Skeletal muscle contraction stimulates capillary recruitment and glucose uptake in insulin-resistant obese Zucker rats

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Wheatley, Catherine M., Stephen Rattigan, Stephen M. Richards, Eugene J. Barrett, and Michael G. Clark. Skeletal muscle contraction stimulates capillary recruitment and glucose uptake in insulin-resistant obese Zucker rats. Am J Physiol Endocrinol Metab 287:E804–E809, 2004. — Exercise and insulin increase muscle glucose uptake by different mechanisms and also increase capillary recruitment, which is proposed to facilitate access for hormones and nutrients. The genetically obese Zucker rat shows impaired insulin- but not contraction-mediated glucose uptake in muscle. Recently, we have shown the genetically obese Zucker rats to have impaired insulin-mediated capillary recruitment and proposed that this contributes to the insulin resistance of muscle in vivo. Because this might imply a general loss of recruitable capillaries, we now assess responses to contraction in muscles of 18 ± 3-wk-old lean and obese Zucker rats in vivo. Field stimulation (2 Hz, 0.1 ms) was conducted for 1 h on one leg of anesthetized instrumented rats, and measurements were made of femoral blood flow (FBF), heart rate (HR), blood pressure (BP), hindleg metabolism of 1-methylxanthine (a measure of capillary recruitment), hindleg glucose uptake (HGU), and lower leg muscle glucose uptake by 2-deoxyglucose (R′g). Lean animals (311 ± 9 g) developed tension at 219 ± 27 g/muscle with no change in BP but with significant increases in HR, FBF, HGU, 1-MX metabolism, and R′g (P < 0.05), compared with nonstimulated control leans. Obese animals (469 ± 7 g) developed tension at 265 ± 31 g/muscle with no change in HR or BP but with significant increases in FBF, HGU, 1-MX metabolism, and R′g (P < 0.05) compared with nonstimulated control obeses. Muscle contraction of lean animals led to a greater increase in lower leg R′g, similar responses in HGU and 1-MX, and a smaller increase in FBF than in obese animals. A tight correlation between FBF and capillary recruitment was noted for all data (P < 0.001). It is concluded that contraction-mediated muscle capillary recruitment and glucose uptake are essentially normal in the obese Zucker rat and that control of FBF and capillary recruitment in exercise is closely linked.

muscle blood flow; insulin and glucose delivery to muscle; exercise; insulin resistance; capillary flow; glucose disposal

THE OBESE ZUCKER (fa/fa) RAT WAS SHOWN IN THE 1960S (39) TO HAVE CHRONICALLY ELEVATED BLOOD GLUCOSE AND, LATER, TO HAVE A GENETIC MUTATION IN THE LEPTIN RECEPTOR GENE (26) AND TO BE HYPERPHAGIC (39). CONSEQUENTLY, OBESITY ENSUES ALONG WITH DYSLIPIDEMIAS, HYPERINSULINEMIA, HYPERGLYCEMIA, AND INSULIN RESISTANCE IN THE MUSCLE (10) AND LIVER. THE INSULIN RESISTANCE OF MUSCLE MANIFESTS AS AN IMPAIRED INSULIN-STIMULATED GLUCOSE TRANSPORT (6, 29) AND GLUCOSE METABOLISM (10, 29) IN VIVO. A CELLULAR BASIS OF THE INSULIN RESISTANCE HAS ALSO BEEN DEMONSTRATED BY REMOVING THE MUSCLES AND CONDUCTING INSULIN DOSE-RESPONSE CURVES UNDER CONDITIONS OF INCUBATION (10). THUS INSULIN-MEDIATED GLUCOSE UPTAKE IS DECREASED AT ALL CONCENTRATIONS OF INSULIN COMPARED WITH MUSCLE FROM LEAN LITTERMATES (10). IN CONTRAST, CONTRACTION-MEDIATED GLUCOSE UPTAKE BY MUSCLES OF THE OBESE ZUCKER RAT APPEARS TO BE RELATIVELY NORMAL (2, 22), SUGGESTING THAT DEFECTS PARTICULAR TO INSULIN SIGNALING MAY EXPLAIN THE INSULIN RESISTANCE.

