

## Tissue distribution of beta 3-adrenergic receptor mRNA in man.

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### Research Article

Expression of mRNA for beta 1-, beta 2-, and beta 3-adrenergic receptors (beta 1-, beta 2-, and beta 3-AR) was investigated in human tissues. beta 1- and beta 2-AR mRNA distribution correlated with that of the cognate receptors established by pharmacological studies. beta 3-AR transcripts were abundant in infant perirenal brown adipose tissue, characterized by the presence of uncoupling protein (UCP) mRNA. In adult whole adipose tissues, beta 3-AR mRNA levels were high in deep deposits such as perirenal and omental, and lower in subcutaneous. In these deposits, UCP mRNA levels paralleled those of beta 3-AR. However, isolated omental and subcutaneous adipose cells, enriched in white adipocytes, expressed beta 3-AR but no UCP transcripts. beta 3-AR mRNA was highly expressed in gallbladder, and to a much lower extent in colon, independently of UCP mRNA. Quadriceps or abdominal muscles, heart, liver, lung, kidney, thyroid, and lymphocytes did not express intrinsic beta 3-AR mRNA. This study demonstrates that substantial amounts of brown adipocytes exist throughout life in adipose deposits, which are generally classified as white. These deposits are the main sites of beta 3-AR expression, which also occurs in gallbladder and colon. beta 3-AR may thus be involved in the control of lipid metabolism, possibly from fat assimilation in the digestive tract, to triglyceride storage and mobilization in adipose tissues.

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Tissue Distribution of  $\beta$ 3-adrenergic Receptor mRNA in Man

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## Abstract

Expression of mRNA for  $\beta$ 1-,  $\beta$ 2-, and  $\beta$ 3-adrenergic receptors ( $\beta$ 1-,  $\beta$ 2-, and  $\beta$ 3-AR) was investigated in human tissues.  $\beta$ 1- and  $\beta$ 2-AR mRNA distribution correlated with that of the cognate receptors established by pharmacological studies.  $\beta$ 3-AR transcripts were abundant in infant perirenal brown adipose tissue, characterized by the presence of uncoupling protein (UCP) mRNA. In adult whole adipose tissues,  $\beta$ 3-AR mRNA levels were high in deep deposits such as perirenal and omental, and lower in subcutaneous. In these deposits, UCP mRNA levels paralleled those of  $\beta$ 3-AR. However, isolated omental and subcutaneous adipose cells, enriched in white adipocytes, expressed  $\beta$ 3-AR but no UCP transcripts.  $\beta$ 3-AR mRNA was highly expressed in gallbladder, and to a much lower extent in colon, independently of UCP mRNA. Quadriceps or abdominal muscles, heart, liver, lung, kidney, thyroid, and lymphocytes did not express intrinsic  $\beta$ 3-AR mRNA. This study demonstrates that substantial amounts of brown adipocytes exist throughout life in adipose deposits, which are generally classified as white. These deposits are the main sites of  $\beta$ 3-AR expression, which also occurs in gallbladder and colon.  $\beta$ 3-AR may thus be involved in the control of lipid metabolism, possibly from fat assimilation in the digestive tract, to triglyceride storage and mobilization in adipose tissues. (*J. Clin. Invest.* 1993. 91:344–349.) Key words: adipose tissue • gallbladder • obesity • uncoupling protein • polymerase chain reaction

## Introduction

The early classification of  $\beta$ -adrenergic receptors ( $\beta$ -ARs)<sup>1</sup> into  $\beta$ 1 and  $\beta$ 2 subtypes was soon insufficient to account for the non- $\beta$ 1- and non- $\beta$ 2-adrenergic responses of rat brown and white adipose tissues (BAT and WAT, respectively) (reviewed

in references 1 and 2). This led to the concept that another, atypical,  $\beta$ -AR mediates thermogenesis in BAT and lipolysis in WAT. Atypical  $\beta$ -ARs, pharmacologically similar to those of adipocytes, have also been suggested to exist in ileum, colon, and gastric fundus (3–6), skeletal muscle (7), and heart (8) of rodents. The characterization of a gene coding for a third  $\beta$ -AR, termed  $\beta$ 3-AR, in humans (9) and rodents (10–12) provided a molecular basis for the existence of an atypical  $\beta$ -AR and identified BAT and WAT as principal sites for  $\beta$ 3-AR expression.

