

In Vivo* Insulin-Dependent Glucose Uptake of Specific Tissues Is Decreased during Aging of Mature Wistar Rats

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ABSTRACT

Aging has been associated with peripheral insulin resistance in both humans and rats. However, the specific tissues that become insensitive to insulin before glucose homeostasis is altered remain to be elucidated. In the present work we studied the glucose metabolic index of a number of tissues known to be insulin sensitive in 3- and 24-month-old Wistar rats by measuring 2-deoxy-D-[1-³H]glucose uptake both under euglycemic-hyperinsulinemic conditions and in the basal state. Analysis of the glucose infusion rate to maintain normoglycemia during the clamp confirmed that the old rats show overall insulin resistance at both saturating and subsaturating insulin concentrations. The maximal response of glucose uptake to insulin as well as insulin sensitivity in red and white quadriceps were unaltered

in old rats. In contrast, glucose uptake by soleus and diaphragm was poorly stimulated in old animals, and a marked decrease in insulin sensitivity was observed in both tissues. In heart, only the sensitivity to the hormone, not the maximal response, was impaired in old rats. In white adipose tissue, no significant stimulation was detected. We conclude that during aging in Wistar rats and before fasting plasma insulin and glucose levels become altered, specific tissues develop insulin resistance, whereas other remain insulin sensitive. We postulate that fat tissue plays a qualitative important role in eliciting the insulin resistance in old animals. Due to the metabolic characteristics of the aged Wistar rat, the changes reported might reflect what occurs in nonobese elderly humans, nongenetically committed to develop type 2 diabetes. (*Endocrinology* **138**: 49–54, 1997)

ASSOCIATION of impaired glucose tolerance with advancing age in humans has been well documented by many investigators using either an oral or iv glucose tolerance test (1). The use of the euglycemic-hyperinsulinemic clamp technique allowed demonstration that the glucose intolerance observed with aging is due to peripheral insulin resistance (2–4). In rats, increases in insulin resistance *in vivo* with aging have also been reported (5–8), and in a study in which the euglycemic-hyperinsulinemic clamp was used, it was concluded that decreased peripheral insulin sensitivity is the main cause of the insulin resistance during aging in mature animals (7).

As skeletal muscle is the main tissue responsible for insulin-induced glucose disposal, most studies have explored possible cellular changes in muscle as a cause of the peripheral insulin resistance in the aged rat (5, 6, 9–13). However, the specific contribution of the different tissues that behave as targets of insulin action to the overall insulin resistance is as yet unknown. In the present work we studied the utilization of glucose by a number of tissues (brain, lung, white

adipose, and several muscles) under euglycemic-hyperinsulinemic clamp conditions in young adult (3 months) and old (24 months) rats. The age of the animals was selected to analyze the effect of aging in mature animals and not the effects of growth or maturation (8, 12), as is the case in most studies in aging research that compare juvenile (1–2 month) and either adult (4–12 month) or old (>20 month) animals (6, 11, 14, 15).

Another major concern in aging research in rodents is the rat strain to be used. Thus, to analyze changes in insulin sensitivity of primary character it is important that the aged rat is able to preserve glucose homeostasis without increases in the plasma insulin concentration. On the other hand, age-associated changes in body composition, such as increased adiposity and body weight, are known to result in insulin resistance, a fact that precludes the use of rat strains with age-associated obesity. The Fisher 344 rat shows a small increase in body weight at 24 months of age and, therefore, has been used in many studies (8, 10, 11, 13, 16). However, in this strain, fat cell size increases sharply, and fasting plasma insulin concentrations are slightly higher in aged animals, a situation that does not allow the determination of whether changes in insulin sensitivity are due to the aging process or to hyperinsulinemia. This last problem is also shared by the Sprague-Dawley strain, which, in turn, shows an acute increment in body weight during aging (5, 6, 17). On the contrary, the 24-month-old Wistar rat shows fasting plasma insulin concentrations identical to those of the adult 3-month-old rat and, despite an obvious increase in body

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weight and fat tissue mass, the size of adipose cells remains constant (18), suggesting that the higher adipose tissue mass is a consequence of adipocyte proliferation. Consequently, studies in this report were conducted using the Wistar rat strain, which we consider more appropriate to analyze aging-associated primary alterations in peripheral insulin sensitivity. This strain has also been considered an adequate model for aging studies by other researchers (9).

