Decreased contraction-stimulated glucose transport in isolated epitrochlearis muscles of pregnant rats

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Sancho, Raquel, Junghoon Kim, and Gregory D. Cartee. Decreased contraction-stimulated glucose transport in isolated epitrochlearis muscles of pregnant rats. J Appl Physiol 98: 1021–1027, 2005. First published November 5, 2004; doi:10.1152/japplphysiol.00953.2004.—Late pregnancy is characterized by insulin resistance for glucose transport in skeletal muscle. The main purpose of this study was to investigate the effect of late pregnancy on contraction-stimulated glucose transport in isolated rat skeletal muscle after in vitro electrical stimulation. Isolated epitrochlearis muscles of 19-day pregnant and aged-matched nonpregnant control rats were studied. One muscle from each rat was stimulated to contract, and the contralateral muscle served as a resting control. Tension developed during contractile activity, 3-O-methyl glucose (3-MG) transport rate, and glycogen concentration were determined. Epitrochlearis muscles from other rats were used to measure insulin-stimulated 3-MG transport. There was no detectable difference between the nonpregnant and pregnant groups for contractile performance (peak tension, total tension, or fatigue). Pregnancy was not associated with significant changes in muscle glycogen concentration (resting or after contractile activity) or the contraction-stimulated decrement in glycogen concentration. For muscles from pregnant vs. nonpregnant groups, there was a 22% reduction (P ≤ 0.05) in contraction-stimulated glucose transport, a 28% decrease (P ≤ 0.05) in insulin-stimulated glucose transport, and unchanged basal glucose transport. In conclusion, isolated epitrochlearis muscles from pregnant vs. nonpregnant rats had a relative decrement in contraction-stimulated glucose transport that was similar to the relative decline in insulin-stimulated glucose transport. The decrement in contraction-stimulated glucose transport was not attributable to pregnancy-related changes in tension development or glycogen levels. The similar relative decline in insulin- and contraction-stimulated glucose transport raises the possibility that pregnancy impairs a distal process that is common to mechanisms whereby each stimulus activates glucose transport.

LATE PREGNANCY (gestational days 18–22 in the rat, third trimester in humans) is characterized by whole body insulin resistance (8, 27). Insulin-stimulated glucose disposal by skeletal muscle during a euglycemic-hyperinsulinemic clamp is reduced in pregnant compared with nonpregnant rats (27). Insulin resistance is also a normal characteristic of late pregnancy in humans, presumably to ensure adequate availability of maternal nutrients to the fetus during this period of rapid growth (5, 25). Toyoda et al. (36) reported decreased insulin-stimulated glucose transport in isolated epitrochlearis muscles of late pregnant rats compared with nonpregnant controls, demonstrating a decrement in the intrinsic capacity for insulin-stimulated glucose transport in skeletal muscle, independent of systemic factors that influence whole body glucose regulation (e.g., pregnancy-related changes in circulating levels of lipids and hormones). Skeletal muscle accounts for a major portion of insulin-stimulated glucose disposal (7). Therefore, decreased insulin sensitivity in skeletal muscle has a great impact on whole body glucose homeostasis.

Although insulin-stimulated glucose transport into skeletal muscle is impaired in late gestation, skeletal muscle glucose transport can also be stimulated by contractile activity or exercise via an insulin-independent pathway (14, 21). Previously published studies have evaluated the influence of pregnancy on glucose uptake by skeletal muscle during electrically stimulated contractile activity in perfused rat hindlimb preparations (11) and during an intravenous glucose tolerance test that followed a bout of treadmill running by rats (37). In light of the pregnancy-induced insulin resistance, it was surprising that skeletal muscle glucose uptake rate was not reduced for pregnant compared with nonpregnant controls in these studies. However, in each study, there were pregnancy-related changes in systemic factors, e.g., altered muscle blood flow distribution (11) or increased plasma insulin concentration (37), which would be expected to influence glucose uptake. Thus it is unclear whether pregnancy alters the intrinsic capacity of skeletal muscle, independent of systemic factors, to increase glucose transport in response to contractile activity. In the context of previous published results, we hypothesized that insulin-stimulated, but not contraction-stimulated, glucose transport would be lower in isolated epitrochlearis muscles from pregnant compared with age-matched nonpregnant control rats.

