Age-related changes in insulin receptor mRNA and protein expression in genetically obese Zucker rats

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Abstract

Aim. – The mechanisms underlying the age-related decrease in insulin-receptor (IR) binding in genetically obese Zucker rats are not well understood. For this reason, the present study analyzed the expression of IR mRNA and protein in selected tissues from 1- to 4-month-old obese (fa/fa) Zucker rats and lean (Fa/–) age-matched controls.

Methods. – The following parameters were evaluated: (1) IR mRNA level, and proportion of isotypes A (exon 11–) and B (exon 11+) of IR mRNA in liver, brain and kidney; (2) level, molecular size and tyrosine phosphorylation of IR-β subunit in liver subcellular fractions; and (3) stability of liver IR based on sensitivity in vivo of insulin-binding activity and IR-β levels in response to tunicamycin, a glycosylation inhibitor.

Results. – At one month, IR mRNA level was increased in liver and brain, but decreased in kidneys and, at four months, both mRNA level and isotype B proportion were decreased in liver. From age two months, the following changes in liver IR protein expression were observed: (1) decreased IR-β level in whole homogenates, but increased IR-β levels in endosomal fractions; (2) increased IR-β tyrosine phosphorylation; and (3) at four months, increased levels of both intact IR-β (95 kDa) and IR-β fragments (72 and 52 kDa) in lysosomal fractions, along with decreased stability in vivo of the IR.

Conclusion. – These data show that obese Zucker rats display age-related alterations of IR gene expression at both pre- and post-translational stages and, in particular, increased endocytosis and degradation of IR protein.

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Keywords: Insulin; Insulin receptor; Liver; Rat; Obesity

Résumé

Modifications de l’expression du récepteur de l’insuline et de son ARNm en rapport avec l’âge chez des rats génétiquement obèses Zucker.

Objectifs. – Les mécanismes qui sous-tendent la diminution de la capacité de liaison du récepteur de l’insuline (IR) en rapport avec l’âge chez le rat génétiquement obèse Zucker sont mal compris. Nous avons analysé l’expression du IR et de son ARNm dans plusieurs tissus de rats obèses (fa/fα) Zucker âgés de un à quatre mois et de leurs témoins non obèses (Fa/–).

Méthodes. – Les paramètres suivants ont été étudiés : (1) taux de l’ARNm du IR et proportion de ses isotypes A (exon 11–) et B (exon 11+) dans le foie, le cerveau et le rein ; (2) taux, taille moléculaire et phosphorylation de la sous-unité β du IR dans des fractions subcellulaires de foie ; et (3) stabilité du IR hépatique jugée sur la sensibilité in vivo de la liaison de l’insuline et du taux de la sous-unité β à la tunicamycine, un inhibiteur de la glycosylation.

Résultats. – À l’âge d’un mois, le taux de l’ARNm du IR est augmenté dans le foie et le cerveau mais diminué dans le rein, et à quatre mois le taux de l’ARNm et la proportion de l’isotype B sont diminués dans le foie. À partir de deux mois, les modifications suivantes de l’expression
du IR dans le foie sont observées : (1) une diminution du taux de IR-β dans les homogénats mais une augmentation de celui-ci dans les fractions endosomales ; (2) une augmentation de la phosphorylation de IR-β ; et (3) à quatre mois, une augmentation du taux de IR-β intact (95 kDa) et des fragments d’IR-β (72 et 52 kDa) dans les fractions lysosomales, ainsi qu’une diminution de la stabilité in vivo du IR.

**Conclusion.** Ces données montrent que les rats obèses Zucker présentent des altérations en rapport avec l’âge de l’expression du gène du IR à des étapes pré- et post-traductionnelles, notamment une augmentation de l’endocytose et de la dégradation du IR.

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**Mots clés :** Insuline ; Récepteur de l’insuline ; Foie ; Rat ; Obésité

1. Introduction

The insulin receptor (IR) is a heterotetrameric membrane protein composed of two extracellular α subunits, which bind insulin, and two transmembrane β subunits, which possess in their cytoplasmic domain an insulin-sensitive tyrosine kinase. Each α−β heterodimer is encoded by a single 120-kb gene, comprising 22 exons, and transcribed into several mRNA species. Alternative splicing of exon 11 results in the generation of two IR isoforms that differ by the presence (isotype B) or absence (isotype A) of 12 amino acids at the C terminus of the α subunit.