Materials and Methods

Animals

Three- and 24-month-old male Wistar rats fed *ad libitum* standard laboratory chow and water were used throughout this study. Animals were obtained from the colony maintained at the Center for Molecular Biology (Madrid, Spain) where they were housed two per cage in climate-controlled quarters with a 12-h light cycle. Animal facilities fulfill the requirements of the European laws on the protection of animals.

Euglycemic-insulin clamp

These studies were performed during the postabsorptive period, 15 h after removal of food. Rats were anesthetized with pentobarbital (4 mg/100 g BW), and after tracheotomy (to avoid respiratory problems), one carotid artery was catheterized for blood sampling. Once glycemia returned to the level observed in blood withdrawn from the tail vein of the awake animals (40 min), insulin (Actrapid, Novo, Copenhagen, Denmark) was infused through a saphenous vein at a constant rate to reach an insulin dose of 0.3 or 0.6 U/h·kg, respectively. A solution of 30% glucose was also infused through the other saphenous vein beginning 5 min after the start of hormone infusion. The infusion rate was adjusted to clamp blood glucose at the level present in awake animals (19). To achieve this, blood samples were taken every 5 min from the carotid artery, blood glucose was determined within 2 min using a RefloLux II Glucose Analyzer (Boehringer Mannheim, Mannheim, Germany) (20), and the pump dial was adjusted according to the changes in the blood glucose level. Within 40 min of starting the clamp, plasma insulin and glucose levels remained constant without further adjusting the pump dial. At this steady state, insulin infusion should equal insulin clearance, and the overall glucose utilization should reach a constant value. The rate of glucose infusion at steady state normalized to the body weight was used to determinate the glucose disposal rate (M) as an index of insulin sensitivity.

Estimate of glucose utilization by individual tissues

As required by the theoretical model (21) a bolus of 80 μ Ci 2-deoxy-D-[1- 3 H]glucose (Amersham, Aylesbury, UK) was injected iv into animals under steady state conditions. The same bolus was administered to rats not infused with insulin to study basal glucose utilization. Blood was sampled via arterial catheter for determination of insulin and glucose concentrations and 2-deoxy-D-[1- 3 H]glucose radioactivity. Measurements of plasma glucose and insulin levels before injecting the radioactive bolus and at the end of the experiment (60 min) confirmed that steady state conditions were maintained throughout the test. At the end of the experiment, rats were killed by cervical dislocation, and pieces of brain, lungs, white adipose tissue (mixture of epididymal and retroperitoneal), heart (ventricular portion), diaphragm, red and white quadriceps, and soleus muscle were rapidly removed and frozen until processing. Tissue samples were digested at 60°C for 45 min in plastic tubes containing 1 M NaOH and neutralized with 1 M HCl. The 2-deoxy-D-[1- 3 H]glucose-6-phosphate content was determined as described previously (22). This method is based on the fact that both 2-deoxyglucose and 2-deoxyglucose-6-phosphate remain soluble in 6% HClO₄ extracts, whereas 2-deoxyglucose-6-phosphate precipitates in the Somogyi reagent [BaSO₄/Zn(OH)₂]. The rate of glucose utilization by each tissue was calculated by dividing the disintegrations per min of 2-deoxy-D-[1- 3 H]glucose-6-phosphate in the tissue by the calculated integral of the ratio of arterial blood 2-deoxy-D-[1- 3 H]glucose to glucose concentration. The data obtained are usually referred to as the glucose metabolic index, and they can be regarded as an index of glucose utilization at different

insulin levels (23). As the protein content of the tissues tested was not different between old and young rats, the glucose utilization was expressed as milligrams per min/kg tissue.

Other analytical procedures

Plasma insulin was determined by RIA, using rat insulin as standard (Incstar Corp., Stillwater, MN). Ketone bodies were determined according to (24). Serum FFA were determined by an enzymatic kit (NEFA C, Wako Chemicals, Neuss, Germany). Serum triglyceride were measured with an enzymatic kit (ITC Diagnostics, Izasa, Spain).

Expression of the results

Results are expressed as the mean \pm SD. Statistical comparisons were made using Student's *t* test.

Results

Characteristics of the animals

The characteristics of the two groups of animals are shown in Table 1. Young rats had plasma insulin and glucose concentrations identical to those observed in old animals, indicating that they were mature with regard to the preservation of glucose homeostasis. Changes in body composition were reflected by increased body weight and fat tissue mass (18), in agreement with data from others (7). Old animals showed significantly elevated triglyceride levels, a characteristic also observed in noninsulin-dependent diabetic patients and normoglycemic/hyperinsulinemic individuals (25) as well as in other rat strains in association with aging (26). In contrast, although a relationship between fasting triglyceride and FFA concentrations has been previously shown (25), FFA levels in older rats were significantly lower. Ketone bodies were also lower in old animals in concordance with the lower FFA concentrations.

