

# Differential gene expression of insulin receptor isoforms A and B and insulin receptor substrates 1, 2 and 3 in rat tissues: modulation by aging and differentiation in rat adipose tissue

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## Abstract

The insulin receptor (IR) occurs as two alternatively spliced isoforms, IR-A (exon 11-) and IR-B (exon 11+), which exhibit functional differences and are expressed in a tissue-specific manner. The IR substrate (IRS) proteins 1, 2 and 3 also differ in function and tissue distribution. Here we show the differential gene expression of IRs and IRSs in several rat target tissues of insulin action. IR-B is significantly higher than IR-A in epididymal white adipose tissue and adipogenesis induces a shift in the alternatively spliced species of IR from the A to the B isoform. Moreover, since aging in the rat is associated with the development of insulin resistance we looked for alterations of expression of these proteins in adipocytes from old rats. Our results reveal that there is a specific decrease in the expression of the IR-B isoform, as well as both mRNA and protein levels of IR, IRS-1 and IRS-3 being significantly decreased, in epididymal adipose tissue from old compared with adult rats. It is concluded that the down-regulation of early components of the insulin transduction pathway in a primary insulin target tissue could be related to the insulin resistance of aging.

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## Introduction

The insulin receptor (IR) is expressed as two mRNA species derived from alternative splicing of exon 11 of the IR gene. Exon 11 consists of 36 nucleotides, which encode 12 amino acids at the C-terminus of the  $\alpha$ -subunit (Seino & Bell 1989).

The relative abundance of the mRNAs encoding isotypes A (exon 11-) and B (exon 11+) of the IR is regulated in a tissue-specific manner in both humans and rats (Møller *et al.* 1989, Goldstein & Dudley 1990). It is also regulated by stage of development and by cell differentiation, with IR-A being the predominant IR isoform in fetal tissues and cancer cells (Frasca *et al.* 1999).

The two IRs have been reported to exhibit distinct functional properties. IR-A shows a higher affinity for insulin and a higher internalization rate than IR-B (Vogt *et al.* 1991, Yamaguchi *et al.* 1991), whereas IR-B is considered to transmit the insulin signal more efficiently than IR-A as long as it has a greater kinase activity (Kellerer *et al.* 1992, Kosaki *et al.* 1995). Besides, recent studies have shown that IR-A and IR-B activate different downstream pathways. In pancreatic  $\beta$ -cells, IR-A

regulates insulin gene expression and IR-B does the same with  $\beta$ -glucokinase but using different classes of phosphatidylinositol 3-kinase (PI3K) (Leibiger *et al.* 2001). In 32D cells, a murine hematopoietic cell line, IR-A sends mitogenic, antiapoptotic signals in response to insulin-like growth factor-II, whereas IR-B is more effective in inducing differentiation (Sciaccia *et al.* 2003).

An altered relative expression of IR isoforms may contribute to the development of insulin resistance. In this regard, it has been demonstrated that a deregulated IR alternative splicing, resulting in a switch to IR-A, correlates with insulin resistance in myotonic dystrophy type I (Savkur *et al.* 2001).

The IR substrate (IRS) proteins are important mediators in insulin signaling. Several members of this family have been identified that differ as to tissue distribution. A reduction in IRS-1 expression has been reported in insulin-resistant states such as obesity and type 2 diabetes (T2DM) (reviewed in Sesti *et al.* 2001a).

Aging has been classically associated with impaired glucose tolerance and/or peripheral insulin resistance (De Fronzo 1981, Narimiya *et al.* 1984). Aged Wistar rats are characterized by fasting normoglycemia and normoinsulinemia and show overall insulin resistance as

**Table 1** Sequences of PCR primers

	PCR primers	Nucleotide number	Size of PCR product (bp)	GenBank accession number
<b>Gene</b>				
Insulin receptor	5'-TTCATTTCAGGAAGACCTTCGA-3' 5'-AGGCCAGAGATGACAAGTGAC-3'	2528–2548 2785–2765	258/222	M29014
IRS-1	5'-GCCAATCTTCATCCAGTTGC-3' 5'-CATCGTGAAGAAGGCATAGG-3'	1382–1401 1718–1699	337	X58375
IRS-2	5'-GCAGTTCAGGTCGCCTCTGC-3' 5'-GGAGCCACACCACATTCCGA-3'	338–357 753–734	416	AF050159
IRS-3	5'-GAGACCGTCTGGCTGCCAT-3' 5'-ATCCACATGTAAGCAGCAGC-3'	969–988 1282–1263	314	U93880
$\beta$ -Actin	5'-GGTATGGAATCCTGTGGCATCCATGAAA-3' 5'-GTGTA AACGCAGCTCAGTAACAGTCCG-3'	2684–2711 3163–3136	356	V01217

