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Insulin resistance for glucose metabolism in disused soleus muscle of mice

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SEIDER, MICHAEL J., WILLIAM F. NICHOLSON, AND FRANK W. BOOTH. *Insulin resistance for glucose metabolism in disused soleus muscle of mice.* Am. J. Physiol. 242 (Endocrinol. Metab. 5): E00-E00, 1982.—Our hypothesis was that insulin resistance for carbohydrate metabolism develops after a single day of muscular disuse. The immobilization of the mouse hind-limb for 24 h was used to produce muscular disuse (group C). As food intake was voluntarily decreased during the immobilization, two additional groups were used: group A was untreated and ate ad libitum, whereas group B was anesthetized with group C and was fed amounts of food similar to those eaten by group C. Because groups B and C differed only by limb immobilization, group B was used as the reference group. When insulin was present in the incubation media, the rates of 2-deoxyglucose uptake and glycogen synthesis were always significantly decreased in soleus muscles from group C (anesthetized, ate less, immobilized) as compared to group B (anesthetized, pair-fed food that group C ate). Significant interaction between the factors of insulin and muscular disuse for the rates of 2-deoxyglucose uptake and glycogen synthesis support the concept that disuse of skeletal muscle attenuates insulin action. These observations are a direct demonstration of a decrease in insulin responsiveness in skeletal muscle as the result of a single day of muscular inactivity. Because plasma insulin concentrations were significantly lower in groups B and C than in the untreated group, it seems likely that the development of insulin resistance in disused skeletal muscle is independent of plasma insulin levels.

limb immobilization; muscle atrophy; skeletal muscle; insulin responsiveness; corticosterone

INSULIN SENSITIVITY for glucose uptake decreases across the forearm of healthy people after a few days of continuous bed rest (21, 22). Indeed, one textbook of internal medicine offers the advice that physicians should be aware that false positive tests for glucose intolerance and diabetes can occur in humans just because of bed rest (34). The molecular bases of these observations are unknown. Because a direct measurement of insulin resistance in disused skeletal muscle has not previously been

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limb immobilization: muscle atrophy; skeletal muscle; insulin responsiveness; corticosterone

INSULIN SENSITIVITY for glucose uptake decreases across the forearm of healthy people after a few days of continuous bed rest (21, 22). Indeed, one textbook of internal medicine offers the advice that physicians should be aware that false positive tests for glucose intolerance and diabetes can occur in humans just because of bed rest (34). The molecular bases of these observations are unknown. Because a direct measurement of insulin resistance in disused skeletal muscle has not previously been demonstrated, we studied indices of glucose metabolism in the isolated mouse soleus muscle. Disuse was produced by limb immobilization, so that the soleus muscle was fixed at a length that was less than its normal resting length. This procedure produces atrophy of the soleus muscle (6). The purposes of this study were to determine whether insulin resistance for carbohydrate metabolism occurs in skeletal muscle after a single day of immobilization and whether development of insulin resistance in muscle was related to changes in either plasma insulin or corticosterone levels.

MATERIALS AND METHODS

Animal care and experimental groups. Male, outbred, albino mice, weighing 20-25 g, were obtained from Texas Inbred Mouse Co. (Houston). They were housed in a constant temperature (22°C) animal room with a 12:12-h light:dark cycle for at least 1 wk prior to usage. They received food (Purina mouse chow) and water ad libitum prior to the experiment.

On the day that the experiment started, mice were randomly divided into three groups, and their body weights were measured. The three experimental groups were: A, untreated; B, anesthetized and food-restricted (fed an amount of food equivalent to that of group C);

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Fig 1

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and C, anesthetized while undergoing limb immobilization and given food and water ad libitum (Fig. 1). Further descriptions of the treatments that some of these groups received follow. Groups B and C were anesthetized (50 mg sodium pentobarbital/kg body wt) 1 day before removal of their soleus muscle. Group C had both of its hindlimbs immobilized with plaster of paris so that feet were in plantar flexion while the mice were anesthetized 1 day before removal of the soleus muscle. The immobilization procedure was a modification of that previously described for rats (6). Thus, the hindlimbs of group C were immobilized for 24 h. Pilot experiments established the amount of food eaten by group C during the 1st day of limb immobilization. This amount of food was given to group B on the day before removal of their soleus muscles.