Euglycemic-hyperinsulinemic clamp

Table 2 shows the plasma insulin and glucose concentrations reached at steady state, 45 min after starting the clamp. In both groups, glucose concentrations were clamped at identical levels, which were not different from those determined in animals not infused with insulin. Interestingly, the plasma insulin concentrations at steady state were significantly higher in old animals at both insulin infusion rates. This has been previously observed in elderly humans at insulin infusion rates leading to submaximal insulin concentrations during euglycemic clamp (3, 4), and the same can be

TABLE 1. Characteristics of the animals

	3-Month-old animals	24-Month-old animals
BW (g)	424 \pm 24	727 \pm 76 ^a
Fasting plasma glucose (mg/dl)	93.9 \pm 3.9	92.0 \pm 6.2
Fasting plasma insulin (μ U/ml)	45 \pm 16	39 \pm 13
Fasting plasma ketone bodies (μ mol/ml)	0.94 \pm 0.48	0.29 \pm 0.04 ^b
Fasting serum FFA (mEq/liter)	1.09 \pm 0.33	0.73 \pm 0.2 ^c
Fasting serum triglyceride (mg/dl)	82.7 \pm 31.0	196.7 \pm 63.3 ^a

Data are the mean \pm SD of 7–15 separate determinations.

^a *P* < 0.001 vs. 3-month-old animals.

^b *P* < 0.01 vs. 3-month-old animals.

^c *P* < 0.05 vs. 3-month-old animals.

TABLE 2. Metabolic characteristics of the animals during euglycemic-hyperinsulinemic clamp

	Age (months)	Insulin infusion rate (U/h·kg)	
		0.3	0.6
Steady state plasma glucose (mg/dl)	3	89.8 ± 21.0	96.8 ± 15.6
	24	86.7 ± 1.4	92.3 ± 11.4
Steady state plasma insulin (μU/ml)	3	207 ± 18	508 ± 32
	24	384 ± 114 ^a	1037 ± 158 ^b
M (mg/min·kg)	3	14 ± 0.9	17.4 ± 2.9
	24	9.6 ± 0.8 ^b	10.9 ± 2.2 ^b

Data were obtained from five or six different clamps performed with each group and represent the mean ± SD.

^a $P < 0.01$ vs. 3-month-old rats.

^b $P < 0.001$ vs. 3-month-old rats.

concluded from studies using aged Wistar rats (7). An aging-associated decrease in the MCR of insulin seems likely to be the cause of these findings (27–30).

The rates of glucose infusion per kg BW required to maintain blood glucose concentrations during the clamp (M) were markedly lower in the 24-month-old rats than in the young controls (Table 2) at both insulin infusion rates, indicating the insulin-resistant condition of the aged animals. At the highest plasma insulin levels reached during the clamp in young and old rats, Nishimura *et al.* (7) reported that hepatic glucose production was fully suppressed. Therefore, it can be assumed that the rate of glucose infusion during the clamp is equal to the rate of glucose utilization, and the differences observed between young and old animals are probably due to differences in glucose consumption by tissues and not to higher hepatic glucose production in the older rats. However, at the lower insulin infusion rate used in these studies, the insulin concentrations reached were reported to elicit a lesser suppression of hepatic glucose production in old than in adult animals (7), and it could consequently be argued that differences in M in this case may be due to a higher hepatic glucose production in older rats and not to the existence of insulin resistance. However, the levels of glucose infused into old rats at both insulin infusion rates are very similar, suggesting that the production of glucose by the liver at the lower insulin dose represents only a small part of the overall glucose utilization that could not account by itself for the decrease in M found in old rats.

Considering that fat tissue mass increases with aging, many investigators have proposed to express glucose disposal rates per kg lean body mass. In this work we have not determined the fat-free mass of the rats, but from the similarity in body weight between the animals in this study and those used previously (7), we can tentatively assume that the fat-free mass amounts to 62% and 55% of body weight in young and old rats, respectively. This would result in glucose disposal rates of 28 and 20 mg/min·kg fat-free mass, for young and old rats, respectively, at the highest insulin level, indicating that old animals also show insulin resistance when glucose disposal is calculated on a fat-free mass basis, in agreement to similar findings in humans (27).