Anti-obesity drugs, which in animals seem to selectively act on adipose tissue  $\beta$ 3-ARs, are less efficient in humans, where they display  $\beta$ 1- and  $\beta$ 2-AR-mediated side effects (13–15). Moreover, although WAT and BAT are clearly distinct in human neonates, the persistence of BAT in adults is still controversial (reviewed in reference 16). These observations question the presence and the functionality of  $\beta$ 3-ARs in humans and the role of a quantitative thermogenically active BAT in adults.

In this work, tissue distribution of  $\beta$ 3-AR mRNA was investigated in humans together with that of the mitochondrial uncoupling protein (UCP), which is specific for BAT, where it is responsible for heat production (17, 18). The use of PCR assay allowed such a study to be performed on small tissue biopsies available from humans.

In man,  $\beta$ 3-AR mRNA is present in BAT and WAT. It is also expressed in gallbladder and to a lesser extent in colon. Moreover, UCP mRNA, always associated with  $\beta$ 3-AR mRNA, is present in infant and adult whole adipose mass, suggesting that significant amounts of brown adipocytes persist throughout life.  $\beta$ 3-ARs may thus participate in the sympathetic control of body fat mass by regulating not only the utilization of fat stores but also the rate of lipid assimilation during digestion.

## Methods

**Subjects and tissues.** The study included 7 male and 21 female adult patients (Karolinska Institute Hospital, Huddinge, Sweden and Broussais Hospital, Paris, France) and 5 male and 3 female child patients (Saint Joseph Hospital and Saint Vincent de Paul Hospital, Paris, France) undergoing elective surgery. Adults' ages ranged from 19 to 75 years (mean  $\pm$  SEM = 50  $\pm$  3 yr), and their body mass index (ratio of body mass [kilograms] to square height [meters]) from 19.4 to 35.5 (mean  $\pm$  SEM = 25.5  $\pm$  1.0). Children's ages were 2 wk ( $n$  = 1), 8 wk ( $n$  = 4), 20 mo ( $n$  = 1), 3 yr ( $n$  = 1), and 12.5 yr ( $n$  = 1). None of the patients, except those with pheochromocytoma, had diseases or medications known to interfere with the  $\beta$ -AR system. Tissue biopsies and isolated adipocytes (19) were immediately frozen and stored in liquid nitrogen for subsequent RNA preparation.

**RNA analysis.** Total RNA was prepared (20) from at least two to three patients for each tissue examined. For PCR analysis, RNA was treated for 1 h at 37°C with 6 U of RNase-free DNase I per  $\mu$ g of RNA in 100 mM Tris-HCl, pH 7.5, and 50 mM MgCl<sub>2</sub> in the presence of 2

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1. Abbreviations used in this paper:  $\beta$ -AR,  $\beta$ -adrenergic receptors; BAT, brown adipose tissue; UCP, uncoupling protein; WAT, white adipose tissue.

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U/ $\mu$ l of placenta RNase inhibitor. After phenol extraction and ethanol precipitation, 1  $\mu$ g of RNA was treated with 400 U of Maloney murine leukemia virus reverse transcriptase (Gibco BRL, Cergy Pontoise, France) in 80  $\mu$ l of PCR buffer (67 mM Tris-HCl, pH 8.4, 6.7 mM MgCl<sub>2</sub>, 6.7  $\mu$ M EDTA, 10 mM  $\beta$ -2-mercaptoethanol, 16 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, and 0.1 mg/ml gelatin) containing 0.4 mM each dNTP, 10  $\mu$ M random hexanucleotides, and 2 U/ $\mu$ l RNase inhibitor. A control without reverse transcriptase was done to verify that amplification did not proceed from residual genomic DNA.  $\beta$ 1-,  $\beta$ 2-, and  $\beta$ 3-AR cDNA was amplified by 29 cycles of temperature (92°C, 1 min; 57°C, 1.5 min; 72°C, 1.5 min) followed by 7 min of extension at 72°C in a temperature cycler (LEP-PREM, LEP Scientific Limited, Milton Keynes, United Kingdom) in 100  $\mu$ l of PCR buffer containing 2.5 U of thermophilus aquaticus polymerase (Perkin-Elmer Cetus, Norwalk, CT), 125 nM each sense and antisense oligonucleotide primers, 125  $\mu$ M each dNTP, and 10% (vol/vol) dimethylsulfoxide. 5% formamide (vol/vol) was also added for  $\beta$ 1-AR amplification. UCP cDNA was amplified as described (21).