assessed by the euglycemic–hyperinsulinemic clamp technique (Nishimura *et al.* 1988, Escrivá *et al.* 1997). *In vivo* studies on glucose uptake by different tissues of old rats have revealed that the white adipose tissue is highly resistant to the action of insulin (Escrivá *et al.* 1997). Moreover, recent experimental evidence suggests that the adipose tissue might play an important role in the genesis of the insulin resistance (Smith 2002).

At the molecular level, the insulin resistance of adipocytes from aged Wistar rats appears to be attributable to a postbinding defect. Previous data of our group have shown that, when using solubilized receptors from adipocyte plasma membrane, there is no modification in either affinity or receptor number in old rats. However, there is a marked impairment of both IR autophosphorylation and its intrinsic tyrosine kinase activity during aging (Ruiz *et al.* 1992). Moreover, in isolated adipocytes from old rats we have also shown that insulin stimulation of IRS-1-associated PI3K, glucose uptake and glucose transporter 4 (GLUT4) translocation to the plasma membrane is reduced (Molero *et al.* 1998).

The aim of the present study was to examine the mRNA expression of IR isoforms and IRSs in several rat tissues and to further investigate its regulation by aging and differentiation in rat adipose tissue.

## Materials and methods

### Experimental animals

Adult (3 months) and old (22–24 months) male Wistar rats with free access to water and standard laboratory chow were used throughout this study. Rats were obtained from the colony of the Center of Molecular Biology Severo Ochoa (Madrid, Spain).

Animal facilities fulfilled the requirements of the European Union and the National Institutes of Health guidelines. Special care was taken to minimize animal suffering and to reduce the number of animals used.

### Cell culture

Primary rat preadipocytes were obtained from the stromal–vascular fraction of epididymal adipose tissue by collagenase digestion (Cabrero *et al.* 2001). Cells were plated in DMEM containing 10% fetal bovine serum at a density of  $1\text{--}2 \times 10^4$  cells/cm<sup>2</sup>. At confluence, differentiation was induced by the addition of medium supplemented with 20 nM insulin, 10  $\mu$ g/ml transferrin, 100 nM cortisol and 200 pM triiodothyronine. To potentiate adipose differentiation, 0.5 mM 3-isobutyl-1-methylxanthine and 25 nM dexamethasone were added to the adipogenic medium during the first 3 days. Medium was changed every 48–72 h. Within 14 days of culture, cells were regarded as differentiated by morphological criteria when, after acquiring a round shape, their cytoplasm was completely filled with multiple lipid droplets (assessed by Oil Red O staining). RNA was extracted from the cells as described below.

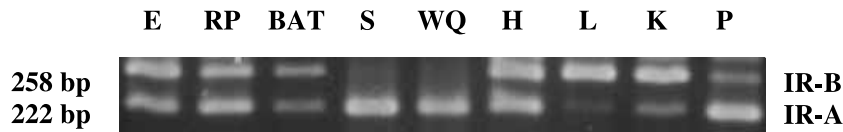
### RNA extraction and RT-PCR analysis

Total RNA from white adipose tissue was isolated using RNeasy Mini Kit (Qiagen). Total RNA from the rest of the tissues analyzed (brown adipose tissue, soleus and white quadriceps skeletal muscle, heart, liver, kidney and pancreas) and from preadipocytes, before and after differentiation, was isolated by acid guanidinium thiocyanate/phenol/chloroform extraction (Chomczynsky & Sacchi 1987).