Glucose utilization in various tissues

The glucose metabolic index was estimated in control animals (*i.e.* without insulin infusion) and in rats under eugly-

cemic-hyperinsulinemic conditions. Table 3 summarizes the data obtained in eight tissues at three different insulin levels.

In white adipose tissue, basal glucose uptake was similar in young and old animals. Maximal stimulation of glucose utilization in young animals (3.75 fold) was reached at the lower insulin dose used, with a slight decrease at higher concentrations of the hormone, reflecting the elevated insulin sensitivity of this tissue. In old rats, however, only a poor insulin effect was observed at both insulin doses, suggesting that adipose tissue becomes extremely insulin resistant with aging.

In soleus muscle and diaphragm, basal glucose uptake was also similar in both groups of rats. However, insulin-induced stimulation of glucose utilization was markedly lower in old than in young animals at both insulin infusion rates, indicating that these two tissues as well as white adipose contribute actively to the insulin-resistant state of the old rats. The increment in the glucose metabolic index at the lower insulin infusion rate over that observed under basal conditions can be compared with the maximal increment obtained at saturating insulin concentrations to obtain a raw estimation of the insulin sensitivity of the tissues. For the soleus muscle and diaphragm, the stimulatory effect elicited by this intermediate insulin concentration represents 75% and 69%, respectively, of the maximal stimulation observed in young animals, whereas in aged rats, this increment reaches only 33% and 26% of the maximal effect. These data clearly indicate that the insulin sensitivity of both tissues is diminished in old animals.

Uptake of glucose by the heart at saturating insulin concentrations was similar in young and old animals, suggesting that the maximal capacity of glucose utilization remains unaltered with aging. However, at submaximal insulin concentrations, glucose uptake was significantly higher in young rats, which indicates that at those intermediate insulin levels the heart might also contribute to the decreased glucose disposal observed in old animals. In contrast, under basal conditions, glucose utilization was significantly elevated in old rats. These data indicate that the insulin sensitivity of the heart appears to decrease as a consequence of aging. An approximate estimation from data in Table 3 suggest that the insulin concentration necessary to induce the half-maximal stimulation of glucose uptake in young animals should be lower than 100 μU/ml, whereas in old animals it is around 200 μU/ml.

TABLE 3. Glucose metabolic index of different tissues of young and old rats under basal conditions and at two different insulin concentrations during euglycemic-hyperinsulinemic clamp

Tissue	Age (months)	Insulin infusion rate (U/h·kg)		
		0	0.3	0.6
White adipose tissue	3	0.8 ± 0.4	3.0 ± 0.7 ^a (3.75)	2.3 ± 0.6 ^a (2.87)
	24	1.1 ± 0.4	1.7 ± 0.9 ^b (1.54)	1.6 ± 0.6 ^b (1.45)
Soleus	3	2.7 ± 0.2	11.4 ± 4.3 ^c (4.22)	14.2 ± 2.8 ^a (5.25)
	24	2.9 ± 0.8	4.5 ± 1.6 ^b (1.55)	7.7 ± 3.5 ^{b,d} (2.65)
Diaphragm	3	10.8 ± 4.8	59.9 ± 19.1 ^a (5.54)	81.5 ± 10.9 ^a (7.54)
	24	18.9 ± 7.9	24.9 ± 8.9 ^b (1.31)	40.7 ± 11.1 ^{d,e} (2.15)
Heart	3	5.5 ± 2.2	86.2 ± 20.9 ^a (15.67)	87.9 ± 9.4 ^a (15.98)
	24	22.2 ± 15.3 ^f	65.1 ± 11.2 ^{c,f} (2.93)	78.5 ± 14.2 ^c (3.53)
Red quadriceps	3	5.5 ± 0.9	21.5 ± 5.6 ^a (3.9)	30.5 ± 10.2 ^a (5.54)
	24	6.8 ± 1.7	17.6 ± 5.0 ^c (2.6)	26.2 ± 11.5 ^c (3.85)
White quadriceps	3	3.2 ± 0.7	12.8 ± 4.1 ^c (4.0)	22.5 ± 5.2 ^a (7.03)
	24	4.1 ± 0.5	11.7 ± 4.9 ^d (2.85)	16.0 ± 4.7 ^c (3.9)
Brain	3	23.9 ± 4.1	20.5 ± 8.6	21.3 ± 3.1
	24	19.5 ± 2.2	16.7 ± 2.3	20.8 ± 1.9
Lung	3	12.4 ± 1.7	10.9 ± 6.0	12.7 ± 0.9
	24	7.1 ± 1.0 ^b	8.8 ± 1.1	7.8 ± 1.2 ^e

Data are expressed as milligrams per min/kg and are the mean ± SD of four to eight independent determinations in each case. Values in parentheses indicate the fold stimulation of glucose utilization elicited by insulin infusion with respect to that observed under basal conditions.