Amplification of cDNA was linear up to 500 ng of initial RNA for  $\beta$ 1- and  $\beta$ 3-ARs and up to 250 ng for  $\beta$ 2-ARs and UCP. Although our goal was not to quantify mRNA expression, the use of 250 ng of RNA for  $\beta$ 1- and  $\beta$ 3-AR amplifications, and 125 ng for  $\beta$ 2-ARs and UCP allowed us to appreciate the relative levels of expression of each mRNA.

Sequences of the sense and antisense oligonucleotides, respectively, were: 5'-TAGGTATAAAGGTGTCCTGG-3' and 5'-CACTTTGTACTGTCCTGGTGG-3' (amino acids 53–249) for UCP, 5'-TCGTGTGCACCGTGTGGCC-3' and 5'-AGGAAACGGCGCTCGCAGCTGTCG-3' (amino acids 178–265) for  $\beta$ 1, 5'-GCCTGCTGACCAGAATAAGGCC-3' and 5'-CCCATCCTGCTCCACCT-3' (amino acids 143–252) for  $\beta$ 2, and 5'-GCTCCGTGGCCTCAGAGAA-3' and 5'-CCCAACGGCCAGTGGCCAGTCAGCG-3' (amino acids 2–106) for  $\beta$ 3. Using these primers, the lengths of the fragments, calculated from the structure of the corresponding genes, were 590, 265, 329, and 314 bp for UCP,  $\beta$ 1-,  $\beta$ 2-, and  $\beta$ 3-ARs, respectively.

PCR products (10- $\mu$ l aliquots) were visualized by electrophoresis through 2% agarose gels. Gels were blotted onto nitrocellulose filters which were hybridized to probes obtained by PCR amplification of cloned human  $\beta$ 1-,  $\beta$ 2-, and  $\beta$ 3-AR genes with the above primers, and to a human UCP probe (22). Filters were submitted to a series of 30-min washes: three times in 2 $\times$  SSC (1 $\times$  SSC is 150 mM NaCl and 15 mM sodium citrate), 0.1% SDS at 25°C, and once at 60°C; and three times in 0.1 $\times$  SSC, 0.05% SDS at 60°C. Nitrocellulose membranes were exposed for 8 h to NIF RX-100 films (Fuji) with intensifying screens.

Direct sequencing of PCR-generated products was performed as described (23) using the sense and anti-sense PCR oligonucleotides as primers for each respective strand.

Northern blots of RNA were successively hybridized to human  $\beta$ 3-AR (24) and UCP (22) probes and washed as above.

## Results

We have studied the tissue distribution of  $\beta$ 3-AR mRNA in humans. The presence of UCP mRNA was investigated to help identify the brown or white nature of adipose tissues. To appreciate the physiological significance of transcript detection using a PCR assay, we analyzed the distribution of mRNA for  $\beta$ 1- and  $\beta$ 2-ARs, receptors that have already been pharmacologically characterized in most tissues.

