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

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1. The effects of aging on the sensitivity and responsiveness of glucose transport, lactate formation and glycogen synthesis to insulin were studied in the incubated stripped soleus muscle isolated from aging Sprague–Dawley and Wistar rats. 2. As Sprague–Dawley rats aged from 5 to 13 weeks, there were marked increases in the concentrations of insulin that were required for half-maximal stimulation (i.e. EC_{50} value, which is a measure of sensitivity) of glucose transport, lactate formation and glycogen synthesis. 3. In marked contrast, there were no alterations in sensitivities of any of these processes to insulin in soleus muscle prepared from Wistar rats aged between 6 and 12 weeks. 4. However, in soleus muscles from 85-week-old Wistar rats the rates of glycogen synthesis in response to basal, sub-maximal and maximal concentrations of insulin were markedly decreased. The insulin EC_{50} value of glycogen synthesis was increased 4-fold, but was unchanged for lactate formation. 5. The insulin-stimulated rates of glucose transport in soleus muscles from 5- or 85-week-old Wistar rats were not significantly different.

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

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14 h period before isolation of muscles, when food was withdrawn. Skeletal muscles were routinely prepared between 09:00 and 10:00 h. The values of age (weeks)/body wt. (g) [means ± S.E.M. (no. of observations in parentheses)] are: Sprague–Dawley, 5/158 ± 6 (21), 7/263 ± 4 (10), 8/299 ± 2 (11) and 13/389 ± 4 (12); Wistar, 6/170 ± 4 (27), 12/290 ± 6 (11) and 85/568 ± 25 (11). The older Wistar rats were between 70 and 100 weeks of age, which is termed '85 weeks' for convenience; rats that suffered from any obvious illness, including infections, tumour growth and skin disorders, were excluded from the study.

Materials

All enzymes, biochemicals and radiochemicals were obtained from sources previously given [10,20,21].

Isolation and incubation procedure

Strips of soleus muscle were isolated as originally described by Crettaz *et al.* [9] and tied under tension to stainless-steel clips. For animals over 200 g body wt. only the outer strips of the soleus were used, and these muscle weights were kept in the previously specified limits [9]. Preliminary studies showed that these muscle strips were biochemically viable [22,23]. After a 30 min pre-incubation in Krebs–Ringer bicarbonate buffer (pH 7.4) containing 5.5 mM-glucose, 4 mM-pyruvate and 1.5% (w/v) defatted bovine serum albumin, muscles were transferred to flasks that contained identical medium (except for pyruvate) plus 0.3 μCi of [U-^{14}C]glucose/ml and insulin at the concentrations given in the Results and discussion section (the details of the protocol have been previously given; see [24,25]). After 60 min of incubation, muscles were removed and frozen in liquid N_2; the concentration of lactate in the incubation medium [26] and the rates of incorporation of [^{14}C]glucose into glycogen [27] were measured. Under the conditions of our experiments, the amount of glucose being oxidized is small [21]. However, the major effects of insulin on glucose utilization in the stripped soleus-muscle preparation on glucose transport and glycogen synthesis [20,22].

The rates of transport of the non-metabolizable glucose analogue 3-*O*-methyl-D-glucose were measured as described previously [28,29], with some modifications [21]. Briefly, soleus-muscle strips were incubated in pre-incubation buffer that lacked glucose but contained 4 mM-pyruvate before transfer to flasks containing Krebs–Ringer buffer, 4 mM-pyruvate, 8 mM-3-*O*-methyl-D-glucose plus 0.1 μCi of [^{14}C]sucrose/ml (to enable the extracellular space to be measured) and either 1 or 2 μCi of 3-*O*-methyl-D-[^{3}H]glucose/ml (see the legend to Table 3). All buffers were gassed continuously with O_2/CO_2 (19:1). Muscles were removed after 20 min incubation and rapidly blotted before being frozen in liquid N_2. After digestion in 0.5 ml of 1 M-KOH, the extracts were transferred to scintillation vials, and 10 ml of scintillation fluid and then 0.17 ml of acetic acid were added. The vials were counted for ^{3}H and ^{14}C radioactivity in a Beckman LS 7500 liquid-scintillation counter. Accumulation of 3-*O*-methyl[^{3}H]glucose in the interstitial space was corrected from the [^{14}C]sucrose measurements. The rate of 3-*O*-methyl-D-glucose accumulation was linear with time (G. D. Dimitriadis, unpublished work).

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 (EC_{50} value) increased with age (Table 1). In contrast, in muscles from Wistar rats there was little change in the EC_{50} 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 EC_{50} 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 EC_{50} 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 EC_{50} 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-[^{3}H]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 [a] ($P < 0.05$), [b] ($P < 0.01$) or [c] ($P < 0.001$).