^a $P < 0.001$ vs. basal.

^b $P < 0.01$ vs. young.

^c $P < 0.01$ vs. basal.

^d $P < 0.05$ vs. basal.

^e $P < 0.001$ vs. young.

^f $P < 0.05$ vs. young.

Concerning red and white quadriceps, the data in Table 3 indicate that there were no significant differences in glucose uptake between young and old animals at the insulin concentrations tested, suggesting that these tissues are not involved in the insulin resistance associated with aging. In young animals, the increment in glucose uptake at intermediate insulin levels over that observed under basal conditions represents 62% and 50% of the maximal increment for red and white quadriceps, respectively, a percentage very similar to the 58% and 62% of the maximal increment observed in both tissues from aged animals. Thus, from these data it can be postulated that the insulin sensitivity of both tissues remains unchanged in old animals.

Finally, Table 3 shows that glucose utilization by brain and lung was not stimulated by insulin in either group of rats. Interestingly, whereas the glucose metabolic index of brain is not influenced by the age of the animals, glucose utilization by lung was decreased in aged rats at all three insulin concentrations tested.

Discussion

Whereas a marked impairment of peripheral insulin action is well known to occur during maturation in rodents and humans, aging of mature individuals has been associated with a rather moderate decrease in peripheral insulin sensitivity (3–8, 11–13). This last phenomenon was clearly shown in humans, whereas in rats, different results were obtained depending on the rat strain and the experimental approach used. In this work we analyzed the *in vivo* glucose disposal rate of mature young (3 months) and old (24 months) Wistar rats, using the euglycemic-hyperinsulinemic

clamp at two different insulin infusion rates. Our data clearly demonstrate a significant decrease in overall glucose utilization in the aged animals at both saturating and subsaturating insulin concentrations, indicating that aging of mature Wistar rats is associated with a decrease in peripheral insulin action. At the higher insulin infusion rate used in this work, the steady state insulin concentrations reached during the clamp in both young and old animals have been previously shown to induce a maximal glucose disposal rate in the Wistar rat (7). Consequently, we can tentatively speculate that in aged rats, the maximal response to the hormone is decreased. These results confirm previous observations by Nishimura *et al.* (7), although in our study the differences in glucose disposal rate reach statistical significance, whereas in their study (7) this was the case only at lower insulin concentrations. Additionally, these data are consistent with the findings in elderly humans (3, 4, 27) who lack genetic commitments to develop noninsulin-dependent diabetes.

Some researchers have claimed that peripheral insulin resistance in aged rats is simply a consequence of increases in body weight and fat mass or is due to physical inactivity. Although it is well documented that these factors affect insulin sensitivity, there is considerable experimental evidence demonstrating that insulin resistance in aged animals and humans also occurs in the absence of increased adiposity and body weight (17, 27) or after training protocols that are usually effective in improving insulin sensitivity (10, 13).

In the present work we also explored the effect of aging on the glucose metabolic index of several insulin target tissues at different plasma insulin concentrations. As the 24-month-old rats are normoinsulinemic and normoglycemic, these

studies allowed us to determine which tissues contribute primarily to the development of insulin resistance. From our data it becomes evident that white adipose tissue is extremely insulin resistant in the old rats. These results are consistent with our previous observations in isolated adipocytes (18, 31) that showed a decreased insulin stimulation of several metabolic functions as well as a reduced insulin receptor kinase activity and with observations by others in human adipocytes (27). Even though adipose tissue uses only a minor part of the glucose available, it should be pointed out that a greater proportion of fat mass is present in the older animals, making its contribution to the overall insulin resistance more relevant. Reaven (25) suggested that the insulin resistance of adipose tissue in type 2 diabetics could be relevant not quantitatively, in terms of glucose utilization, but, rather, qualitatively. Thus, the lack of an antilipolytic effect of insulin or a decreased stimulation of adipocyte-associated lipoprotein lipase could lead to increases in plasma FFA and triglyceride levels that might, in turn, induce a decrease in glucose utilization by other tissues. In fact, fasting serum triglyceride levels were elevated in the old rats used in this work, in agreement with previous reports using Fischer-344 rats (26). In contrast, the serum FFA concentration appears to be decreased in old animals. More experimental work is required to elucidate whether this is due to decreased adipocyte fatty acid production or increased liver fatty acid utilization for triglyceride synthesis. Our results here indicate that the insulin insensitivity of adipose tissue could be a primary defect associated with aging, and it is of great interest to establish at which age this resistance is detectable.