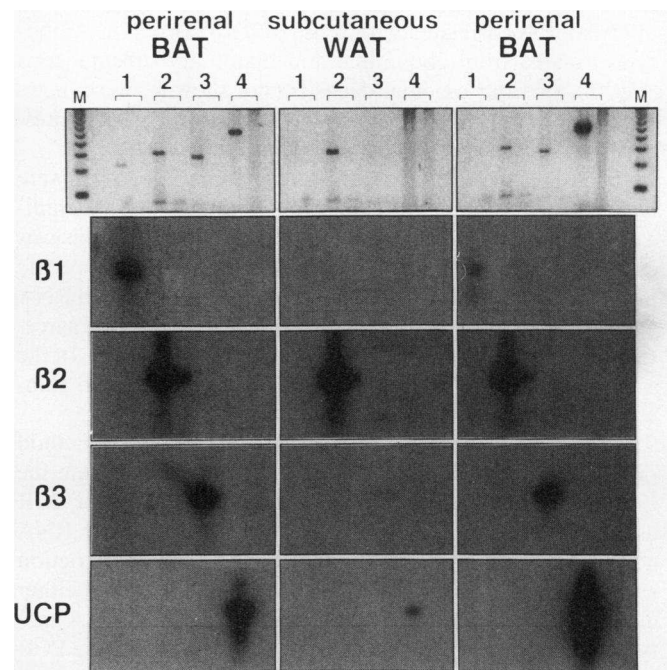
**Identification of the PCR-amplified products.** The lengths of PCR-amplified products using  $\beta$ 1-,  $\beta$ 2-, and  $\beta$ 3-ARs and UCP primers agreed with those expected from the structure of the corresponding genes. Hybridization to specific probes obtained from the cloned genes confirmed the identities of the fragments and improved detection sensitivity over that obtained by ethidium bromide staining of agarose gels. A weak

cross-hybridization of the  $\beta$ 2 probe to  $\beta$ 1 fragments was occasionally observed but was not significant compared with hybridization to  $\beta$ 2-AR products.

Expression of  $\beta$ 3-AR and UCP mRNA was further confirmed by using at least one additional pair of sense and antisense primers, which again yielded fragments of the expected sizes (not shown). Identity of the PCR-amplified products was unambiguously established by partial determination of their nucleotide sequences (~200–250 bp), which were almost 100% identical to the corresponding sequences of the cloned genes. The observed differences (1–3 bp) may result from polymerase errors or reflect minor polymorphism of the genes.

**$\beta$ 3-AR and UCP mRNA expression in adipose tissues.** In infant perirenal adipose deposits, visibly brown,  $\beta$ 3-AR and  $\beta$ 1- and  $\beta$ 2-AR mRNA were expressed at comparable levels in samples from six individuals (Fig. 1, left). In two additional samples,  $\beta$ 1-AR mRNA expression was much lower and could be demonstrated only by hybridization to a  $\beta$ 1-AR probe (Fig. 1, right). The brown character of the tissues was confirmed by the presence of abundant UCP transcripts which, in contrast, were in very low quantities in subcutaneous WAT (Fig. 1, middle).

In each adipose location of adults, where  $\beta$ 1- and  $\beta$ 2-ARs have already been pharmacologically characterized, comparable and constant amounts of the corresponding RNA occurred for almost all individuals examined (Fig. 2). Levels of  $\beta$ 3-AR



**Figure 1.** Distribution of  $\beta$ 1-,  $\beta$ 2-, and  $\beta$ 3-AR and UCP mRNA in infant BAT and WAT. Top panels display negatives of agarose gel electrophoresis pictures of  $\beta$ 1- (1),  $\beta$ 2- (2), and  $\beta$ 3-ARs (3), and UCP (4) cDNA fragments amplified by PCR from infant adipose deposits. Each horizontal bracket delimits two lanes containing PCR samples obtained from RNA treated (left lane) or untreated (right lane) with reverse transcriptase. Size markers (M) are the 123-bp DNA ladder (Gibco BRL). Lower panels display corresponding Southern blots hybridized as indicated to  $\beta$ 1,  $\beta$ 2,  $\beta$ 3, and UCP probes. From left to right, results are shown that are representative of two experiments performed on perirenal BAT ( $n = 6$ ), subcutaneous WAT ( $n = 1$ ), and perirenal BAT samples ( $n = 2$ ) from several individuals.