In vitro incubation. Mice were anesthetized with sodium pentobarbital (50 mg/kg body wt ip). Plaster casts, if present, were removed and the soleus muscles dissected. The proximal tendon of the soleus muscle was isolated and ligated with 6-0 silk. The muscle was then removed and quickly placed into a tissue bath (37°C) containing oxygenated Krebs-Ringer bicarbonate buffer (pH, 7.4) and 5 mM glucose and 1% fatty-acid-free bovine serum albumin. While in this bath, the muscle was placed on a Dow Corning Silastic strip by placing 27-gauge needles through the Achilles and proximal tendons of the soleus muscle. The muscle was stretched to its in vivo resting length when pinned to the Silastic block, and a gap of approximately 3 mm was left between the block and muscle. The block with its pinned muscle was then transferred from the tissue bath to a plastic test tube for preincubation as described for each specific experiment.

Assay procedures. The same muscle was used to measure glucose oxidation and glycogen synthesis. For glucose oxidation, muscles pinned to Silastic blocks were preincubated for 30 min at 37°C in 2.5 ml of Krebs-Ringer bicarbonate buffer (pH, 7.4) containing 5 mM glucose, 1% fatty-acid-free bovine serum albumin, and insulin as indicated. The tubes containing muscles and medium were placed into a gyrotory shaker bath and were continuously gassed with O₂:CO₂ (19:1). After preincubation, muscles with their Silastic blocks were transferred to a second set of plastic tubes containing the incubation medium. This medium was identical to the preincubation medium except that 0.2 μCi [¹⁴C]glucose (uniformly labeled, New England Nuclear) per milliliter of medium was included for the measurement of glucose oxidation and glycogen synthesis rates. Before the addition of the muscle to the incubation tubes, tubes were continuously gassed with O₂:CO₂ (19:1) for 1 h followed by an additional minute of gassing after the addition of muscle and before sealing the tube with a rubber stopper for the duration of the subsequent 1-h incubation. Following the

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preincubation as described for each specific experiment.

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Muscles in which glucose oxidation was measured were also utilized for assessment of glycogen synthesis. The formation of glycogen from radiolabeled glucose was determined using modifications of the methods of Stauffer and Renold (33) and Cuendet et al. (10). After the muscles had been removed from the incubation medium, they were placed in preweighed tubes containing 0.5 ml of 1 N NaOH and 10 mg of carrier glycogen. Tubes were then reweighed and placed in boiling water for 10 min. A 50-μl aliquot of the resulting hydrolysate was removed for protein determination by the method of Bradford (7). Then 1.6 ml of absolute ethanol were added to the

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178 remaining hydrolysate, and glycogen was allowed to pre-
179 cipitate overnight at -80°C . The glycogen precipitate
180 was then washed twice with 1 ml of 66% ethanol. The
181 washed precipitate was dissolved in 0.5 ml water and
182 added to Aquasol (New England Nuclear) for liquid
183 scintillation counting. Correction of counts per minute to
184 disintegrations per minute was made via a quench curve.

185 Procedures for the measurement of 2-deoxyglucose
186 uptake into soleus muscles differed from the previous
187 measurements. The preincubation medium consisted of
188 Krebs-Ringer bicarbonate buffer (pH, 7.4) with 2 mM
189 pyruvate and insulin as indicated. In addition to these
190 modifications, the incubation medium contained 5 mM
191 2-deoxyglucose, $0.5 \mu\text{Ci } 2\text{-}[^3\text{H}]\text{deoxy-D-glucose}$ per milli-
192 liter of medium, and $0.1 \mu\text{Ci } [^{14}\text{C}]\text{inulin}$ per milliliter of
193 medium. Experimental procedures performed during
194 preincubation and incubation were identical to that de-
195 scribed above except that the duration of incubation was
196 15 min with continuous gassing with $\text{O}_2:\text{CO}_2$ (19:1) during
197 this time. Following incubation, muscles were removed,
198 blotted, and then placed in preweighed tubes containing
199 0.5 ml of 1 N NaOH and reweighed. Muscles were then
200 hydrolyzed in the NaOH, and a sample of the hydrolysate
201 was counted. Extracellular amounts of $2\text{-}[^3\text{H}]\text{deoxy-D-}$
202 glucose were estimated from inulin-accessible spaces, and
203 intracellular $2\text{-}[^3\text{H}]\text{deoxy-D-glucose}$ was obtained by sub-
204 tracting the extracellular amount from the total in the
205 muscle.