Accordingly, the primary purpose of the present study was to determine whether late pregnancy affects contraction-stimulated glucose transport in rat skeletal muscle. In this study, isolated epitrochlearis muscles from pregnant and nonpregnant rats were stimulated to contract by the same stimulation protocol. In addition, insulin-stimulated glucose uptake was determined for epitrochlearis muscles so that the magnitude of pregnancy effects on the two major pathways for activating muscle glucose transport could be compared in the same study.

Under resting conditions, skeletal muscle glycogen concentration is similar or higher for pregnant compared with nonpregnant rats (17, 18, 28). However, the effect of contractile activity on glycogen depletion during pregnancy has apparently not been previously reported. Because glycogen concentration has been implicated as a possible regulator of contractile activity, demonstrating a decrement in the intrinsic capacity for insulin-stimulated glucose transport in skeletal muscle, independent of systemic factors that influence whole body glucose regulation (e.g., pregnancy-related changes in circulating levels of lipids and hormones). Skeletal muscle accounts for a major portion of insulin-stimulated glucose disposal (7). Therefore, decreased insulin sensitivity in skeletal muscle has a great impact on whole body glucose homeostasis.

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tion-stimulated glucose transport into skeletal muscle (2, 15), we also evaluated the effect of pregnancy on resting and postcontraction glycogen concentration.

Contraction-stimulated glucose transport has been reported to increase as the amount of tension developed by the muscle increases (19, 20). In apparently the only published description of pregnancy effects on skeletal muscle contractile performance, Gorski et al. (11) found more rapid fatigue in skeletal muscle from pregnant compared with nonpregnant rats during contractile activity induced by electrical stimulation via the nerve. Therefore, we also evaluated tension development and fatigue during contractile activity in muscles from pregnant and nonpregnant rats.

METHODS

Animal treatment. Animal care and treatment were performed according to a protocol that was approved by the University of Wisconsin-Madison Animal Care and Use Committee. Female Sprague-Dawley rats, pregnant and aged-matched nonpregnant controls, were obtained from Harlan (Oregon, WI) at 11 wk old (gestational day 14 for pregnant rats). Rats were provided with rat chow (Harlan Teklad Laboratory Rodent Diet, Madison, WI) and water ad libitum, until 1700 on the day before an experiment, at which time food was restricted to 4 g. On the day of an experiment, between 0900 and 1300, pregnant (day 19 of pregnancy) and nonpregnant control rats were anesthetized by an intraperitoneal injection of pentobarbital sodium (5 mg/100 g body wt), and both epitrochlearis muscles were carefully dissected out.

Muscle incubation for experiments with insulin. Earlier research demonstrated that glucose transport in insulin-stimulated epitrochlearis muscles was lower for pregnant compared with nonpregnant rats (36). The insulin dose used was known to be maximally effective for muscle from nonpregnant rats, but previous studies had not confirmed that the insulin dose was sufficient for maximally activating glucose transport by epitrochlearis muscles from insulin-resistant pregnant rats. Therefore, for experiment 1, only pregnant rats were studied to establish a maximally effective concentration in epitrochlearis muscles from pregnant rats by comparing the ability of two, supraphysiologically elevated insulin concentrations to increase glucose transport in paired muscles from pregnant rats. After dissection, muscles were incubated in 25-ml Erlenmeyer flasks containing 3 ml of Krebs-Henseleit buffer (KHB), 0.1% BSA, 2 mM sodium pyruvate, and 6 mM mannitol, supplemented with insulin (either 2,000 μU/ml or 10,000 μU/ml) for the contralateral muscle) at 30°C for 30 min. Muscles from other pregnant rats were incubated identically except that the incubation solution was not supplemented with insulin (basal). Flasks were gassed continuously with 95% O2-5% CO2.

For experiment 2, paired epitrochlearis muscles from pregnant and nonpregnant rats were studied. One muscle from each rat was incubated without insulin, and the contralateral muscle was incubated with insulin by using a concentration of insulin found to be maximally effective in experiment 1. The incubations were performed as described for experiment 1. For each rat, insulin-stimulated glucose transport was calculated as the difference between the insulin-treated and basal (no insulin) values.