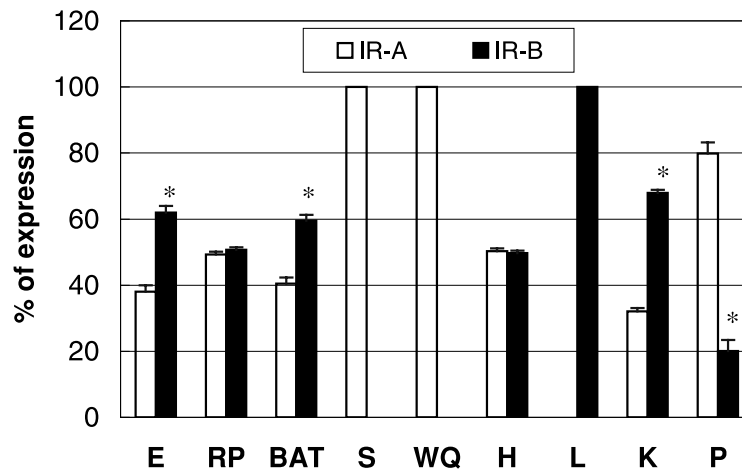
The cDNA was synthesized from 5  $\mu$ g DNase-treated RNA (Sambrook *et al.* 1989) with the reverse transcriptase (RT) activity from Moloney murine leukemia virus (GibcoBRL), and pd(N)<sub>6</sub> (Roche) as random primer.

PCR was performed in a total volume of 50  $\mu$ l using one-fourth (one-tenth for actin) of the RT sample as template, in medium containing 1 $\times$ PCR buffer (50 mM KCl, 2 mM MgCl<sub>2</sub>, 20 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 75 mM

(A)



(B)



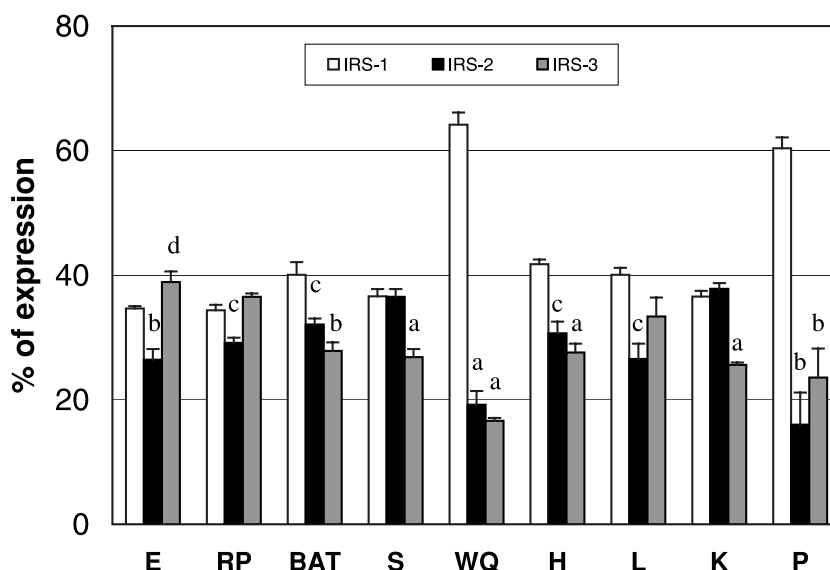
**Figure 1** Tissue-specific expression of IR isoforms in adult rats. The relative abundance of IR isoforms was analyzed by RT-PCR using oligonucleotides flanking the alternatively spliced exon 11 of the rat IR cDNA sequence. (A) A representative experiment is shown. The two PCR amplification products obtained, IR-A (exon 11-) and IR-B (exon 11+), are indicated. The sources of RNA from 3-month-old rat tissues were E: epididymal white adipose tissue, RP: retroperitoneal white adipose tissue, BAT: brown adipose tissue, S: soleus, WQ: white quadriceps, H: heart, L: liver, K: kidney, P: pancreas. (B) Quantification of the amplified products was performed as described in Methods. Percentage of IR-A and IR-B for each tissue is presented as the mean  $\pm$  S.E.M. of three to seven animals. \* $P < 0.001$  vs IR-A.

Tris-HCl pH 9.0), 0.2 mM each of dNTP, 50 pmol of specific primers (20 pmol for actin) and 1.25 units of thermostable DNA polymerase (Biotools B & M Labs, Madrid, Spain). The sequences of the specific primers used for amplification are shown in Table 1. After 5 min at 94 °C, the PCR mixtures were subjected to 30 cycles of amplification with a cycle profile including denaturation for 30 s at 94 °C, annealing for 45 s at 60 °C and elongation for 1 min at 72 °C. Reactions were finished with a final extension of 7 min at 72 °C. PCR amplification was carried out in a DNA thermal cycler (Perkin-Elmer Corp., Madrid, Spain). The number of PCR cycles was selected within the linear range of detection to reflect relative levels of expression. Products of PCR were resolved by electrophoresis on 3% (for IR isoforms) or 2%

(for IRSs) agarose gels, stained with ethidium bromide and photographed. The band densities were quantified by scanning densitometry. Levels of amplification were expressed relative to that of  $\beta$ -actin as internal control of the amount of cDNA in each reaction. Only the relative expression of the mRNAs, and not their actual concentrations, is given by this procedure.

