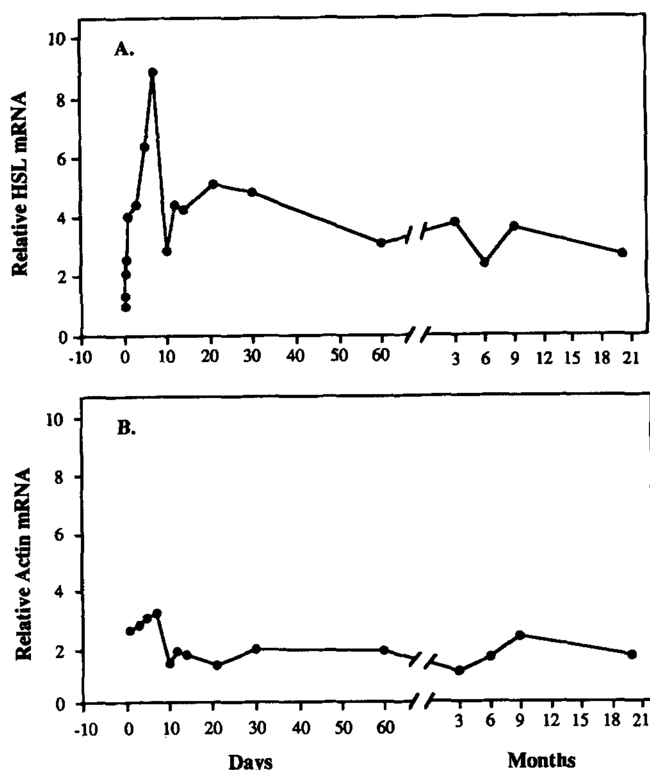


Fig. 4. Developmental expression of HSL mRNA (A) and β-actin mRNA (B) in rat adrenal. Total RNA was isolated and specific mRNA quantitated as described in Fig. 2 and in Methods. The results are expressed relative to the lowest value of HSL mRNA (A) and β-actin mRNA (B) in adrenal, respectively.

size, a 3.5 kb species in heart, two species of 3.3 and 3.5 kb sizes in ovary, and a 3.9 kb HSL mRNA in testis (19). In those studies HSL mRNA was found primarily in adipose tissue with lower amounts in steroidogenic tissues and still lower amounts in heart and muscle (19). Although the relative distribution of HSL mRNA in tissues was not quantitated in those studies, other studies that measured HSL activity and HSL immunoreactive protein observed adipose tissue to have approximately 5- to 10-fold more HSL than steroidogenic tissues, and 50- to 100-fold greater amounts than heart and muscle (11). In the present studies, a 3.3–3.5 kg HSL mRNA species was observed in adipose tissue, heart, adrenal, and testis. When the distribution of HSL mRNA was examined, HSL mRNA was found to be most abundant in adipose tissue, being approximately 2.5-fold greater than in adrenal, 4-fold greater than in testis, and 15-fold greater than in heart. The reasons for the discrepancies between the size and distribution of HSL mRNA in the present studies and previous observations are not clear. Although the HSL cDNA probe used in the two studies was identical, the hybridization conditions used were slightly different and might have contributed to the minor differences in resolution observed. Nonetheless, the present studies confirm, for the most part, the size of the predominant HSL mRNA species and the relative distribution of HSL mRNA in tissues.

Dramatic metabolic changes occur in the rat from late fetal development through the first several weeks of life. The fetal rat uses carbohydrate and free fatty acids derived from the maternal circulation for its energy requirements (26), while after birth it is dependent on suckling and oral intake. The first few weeks after birth are associated with a large increase in the number of adipocytes, without a significant increase in cell size (27). The number of adipocytes plateaus between 2 to 3 months, and fat cell size increases steadily thereafter (27, 28). When the rate of basal lipolysis was assessed, it was found to be high at 2 weeks of age and to decrease 75% by 42 weeks (28). Basal lipolysis then remains low until it increases after 3 months in parallel to the well-described positive correlation between lipolysis and fat cell size (28, 29). In addition to these alterations in basal lipolysis reported with development and aging, hormone-stimulated lipolysis has been found to decline with age (30–32). Although multiple mechanisms have been proposed to explain this effect, an increase in the sensitivity of fat cells to the inhibitory actions of adenosine with age appears responsible for the decline in hormone-stimulated lipolysis since maximal responsiveness of lipolysis to stimulation by β-agonists is not altered with age if the actions of antilipolytic factors are removed (33). While we were not