206 For the determination of whole muscle ATP and gly-
207 cogen levels, soleus muscles were quick-frozen with liquid
208 nitrogen-cooled Wollenberger tongs. Glycogen and ATP
209 were determined with the fluorometric procedure of
210 Lowry (24). The concentration of ATP in the mouse
211 soleus muscle was not significantly changed from in vivo
212 levels by 90 min of incubation in vitro. There was 25.5
213 $\pm 1.9 \text{ nmol ATP/mg protein}$ in soleus muscles frozen in
214 liquid nitrogen-cooled Wollenberger tongs immediately
215 after the removal of the muscle from the anesthetized
216 mouse, and there was $26.5 \pm 3.3 \text{ nmol ATP/mg protein}$
217 in soleus muscles after 90 min of in vitro incubation. This
218 demonstrated the viability of these muscles during in-
219 cubation.

220 Blood samples for the measurement of plasma glucose
221 and immunoreactive insulin (IRI) were obtained from
222 anesthetized mice via cardiac puncture into heparinized
223 syringes. These mice were not the same animals used for
224 muscle studies. Blood was immediately centrifuged and
225 the plasma stored at -80°C until assayed. Plasma IRI
226 measurements were made with an insulin radioimmu-
227 noassay kit (Amersham) using human insulin as stan-
228 dard. Plasma glucose was determined with a colorimetric
229 procedure (29).

230 Mice for the glucocorticoid study were brought daily
231 to the laboratory and were handled for a period of 10

209 were determined with the fluorometric procedure of
210 Lowry (24). The concentration of ATP in the mouse
211 soleus muscle was not significantly changed from in vivo
212 levels by 90 min of incubation in vitro. There was $25.5 \pm$
213 1.9 nmol ATP/mg protein in soleus muscles frozen in
214 liquid nitrogen-cooled Wollenberger tongs immediately
215 after the removal of the muscle from the anesthetized
216 mouse, and there was 26.5 ± 3.3 nmol ATP/mg protein
217 in soleus muscles after 90 min of in vitro incubation. This
218 demonstrated the viability of these muscles during in-
219 cubation.

220 Blood samples for the measurement of plasma glucose
221 and immunoreactive insulin (IRI) were obtained from
222 anesthetized mice via cardiac puncture into heparinized
223 syringes. These mice were not the same animals used for
224 muscle studies. Blood was immediately centrifuged and
225 the plasma stored at -80°C until assayed. Plasma IRI
226 measurements were made with an insulin radioimmu-
227 noassay kit (Amersham) using human insulin as stan-
228 dard. Plasma glucose was determined with a colorimetric
229 procedure (29).

230 Mice for the glucocorticoid study were brought daily
231 to the laboratory and were handled for a period of 10
232 days to acclimate them. One-half of the mice had hind-
233 limbs immobilized the day before decapitation, and the
234 remainder were controls. Following decapitation, blood
235 was collected in a heparinized tube, centrifuged, and then
236 frozen until assayed. Plasma from two mice were pooled
237 for a single observation. Plasma corticosterone was meas-
238 ured by the fluorometric procedure of Silber et al. (32).

239 Statistical analysis was performed with an unpaired
240 Student's *t* test or with factorial analysis of variance
241 (ANOVA). Tukey's least-significant difference and
242 Fisher's *F* tests were used to identify significant differ-
243 ences among groups and treatments. A probability level
244 of 0.05 was designated as significantly different.
245

246 RESULTS

247 Following the treatment period, there were no signifi-
248 cant differences among groups A, B, and C for the wet
249 weight and the total protein content of the soleus muscle
250 (Table 1). Food intake and body weight, however, were
251 significantly decreased in group B (anesthetized with
252 food restriction) as compared to the untreated group
253 (group A) (Table 1). Adding the treatment of limb
254 immobilization contributed no further significant differ-
ence in either food intake or final body weight. Muscle

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Table 1

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glycogen was significantly decreased in *group B* (Table 1). Food restriction is known to decrease muscle glycogen (19) so that the difference between *group A* (untreated) and *group B* (anesthetized, food-restricted) is not surprising. However, *groups B* and *C* ate similar amounts of food on the treatment day; yet, *group C* had approximately 2 times greater concentration of muscle glycogen than did *group B* (Table 1). Because the only treatment difference between these groups was limb immobilization, the reduction in the usage of the soleus muscle by limb immobilization may have resulted in a decreased rate of glycogenolysis in *group C*. Values for body weight, muscle wet weight, muscle total protein content, and muscle glycogen concentration of the untreated group are similar to that observed by others (19).