#### Adipocyte isolation and fractionation

Adipocytes were isolated from epididymal fat pads of adult and old rats by collagenase digestion (Rodbell 1964). Cells were then homogenized with a Dounce tissue grinder in buffer containing 0.1 M sucrose, 50 mM TES-NaOH (pH 7.4), 1 mM EDTA, 2 mM



**Figure 2** Tissue-specific expression of IRSs in adult rats. The relative abundance of IRSs was analyzed by RT-PCR using oligonucleotides specific for each substrate. The sources of RNA from 3-month-old rat tissues are the same as those in Fig. 1. Quantification of the amplified products was performed as described in Methods. Percentage of IRS-1, IRS-2 and IRS-3 for each tissue is presented as the mean  $\pm$  S.E.M. of three to seven animals. <sup>a</sup> $P < 0.001$ , <sup>b</sup> $P < 0.005$ , <sup>c</sup> $P < 0.01$ , <sup>d</sup> $P < 0.05$  vs IRS-1.

EGTA, 1 mM dithiothreitol, 5 mM NaF, 0.05 M  $\text{Na}_3\text{VO}_4$ , 1 mM phenylmethylsulfonyl fluoride, 10  $\mu\text{g/ml}$  leupeptin, 10  $\mu\text{g/ml}$  antipain and 1  $\mu\text{g/ml}$  pepstatin. For preparation of a total membrane fraction, the homogenate was first centrifuged at 800  $g$  for 5 min in order to remove the upper lipid layer, and the infranatant was then centrifuged at 200 000  $g$  for 1 h. The resulting pellet contains the whole particulate fraction of the adipocyte.

### Western blot analysis

After protein content determination, equal amounts of protein were separated on a 10% SDS-PAGE gel under reducing conditions, transferred to nitrocellulose (0.2  $\mu\text{m}$ ) (Bio-Rad) and incubated overnight at 4  $^\circ\text{C}$  with anti-IR  $\beta$ -subunit diluted 1:500 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-IRS-1 diluted 1:1000 (Cell Signaling Technology, Beverly, MA, USA) or anti-IRS-3 diluted 1:200 (Santa Cruz Biotechnology). The bound antibody was detected by using the ECL Western-blotting detection system (Amersham) and quantified by scanning densitometry of autoradiographs with exposure in the linear range.

### Statistical methods

Data are expressed as means  $\pm$  S.E.M. The significance of differences between two groups was determined by an

unpaired Student's *t*-test. Differences were considered significant at  $P < 0.05$ .

## Results and discussion

### Heterogeneity of IR and IRS mRNA expression

The relative amounts of the alternatively spliced IR isoforms, IR-A and IR-B, were analyzed by RT-PCR in several rat tissues (Fig. 1). Rat muscles (soleus and white quadriceps) express mostly the shorter transcript, IR-A, whereas liver expresses mostly the longer isoform, IR-B. The rest of the tissues analyzed express both isoforms, IR-B mRNA levels being higher in epididymal white adipose tissue, brown adipose tissue and kidney. IR-A isoform predominates in pancreas. Retroperitoneal white adipose tissue and heart express both species at the same level.

In human adult tissues there is a predominant expression of IR-B (Moller *et al.* 1989); in adult rat, however, some tissues such as muscle, pancreas, brain, placenta and spleen express preferentially IR-A (Goldstein & Dudley 1990, Sugimoto *et al.* 2000, this work). It is not known thus far the reason for this difference between rats and humans.