In vitro contractile activity. The effects of contractile activity on isolated epitrochlearis muscles from pregnant and nonpregnant rats were evaluated in experiment 3. After dissection, both epitrochlearis muscles from pregnant and nonpregnant rats were carefully mounted in an in vitro electrical stimulation apparatus (Radnoti, Monrovia, CA). The distal end of the muscle was attached, with a modified muscle clip (Kent Scientific, Litchfield, CT), to a vertical glass rod suspended in an 8-ml glass bath containing two platinum electrodes.

The proximal end of the muscle was attached, with another modified muscle clip, to an isometric force transducer (Radnoti). The muscles were suspended in a solution containing KHB, 8 mM glucose, and 2 mM mannitol, at 37°C and gassed with 95% O2-5% CO2. The resting tension was adjusted to 0.4 g. Muscles were allowed to recover from surgery and mounting procedures for 5 min. One muscle was electrically stimulated to contract with supramaximal square-wave pulses by use of a Grass S48 Stimulator (Grass Instruments, Quincy, MA). Ten tetanic isometric contractions were produced at a rate of one tetanus per minute at 100 Hz for 10 s, with a pulse duration of 0.5 ms, for a total of 10 min. The contralateral muscle was mounted identically, with resting tension equal to 0.4 g, but not stimulated to contract.

After the contractile activity (or equivalent time period for the resting control muscles), muscles were transferred to 25-ml Erlenmeyer flasks containing 3 ml of KHB, 2 mM sodium pyruvate, and 6 mM mannitol at 30°C for 10 min.

3-O-methylglucose transport measurement. After the initial 30-min incubation period for muscles in the insulin-stimulation experiments and the postcontraction, 10 min incubation period for muscles in the contraction experiment, muscles were transferred to a flask containing 3 ml of KHB, 8 mM 3-O-methylglucose (3-MG; 0.25 mCi/mmol 3H-labeled 3-MG), and 2 mM mannitol (6.25 mCi/mmol D-1,4-C[mannitol]) at 30°C in a shaking water bath for 15 min. Insulin and BSA concentrations were identical to the preceding incubation step. Flasks were gassed continuously with 95% O2-5% CO2. Muscles were then rapidly trimmed, frozen by using aluminum tongs cooled in liquid nitrogen, and stored at −80°C until processed. Frozen muscles were weighed and then homogenized using glass-on-glass grinding tubes in 1 ml of 0.3 M perchloric acid at 4°C. A portion of the muscle homogenate and of the incubation media was used to determine the rate of 3-MG transport as described (4). Values were expressed as micromoles 3-MG accumulated per gram per 15 min. Insulin-stimulated (experiment 2) and contraction-stimulated (experiment 3) glucose transport above basal controls were calculated as the difference between the stimulated (insulin or contraction, respectively) value and the unstimulated value in the contralateral muscle. The purpose of these calculations was to allow evaluation of the specific effects of insulin or contraction on glucose transport distinct from basal glucose transport rates.

Glycogen concentration. Glycogen concentration was determined in perchloric acid homogenates via the amyloglucosidase method described by Passonneau and Lauderdale (32). For each animal, glycogen depletion was estimated as the difference between the contracted and the noncontracted values.

Tension development. Tension was measured on the same muscles that were examined during the contraction-stimulation glucose transport studies. Tension development data, measured by an isometric force transducer during the 10-min contracting period, was recorded and analyzed using a MP-100 unit and MP-100 software (BIOPAC Systems, Santa Barbara, CA). For each tetanic contraction, tension development was calculated as the highest tension achieved during that tetanus minus baseline tension. Peak tension was defined as the maximal tension developed during the 10-min contractile period. For each tetanic contraction, a fatigue index was calculated as follows: (tension developed + peak tension) × 100%. For each tetanus, except the tetanus producing peak tension, we compared the mean fatigue index of the pregnant group with the mean fatigue index of the nonpregnant group. In addition, the area under the curve (AUC), which represents the total tension developed in a tetanus, was calculated for each of the 10 tetanic contractions performed by muscles of pregnant and nonpregnant rats.