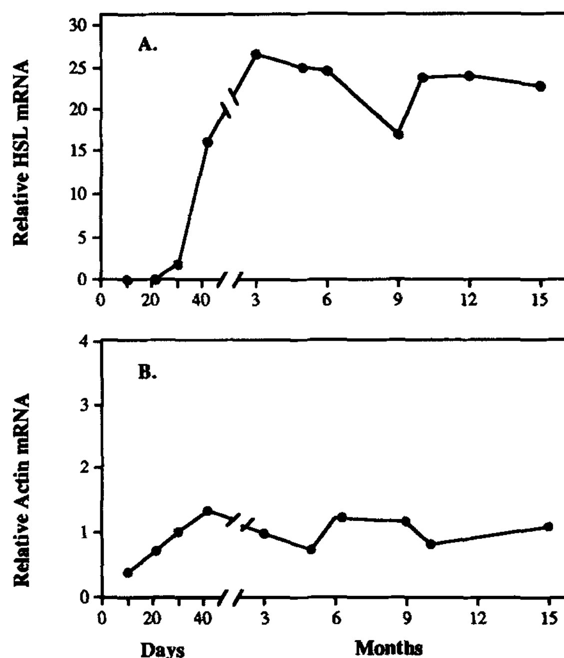


Fig. 5. Developmental expression of HSL mRNA (A) and β-actin mRNA (B) in rat testis. Total RNA was isolated and specific mRNA quantiated as described in Fig. 2 and in Methods. The results are expressed relative to the lowest value of HSL mRNA (A) and β-actin mRNA (B) in testis, respectively.



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able to obtain sufficient adipose tissue from rats less than 3 weeks old in order to measure HSL mRNA, the abundance of HSL mRNA did not change from 3 weeks through 2 years of age. This long period spans the time during which obesity and insulin resistance (34) are observed in the rat with the concomitant development of marked alterations in both basal and hormone-stimulated lipolysis. Thus, our findings are consistent with the observation that maximal lipolytic capacity is not altered with age (33). Furthermore, under the conditions used in the present studies, it would appear that HSL activity in adipose tissue is probably controlled principally by phosphorylation-dephosphorylation; nonetheless, our results do not preclude the possibility that alterations in HSL gene expression might influence lipolysis.

The rat heart is not fully developed at birth and completes its differentiation by the third to fourth postnatal week (35). Although the mechanisms involved in maturational events are not fully understood, it has been suggested that thyroid hormone and glucocorticoids are primarily involved in many of the changes (36). Heart HSL mRNA levels were relatively low in fetal life, and increased slightly during the first day of life. Levels gradually increased thereafter, reaching final adult levels by 3 weeks of age, the time of weaning. These changes in heart HSL mRNA parallel the increased expression of lipoprotein lipase mRNA (37-39), another neutral lipase that hydrolyzes circulating triglyceride-rich lipoproteins to free fatty acids for uptake by the tissue. Although the magnitude of the increase in HSL mRNA (3-fold) is much smaller than that observed with lipoprotein lipase mRNA (12- to 300-fold), the temporal relationship between HSL and lipoprotein lipase expression suggests coordinate regulation by a common factor(s).

The pattern of expression of HSL mRNA levels was very different in the adrenals. The amount of HSL mRNA expressed in the adrenals increased dramatically during the first week of life before rapidly falling and then increasing slightly after 14 days. The early rise in HSL mRNA levels coincides with the stress of birth and early suckling; however, it does not correlate with concentrations of plasma corticosterone which are highest 1 day before birth and rapidly decline postnatally to reach a nadir by day 4 (40). Plasma corticosterone concentrations remain low until day 14 when they gradually rise to adult values by 24 days (41). Thus, there appears to be an initial inverse relationship between corticosterone values and adrenal HSL mRNA levels that becomes parallel after 2 weeks. Therefore, the relationship between glucocorticoid production and HSL expression is not straightforward.

The most dramatic changes in HSL mRNA levels occurred in the testis, where HSL mRNA began to increase during sexual development and rose 25-fold by sexual maturity. It is tempting to speculate that the increased expression of HSL is required to provide the necessary cholesterol substrate for the synthesis of testosterone and that the HSL gene is induced by the increased pulsatile secretion of LH and FSH that heralds the onset of testicular maturation. However, in view of the complex relationship between glucocorticoid levels and HSL expression in the adrenal, it is possible that androgens or other factors are responsible for regulation of HSL expression in the testis.

Finally, the results of the experiments with $\beta$-actin demonstrate that $\beta$-actin mRNA levels are not static in all tissues during aging in the rat. Previous studies have shown that the expression of actin isoforms and, specifically, $\beta$-actin varies in muscle (42, 43) and testes (44) during development. While the changes in $\beta$-actin mRNA levels in adrenals and testes in the present studies were not as large as those observed with HSL mRNA levels, the findings reemphasize the potential problems when $\beta$-actin is utilized as a standard in developmental studies.

While HSL activity is rapidly modulated by hormones via phosphorylation-dephosphorylation of the HSL protein, results from the present studies suggest that during subacute or prolonged metabolic perturbation enzyme activity may be dependent upon earlier events such as transcriptional regulation of the HSL gene or changes in the stability or processing of HSL mRNA. Furthermore, it is interesting that during development and aging HSL mRNA levels were constant (fat) or only slightly increased (heart) in tissues where HSL acts primarily to hydrolyze intracellular triglycerides, but were markedly increased in steroidogenic tissues (adrenal, testis) where HSL functions as a cholesteryl ester hydrolase. This finding suggests that the mechanisms regulating the activity of HSL might differ among tissues with posttranslational modification of the enzyme being the predominant, or the only, mechanism involved in the regulation of HSL in tissues where it functions as a triglyceride lipase, while pretranslational events might be more important in controlling HSL in tissues where it is responsible for cholesteryl ester hydrolysis.

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