Insulin concn. (μunits/ml)	Age (weeks)...	Rate of lactate formation (μmol/h per g wet wt.)			
		5	7	8	13
1		9.67 ± 0.38 (18)	8.53 ± 0.81 (7)	8.62 ± 0.54 (10)	9.11 ± 0.06 (8)
10		10.99 ± 0.49 (19)	9.03 ± 0.88 (8)	8.98 ± 0.60 (10)[a]	10.11 ± 0.36 (9)
100		14.99 ± 0.70 (19)	10.44 ± 0.86 (8)[c]	10.37 ± 0.52 (10)[c]	9.93 ± 0.54 (11)[c]
1000		15.32 ± 0.58 (18)	11.94 ± 0.92 (8)[b]	11.81 ± 0.66 (9)[c]	13.08 ± 0.61 (9)[b]
10000		14.70 ± 0.32 (12)	12.54 ± 0.50 (8)[b]	12.57 ± 0.60 (9)[c]	12.17 ± 0.52 (9)[c]
EC_{50} (μunits/ml)...		24	119	150	237
		Rate of glycogen synthesis (μmol of 'glucosyl units'/h per g)			
	Age (weeks)...	5	7	8	13
1		1.80 ± 0.14 (19)	1.93 ± 0.31 (8)	2.00 ± 0.16 (10)	1.88 ± 0.19 (9)
10		2.06 ± 0.13 (14)	1.97 ± 0.28 (8)	1.96 ± 0.25 (10)	2.21 ± 0.10 (9)
100		4.04 ± 0.26 (19)	2.73 ± 0.23 (8)[c]	2.96 ± 0.39 (10)[a]	2.67 ± 0.18 (11)[c]
1000		5.41 ± 0.34 (19)	4.37 ± 0.90 (8)	5.58 ± 0.81 (9)	5.91 ± 0.37 (9)
10000		5.80 ± 0.47 (12)	5.69 ± 0.43 (8)	6.95 ± 0.38 (8)	6.14 ± 0.24 (9)
EC_{50} (μunits/ml)...		80	447	376	266

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 [a] ($P < 0.05$), [b] ($P < 0.001$) or [c] ($P < 0.001$).

Insulin concn. (μunits/ml)	Age (weeks)...	Rate of lactate formation (μmol/h per g wet wt.)		
		6	12	85
1		8.60 ± 0.33 (20)	8.10 ± 0.17 (9)	12.26 ± 0.79 (9)[c]
10		10.18 ± 0.48 (21)	8.00 ± 0.45 (9)[b]	9.96 ± 0.48 (8)
100		12.64 ± 0.45 (24)	12.32 ± 0.93 (9)	13.87 ± 0.96 (9)
1000		16.42 ± 0.58 (21)	13.80 ± 1.04 (9)	14.66 ± 0.67 (9)
10000		17.18 ± 0.57 (20)	12.75 ± 0.50 (8)[c]	16.10 ± 0.80 (8)
EC_{50} (μunits/ml)...		100	50	65
		Rate of glycogen synthesis (μmol of 'glucosyl units'/h per g)		
	Age (weeks)...	6	12	85
1		2.28 ± 0.30 (20)	1.92 ± 0.17 (9)	1.28 ± 0.37 (9)[a]
10		2.26 ± 0.24 (17)	1.99 ± 0.16 (9)	1.06 ± 0.29 (9)[b]
100		4.11 ± 0.40 (17)	3.39 ± 0.28 (9)	1.68 ± 0.41 (9)[c]
1000		6.25 ± 0.65 (14)	6.26 ± 0.50 (9)	2.95 ± 0.51 (9)[c]
10000		6.51 ± 0.55 (17)	5.48 ± 0.55 (8)	4.10 ± 0.73 (8)[a]
EC_{50} (μunits/ml)...		126	122	530

Table 3. Effects of aging on insulin-stimulated rates of 3-*O*-methyl-D-[^{3}H]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-[^{3}H]glucose/ml respectively.

	Rate of 3-*O*-methyl-D-glucose transport (d.p.m./20 min per mg wet wt.)	
Insulin concn. (μunits/ml)	Sprague–Dawley (5 weeks)	Sprague–Dawley (13 weeks)
10	984 ± 110	926 ± 72
100	1465 ± 75	940 ± 69*
1000	1683 ± 211	1567 ± 89
	Wistar (6 weeks)	Wistar (85 weeks)
10	465 ± 33	459 ± 33
100	633 ± 36	686 ± 65
1000	742 ± 29	844 ± 71

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 nM human pancreatic amylin 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 amylin 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 amylin or CGRP cause insulin resistance in skeletal muscle in aging animals warrants further investigation.

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