Although the IR gene is ubiquitously expressed, the levels of IR mRNA and protein, as well as the relative expression of the A and B receptor isoforms, vary among cells and tissues, and are regulated by different agents and environmental conditions. Depending on the agent, regulation of IR gene expression occurs at transcriptional, post-transcriptional and/or post-translational stages. For instance, glucocorticoid hormones upregulate the IR by stimulating IR gene transcription [1], whereas insulin downregulates the IR primarily by increasing IR protein degradation [2], but also by inhibiting IR gene transcription [3].

The genetically obese Zucker (fa/fa) rat, which displays a mutant and functionally defective leptin receptor (I4), and its references], has been widely used to assess insulin-dependent regulation of IR expression and activity in vivo. In a number of studies, insulin binding to liver subcellular fractions [5–8] and isolated hepatocytes [9,10] has been shown to be decreased in hyperinsulinemic obese rats after three months of age. A decrease in insulin-binding activity in skeletal muscle [11], cardiomyocytes [12], brain [13] and brain capillaries [14] of obese Zucker rats has also been reported. Although these changes appear to result from a decrease in IR number, there is limited information on the expression of IR protein in tissues of obese Zucker rats [15–18], and studies of insulin-stimulated autophosphorylation and tyrosine-kinase activity of liver IR [8,16,17,19,20] have found conflicting results. In addition, while the reduced IR expression observed in obese Zucker rats is thought to be a consequence of hyperinsulinemia, the underlying mechanisms are not well understood. At least one mechanism may be increased proteolytic degradation of receptor protein, as prolonged administration of E64, a thiol protease inhibitor, has been shown to normalize immunodetectable IR-β levels in liver, adipose tissue and skeletal muscle in 6-month-old obese rats [15]. However, this observation does not exclude additional, age-dependent, changes affecting pretranslational stages of expression of the IR gene.

To address these issues, 2-week- to 4-month-old obese rats and their age-matched lean littermates were examined for: (1) IR mRNA level and proportion of isotypes A (exon 11−) and B (exon 11+) of IR mRNA in the liver and brain, as well as kidneys, tissues in which the expression of IR protein is reduced [18]; (2) level, molecular size and tyrosine phosphorylation of the IR-β subunit in liver subcellular fractions; and (3) stability in vivo of liver IR protein.

2. Materials and methods

2.1. Animals

Animal studies were conducted in accordance with the institutional guidelines for the use and care of experimental animals. Two-week to 4-month-old male obese (fa/fa) and lean (Fa−/) Zucker rats, bred at INSERM U465 (Centre for Biomedical Research of the Cordeliers, Paris, France), were kept under a 12-h light–dark cycle, and allowed free access to food and water. Unless otherwise indicated, food was withdrawn 16 h before the animals were sacrificed by decapitation. Blood was collected and the plasma stored at −20 °C for determination of glucose (glucose-oxidase method), insulin (radioimmunoassay) and corticosterone (radioimmunoassay). A portion of the liver, the whole brain and kidneys were rapidly removed, immersed in liquid nitrogen and stored at −80 °C for RNA extraction; the remainder of the liver underwent subcellular fractionation.

2.2. RNA isolation and analysis of insulin receptor mRNA and its isoforms

Total RNA was extracted by guanidinium isothiocyanate [21] and subjected to Northern blot analysis, as described elsewhere [22,23]. Blots were hybridized with a [32P]-labelled IR antisense RNA probe, prepared by transcription in vitro of a 2.3-kb cDNA fragment coding for rat IR-α subunit (a gift from Dr Barry Goldstein, Thomas Jefferson University, Philadelphia, PA, USA), and with a [32P]-labelled probe specific for rRNA 18S. Hybridization signals were detected by autoradiography and quantified by scanning densitometry.