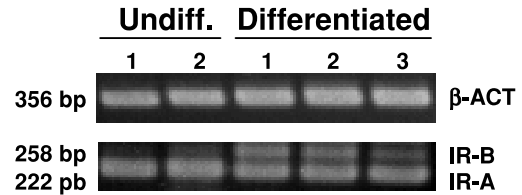
PCR measurements of mRNA obtained from the rat tissues described above revealed IRSs as being differentially expressed as well (Fig. 2). White quadriceps and pancreas are characterized by a larger percentage of

IRS-1 expression as compared with the two other substrates. In addition, it is interesting to note that in these tissues IR-A is preferentially expressed. IRS-3 mRNA levels were higher than those of IRS-1 only in white adipose tissue (IRS-1 vs IRS-3  $P < 0.05$ ). The physiological role of IRS-3 is controversial because targeted disruption of its gene has no effect on either growth or glucose homeostasis (Liu *et al.* 1999). Moreover, humans lack a functional IRS-3 gene (Björnholm *et al.* 2002). Nevertheless, it has been shown that combined deficiency of IRS-1 and IRS-3 in mice results in severe deficiency of white adipose tissue, providing evidence that both substrates are critical for adipogenesis (Laustsen *et al.* 2002).

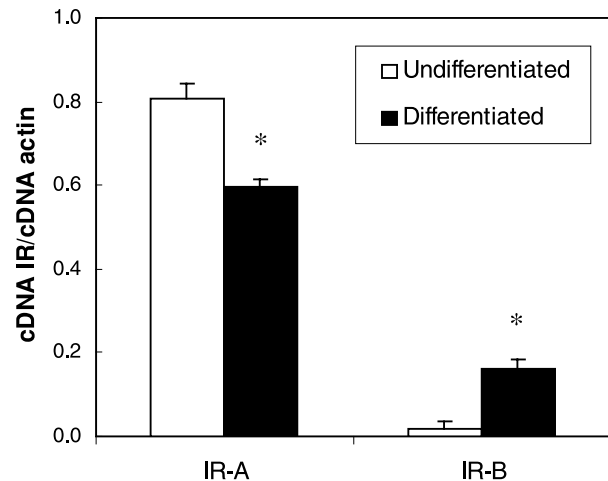
The manifest heterogeneity of IR may contribute to tissue-specific differences in insulin action. Recent studies have shown that signaling through both IR isoforms selectively activates different pathways. In pancreatic  $\beta$ -cells, signaling through IR-A, PI3K-Ia and p70s6k promotes insulin gene transcription, whereas signaling through IR-B, PI3K-II and protein kinase B (PKB) promotes  $\beta$ -glucokinase gene transcription (Leibiger *et al.* 2001). In a murine hematopoietic cell line, IR-A preferentially sends mitogenic, antiapoptotic signals, whereas IR-B tends to send differentiation signals (Sciaccia *et al.* 2003). It seems conceivable that the existence of two different IR isoforms could represent a mechanism for selective insulin action. In concordance with this hypothesis, a more recent work shows a different localization of the IR isoforms in the plasma membrane (Uhles *et al.* 2003). In contrast to these observations, it has recently been reported that both IR isoforms were equivalent in their ability to activate signaling through PKB and MAPK, when expressing either IR-A or IR-B in IR-deficient mice brown preadipocytes (Entigh *et al.* 2003). Thus, beyond the functional differences of both IR isoforms in cultured cell lines, the individual contribution of each isoform in mature tissues, where expression of both receptors occurs naturally, remains to be established in order to understand the function of heterogeneous expression of these IR isoforms in insulin-sensitive tissues.

With regard to the substrates, in this study we were able to detect for the first time the relative mRNA expression of IRSs (IRS-1, -2 and -3) in all rat tissues tested. Previous work in the mouse failed to detect IRS-3 and IRS-2 mRNA expression in skeletal muscle (Sciaccitano & Taylor 1997) and fat (Kulkarni *et al.* 1999) respectively. The heterogeneity of IRS expression may also contribute to tissue-specific differences in insulin action. In fact, studies of knockout mice lacking IRSs indicate that each IRS protein has distinct physiological roles in mediating insulin action in target tissues (reviewed in Sesti *et al.* 2001a).