Significant hypoinsulinemia was observed in *groups B* and *C* with respect to *group A* (Fig. 2). There were, however, no significant differences between *groups B* and *C* in plasma IRI. Food restriction has been shown to produce hypoinsulinemia in mice (19). Because we decided to measure carbohydrate metabolism in muscles from *groups A-C* at the same time, the quantity of food given *group B* was based on a pilot experiment during which food intake was measured during the 1st day of hindlimb immobilization and was shown to be 1.2 g/day. However, mice whose muscles were used in measurements of carbohydrate metabolism in *group C* ate one-third less than those mice in the pilot experiment. To test whether this difference in food intake would effect plasma IRI, a third set of mice was used. No significant difference existed in plasma IRI between the groups of mice eating either 1.2 or 0.8 g/day during the 1st day of hindlimb immobilization (Fig. 2). These values also did not differ from a group of mice, not immobilized, but eating 1.2 g of food (Fig. 2). Thus, the difference in food intake from 1.2 to 0.8 g was not sufficient to cause a further significant change in plasma IRI. Values for plasma IRI in mice are similar to published values (14, 19, 25).

Plasma corticosterone was significantly increased ($P < 0.05$) after 1 day of hindlimb immobilization ($30.8 \pm 3.0 \mu\text{g}$ corticosterone/dl plasma) as compared to control values (21.2 ± 2.2). There were five to six observations per mean. Values for plasma glucose in *group B* ($63.4 \pm 7.8 \text{ mg glucose/dl}$) were not significantly different from either *group A* (72.9 ± 11.5) or *group C* (51.2 ± 5.7). There were three to seven observations per mean.

Significant differences among *groups A, B, and C* existed for 2-deoxyglucose uptake, glycogen synthesis, and glucose oxidation (as shown by the F value for the factor of muscular usage in the factorial ANOVA, Table 2). To determine which of these means were different,

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Fig 2

Table 2

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286 mice eating either 1.2 or 0.8 g/day during the last day of
287 hindlimb immobilization (Fig. 2). These values also did
288 not differ from a group of mice, not immobilized, but
289 eating 1.2 g of food (Fig. 2). Thus, the difference in food
290 intake from 1.2 to 0.8 g was not sufficient to cause a
291 further significant change in plasma IRI. Values for
292 plasma IRI in mice are similar to published values (14,
293 19, 25).

294 Plasma corticosterone was significantly increased (P
295 < 0.05) after 1 day of hindlimb immobilization ($30.8 \pm$
296 $3.0 \mu\text{g corticosterone/dl plasma}$) as compared to control
297 values (21.2 ± 2.2). There were five to six observations
298 per mean. Values for plasma glucose in group B ($63.4 \pm$
299 $7.8 \text{ mg glucose/dl}$) were not significantly different from
300 either group A (72.9 ± 11.5) or group C (51.2 ± 5.7).
301 There were three to seven observations per mean.

302 Significant differences among groups A, B, and C
303 existed for 2-deoxyglucose uptake, glycogen synthesis,
304 and glucose oxidation (as shown by the F value for the
305 factor of muscular usage in the factorial ANOVA. Table
306 2). To determine which of these means were different,
307 Tukey's multiple-range test was used. Treatment with
308 sodium pentobarbital and food restriction for only 1 day
309 prior to measurement resulted in some significant differ-
310 ences in carbohydrate metabolism. 2-Deoxyglucose up-
311 take rate at 0.5 nM insulin in the incubation medium was
312 significantly increased in group B versus group A, as was
313 glycogen synthesis rate at 5 and 10 nM medium insulin
314 (Figs. 3 and 4). Glucose oxidation rate at 5 nM medium
315 insulin was decreased in group B versus group A (Fig. 5).
316 Because the only treatment difference between groups B
317 and C was the existence of hindlimb immobilization in
318 group C, group B was chosen as the reference group for
319 group C when determining whether or not statistical
320 differences existed solely as a result of the treatment of
321 hindlimb immobilization.

322 In the absence of insulin, there were no significant
323 differences between groups B and C in any indices of
324 carbohydrate metabolism utilized (Figs. 3-5). In the pres-
325 ence of all concentrations of insulin, however, 2-deoxy-
326 glucose uptake and glycogen synthesis were significantly
327 depressed in group C versus group B (Figs. 3 and 4). This
328 finding suggests decreased responsiveness and sensitivity
329 to insulin in the soleus muscle as a consequence of limb
330 immobilization. On the other hand, there was no signifi-
331 cant difference in glucose oxidation in soleus muscle
332 between groups B and C.