Analysis of IR mRNA isoforms was achieved by reverse transcription of RNA, followed by radioactive polymerase chain reaction (PCR) amplification of IR cDNA, as described elsewhere [24]. As primers, oligonucleotides spanning sequences 2530–2550 in exon 10 (sense primer) and 2822–2843 in exon 12 (antisense primer) of rat IR cDNA were used. The 313-bp and 277-bp products, corresponding to the B (exon 11+) and A...
2.3. Liver subcellular fractionation

Livers were homogenized in 0.25 M sucrose containing 1 mM of Na3VO4, 0.5 mM of phenylmethylsulphonyl fluoride (PMSF), 1 mM of benzamidine, 0.2 mg/mL of bacitracin, 20 μg/mL of aprotinin, 5 μg/mL of leupeptin and 2.5 μg/mL of pepstatin, using a Dounce homogenizer. Nuclear, mitochondrial, microsomal and cytosolic fractions were prepared by differential centrifugation. A plasma membrane (PM) fraction [25], lysosomal fraction [26], and combined (GE) or separate intermediate (GEi) and heavy (GEh) endosomal fractions [27] were isolated by flotation from the nuclear, mitochondrial and microsomal fractions, respectively. Fractions were stored at –80°C until they were assayed for protein [28], immunodetectable IR-β and phospho-IR-β, and insulin-binding activity [7].

2.4. Western blot analysis of insulin receptor-β subunit and tyrosine-phosphorylated β subunit

Western blot analysis of the IR-β subunit was carried out using rabbit polyclonal anti-IR-β antibody C19 (Santa Cruz Biotechnology), as described elsewhere [29]. Equal amounts of cell fraction protein (10–20 μg) in Laemmli sample buffer were subjected to sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), followed by transfer to nitrocellulose membranes. Immunoreactive proteins were revealed using horseradish-peroxidase (HRP)-conjugated goat anti-rabbit IgG (Biorad) and enhanced chemiluminescence (ECL) reagents (Amersham Pharmacia Biotech), as described elsewhere [29], immunodetectable IR-β and phospho-IR-β, and insulin-binding activity [7].

This was estimated from the decrease in liver insulin-binding activity and IR-β levels induced by tunicamycin, an inhibitor of protein N-linked glycosylation, which blocks the maturation of newly synthesized receptors. Obese and lean rats were sacrificed 12 h after intraperitoneal (IP) injection of tunicamycin (0.2 mg/100 g body weight), conditions previously shown to result in a 50% decrease in the number of insulin-binding sites in liver plasma membranes of Sprague–Dawley rats [30].

2.6. Statistical analysis

Data are presented as means ± SEM, and analyzed by ANOVA using StatView software.

3. Results

3.1. Animals’ characteristics

The main characteristics of the 1- to 4-month-old fasted Zucker rats are shown in Table 1. Compared with lean animals, obese rats showed a significant increase in body weight, liver weight and plasma insulin concentration, which was detectable at one month and became more pronounced with age. Obese rats also showed a moderate, albeit significant, increase in plasma corticosterone at one month, and in plasma glucose at four months. Body weight, liver weight and plasma insulin were also increased in 1- to 3-month-old fed obese rats, but were unchanged in 2-week-old suckling preobese animals (data not shown).

3.2. Tissue insulin receptor mRNA levels and isotype expression

IR mRNA expression in Zucker rat tissues was characterized and quantified by Northern blot analysis. In liver, two major transcripts (9.5 kb and 7.5 kb) were identified, regardless of age and phenotype, a pattern typical of rodent species (Fig. 1A). Compared with lean rats, the abundance of these transcripts in

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Body weight (g)</th>
<th>Liver weight (g)</th>
<th>Plasma insulin (ng/mL)</th>
<th>Plasma glucose (mmol/L)</th>
<th>Plasma corticosterone (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 L 64.2 ± 2.3</td>
<td>3.39 ± 0.13</td>
<td>0.25 ± 0.04</td>
<td>6.70 ± 0.60</td>
<td>86 ± 28</td>
<td></td>
</tr>
<tr>
<td>1 Ob 78.6 ± 1.5***</td>
<td>4.39 ± 0.09***</td>
<td>0.38 ± 0.04*</td>
<td>6.59 ± 0.21</td>
<td>106 ± 22*</td>
<td></td>
</tr>
<tr>
<td>2 L 230 ± 11</td>
<td>9.75 ± 0.81</td>
<td>0.33 ± 0.06</td>
<td>8.28 ± 0.18</td>
<td>282 ± 169</td>
<td></td>
</tr>
<tr>
<td>2 Ob 311 ± 11**</td>
<td>11.8 ± 0.67*</td>
<td>1.02 ± 0.08**</td>
<td>8.69 ± 0.40</td>
<td>464 ± 140</td>
<td></td>
</tr>
<tr>
<td>3 L 315 ± 2</td>
<td>9.29 ± 0.38</td>
<td>0.50 ± 0.05</td>
<td>8.01 ± 0.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Ob 443 ± 22**</td>
<td>14.7 ± 1.39**</td>
<td>1.75 ± 0.10**</td>
<td>9.02 ± 0.71</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 L 360 ± 23</td>
<td>9.67 ± 0.3</td>
<td>1.30 ± 0.17</td>
<td>7.26 ± 0.21</td>
<td>644 ± 51</td>
<td></td>
</tr>
<tr>
<td>4 Ob 524 ± 38**</td>
<td>16.3 ± 1.9*</td>
<td>4.71 ± 0.36**</td>
<td>9.82 ± 0.32***</td>
<td>786 ± 41</td>
<td></td>
</tr>
</tbody>
</table>