(A)



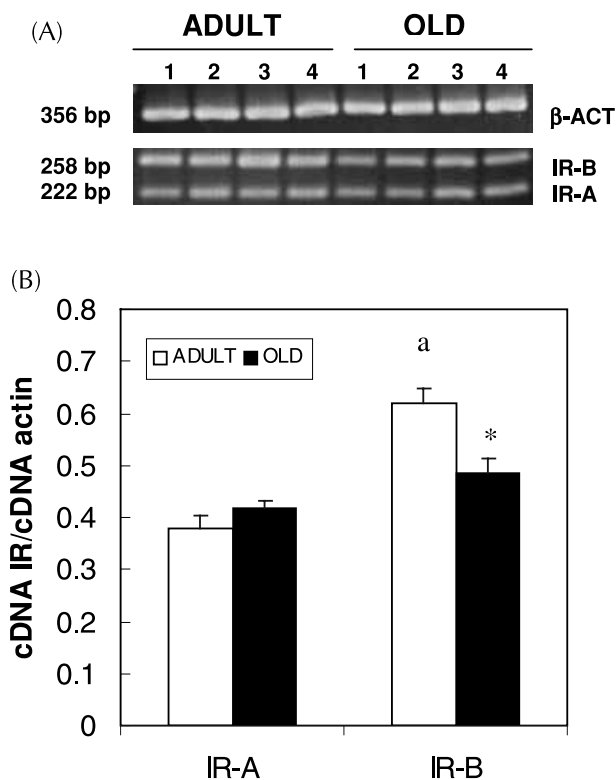
(B)



**Figure 3** Effect of preadipocytes differentiation on the relative expression of the A and B IR mRNA isoforms. RT-PCR of IR isoforms was performed before and after differentiation of preadipocytes in primary culture. RNA was extracted at day 0 (undifferentiated) and at day 14 after induction of differentiation (differentiated). Products of PCR amplification were quantified as the ratio IR cDNA/ $\beta$ -actin cDNA for both undifferentiated and differentiated preadipocytes. Values are means  $\pm$  S.E.M. of two or three determinations on RNA samples derived from separate primary cultures. \* $P < 0.02$  vs undifferentiated preadipocytes.

#### Effect of differentiation on IR gene expression in white adipose tissue

As shown in Fig. 1, IR-B is significantly higher than IR-A in epididymal white adipose tissue (IR-B vs IR-A  $P < 0.001$ ). To investigate whether alternative splicing of IR is regulated by adipogenesis, we carried out differentiation of stromal-vascular preadipocytes isolated from epididymal white adipose tissue, in primary culture. RT-PCR assays for IR isoforms expression was performed with RNA extracted from undifferentiated (day 0) and differentiated (day 14 after induction of differentiation) preadipocytes. In Fig. 3 we show that level of IR-A decreases during preadipocytes differentiation, whereas the expression of IR-B increases significantly. Our results in primary cultured white preadipocytes are consistent with previous observations in 3T3-L1 cells, where a similar isoform shift from IR-A

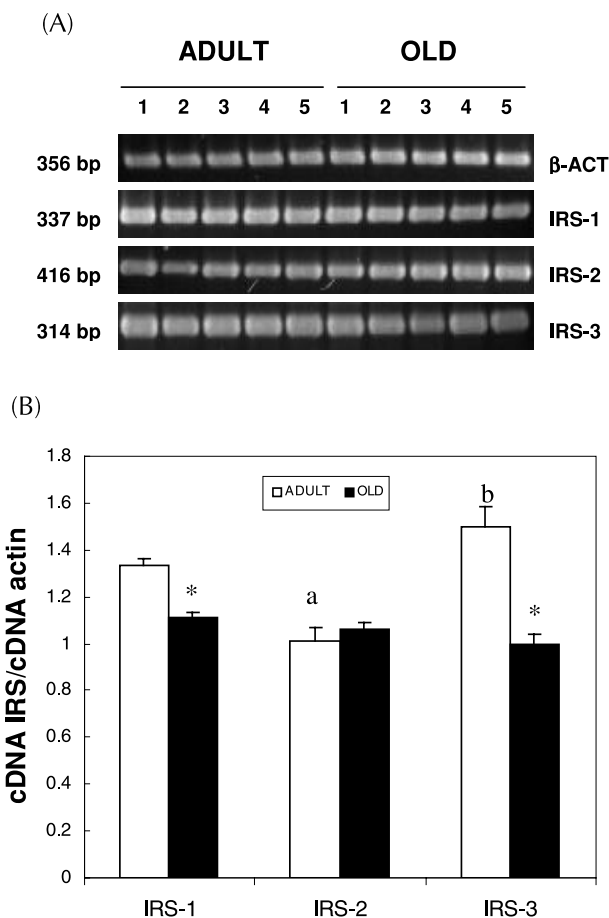


**Figure 4** Effect of aging on the relative expression of the A and B IR mRNA isoforms in rat epididymal adipose tissue. (A) PCR amplification products corresponding to IR-A (222 bp) and IR-B (258 bp), along with  $\beta$ -actin (356 bp) as internal control, were resolved by electrophoresis and visualized under UV light. (B) Products were quantified as the ratio IR cDNA/ $\beta$ -actin cDNA for both adult and old rats. Values are mean  $\pm$  S.E.M. of four determinations on RNA samples derived from separate animals. \* $P < 0.01$  vs adult rats, <sup>a</sup> $P < 0.001$  vs IR-A.