Statistical analysis. An unpaired Student’s t-test was used to compare values from pregnant and nonpregnant rats. A paired t-test was used when comparing paired muscles from the same rat in the experiment designed to determine a maximally effective insulin concentration in muscles from pregnant rats. A P value ≤0.05 was considered statistically significant. Data are presented as means ± SE.
RESULTS

Body weight. As expected, the body weight of 19-day pregnant rats was significantly increased compared with the age-matched nonpregnant rats (means ± SE for pooled data from all three experiments: 293.8 ± 2.9 g, n = 27, and 206.5 ± 1.2 g, n = 22, respectively, P < 0.001).

Effect of two supraphysiological insulin concentrations on glucose transport by muscles from pregnant rats. In pregnant animals, there was no difference between the 3-MG transport values obtained for paired epitrochlearis muscles (n = 6) incubated with 2,000 µU/ml (0.589 ± 0.088 µmol × g⁻¹ × 15 min⁻¹) compared with 10,000 µU/ml (0.544 ± 0.052 µmol × g⁻¹ × 15 min⁻¹) of insulin. The lack of higher glucose transport with 10,000 vs. 2,000 µU/ml insulin despite a five-fold increase in insulin concentration confirms that 2,000 µU/ml insulin is sufficient to maximally activate insulin-stimulated glucose transport of insulin resistant epitrochlearis muscles from pregnant rats. Basal 3-MG transport in muscles from other pregnant rats (n = 3) was 0.066 ± 0.011 µmol × g⁻¹ × 15 min⁻¹.

Insulin-stimulated glucose transport for muscles from nonpregnant and pregnant rats. 3-MG transport values under the basal (no insulin) condition were not significantly different between the pregnant (n = 6) and nonpregnant (n = 7) groups (Fig. 1). Glucose transport values in muscles incubated with insulin were 25% lower (P < 0.05) for the pregnant group compared with the nonpregnant group. Insulin-stimulated glucose transport expressed as the delta value (insulin-treated value minus basal value from paired muscles) was decreased by 28% in the pregnant group compared with the nonpregnant group (P < 0.05).

Contraction-stimulated glucose transport. The 3-MG transport values in the resting muscles were similar for the pregnant (n = 12) and nonpregnant (n = 15) groups (Fig. 2). The 3-MG transport values for contracted muscles were 16% lower (P < 0.05) in the pregnant group compared with the nonpregnant group. Contraction-stimulated glucose transport expressed as the delta value (postcontraction value minus paired, resting value) was 22% lower (P < 0.05) in the pregnant compared with the nonpregnant group (Fig. 2).

Glycogen concentration. Resting (no contraction) glycogen values were not significantly different for muscles from pregnant (n = 12) and nonpregnant (n = 15) rats (Fig. 3). Likewise, glycogen values from contracted muscles were similar for pregnant and nonpregnant muscles. Contraction-induced glycogen depletion (postcontraction value minus paired, resting value) was also not different between pregnant and nonpregnant groups.

![Fig. 1. 3-O-methylglucose (3-MG) transport in epitrochlearis muscles from pregnant (n = 6) and nonpregnant (n = 7) rats. For each rat, 1 muscle was treated with no insulin (basal; open bars) and the contralateral muscle was treated with 2,000 µU/ml of insulin (hatched bars). For paired muscles from each rat, insulin-stimulated above basal (crosshatched bars) was calculated as the difference between the value of the insulin-treated muscle and the value from the contralateral muscle that was incubated without insulin. Values are means ± SE. *Significantly different from nonpregnant with same insulin concentration (P ≤ 0.05).](http://jap.physiology.org/)

![Fig. 2. 3-MG transport in epitrochlearis muscles from pregnant (n = 12) and nonpregnant (n = 15) rats. For each rat, 1 muscle was stimulated to contract whereas the contralateral muscle was not contracted (basal). For paired muscles from each rat, contraction-stimulated above basal (crosshatched bars) was calculated as the difference between the value of the contracted muscle (hatched bars) and the value from the contralateral muscle that was not contracted (open bars). Values are means ± SE. *Significantly different from nonpregnant with same contraction protocol (P ≤ 0.05).](http://jap.physiology.org/)