Rats were sacrificed after a 16-h fast; values are expressed as the mean ± SEM of five to eight determinations using different rats; *P < 0.05; **P < 0.01; ***P < 0.001 (between L and Ob rats).
Fig. 1. Age-related expression of liver IR mRNA and isotypes in obese (Ob) and lean (L) Zucker rats: (A) RNA was subjected to Northern blot analysis, using \([^{32}P]\)-labelled probes for IR and 18S rRNA; these typical autoradiograms show the 9.5-kb and 7.5-kb IR transcripts, and 1.9-kb 18S rRNA. Quantification of the IR transcripts has been normalized to 18S rRNA and expressed relative to the mean value of the 9.5-kb transcript in L rats; (B) partial sequences of A (exon 11–) and B (exon 11+) IR cDNA isotypes were generated, separated and quantified, with autoradiograms showing the 277-bp and 313-bp products corresponding to those respective isotypes. The amount of A product is expressed as the percentage of total products. Results are presented as the mean \(\pm\) SEM of at least five determinations using different livers. *\(P<0.05\) (between L and Ob rats).

Obese rats was significantly increased at one month, unchanged at two and three months, and significantly decreased at four months. In brain and kidneys, two IR transcripts (9.5 kb and 7.5 kb) were also identified (results not shown). The abundance of these transcripts in obese rats was significantly increased in brain and decreased in kidneys at one month, but was unchanged in the other age groups (Table 2).

The relative expression of IR mRNA encoding the A (exon 11–) and B (exon 11+) IR isotypes was assessed from the proportions of 277-bp and 313-bp products (corresponding to the A and B isotypes, respectively) generated from IR mRNA by RT-PCR. The liver of lean rats predominantly expressed the B isoform, with the A isoform accounting for only about 12% of total products at one month, and 8% at 2–4 months (Fig. 1B). Compared with lean rats, the proportion of A isotype in liver of obese rats was significantly increased at four months, but unchanged in the other age groups. Kidney predominantly expressed the B isoform, albeit to a lesser extent than the liver, whereas brain predominantly expressed the A isoform (Table 2). Regardless of age, the proportion of A isoform in brain and kidneys was similar in both lean and obese rats.

3.3. Western blot analysis of liver insulin receptor-β subunit and tyrosine-phosphorylated β subunit

Liver subcellular fractions from 1-to 4-month-old rats were subjected to Western blot analysis, using an antibody against the IR-β subunit (Fig. 2). Regardless of age, phenotype and fraction, a major protein of 95 kDa, the expected size of intact IR-β, was detectable. At one month of age, this protein was...
immunoblotted, using anti-IR-\(^{/H9252}\)

fractions of obese rats were accompanied by changes in IR-\(^{/H9252}\) degradation within, lysosomes.

1.00 ±

2 months L 1.00 ±

1 month L 1.00 ±

3 months L 1.00 ±

increases in intact IR-\(^{/H9252}\) in lysosomes, the lysosomal fraction of 4-month-old rats was observed in obese rats. These results suggest that, in obese rats, intact IR-\(^{/H9252}\) expressed two additional proteins of about 72 kDa and 52 kDa, expressed mainly intact 95-kDa IR-\(^{/H9252}\) subunits in this fraction.

Ob rats).

