Effects of aging on the responsiveness and sensitivity of glucose metabolism to insulin in the incubated soleus muscle isolated from Sprague–Dawley and Wistar rats

Brendan LEIGHTON,* George D. DIMITRIADIS, Mark PARRY-BILLINGS, Fred J. LOZEMAN† and Eric A. NEWSHOLME

Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, U.K.

INTRODUCTION

The ability to control precisely the blood glucose level in man and other experimental animals declines with age. Many studies of elderly subjects have demonstrated impaired tolerance to either oral [1,2] or intravenous [3,4] glucose loads. This impairment appears to begin in mid-life (about 30–40 years [5]). In some studies the plasma insulin level is elevated during the period of glucose intolerance after an oral glucose load, which suggests that insulin resistance, at least in part, may be responsible for the decline in glucose tolerance [6]. Use of the hyperglycaemic/hyperinsulinaemic clamp has indicated that resistance is due to changes in the sensitivity of the periphery, rather than the liver, to insulin [3]; and the peripheral resistance appears to be a post-insulin-receptor defect [4]. However, there is little information as to which aspect of carbohydrate metabolism becomes resistant to insulin with age.

Skeletal muscle is the major site for insulin-stimulated glucose disposal in rat [7] and man [3]. For example, over 70 % of an infused glucose load is disposed of in human skeletal muscle under either euglycaemic or hyperglycaemic/hyperinsulinaemic-clamp conditions [8].

In order to gain some understanding of the mechanism of insulin resistance in skeletal muscle, the effects of insulin on glucose metabolism in the isolated incubated soleus muscle of the rat is commonly investigated [9,10]. However, little consideration has been given to the effects of age or strain of the rats: in particular, Sprague–Dawley and Wistar strains of rats appear to be used in a non-systematic manner [11–14]. As both strains of rats age, they exhibit marked hyperinsulinaemia [13,15–17] and either mild hyperglycaemia [13,17] or normoglycaemia [15,16]. There is abundant evidence which suggests that insulin-stimulated glucose disposal is impaired in vivo in older Sprague–Dawley rats [16,18] in both isolated adipocytes [19] and hindlimb preparations [13,18]. Recent studies on conscious Wistar rats in vivo demonstrated that whole-body insulin-stimulated glucose disposal declines with age [15]. We considered it important to investigate the effect of aging on the responses of glucose transport, lactate release (glycolysis) and glycogen synthesis to various concentrations of insulin in the isolated incubated soleus muscle prepared from either Sprague–Dawley or Wistar rats. Goodman et al. [13] have measured the rates of insulin-stimulated glucose disposal and 2-deoxyglucose transport and phosphorylation in perfused rat hindlimb in aging Sprague–Dawley rats, but the present study differs in the following ways: an isolated incubated muscle preparation was used; the effect of aging on insulin sensitivity in skeletal muscle from two rat strains was measured; the effect of aging on partitioning of insulin-stimulated intracellular glucose metabolism was measured; the rates of insulin-stimulated glucose transport were monitored with the non-metabolizable glucose analogue 3-O-methyl-D-glucose.

MATERIALS AND METHODS

Animals

Male Wistar and Sprague–Dawley rats (Harlan–Olac, Bicester, Oxon., U.K.) were purchased at 3 weeks of age and were kept in the Department’s animal quarters until killed at the ages indicated in the Results and discussion section. The animals were housed in controlled conditions (23 ± 1 °C; 12 h-light/dark cycle) and received standard laboratory chow and water ad libitum, except for the...

* To whom correspondence and reprint requests should be addressed.
† Present address: Howard Hughes Research Laboratories, University of Washington School of Medicine, Seattle, WA 98195, U.S.A.
results and discussion

Two strains of rats were used in the present study: Sprague–Dawley and Wistar. Four groups of Sprague–Dawley and three groups of Wistar rats were used: for each strain, respectively, the youngest were 5 and 6 weeks and the oldest were 13 and 85 weeks. The mean lengths of life of male Sprague–Dawley and Wistar rats (that are not barrier maintained) are about 101 and 107 weeks respectively [30].

There was no effect of aging, in general, on basal responses of lactate formation in insulin in any of the soleus-muscle preparations, except in those muscles from 85-week Wistar rats. In this instance the rate of lactate formation was increased at 1 but not 10 μunits of insulin/ml. In isolated soleus muscle from Sprague–Dawley rats the rates of lactate formation were markedly decreased at 8 and 13 weeks, compared with 6 weeks, at 100, 1000 and 10000 μunits of insulin/ml (Table 1). In stripped soleus muscle from Sprague–Dawley rats the concentration of insulin required to stimulate lactate formation half-maximally (EC₅₀ value) increased with age (Table 1). In contrast, in muscles from Wistar rats there was little change in the EC₅₀ value for insulin (Table 2).