![Fig. 3. Glycogen concentration in epitrochlearis muscles from pregnant (n = 12) and nonpregnant (n = 15) rats. Muscles were stimulated to contract or not contracted. For paired muscles from each rat, contraction-induced glycogen depletion (crosshatched bars) was calculated as the difference between the value of the contracted muscle (hatched bars) and the value of the contralateral muscle that was not contracted (open bars). Values are means ± SE.](http://jap.physiology.org/)
Tension development and fatigue index. Because of computer failures during data collection, tension data were lost for 1 pregnant rat and 2 nonpregnant rats, and sample sizes are reduced accordingly for contractile data (pregnant, n = 11; nonpregnant, n = 13). Peak tension (maximal tension developed during contractile activity) always occurred in the initial tetanus and was similar between the pregnant and nonpregnant groups (32.2 ± 0.3 and 33.7 ± 0.2 g, respectively). Tension development for each subsequent tetanus (i.e., from tetanus 2 to tetanus 10) was also similar and not significantly different between muscles of nonpregnant and pregnant rats. The epitrochlearis mass did not differ significantly between the two groups (pregnant = 33.5 ± 0.3 mg and nonpregnant = 34.1 ± 0.2 mg). Accordingly, there were no significant differences between groups for tension expressed per gram muscle wet weight. Fatigue index (% of peak tension) for tetani 2-10 was also not different between the pregnant and nonpregnant groups (Fig. 4). Resting tension for the resting muscles in both groups was stable throughout the experiment (i.e., at passive tension of 0.4 g).

There were no significant differences between pregnant and nonpregnant rats for total tension developed, represented by the AUC, for any of the 10 tetani performed, expressed in absolute units (g/s) or expressed relative to muscle mass (g × s⁻¹ × g muscle wet wt⁻¹; Fig. 5). Additionally, summed AUC for all 10 tetani was not significantly different between pregnant and nonpregnant rats.

DISCUSSION

The most important new findings of the present study were that in isolated epitrochlearis muscle from late pregnant rats compared with nonpregnant controls there was 1) a decrease (22%) in contraction-stimulated glucose transport that was similar in magnitude to the pregnancy-related reduction (28%) in insulin-stimulated glucose transport; 2) unaltered contractile performance (peak tension, total tension, and fatigue) with direct muscle stimulation; and 3) no significant difference in contraction-stimulated muscle glycogen depletion. These results provide novel insights into the influence of pregnancy on key aspects of skeletal muscle metabolism and contractile function.

The 28% decrease in insulin-stimulated glucose transport (insulin-treated value minus basal value) in epitrochlearis muscles from pregnant vs. nonpregnant rats is very similar to the results of Toyoda et al. (36), who found that insulin-stimulated glucose transport in isolated epitrochlearis muscles of 20-day pregnant rats was ~30% lower than values for nonpregnant rats. Leturque et al. (27) used a euglycemic-hyperinsulinemic clamp in rats, together with injection of radiolabeled 2-deoxyglucose (2-DG), and found reduced in vivo 2-DG uptake of epitrochlearis muscles of 19-day pregnant compared with nonpregnant controls. These results indicate that pregnancy-induced insulin resistance in isolated rat epitrochlearis muscles is preserved in vivo.

Our experiment is apparently the first to assess the effect of pregnancy on contraction-stimulated glucose transport by isolated skeletal muscle. On the basis of the results of previous studies using other models to measure skeletal muscle glucose uptake after exercise or contractile activity, i.e., intravenous glucose tolerance test (IVGTT) with 2-DG injection in vivo (37) and perfused hindlimb (11), we did not expect to find a pregnancy-induced decrement in contraction-stimulated glucose transport. The divergent results in our study compared with these earlier studies are likely related to differences in experimental techniques and, taken together, provide valuable information about skeletal muscle function during pregnancy.

Both Gorski et al. (11) and Treadway and Young (37) studied rat hindlimb muscles. The fiber type composition of the epitrochlearis muscle (75% fast-twitch glycolytic, 17% fast-twitch oxidative glycolytic, and 8% slow-twitch oxidative) is very similar to the average fiber type composition of 32% fast-twitch oxidative glycolytic, 5% slow-twitch oxidative) (1,
Therefore, it is unlikely that the discrepancies between our results and the results by Gorski et al. (11) and Treadway and Young (37) are related to differences in fiber type.