Brain Kidneys Brain Kidneys

<table>
<thead>
<tr>
<th>Age</th>
<th>mRNA levels (arbitrary units)</th>
<th>A isotype mRNA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9.5 kb</td>
<td>7.5 kb</td>
</tr>
<tr>
<td>2 weeks</td>
<td>L</td>
<td>1.00 ± 0.40</td>
</tr>
<tr>
<td></td>
<td>Ob</td>
<td>1.29 ± 0.16</td>
</tr>
<tr>
<td>1 month</td>
<td>L</td>
<td>1.00 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>Ob</td>
<td>1.21 ± 0.06*</td>
</tr>
<tr>
<td>2 months</td>
<td>L</td>
<td>1.00 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>Ob</td>
<td>1.04 ± 0.06</td>
</tr>
<tr>
<td>3 months</td>
<td>L</td>
<td>1.00 ± 0.39</td>
</tr>
<tr>
<td></td>
<td>Ob</td>
<td>0.79 ± 0.37</td>
</tr>
</tbody>
</table>

Rats were sacrificed in the fed state; levels of 9.5-kb and 7.7-kb species of IR mRNA, and proportion of A (exon 11–) isotype of IR mRNA were determined as described in the legend to Fig. 1; all values are expressed as the means ± SEM of five to six determinations using different rats; *P < 0.05; **P < 0.01 (between L and Ob rats).

expressed at similar levels in lean and obese rats. After two months, however, IR-β level in obese rats underwent progressive changes compared with their age-matched lean rats, albeit variable, depending on the fraction. IR-β level decreased in homogenates, reaching about 65% of control values at three months and 45% at four months, as well as in the microsomal fraction, reaching about 75% of control values at three months. However, IR-β levels increased markedly (eightfold at three months) in the endosomal fraction and moderately (60%) in the PM fraction. These results indicate an age-related decrease in total cellular IR-β in the liver of obese rats, along with increased IR-β levels in endosomes.

Consistent with previous studies [5,7,8], the age-related decrease in IR-β level seen in the homogenates and microsomal fractions from obese rats was matched by a parallel decrease in insulin-binding activity, albeit less marked (Fig. 2). Likewise, the eightfold increase in IR-β in the endosomal fraction of 3-month-old obese rats was associated with a fourfold increase in insulin-binding activity. These results suggest changes in IR-α expression comparable to those affecting IR-β. Unexpectedly, however, the 60% increase in IR-β in the PM fraction of 3-month-old obese rats occurred in the face of a 25% decrease in insulin-binding activity, suggesting differential expression of the IR-α and IR-β subunits in this fraction.

As degradation of the internalized IR is thought to occur in lysosomes, the lysosomal fraction of 4-month-old rats was immunoblotted together with the endosomal fraction using anti-IR-β antibody (Fig. 3). Unlike the endosomal fraction, which expressed mainly intact 95-kDa IR-β, the lysosomal fraction expressed two additional proteins of about 72 kDa and 52 kDa, which were probably proteolytic fragments of IR-β. Significant increases in intact IR-β in the endosomal fraction, and of both intact IR-β and IR-β fragments in the lysosomal fraction, were observed in obese rats. These results suggest that, in obese rats, internalized IR-β undergoes an increased association with, and degradation within, lysosomes.

To assess whether the changes in IR-β level in liver-cell fractions of obese rats were accompanied by changes in IR-β tyrosine phosphorylation, IRSs were immunoprecipitated and immunoblotted, using anti-IR-β and anti-phosphotyrosine antibodies (Fig. 4). When normalized to IR-β, phospho-IR-β levels were significantly increased in 2- and 3-month-old obese rats, reaching maximum at three months (an approximately three- to fourfold increase in the homogenate and PM fractions, and a two- to threefold increase in the endosomal fraction). These changes presumably were the result of the increase in plasma insulin concentrations that occurs with age in obese rats.