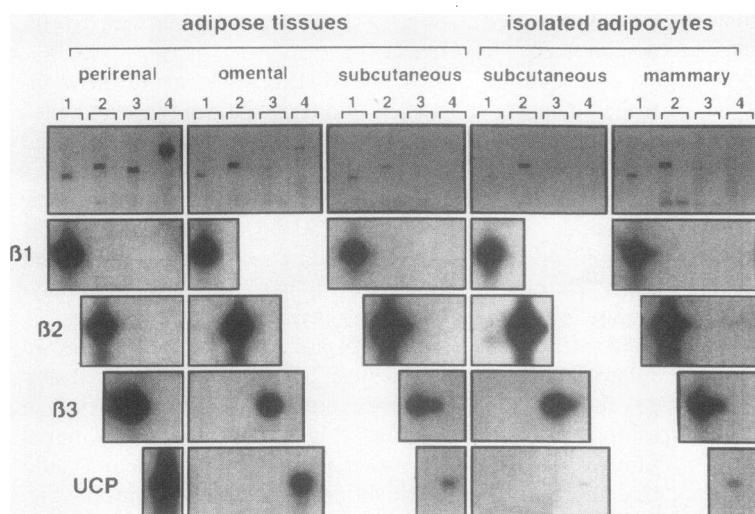


Figure 2. Distribution of  $\beta_1$ -,  $\beta_2$ -, and  $\beta_3$ -AR and UCP mRNA in adult adipose deposits. Organization of the figure is as in Fig. 1. Representative results obtained from indicated adipose tissue deposits and isolated adipocytes are shown. For each deposit and isolated cells, at least two experiments were performed on samples from three to five individuals (except mammary adipocytes,  $n = 1$ ). The results presented for the subcutaneous deposit and isolated adipocytes were obtained with material prepared from the same biopsy.

mRNA varied according to the adipose deposit analyzed: they were generally high in perirenal, low in subcutaneous, and intermediate in omental fat. In subcutaneous deposits from two individuals,  $\beta_3$ -AR mRNA levels were similar to those observed in omental fat, and in the omental biopsy from one patient,  $\beta_3$ -AR transcripts were barely detectable (not shown). In perirenal, omental, and subcutaneous fat deposits, UCP mRNA levels correlated with those of  $\beta_3$ -AR mRNA. Adipocytes isolated from abdominal and mammary subcutaneous (Fig. 2), or from omental deposits (not shown), were, however, essentially devoid of UCP mRNA while retaining the presence of  $\beta_1$ -,  $\beta_2$ -, and  $\beta_3$ -AR mRNA.

$\beta_3$ -AR signals obtained from perirenal deposits were readily visible after ethidium bromide staining of the gels, indicating particularly high levels of mRNA expression. This was corroborated by Northern blot analyses using a  $\beta_3$ -AR-specific probe that identified 2.3-kb transcripts in polyadenylated RNA from perirenal BAT samples (Fig. 3). This size is in good agreement with that of  $\beta_3$ -AR transcripts in rodents (10–12). In the same samples, a human UCP probe revealed a 1.9-kb transcript as already reported (22).

Preliminary quantitation experiments, based on a method previously described (25), were performed to appreciate the biological relevance of  $\beta_3$ -AR mRNA detection by our PCR assay. Varying amounts (0.05–100 amol) of synthetic RNA containing a 2-bp mutation that created a BamHI restriction site in between the two  $\beta_3$ -AR primers were amplified either alone or together with 250 ng of the omental WAT RNA analyzed in Fig. 2. After visualization on agarose gels of the PCR products digested with BamHI (not shown), it was estimated that  $\beta_3$ -AR signals obtained from this omental WAT sample corresponded to  $\sim 2$  amol of tissular  $\beta_3$ -AR mRNA per microgram of total RNA.

**$\beta_3$ -AR expression in nonadipose tissues.** Atypical  $\beta$ -ARs, which are pharmacologically related to the  $\beta_3$ -ARs of BAT and WAT, but which remain to be fully characterized, have been suggested to exist in the digestive tract, skeletal muscles, and heart of rodents (3–8). To examine if this is the case in humans, we extended the analysis of  $\beta_3$ -AR mRNA expression to a variety of tissues. Because of the presence of UCP mRNA in all adipose deposits, these are likely to contain a mixture of brown and white adipocytes. Since biopsies may occasionally

be contaminated, or in some cases normally infiltrated with fat cells, UCP mRNA expression was investigated in parallel in each tissue.