Gorski et al. (11) found no significant effect of pregnancy on glucose uptake by perfused hindlimb muscles during in situ contractions. Although total perfusate flow was similar for pregnant and nonpregnant animals, perfusion pressure was significantly higher for pregnant compared with pregnant rats. The greater 2-DG uptake values in the white gastrocnemius of pregnant rats, compared with the nonpregnant rats, may reflect increased load and possibly differences in muscle recruitment.

The decrement of contraction-stimulated glucose transport demonstrated in isolated rat skeletal muscle during late pregnancy may be an adaptation that serves to protect the nutrient supply to the fetus. This interpretation is highly speculative, and therefore caution is appropriate when considering the possible relevance of these results for understanding the influence of exercise on glucose metabolism in pregnant women.

Previous studies suggested tension development as a possible modulator of contraction-stimulated glucose transport (19, 20, 22), and the results from Gorski et al. (11) indicated that pregnancy might influence contractile performance. Therefore, tension development was measured to gain insights into the possible mechanisms regulating contraction-stimulated glucose transport in late pregnancy. Peach tetanic tension development, total tension development, and fatigue during in vitro contractile activity were not different between the pregnant and nonpregnant groups. Gorski et al. measured tension development by hindlimb muscles during in situ stimulation via the nerve in late pregnant and nonpregnant rats and, consistent with our results, found that pregnant and nonpregnant muscles achieved similar peak tensions, whether expressed in absolute terms or relative to muscle mass. However, in contrast to our results, they found that muscles from pregnant rats exhibited more rapid fatigue than nonpregnant controls. Although the present study used a stimulation protocol different from the protocol used by Gorski et al., both stimulation protocols produced intense muscular contractions: the force developed near the end of the contracting period was only 10–35% of initial peak tension developed. The discrepancies in fatigue are possibly related to the mode of stimulation: Gorski et al. stimulated the hindlimb muscles through the nerve, and we stimulated the muscles directly. Perhaps the greater fatigue was attributable to the effect of pregnancy on neural factors rather than changes in the intrinsic ability of the muscle to produce tension.

Results from several studies suggest that late pregnancy may increase skeletal muscle resting glycogen concentrations (17, 18, 28), and previous studies have implicated precontraction glycogen concentration as a regulator of contraction-induced glycogen depletion and glucose transport (15, 22, 23). Accord-
ingly, we assessed glycogen levels and found that pregnancy was associated with a nonsignificant trend for 13% increase in resting glycogen concentrations in epitrochlearis muscles, a result similar to the data of Holness and Sugden (17, 18). Our results also indicated that pregnancy did not alter the amount of contraction-induced glycogen depletion. These findings indicate that the pregnancy-induced decline in contraction-stimulated glucose transport cannot be attributed to pregnancy-related changes in resting glycogen concentrations, postcontraction glycogen concentration, or contraction-induced glycogen depletion.