We also found that insulin-stimulated glucose uptake by the diaphragm is markedly reduced in old rats at saturating and subsaturating insulin concentrations, whereas basal utilization remains unaltered. Furthermore, from our data it can be postulated that the insulin sensitivity of the diaphragm is decreased in the aged rat. A previous report using isolated diaphragm from Wistar rats claimed that insulin resistance occurs during maturation, but no further decrease in insulin action was observed with aging (32). As our data were obtained *in vivo*, they cannot be directly compared with the former. It is possible that *in vitro* studies with isolated diaphragm do not fully reproduce the conditions existing *in vivo*, resulting in a loss of its insulin resistance.

In contrast to the former tissues, the glucose metabolic index of heart in the presence of saturating insulin reaches similar levels in young and old animals, indicating that the metabolic capacity of heart is not influenced by aging. However, at subsaturating insulin concentrations, glucose utilization is lower in the old animals, whereas in the presence of physiological plasma insulin concentrations, glucose utilization is higher in old rats. Taken together, these data suggest that in the hearts of old animals the insulin sensitivity is decreased without changes in the maximal response to the hormone.

Finally, concerning skeletal muscles, it appears evident from our data that insulin resistance develops mainly in certain muscle types. Thus, whereas decreases in old rats in glucose utilization by the red and white portions of the quadriceps at both insulin infusion rates are rather low, the stimulation by insulin of glucose utilization in the soleus is

greatly reduced in aged animals. Furthermore, the insulin sensitivity of red and white quadriceps is unaltered with aging, in contrast to the acute decrease observed in the soleus muscle of old rats. These three muscle tissues differ in fiber type composition, with the soleus being mainly constituted by type 1 fibers, and the white and red quadriceps by type 2b and 2a fibers, respectively (12). The type 1 fibers of the soleus are oxidative and can use fatty acids as fuel, whereas type 2b fibers are extremely glycolytic and cannot oxidize it (33). Thus, it can be speculated that the insulin-resistant adipose tissue of the older rats incorporates a lesser amount of fatty acids from lipoprotein triglyceride that remain available as an alternative fuel to glucose for oxidative muscles such as soleus or even heart. The greater availability of fatty acids, however, would not influence glucose utilization by glycolytic muscles such as quadriceps.

Taking into account that slow twitch fibers represent only about 10% of the rat muscle mass, it appears difficult to reconcile the observed decrement in overall glucose disposal with the development of insulin resistance in the oxidative muscles and diaphragm. In this regard there are several considerations. Firstly, the lower glucose utilization by lungs in old animals reported here could partially contribute to the decreased glucose disposal. On the other hand, it has been proposed that aging is associated with a preferential loss of fast twitch fibers and a progressive increase in the proportion of aerobic type 1 fibers that would be insulin resistant (33). Finally, it cannot be ruled out that other tissues are contributing to the decreased glucose disposal or even that, as experiments were performed with anesthetized rats, the contribution of diaphragm to the total glucose utilization might be overestimated, resulting in a higher decrement in glucose disposal.

The development of tissue-specific insulin resistance could also be due to regional changes in blood flow. However, although insulin stimulation of peripheral blood flow has been shown to be impaired in several cases of insulin resistance (34–36), considerable experimental evidence has dissociated the increase in glucose utilization from the increment in regional blood flow (37). Thus, it seems unlikely that specific changes in blood flow are the ultimate cause of the tissue-specific decrease in glucose uptake.

To summarize, our data demonstrate that normoglycemic and normoinsulinemic 24-month-old Wistar rats show insulin resistance *in vivo*, as manifested by the decreased glucose disposal rates under hyperinsulinemic conditions. Some insulin target tissues, such as diaphragm, soleus muscle, heart, and white adipose tissue, play a primary role in development of the insulin-resistant condition associated with aging, whereas the other mainly glycolytic muscles remain sensitive to the hormone.

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