Table 2

Figs 3 & 4
Fig 5

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responsiveness

333 The ANOVA test also indicated that the levels of 2-
 334 deoxyglucose uptake and glycogen synthesis were de-
 335 pendent on insulin, but that the amount of glucose oxi-
 336 dation was independent of the level of insulin. In addi-
 337 tion, significant interaction existed between the factors
 338 of insulin and muscular usage for the processes of 2-
 339 deoxyglucose uptake and glycogen synthesis rates (Table
 340 1). Thus, muscular disuse attenuated the effect of varied
 341 concentrations of insulin on 2-deoxyglucose uptake and
 342 glycogen synthesis rates. No significant interaction be-
 343 tween these factors existed for glucose oxidation rate.
 344 The rates of 2-deoxyglucose uptake, glycogen synthesis,
 345 and glucose oxidation under control conditions observed
 346 in our present study are similar to previously published
 347 data (10, 20).
 348

DISCUSSION

349 Insulin sensitivity and responsiveness for indices of
 350 glucose metabolism are decreased in the soleus muscle of
 351 mice whose hindlimbs had been immobilized during the
 352 previous day. Compared to previous reports, this change
 353 is noteworthy both in the degree of lost insulin respon-
 354 siveness and in the rapidity of this loss. First, the loss of
 355 insulin responsiveness is greater in muscles from immo-
 356 bilized limbs. For example, indices of glucose metabolism
 357 in the soleus muscle of obese animals do not show the
 358 degree of loss in insulin sensitivity that was observed in
 359 the soleus muscles of immobilized limbs. Insulin respon-
 360 siveness for 2-deoxyglucose uptake is 60-100% of normal
 361 values in the soleus muscle in vitro from obese animals
 362 (9, 10, 11, 15). In these same studies, the insulin respon-
 363 siveness was 30-60% of normal for glycogen synthesis
 364 and 40-65% of normal for glucose oxidation in soleus
 365 muscles from obese animals. In contrast, there was es-
 366 sentially no insulin responsiveness of soleus muscles from
 367 immobilized mouse limbs in the present study. Second,
 368 the onset of the loss of insulin sensitivity and responsive-
 369 ness is more rapid in soleus muscles from immobilized
 370 limbs than from limbs of starved or obese mice (15, 19).
 371 For example, insulin sensitivity and responsiveness for
 372 glucose oxidation in the soleus muscle increase after 1
 373 day of fasting, but insulin sensitivity and responsiveness
 374 for glucose uptake and glycogen synthesis rates did not
 375 increase until the 2nd day of fasting (19). On the other
 376 hand, a marked insulin resistance for 2-deoxyglucose
 377 uptake and glycogen synthesis was present in the soleus
 378 muscle after 1 day of muscular disuse.

379 These observations on the more rapid and greater loss
 380 of insulin responsiveness in soleus muscles from immo-
 381 bilized limbs as compared to obese animals suggest that
 382 some process associated with limb immobilization is very
 383 powerful in modulating the sensitivity of the muscle to

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and 40-65% of normal for glucose oxidation in soleus muscles from obese animals. In contrast, there was essentially no insulin responsiveness of soleus muscles from immobilized mouse limbs in the present study. Second, the onset of the loss of insulin sensitivity and responsiveness is more rapid in soleus muscles from immobilized limbs than from limbs of starved or obese mice (15, 19). For example, insulin sensitivity and responsiveness for glucose oxidation in the soleus muscle increase after 1 day of fasting, but insulin sensitivity and responsiveness for glucose uptake and glycogen synthesis rates did not increase until the 2nd day of fasting (19). On the other hand, a marked insulin resistance for 2-deoxyglucose uptake and glycogen synthesis was present in the soleus muscle after 1 day of muscular disuse.

These observations on the more rapid and greater loss of insulin responsiveness in soleus muscles from immobilized limbs as compared to obese animals suggest that some process associated with limb immobilization is very powerful in modulating the sensitivity of the muscle to insulin. Glucose intolerance associated with insulin resistance exists in a number of clinical states, such as obesity, nonketotic diabetes mellitus, chronic renal failure, and states of adrenal corticoid excess (30). Hyperinsulinemia and/or increased blood glucocorticoids play roles in insulin resistance in certain of the above clinical conditions (30).