Both insulin and exercise/contractile activity induce an increase in skeletal muscle glucose transport by activating the redistribution of GLUT4 glucose transporters from the cell interior to the cell surface (16). Earlier research demonstrated that skeletal muscle (red quadriiceps, white quadriiceps, soleus, red gastrocnemius, or white gastrocnemius) GLUT4 abundance is not different for pregnant compared with nonpregnant rats (30, 31, 40), indicating that reduced GLUT4 expression is not the cause of the pregnancy-induced effects on glucose transport. Skeletal muscle GLUT4 abundance is also not reduced in humans during pregnancy, whether the women are lean or obese or have gestational diabetes mellitus (10, 35). GLUT4 was not measured in the present study, but there is compelling evidence that skeletal muscle GLUT4 is not reduced in pregnancy, including white gastrocnemius and white quadriiceps muscles with predominantly fast-twitch glycolytic fiber-type composition (1) similar to the epitrochlearis (38). Although insulin and contractile activity ultimately depend on GLUT4 translocation, they use different signaling pathways: insulin relies on a pathway that involves the insulin receptor, insulin receptor substrates (predominantly IRS-1), and phosphatidylinositol 3-kinase (PI3K) (6), and exercise/contraction appears to depend on the combined effects of AMP-activated protein kinase and calcium-mediated processes, e.g., calcium/calmodulin-dependent protein kinase (39). Saad et al. (33) found decreased levels of tyrosine phosphorylated IRS-1 and IRS-1-associated PI3K in insulin-stimulated skeletal muscles of 20-day pregnant rats compared with nonpregnant rats. However, PI3K is not required for contraction-stimulated glucose transport (26), so a decrement in PI3K does not account for the reduced contraction-stimulated glucose transport in skeletal muscle from pregnant rats. One possibility is that pregnancy causes a defect in AMP-activated protein kinase- and/or calcium/calmodulin-dependent protein kinase-related signaling. Alternatively, pregnancy could result in a defect at a distal, shared step in the process whereby insulin and contraction lead to glucose transport. The similarity in the magnitude of the decrease in insulin-stimulated glucose transport (28%) and contraction-stimulated glucose transport (22%) is consistent with, although not proof for, the latter idea. Interestingly, Shao et al. (34) found that insulin-stimulated glucose transport measured in vitro with skeletal muscle strips is lower for pregnant compared with nonpregnant women. Tyrosine phosphorylation of IRS-1 was also lower in muscles from the pregnant women vs. nonpregnant controls, but elimination of the difference in tyrosine phosphorylated IRS-1 using vandate, a phosphatase inhibitor, did not normalize glucose transport in the pregnant group. The authors interpreted these results to suggest that pregnancy acts on sites distal to IRS-1, potentially involved with GLUT4 trafficking. Regardless of the site(s) of the defect(s), pregnancy apparently leads to a reduction in the recruitment, translocation, and/or activity of GLUT4 transporter proteins rather than altered total GLUT4 abundance.

Late pregnancy is not unique with regard to the impaired activation of skeletal muscle glucose transport with either insulin or contractions. Rats consuming high-fat or high-sucrose diets (4–8 wk) compared with animals eating control diets display decrements epitrochlearis glucose transport stimulated by either insulin or contractile activity (12, 13, 24). The diet-induced reductions in glucose transport were attributable to reduced cell surface GLUT4 content with either stimulus even though there was no reduction in total GLUT4 levels (12, 13, 24). In contrast, skeletal muscle from obese compared with lean Zucker rats had a selective decrement in insulin-stimulated glucose uptake and cell-surface GLUT4 with normal contraction-stimulated glucose uptake and cell-surface GLUT4 in skeletal muscle (3, 9). Insulin resistance in obese Zucker rats was found even in muscles with normal GLUT4 levels. Normal insulin-stimulated glucose transport concomitant with attenuated contraction-stimulated glucose transport has also been observed in skeletal muscle from transgenic mice expressing a dominant inhibitory mutant of AMP-activated protein kinase vs. nontransgenic littermates despite no genotype-related difference for total GLUT4 levels (29). Thus reduced glucose transport in response to insulin and/or contraction has been observed under many conditions that do not reduce total GLUT4 abundance in skeletal muscle.

In conclusion, insulin-resistant epitrochlearis muscles of late pregnant rats have decreased contraction-stimulated glucose transport, compared with nonpregnant rats. The decrease in contraction-stimulated glucose transport cannot be attributed to alterations in resting glycogen concentration, contraction-induced glycogen depletion, peak tension development, or muscular fatigue, because the values for those parameters were similar between the pregnant and nonpregnant groups. Taking together the present demonstration that contraction-stimulated glucose transport is reduced in isolated skeletal muscle from pregnant rats with evidence from earlier studies that indicated that glucose uptake during in situ contractile activity or after in vivo exercise is not reduced in pregnant rats (11, 37) suggests the possibility that during in vivo exercise or in situ contractile activity, compensation by systemic factors (e.g., altered blood flow, increased muscle loading and/or recruitment, and/or increased circulating insulin) may oppose the intrinsic decline in the activation of skeletal muscle glucose transport in late pregnancy.

GRANTS

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REFERENCES