3.4. Liver insulin receptor protein stability

To assess this, the effects of tunicamycin treatment on liver insulin-binding activity (Fig. 5A) and IR-β level (Fig. 5B) in 4-month-old Zucker rats were examined. Compared with their lean littersmates, untreated obese rats showed 15–20% lower insulin-binding activity in microsomal and PM fractions, but 40–150% greater binding activity in endosomal fractions. In both groups of rats and regardless of fraction, tunicamycin treatment led to a significant decrease in insulin-binding activity compared with untreated controls. However, the relative decrease was significantly greater in obese rats (75–80% in microsomal and PM fractions; 60–70% in endosomal fractions) than in lean rats (50% in microsomal and PM fractions; 25–30% in endosomal fractions), indicating a shorter receptor half-life in obese rats. With tunicamycin treatment, IR-β level in the endosomal fraction also decreased to a greater extent in the obese compared with lean rats. These findings suggest reduced stability of both IR-α and IR-β subunits in 4-month-old obese Zucker rats.

4. Discussion

The studies presented here demonstrate that obese Zucker rats display age-related changes in the level of IR mRNA in liver, brain and kidneys, and in the proportions of A and B isoforms of IR mRNA in liver. Obese Zucker rats also display, from two months of age, several changes in liver IR protein expression, including: (1) decreased IR-β in whole homogenates, but increased IR-β in endosomal fractions; (2) increased IR-β tyrosine phosphorylation; and (3) at four months, increased levels...
of both intact IR-β and IR-β fragments in lysosomal fractions, along with decreased IR stability in vivo. Taken altogether, these data indicate that obese Zucker rats display age-related changes in IR gene expression at both pre- and post-translational stages and, notably, increased endocytosis and degradation of IR protein. As leptin has been shown to elicit direct effects on cell function and gene expression in insulin-sensitive cells ([31], and its references), the involvement of defective leptin signalling [4] in these changes should be considered. However, no effects of leptin on IR gene expression in isolated cells have so far been described and, thus, the altered expression of this gene in obese Zucker rats is probably not a direct consequence of the lack of functional leptin receptors.

4.1. Insulin receptor mRNA expression

Consistent with previous studies using rodent tissues [32,33], the liver, kidneys and brain of Zucker rats were shown to express, on Northern blot analysis, two major IR mRNA species of 9.6 kb and 7.4 kb. In obese rats, the abundance of these transcripts was increased in liver and brain, but decreased in kidneys at one month, and decreased in liver at four months. Unlike the late decrease in liver IR mRNA, the early increase in liver IR mRNA was not matched by a parallel change in IR protein (see below). Increased IR mRNA in the face of decreased IR protein has been previously observed in the skeletal muscle of 10-week-old obese Zucker rats [34], and in the liver of genetically diabetic (db/db) and obese (ob/ob) hyperinsulinaemic mice [33].

As plasma concentrations of corticosterone and insulin are increased in obese Zucker rats, the early increase in liver and brain IR mRNA levels might be linked to hypercortisoleraemia, and the late decrease in liver IR mRNA to hyperinsulinaemia. Glucocorticoids have been shown to upregulate IR mRNA levels in cultured cells and liver in vivo [32,35,36]. On the other hand, although not affecting IR mRNA levels in IM9 lymphocytes [36], FaO hepatoma cells [24] and primary cultured hepatocytes [37], insulin has been shown to downregulate IR mRNA in HepG2 hepatoma cells [38] and AR42J pancreatic acinar cells [39]. Furthermore, insulin has recently been shown
Consistent with previous studies on rat tissues [41,42], liver and kidney of Zucker rats were found to predominantly express the B (exon 11 +) IR mRNA isotype, while brain predominantly expressed the A (exon 11–) isotype. In addition, a small, but significant, increase in the relative expression of the A isotype was observed in the liver of 4-month-old obese rats. Whether this is linked to the hyperinsulinaemia and/or mild hyperglycaemia (Table 1) displayed by obese rats is unclear, given the lack of reproducible effects of insulin and glucose on IR mRNA isotype expression in hepatocytes and hepatoma cells [24,37,43].

4.2. Insulin receptor protein expression and stability

A fourfold decrease in IR-β subunit level in immunopurified liver lysates has been observed in 6-month-old female obese Zucker rats [15]. Our studies, showing an age-related decrease in IR-β in whole-liver homogenates along with increased IR-β levels in endosomal and lysosomal fractions, confirm and extend this observation. The latter changes presumably reflect increased endocytosis of the IR induced by hyperinsulinaemia, as an increase in IR-β in endosomal fractions has also been observed after bolus administration of insulin in adult Sprague–Dawley rats [29,44]. However, in contrast to what was observed in insulin-injected rats, IR-β level in the PM fraction of obese Zucker rats was not decreased but, instead, increased. This suggests that the effects of hyperinsulinaemia on liver IR endocytosis may differ under acute and chronic conditions.