Hypoinsulinemia for a period of days in starved or streptozotocin diabetic mice is associated with an increased sensitivity to insulin by the soleus muscle (19). However, hypoinsulinemia is associated with a decreased, rather than an increased, sensitivity to insulin in soleus muscles from immobilized limbs. In contrast to the association of hyperinsulinemia and insulin resistance in the soleus muscle of obese mice (9-11, 15, 20, 27), insulin resistance in the soleus muscle after a day of disuse is not associated with hyperinsulinemia. Thus, insulin resistance in the disused soleus muscle is not a result of hyperinsulinemia and probably is not a result of insulin-receptor down-regulation, an event that is associated with hyperinsulinemia (16). On the other hand, levels of plasma corticosterone were most likely high throughout the 1 day of limb immobilization. We observed an increased plasma corticosterone level after 1 day of limb immobilization, which confirms a similar observation made by Ganong et al. (13) in dogs during

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410 limb immobilization. An increase in plasma corticoste-
411 roids might therefore result in insulin resistance as cor-
412 ticosteroids decrease the ability of skeletal muscles to
413 oxidize glucose (18, 31). Part of this action may be caused
414 by a decreased affinity for insulin by insulin receptors of
415 skeletal muscle because a similar postulate has been
416 shown for hepatocytes and adipocytes (17, 28).

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FIG. 1. Treatment: given to the 3 groups of mice used for measurements of carbohydrate metabolism in soleus muscle.

FIG. 2. Plasma immunoreactive insulin (IRI) was measured in 4 groups of mice. These groups were: 1) controls who daily ate 4.1 g of food ad libitum ($n = 12$), 2) anesthetized 1 day before and restricted to 1.2 g of food during next day ($n = 10$), 3) anesthetized while hindlimbs were immobilized 1 day before and restricted to 1.2 g of food during next day ($n = 5$), and 4) anesthetized while hindlimbs were immobilized 1 day before and eating 0.8 g of food during next day ($n = 12$). * $P < 0.05$, treatment group vs. control group. No significant differences existed among treatment groups. A least-significant-difference test was used to establish where significant differences existed among means after a significant F value was obtained from a one-way ANOVA. Values are means \pm SE; n = number of observations. Each observation is pooled blood from 2 mice.

FIG. 3. 2-Deoxyglucose uptake into mouse soleus muscle as a function of insulin concentrations in incubation medium. With Turkey's multiple-range test, a $P < 0.05$ within a single insulin concentration is indicated by * between \circ (group B) and \ominus (group C), + between Δ (group A) and \ominus , and +† between Δ and \circ . Values are means \pm SE.

FIG. 4. Glycogen synthesis rates in mouse soleus muscle as a function of insulin concentrations in incubation medium. A $P < 0.05$ within a single insulin concentration is indicated by * between \circ (group B) and \ominus (group C) and +† between Δ (group A) and \circ . Values are means \pm SE.

FIG. 5. Glucose oxidation rates in mouse soleus muscle as a function of insulin concentrations in incubation medium. A $P < 0.05$ within a single insulin concentration is indicated by + between Δ (group A) and \ominus (group C) and +† between Δ and \circ (group B). Values are means \pm SE.

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Previous reports showed a decreased uptake of glucose across the forearms of healthy people during continuous bed rest (21, 22). In these studies, muscle, skin, and bone were perfused. The existence of insulin resistance in skeletal muscle as a result of disuse was inferred. Our present data suggest a direct insulin resistance in skeletal muscle as a consequence of disuse. The usage of isolated muscles in vitro has the advantage of testing for the direct involvement of skeletal muscle in insulin resistance. This methodology also has potential limitations. One disadvantage is diffusion limitations. Muscles in vitro must rely on the diffusion of substrates and hormones from the external medium instead of on delivery under pressure from capillaries. Utilization of the mouse soleus muscle minimizes this limitation for the following reasons. First, diffusion limitations for glucose uptake (8) and for glucose oxidation and glycogen synthesis (9) do not occur until the wet weight of the soleus muscle exceeds 30 mg (9). Soleus muscles were one-third this weight in the present study. Second, the concentration of ATP in soleus muscles did not differ between in vivo values and levels found at the end of the in vitro incubation period. This suggests that there was no major diffusion limitation for oxygen in isolated muscles of the present study.

The results of the present study on muscular disuse, as well as the results of earlier studies on muscular activity, suggest the following concept: there is a direct relationship between the quantity of contractile activity and the insulin responsiveness of the skeletal muscle. After 1 day of limb immobilization, physiological and pharmacological concentrations of insulin are not effective in stimulating carbohydrate metabolism in the disused soleus muscle. In contrast, lower than physiological concentrations of insulin in the plasma or perfusate are effective in stimulating glucose uptake into contracting skeletal muscle (1, 2). Although numerous studies have shown a decreased glucose tolerance in healthy humans after chronic bed rest (5, 12, 21, 22), an increased insulin sensitivity for glucose removal during glucose tolerance has been shown after physical training in humans (4, 23) and in animals (3, 26). Thus, responsiveness of the muscle to insulin is directly related to its immediate history of usage.