The tissue distribution of  $\beta_1$ - and  $\beta_2$ -AR mRNA (Fig. 4) closely correlated with that of the corresponding receptors determined by pharmacological studies. No  $\beta_3$ -AR mRNA could be detected in quadriceps or abdominal muscles, liver, lung, kidney, thyroid, or lymphocytes, which only expressed  $\beta_1$ - and  $\beta_2$ -AR mRNA. High levels of  $\beta_3$ -AR mRNA, visualized after ethidium bromide staining of agarose gels, were found in gallbladder. Low levels of  $\beta_3$ -AR mRNA, mostly seen after Southern hybridization, were detected in heart atria and ventricles and in colon. In heart,  $\beta_3$ -AR message was associated with that for UCP, whereas this was not the case in gallbladder or colon.

## Discussion

In humans,  $\beta_3$ -AR mRNA is expressed in adipose tissues, in gallbladder, and to a much lower extent in colon. In rat BAT

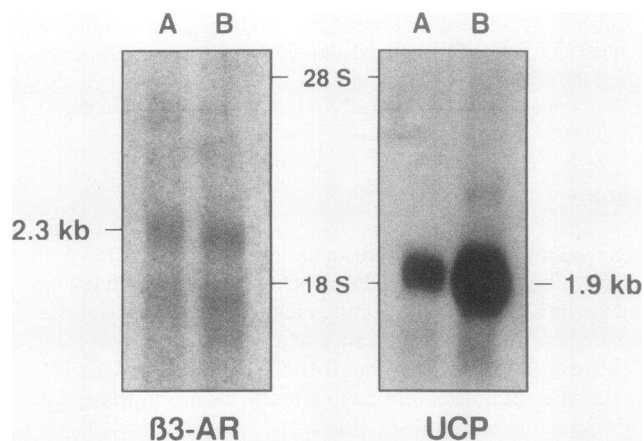


Figure 3. Analysis of  $\beta_3$ -AR and UCP mRNA in human BAT. Northern blots of polyadenylated RNA extracted from perirenal BAT from a 6-wk-old infant (3  $\mu$ g, lane A) and from a 19-yr-old patient with pheochromocytoma (5  $\mu$ g, lane B) were hybridized to  $\beta_3$ -AR (left) and UCP (right) DNA probes. The length (kilobases) of the  $\beta_3$ -AR and UCP transcripts, and the positions of 18 S and 28 S ribosomal RNA are indicated.