to IR-B has been observed when these established cells are differentiated in the presence of dexamethasone (Kosaki & Webster 1993). In addition, recently there has been reported a similar behavior for brown preadipocytes in culture, where a shift in the alternatively spliced forms of IR from the A to the B isoform during differentiation is also observed (Entingh *et al.* 2003). Taken together, these results suggest that during adipogenesis the alternative splicing of IR may be regulated.

#### Effect of aging on IR and IRS gene expression in white adipose tissue

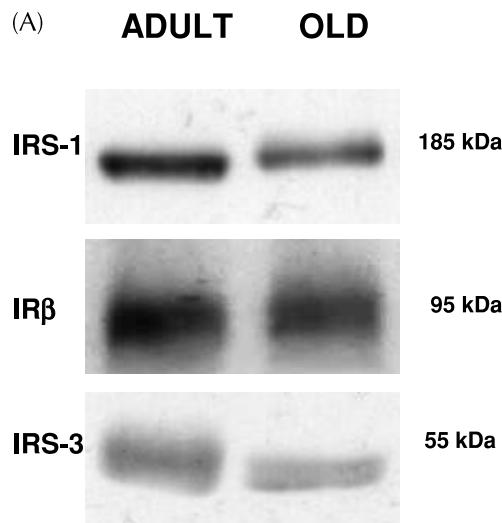
The gene expression of IR isoforms was examined in rat white adipose tissue by RT-PCR in order to investigate whether the insulin resistance of aging is associated with an impaired IR alternative splicing. As shown in Fig. 4, aging causes a significant decrease of IR-B mRNA level (old vs adult  $P < 0.01$ ) in epididymal adipose tissue. On



**Figure 5** Effect of aging on gene expression of IRS-1, 2 and 3 in rat epididymal adipose tissue. (A) PCR amplification products corresponding to IRS-1 (337 bp), IRS-2 (416 bp) and IRS-3 (314 bp), along with  $\beta$ -actin (356 bp) as internal control, were resolved by electrophoresis and visualized under UV light. (B) Products were quantified as the ratio IRS cDNA/ $\beta$ -actin cDNA for both adult and old rats. Values are means  $\pm$  S.E.M. of five determinations on RNA samples derived from separate animals. \* $P < 0.005$  vs adult rats, <sup>a</sup> $P < 0.005$  and <sup>b</sup> $P < 0.05$  vs IRS-1.

the contrary, there was no modification in IR mRNA splicing in retroperitoneal adipose tissue with aging (data not shown).

The effect of aging corresponds to a reduction of the absolute quantity of IR-B, without modification of the IR-A mRNA level. Thus, the ratio IR-A/IR-B increases as occurs in myotonic dystrophy, a different model of insulin resistance (Savkur *et al.* 2001). Changes in expression of IR isoforms have been most extensively studied in T2DM, but in this respect contradictory data have been obtained, with some researchers reporting an increase in IR-B expression in skeletal muscle and adipose tissue of T2DM patients and some others reporting no difference (reviewed in Sesti *et al.* 2001b).



(B)

	IR-β	IRS-1	IRS-3
ADULT	100 ± 2.5	100 ± 2.7	100 ± 7.3
OLD	81.2 ± 4.8 *	67.9 ± 5.5 **	49.7 ± 9.3 *

**Figure 6** Effect of aging on protein expression of IR and IRS-1 and -3 in isolated rat epididymal adipocytes. (A) Representative immunoblot of total protein amount of IR β-subunit, IRS-1 and IRS-3 in adipocytes from adult and old rats; 75 μg total membrane protein (for detection of IR β-subunit) or 75 μg adipocyte homogenates (for detection of IRSs) were subjected to 10% SDS-PAGE under reducing conditions and immunoblotted with specific antibodies. (B) The autoradiographs corresponding to three to six independent experiments were quantified by scanning densitometry. Data are expressed as percentages of that found in adipocytes from adult rats, which were set at 100%, and are means ± S.E.M. \* $P < 0.02$ , \*\* $P < 0.001$  vs adult rats.