In summary, our present results provide the first direct evidence of insulin resistance in skeletal muscle that has undergone a previous period of reduced muscle usage. Furthermore, this lack of responsiveness to insulin developed in 1 day and in the presence of hypoinsulinemia.

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lating carbohydrate metabolism in the discussed skeletal muscle. In contrast, lower than physiological concentrations of insulin in the plasma or perfusate are effective in stimulating glucose uptake into contracting skeletal muscle (1, 2). Although numerous studies have shown a decreased glucose tolerance in healthy humans after chronic bed rest (5, 12, 21, 22), an increased insulin sensitivity for glucose removal during glucose tolerance has been shown after physical training in humans (4, 23) and in animals (3, 26). Thus, responsiveness of the muscle to insulin is directly related to its immediate history of usage.

In summary, our present results provide the first direct evidence of insulin resistance in skeletal muscle that has undergone a previous period of reduced muscle usage. Furthermore, this lack of responsiveness to insulin developed in 1 day and in the presence of hypoinsulinemia. Future studies that utilize the model of hindlimb immobilization to determine the cause(s) of these changes now seem to be justified.

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TABLE 1. Some characteristics of mice after various treatments

| | Group A | Group B | Group C |
|--|---------------------------------|---------------------|--------------------------------|
| Body wt, g | | | |
| Before treatments | 31 ± 1 (18) | 31 ± 1 (24) | 31 ± 1 (24) |
| One day later | 30 ± 1 [‡] (16) | 27 ± 1 (21) | 28 ± 1 (20) |
| Food intake, g/day | 5.0 ± 0.6 [†] (16) | 1.2 ± 0.4 (21) | 0.8 ± 0.2 (20) |
| Soleus wet wt, mg | 9.8 ± 0.5 (18) | 9.4 ± 0.3 (42) | 10.1 ± 0.3 (42) |
| Soleus protein content, mg protein/soleus muscle | 1.52 ± 0.05 (18) | 1.58 ± 0.05 (42) | 1.66 ± 0.06 (42) |
| Soleus ↓ glycogen ↓ concn. μmol/g wet wt/ | 22.6 ± 1.0 [†] (17) | 11.6 ± 2.0 (8) | 23.4 ± 3.0 [‡] (8) |

Values are means ± SE; numbers in parentheses are numbers of the observations. Group A: untreated; group B: anesthetized, food-restricted; group C: anesthetized, ate less, hindlimbs immobilized for 1 day. See METHODS for description of treatments. Statistical analysis was with an unpaired Student's *t* test. * *P* < 0.006 vs. group B; † *P* < 0.001 vs. group B; ‡ *P* < 0.01 vs. group B.

TABLE 2. *F* values for factorial ANOVA for 2-deoxyglucose uptake, glycogen synthesis, and glucose oxidation

| Treatments | <i>F</i> values | | |
|------------------------|-------------------|--------------------|-------------------|
| | 2-Deoxyglucose | Glycogen synthesis | Glucose oxidation |
| Insulin | 7.42* | 8.49* | 1.97 |
| Muscle usage | 13.35* | 43.41* | 40.90* |
| Insulin × muscle usage | 3.00 [†] | 2.97 [‡] | 1.98 |

A 3 × 3 factorial ANOVA was used for 2-deoxyglucose, whereas a 3 × 5 factorial ANOVA was separately employed for glycogen synthesis and glucose oxidation. Application of Fisher's *F* test to *F* values from the factorial ANOVA demonstrated that significant differences among group means for 2-deoxyglucose and for glycogen synthesis occur because of variations in insulin dosage alone and that significant differences among group means occur for all three indices of glucose metabolism because of variation in usage among. The *F* test also indicated that some of the significance among group means is due to interaction between the treatments of insulin muscle usage. In other words, the effects elicited by administration of these two treatments simultaneously attenuated the effect of insulin alone. * *P* < 0.005; † *P* < 0.05; ‡ *P* < 0.01.