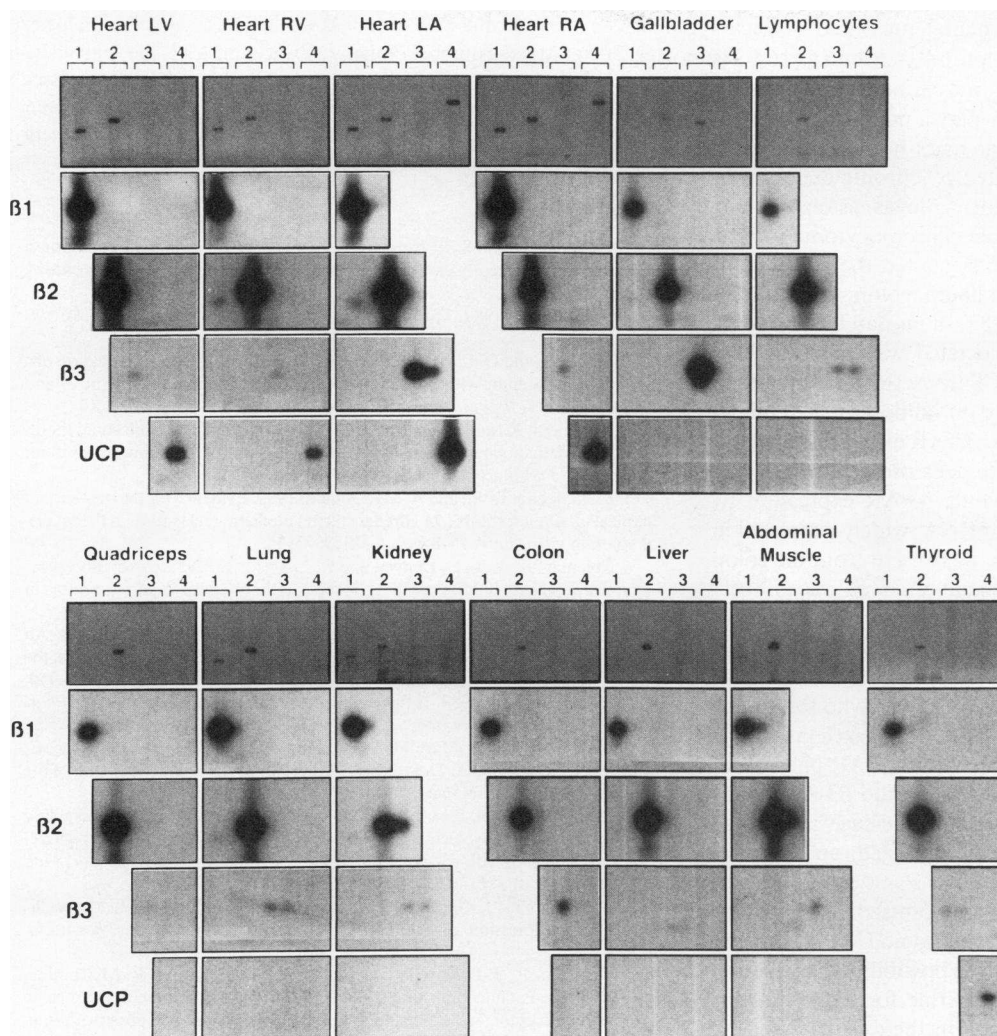


Figure 4. Distribution of  $\beta$ 1-,  $\beta$ 2-, and  $\beta$ 3-AR and UCP mRNA in adult tissues. General organization of the figure is as in Fig. 1. Representative results obtained from the indicated tissues are shown. *Heart LV, RV, LA, and RA*, heart left and right ventricles and atria, respectively. For each tissue, two experiments were performed on biopsy samples from two or three individuals.

and WAT,  $\beta$ 3-AR mRNA levels range from 10 to 60 amol/ $\mu$ g of total RNA (26). Our results suggest that in human omental adipose tissue this value is  $\sim$  2 amol. Since  $\beta$ 3-AR signals observed in perirenal fat were much higher than in omental,  $\beta$ 3-AR mRNA levels in man may reach those reported in rat adipocytes, which indeed express functional  $\beta$ 3-AR (1, 2, 27). Together, these observations support the biological relevance of the  $\beta$ 3-AR transcripts detected here in humans.

In agreement with this report, our preliminary results (Lönqvist, F., S. Krief, L. J. Emorine, A. D. Strosberg, B. Nyberg, and P. Arner, manuscript in preparation) demonstrate the presence of lipolytically active  $\beta$ 3-ARs in omental and, to a lower extent, in subcutaneous adipose cells. Other studies in humans, performed on subcutaneous adipose cells, have suggested either little (28) or no (27) participation of  $\beta$ 3-ARs in agonist-stimulated lipolysis. The difficulties of characterizing  $\beta$ 3-ARs in subcutaneous adipose cells certainly reflect the low intrinsic responses of these cells to  $\beta$ -adrenergic stimuli (29, 30) in addition to the inefficiency in humans of  $\beta$ 3-AR agonists used in animals (10, 31). The discrepancies between the two reports (27, 28) could also reflect interindividual variations in  $\beta$ 3-AR expression as observed here in some fat samples. These variations might represent adaptive or hormonal regulation of  $\beta$ 3-AR expression as seen in cold exposed rats (26) or in glucocorticoid-treated adipose cells (24).

To determine whether  $\beta$ 3-AR mRNA expression in adipose tissues occurred in WAT or in BAT, we also investigated expression of UCP mRNA, a marker unique to BAT. The two tissues are clearly distinct in infants, both on functional and histological bases. During development, concomitant with WAT growth and differentiation, BAT is thought to regress, and either almost disappear from adults (32) or undergo alterations that render it indistinguishable from surrounding WAT (16). In this study,  $\beta$ 3-AR mRNA was found in two BAT samples which are unambiguously classified as such in humans: perirenal fat from infants and from patients with pheochromocytoma (16, 33). The presence of  $\beta$ 3-AR mRNA was also demonstrated in perirenal, omental, and subcutaneous fat of adults up to the age of 74 yr. These deposits are generally considered to be WAT, although we show here that they express mRNA for UCP. Nevertheless, isolated adipose cells, which are essentially white adipocytes, retained  $\beta$ 3-AR but no UCP mRNA.