Unlike the endosomal fractions, in which the 95-kDa β subunit was the major immunodetectable protein, the lysosomal fraction in Zucker rats expressed mostly two proteins of around 72 kDa and 52 kDa, which were presumably proteolytic fragments of IR-β. In addition, the level of both intact IR-β and IR-β fragments in the latter fraction was increased in obese rats, suggesting increased accumulation and degradation of internalized IR in lysosomes. A single 52-kDa protein was also immunodetected in the cytosol (data not shown). These fragments may be related to the 47-kDa and 50-kDa IR-β fragments identified in liver lysosomal fractions of insulin-treated Sprague–Dawley rats [37], and to the 61-kDa fragment detected in the cytosol of insulin-treated 3T3-C2 fibroblasts [45] and 3T3-L1 adipocytes [15]. Interestingly, the 61-kDa fragment has also been detected in tissue lysates of 6-month-old obese Zucker rats, and administration of the protease inhibitor E64 caused a decrease of the level of this fragment in muscle, adipose tissue and kidneys, but a paradoxical increase in liver [15].

Recent Western blot and immunoperoxidase labelling studies have shown that the expression of both IR-α and IR-β subunits is reduced in the kidneys of 12-week-old obese Zucker rats [18]. In our present study, the age-related changes in IR-β level in liver homogenates and endosomal fractions of obese rats were matched by comparable changes in insulin-binding activity, suggesting parallel changes in IR-α and IR-β level in these fractions. In addition, using a polyclonal antibody directed against an internal epitope of IR-α, we have observed, on Western blots, an increased level of IR-α in endosomal fractions, and of both intact and truncated IR-α in lysosomal fractions, in 4-month-old obese Zucker rats (data not shown).
Contrary to expectation, however, the PM fraction of 3-month-old obese rats displayed decreased insulin-binding activity in the face of increased IR-β subunit level. The latter observation suggests differential expression of IR-α and IR-β in the PM fraction, perhaps linked to increased degradation of the IR-α subunit.

The capacity of insulin to accelerate IR protein degradation in isolated cells is well established [2]. The use of tunicamycin, which blocks maturation of the IR, showed that, based on the extent of decreased insulin-binding activity and IR-β levels in cell fractions induced by the drug, the stability of liver IR is markedly reduced in 4-month-old obese rats. This probably reflects long-term hyperinsulinaemia, as neither IR levels [29,46] nor IR half-life, as determined by tunicamycin sensitivity in vivo [46], are affected by bolus insulin injection. Along with the ability of E64 to normalize expression of liver IR-β [15], our results indicate that increased proteolysis of IR protein, presumably induced by hyperinsulinaemia, contributes to its reduced expression in older obese Zucker rats.

4.3. Insulin receptor-β subunit phosphorylation state

Previous studies of insulin-induced IR autophosphorylation and tyrosine-kinase activity in the liver of obese Zucker rats have yielded conflicting results. In some studies, in vitro, insulin-induced IR autophosphorylation and phosphorylation of a synthetic peptide bearing the IR sequence 1142–1153 were decreased [20], and tyrosine phosphorylation of IR-β in response to intraperitoneally injected insulin was also either decreased [17] or unchanged [16]. In one of these studies, increased phosphorylation of IR-β at Ser 994 was implicated in the reduced tyrosine phosphorylation [17]. In two other studies, however, liver IR in obese rats showed a twofold increase in both autophosphorylation and the ability to phosphorylate the synthetic substrate poly(−Glu, Tyr) in response to insulin in vitro, which was reversed on lowering plasma insulin by fasting or with streptozotocin treatment [8,19]. Our present results, which found an age-related increase in tyrosine-phosphorylated IR-β levels in liver subcellular fractions of obese rats, are consistent with those latter two studies. In addition, our findings suggest that the liver insulin resistance displayed by obese Zucker rats may mostly be the result of impairment of insulin-signalling stages downstream of the IR.

Conflict of interest

No potential conflict of interest relevant to this submitted article is reported.

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References