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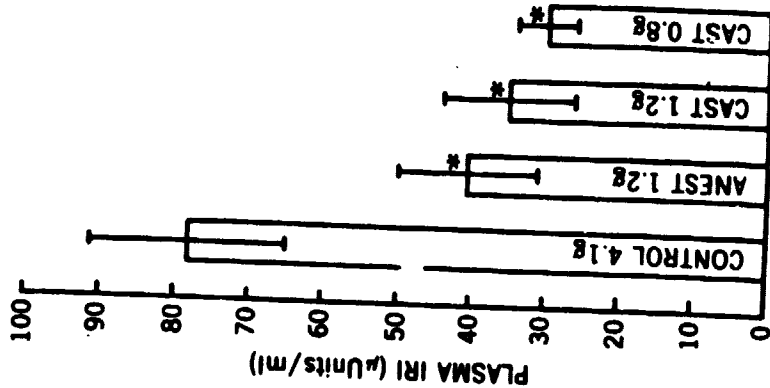
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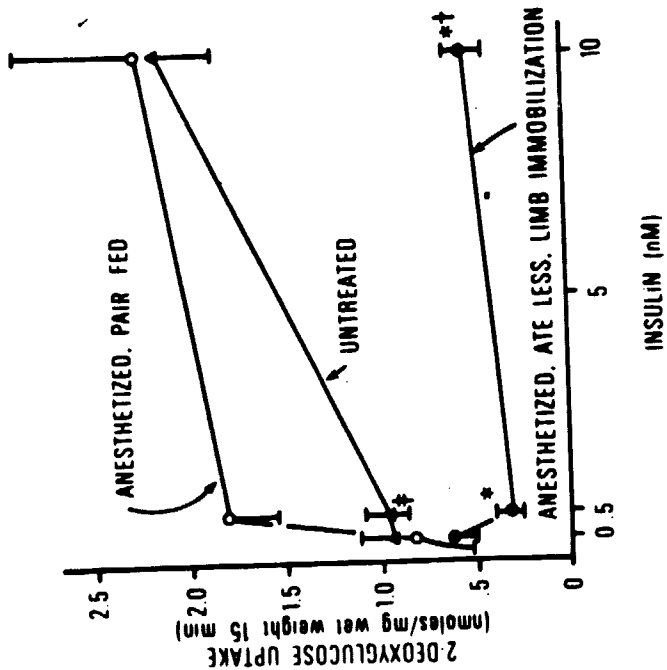
| Treatment | Group A | Group B | Group C |
|---------------------|------------|--------------------------------|------------------------------------|
| Anesthesia | ----- | Anesthetized 1 day prior | Anesthetized 1 day prior |
| Food restriction | ----- | Food restricted 1 day prior | Ate less 1 day prior |
| Limb immobilization | ----- | ----- | Limb immobilization 1 day prior |

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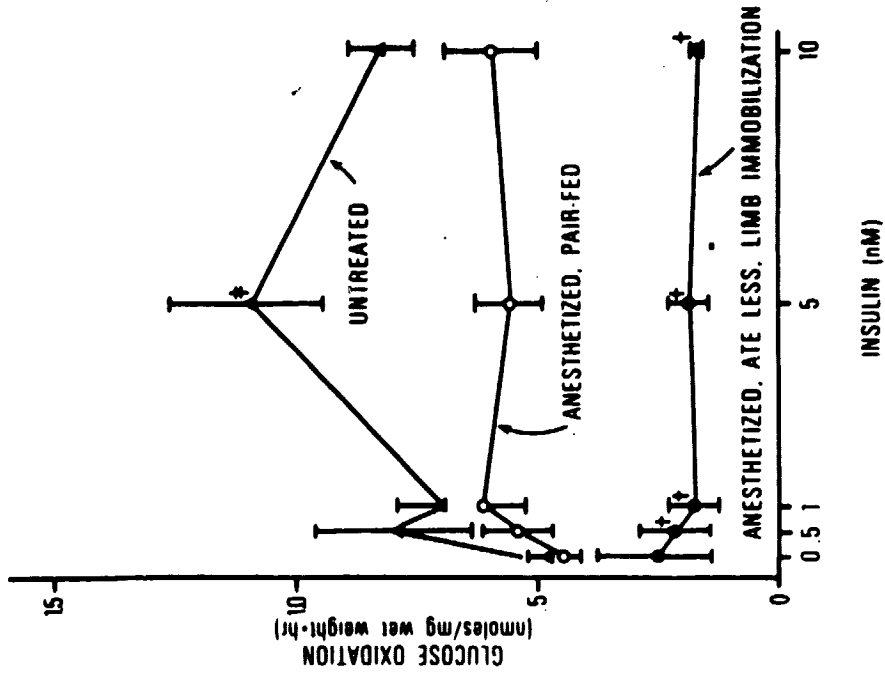
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