Together, these data demonstrate that fat deposits contain white and brown adipocytes, both expressing intrinsic  $\beta$ 3-ARs. UCP mRNA, probably derived from fat cells present in biopsies, was found in heart and thyroid, and occasionally in biopsies from other sites (not shown), indicating a widespread representation of BAT in the body. Our observations agree with the suggestion that quiescent brown adipocytes, or precursory cells, persist throughout life in adipose deposits that are typi-

cally brown in infancy: perirenal, omental, mesenteric, pericardial, intercostal, axillary, cervical, and interscapular (16). As in rodents or infants, adults' BAT may thus represent up to 1–2% of body weight (16) and may play a major role in adaptive thermogenesis and in body fat mass homeostasis, especially when nascent BAT is activated by chronic exposure to catecholamines or to  $\beta$ 3-AR-selective agonists, as observed in animals (34, 35) or humans with pheochromocytoma (33).

Several studies in rodents have suggested the presence of  $\beta$ 3-AR, or related atypical  $\beta$ -AR, in ileum, colon, stomach (3–6), skeletal muscle (7), and heart (8). In human heart,  $\beta$ 3-AR mRNA expression was always associated with that of UCP. The highest levels of  $\beta$ 3-AR and UCP transcripts were found in atria that are covered by the abundant adipose tissue embedding the cardiac great vessels. Thus,  $\beta$ 3-AR expression in heart was probably derived from adipose cells present in biopsies, although one cannot formally exclude  $\beta$ 3-AR expression by some specialized cells. Since UCP mRNA, widely expressed in the whole abdominal adipose mass, was absent from the colon biopsies, the weak  $\beta$ 3-AR mRNA signal observed was unlikely to be due to fat contamination (or if so, from white adipocytes only), but may represent  $\beta$ 3-AR expressed by a few noradrenergic-innervated cells (36).

Our findings correlate with those of others who found low levels of  $\beta$ 3-AR mRNA in rat ileum, but not in skeletal muscle and heart (11), and suggest that the atypical  $\beta$ -AR pharmacologically described in the digestive tract is the  $\beta$ 3-AR. Since neither study was able to detect  $\beta$ 3-AR message in skeletal muscles, it remains possible that, as suggested, an "atypical  $\beta$ 2-AR" exists in this tissue (37).

In gallbladder,  $\beta$ 3-AR mRNA was expressed at levels comparable to those seen in adipose tissues and no UCP transcripts were found. This virtually excludes the possibility that it represents a contamination by fat cells. A role for  $\beta$ 3-AR in this organ has not yet been suggested. As in the digestive tract, it may inhibit contractions of gallbladder smooth muscle, thus preventing release of biliary salts and decreasing lipid assimilation.

Altogether, our studies suggest that in humans  $\beta$ 3-ARs may play a major role in the control of lipid metabolism. In animal models of obesity and type II diabetes, agonists selective for the  $\beta$ 3-AR produce significant weight loss and improve glucose homeostasis. They are less active in man and induce  $\beta$ 1- and  $\beta$ 2-AR-mediated side effects (13–15). Indeed, when tested on eukaryotic cells expressing the corresponding recombinant genes, these compounds were less efficient on human than on murine  $\beta$ 3-ARs (10) and poorly selective of the human  $\beta$ 3- versus  $\beta$ 1- and  $\beta$ 2-ARs (31). In the light of this study, proper human  $\beta$ 3-AR-selective drugs may decrease fat stores, while preserving skeletal muscle mass, by preventing fat assimilation during digestion and by increasing WAT lipolysis and BAT thermogenesis. Agonists selective for the human  $\beta$ 3-AR may thus prove useful in treating obesity and type II diabetes as well as associated diseases.

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