In skeletal-muscle preparations from Sprague–Dawley rats there was a marked decrease in the response of glycogen synthesis to 100 μunits of insulin/ml at 13 weeks compared with 8 weeks (Table 1). Consequently, the insulin EC₅₀ values for glycogen synthesis were higher in muscles from the 7-, 8- and 13-week-old animals compared with those from 5-week-old animals (Table 1). In marked contrast, in muscles from Wistar rats the EC₅₀ values for insulin for glycogen synthesis were not changed from 6 to 12 weeks; it was increased dramatically in the 85-week animals (Table 2). In the present study it was considered that 10000 μunits of insulin/ml gave a near-maximal response. However, the possibility that the rate of glycogen synthesis is higher at a higher concentration of insulin cannot be ruled out. Thus the EC₅₀ value calculated from the present results may be an underestimate.

The effects of aging on insulin-stimulated rates of hexose transport in isolated incubated skeletal-muscle preparations has never previously been reported. Therefore the rates of hexose transport into skeletal muscle were measured with the non-metabolizable glucose analogue 3-O-methyl-d-glucose. The rates of transport of 3-O-methyl-d-[³H]glucose into muscles from 5- and 13-week-old Sprague-Dawley and 6- and 85-week-old Wistar rats are given in Table 3. In soleus muscles from young animals, insulin stimulated the rates of uptake of the analogue in vitro in a concentration-dependent manner. However, insulin at 100 μunits/ml did not stimulate hexose transport in soleus muscle from 13-week-old Sprague–Dawley rats: in contrast, this concentration of insulin stimulated the rate of 3-O-methyl-d-glucose transport in muscles from older Wistar rats to a similar extent to that in the younger rats. These results are qualitatively similar to those obtained for lactate release.

The decrease in peripheral glucose disposal in elderly humans has mainly been attributed both to a post-receptor decrease in the response of glucose metabolism to insulin in peripheral tissues (i.e. skeletal muscle [3,4]) and to diminished muscle mass [31]. There is no decrease
Table 1. Effects of various concentrations of insulin on rates of lactate release and glycogen synthesis in incubated stripped soleus muscles isolated from Sprague–Dawley rats

Soleus muscles were isolated from 14 h-fasted rats. Values are presented as means±S.E.M. for the numbers of separate experiments given in parentheses. The concentration of insulin required for half-maximal stimulation of either process (EC$_{50}$ value) is also shown. The statistical significance (Student's $t$ test) of difference between all groups compared with 5-week-old rats is indicated by * ($P < 0.05$), ** ($P < 0.01$) or *** ($P < 0.001$).

<table>
<thead>
<tr>
<th>Insulin concn. (μunits/ml)</th>
<th>Age (weeks)…</th>
<th>Rate of lactate formation (μmol/h per g wet wt.)</th>
<th>Rate of glycogen synthesis (μmol of glucosyl units)/h per g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>1</td>
<td>9.67±0.38 (18)</td>
<td>8.53±0.81 (7)</td>
<td>8.62±0.54 (10)</td>
</tr>
<tr>
<td>10</td>
<td>10.99±0.49 (19)</td>
<td>9.03±0.88 (8)</td>
<td>8.98±0.60 (10)*</td>
</tr>
<tr>
<td>100</td>
<td>14.99±0.70 (19)</td>
<td>10.44±0.86 (8)*</td>
<td>10.37±0.52 (10)*</td>
</tr>
<tr>
<td>1000</td>
<td>15.32±0.58 (18)</td>
<td>11.94±0.92 (8)*</td>
<td>11.81±0.66 (9)*</td>
</tr>
<tr>
<td>10000</td>
<td>14.70±0.32 (12)</td>
<td>12.54±0.50 (8)*</td>
<td>12.57±0.60 (9)*</td>
</tr>
<tr>
<td>EC$_{50}$ (μunits/ml)…</td>
<td>24</td>
<td>119</td>
<td>150</td>
</tr>
</tbody>
</table>

Table 2. Effects of various concentrations of insulin on rates of lactate release and glycogen synthesis in incubated stripped soleus muscles isolated from Wistar rats

Soleus muscles were isolated from overnight-fasted rats. Values are presented as means±S.E.M. for the numbers of separate experiments given in parentheses. The concentration of insulin required for half-maximal stimulation of either process (EC$_{50}$ value) is also shown. The statistical significance (Student's $t$ test) of differences between all groups compared with 6-week-old rats is indicated by * ($P < 0.05$), ** ($P < 0.001$) or *** ($P < 0.001$).