The controversy might be caused by variations in subject selection and subject grouping (by insulinemia, glycemia and/or obesity) as suggested by Huang *et al.* (1996), since T2DM is a complex heterogeneous disease with multiple genes contributing to the cause of this disorder.

Regulation of the IR alternative splicing depends on the age of the rat, since in 6-month-old rats the ratio IR-A/IR-B was not altered (Vidal *et al.* 1995), but mRNA expression of IR-B decreased in middle-aged 12-month-old rats (Wiersma *et al.* 1997) as occurs in our model of aging (24-month-old rats).

Gene expression of IRS-1, but not of IRS-3, has been extensively studied in several insulin-resistant states, such

as obesity and T2DM, concluding that these are associated with low expression of IRS-1. Regulation of IRS-1, -2 and -3 expression has also been studied in response to high-fat feeding in epididymal adipocytes (Anai *et al.* 1998). It was demonstrated that a high-fat diet, which induces insulin resistance, up-regulates IRS-3 but down-regulates IRS-1 and -2.

No data are, to our knowledge, available on the effect of aging on IRSs gene expression in white adipose tissue. In the present study we have investigated the effect of aging on mRNA levels of IRS-1, -2 and -3 (Fig. 5). IRS-1 mRNA levels were found to be 17% lower in epididymal adipose tissue from old rats compared with

control adult rats. IRS-2 mRNA levels were similar in both groups of rats. Finally, aging significantly decreased mRNA expression of IRS-3 by 33% ( $P < 0.005$ ).

These new findings clearly show that aging not only induces a significant decrease in the IR-B isoform mRNA expression, but also down-regulates gene expression of IRS-3, and to a lesser degree that of IRS-1, in rat adipose tissue. The results presented herein support the hypothesis that alterations in gene expression of IR isoforms and substrates (IRS-1 and -3) in adipocytes may participate in the molecular mechanism of insulin resistance associated with aging.

### Effect of aging on IR and IRS protein expression in isolated rat epididymal adipocytes

A lower expression of IR-B, without changes in IR-A, denotes a decrease of IR mRNA levels in epididymal adipose tissue from old rats. This result is in good agreement with the amount of IR in total membrane fraction of adipocytes from adult and old rats as assessed by Western blotting with specific antibody (Fig. 6).

Likewise, we examined the total amount of IRS-1 and IRS-3 in adipocyte homogenates from adult and old rats. As can be seen in Fig. 6, aging reduces both IRS-1 (by ~32%) and IRS-3 (by ~50%) protein expression. Again, IRS-3 reduction in response to aging is higher than that of IRS-1 in epididymal adipose tissue.

In old rats a low IRS-1 and IRS-3 protein expression is associated with low mRNA levels, as measured by RT-PCR, suggesting that it is partially due to impaired gene transcription. Nevertheless, the decrease in protein is higher than the decrease in gene expression. It could be possible that the reduced levels of IRS-1 and -3 were also due to enhanced protein degradation in old rat adipose tissue. In fact, regulated degradation of IRS proteins has been proposed as a long-term mechanism of insulin resistance (White 2002).

We have previously shown that insulin-resistant adipocytes from old rats are characterized by decreased IR autophosphorylation and by impaired insulin stimulation of IRS-1-associated PI3K activity, GLUT4 translocation to the plasma membrane, glucose uptake and MAPK activity (Carrascosa *et al.* 1989, Molero *et al.* 1998, 2002). These data can partially be explained with the results presented here because insulin signaling is dependent on IR and IRS activation, and we show that aging down-regulates the expression of IR-B (the isoform considered to transmit the insulin signal more efficiently). In addition, both gene and protein levels of IR, IRS-1 and IRS-3 are also decreased in white adipose tissue from aged rats, suggesting that the down-regulation of early components of the insulin transduction pathway could be related to the insulin resistance associated with the process of aging.

In summary, our results highlight the importance of IRS-3 in white adipose tissue, as long as it is more abundant than IRS-1 and is modulated by aging in a higher degree. Our results also demonstrate that alternative splicing of exon 11 of the IR mRNA is regulated in a differentiation-dependent way and is modified with aging.

### Acknowledgements

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