<table>
<thead>
<tr>
<th>Insulin concn. (μunits/ml)</th>
<th>Age (weeks)…</th>
<th>Rate of lactate formation (μmol/h per g wet wt.)</th>
<th>Rate of glycogen synthesis (μmol of glucosyl units)/h per g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6</td>
<td>12</td>
<td>85</td>
</tr>
<tr>
<td>1</td>
<td>8.60±0.33 (20)</td>
<td>8.10±0.17 (9)</td>
<td>12.26±0.79 (9)**</td>
</tr>
<tr>
<td>10</td>
<td>10.18±0.48 (21)</td>
<td>8.00±0.45 (9)*</td>
<td>9.96±0.48 (8)</td>
</tr>
<tr>
<td>100</td>
<td>12.64±0.45 (24)</td>
<td>12.32±0.93 (9)</td>
<td>13.87±0.96 (9)</td>
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<tr>
<td>1000</td>
<td>16.42±0.58 (21)</td>
<td>13.80±1.04 (9)</td>
<td>14.66±0.67 (9)</td>
</tr>
<tr>
<td>10000</td>
<td>17.18±0.57 (20)</td>
<td>12.75±0.50 (8)*</td>
<td>16.10±0.80 (8)</td>
</tr>
<tr>
<td>EC$_{50}$ (μunits/ml)…</td>
<td>100</td>
<td>50</td>
<td>65</td>
</tr>
</tbody>
</table>
### Table 3. Effects of aging on insulin-stimulated rates of 3-O-methyl-d-[3H]glucose transport into stripped soleus muscle from Sprague–Dawley and Wistar rats

Values are presented as means ± S.E.M. for at least four separate experiments. The statistical significance (Student's t test) of the difference between responses of hexose transport muscles for older rats compared with younger animals is indicated by * (P < 0.05). Muscles from Sprague–Dawley and Wistar rats were incubated in the presence of 2 and 1 μCi of 3-O-methyl-d-[3H]glucose/ml respectively.

<table>
<thead>
<tr>
<th>Insulin conc. (μunits/ml)</th>
<th>Rate of 3-O-methyl-d-glucose transport (d.p.m./20 min per mg wet wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sprague–Dawley (5 weeks)</td>
</tr>
<tr>
<td>10</td>
<td>984 ± 110</td>
</tr>
<tr>
<td>100</td>
<td>1465 ± 75</td>
</tr>
<tr>
<td>1000</td>
<td>1683 ± 211</td>
</tr>
<tr>
<td>Wistar (6 weeks)</td>
<td>465 ± 33</td>
</tr>
<tr>
<td>100</td>
<td>633 ± 36</td>
</tr>
<tr>
<td>1000</td>
<td>742 ± 29</td>
</tr>
</tbody>
</table>

in muscle mass in 2-year-old Sprague–Dawley rats, but muscle mass diminishes in Wistar rats between 18 and 28 months (similar to the age range for the rats used in this study) [13,32,33]. Furthermore, the insulin resistance in soleus muscle from Sprague–Dawley rats is exhibited while these rats are rapidly growing (Table 1). Therefore, if diminished glucose disposal in humans in mid-life is indeed due to diminished muscle mass and to unresponsiveness of skeletal muscle to insulin, then Wistar rats appear to be the better model of human aging.

Future studies concerned with the insensitivity of skeletal muscle in older rats to insulin must take into account the strain of rat employed. The factors that lead to development of insulin resistance in skeletal muscle from Sprague–Dawley rats have previously been discussed in detail [16]. Any factors which decrease the sensitivity of glycogen synthesis, but not glucose transport, to insulin in skeletal muscle may explain the insulin-resistant state in aging Wistar rats [15]. The results obtained for soleus muscle from 85-week Wistar rats (the present paper) are strikingly similar to those reported for isolated soleus muscle incubated with either 1 μM human pancreatic amylase or the neuropeptide calcitonin-gene-related peptide (CGRP) [34]. Tissue contents and plasma levels of CGRP-like immunoreactivity (because both peptides are homologous, this may be amylase or CGRP) are reported to be elevated in aging Wistar rats [35,36]. The possibility that increased rates of production and/or release of either amylase or CGRP cause insulin resistance in skeletal muscle in aging animals warrants further investigation.

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### REFERENCES


1989
Effects of aging on insulin sensitivity in skeletal muscle


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