Insulin has both metabolic and hemodynamic effects in vivo. The hemodynamic effects, in particular capillary recruitment, may play a key role in facilitating access for both insulin and nutrients to the muscle cells, although this is not yet certain. Evidence in favor of a contributory role includes recent evidence that capillary recruitment is an early (34) and highly sensitive process (37) for insulin action in vivo. Also, when insulin-mediated capillary recruitment is inhibited by pharmacological means (28), TNF-α (36), or elevated free fatty acids (9), an acute state of insulin resistance occurs, reflected by diminished insulin-mediated glucose uptake. Recently, we (35) have shown that the obese insulin-resistant Zucker rat has markedly impaired insulin-mediated capillary recruitment and have proposed that the muscle insulin resistance results, in part, from this impaired hemodynamic response. However, the failure of insulin to elicit capillary recruitment in the muscle of the obese Zuckers may not result specifically from an impairment in insulin signaling but from a more general issue of fewer recruitable capillaries contributing to other deficiencies in muscle function. Because muscle contraction is another stimulus for capillary recruitment and glucose uptake, we have assessed the hemodynamic and metabolic responses of lean and obese Zuckers to acute muscle stimulation.

RESEARCH DESIGN AND METHODS

Animals. Male 18-wk-old lean (Fa/?) and obese (fa/fa) Zucker rats were purchased from Monash University, Melbourne, Australia. Rats were then housed at a constant temperature of 21 ± 1°C in a 12:12-h light-dark cycle and allowed free access to water and a commercial diet, as previously described (36). All procedures adopted and experiments undertaken were approved by the University of Tasmania Ethics Committee.

Surgery. Each rat was anesthetized using Nembutal (50 mg/kg body wt). Polyethylene cannulas (PE-50, Intramedic) were surgically implanted into the carotid artery for arterial sampling and measurement of blood pressure (pressure transducer Transpac IV, Abbott Critical Systems) and into both jugular veins for continuous administration of insulin and other intravenous infusions. A tracheotomy

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tube was inserted, and the animal was allowed to spontaneously breathe room air throughout the course of the experiment. Small incisions (1.5 cm) were made in the skin overlaying the femoral vessels of both legs, and the femoral artery was separated from the femoral vein and saphenous nerve. The epigastric vessels were then ligated, and an ultrasonic flow probe (Transonic Systems, VB series 0.5 mm) was positioned around the femoral artery of the right leg just distal to the rectus abdominis muscle. The catheter in the leg surrounding the flow probe was filled with lubricating jelly (H-R; Mohawk Medical Supply, Utica, NY) to provide acoustic coupling to the probe. The probe was then connected to the flow meter (model T106 ultrasonic volume flowmeter, Transonic Systems). This was in turn interfaced with an IBM-compatible PC computer, which acquired the data (at a sampling frequency of 100 Hz) for femoral blood flow (FBF), heart rate, and blood pressure with WINDAQ data acquisition software (DATAQ Instruments). The body temperature was maintained using a water-jacketed platform and a heating lamp positioned above the rat.

The surgical procedure generally lasted ~30 min, and then the animals were maintained under anesthesia for the duration of the experiment using a variable infusion of Nembutal (0–0.6 mg·min⁻¹·kg⁻¹) via the left jugular cannula. The femoral vein of the contracting leg was used for venous sampling, using an insulin syringe with an attached 29-gauge needle (Becton Dickinson). A duplicate venous sample was taken only on completion of the experiment (120 min) to prevent alteration of the blood flow from the hindlimb due to sampling, and to minimize the effects of blood loss.

Contraction studies. For electrically induced contraction, one electrode was inserted through the skin above the knee and another around the femoral artery 5, 10, 15, 30, and 45 min after the bolus. Glucose uptake was calculated as described by others (19, 23).

Analytical methods. A glucose analyzer (model 2300 Stat Plus, Yellow Springs Instruments) was used to determine whole blood glucose (by the glucose oxidase method) during the insulin clamp. A blood sample of 25 μl was required for each determination. perchloric acid-treated plasma samples were centrifuged for 10 min, and the supernatant was used to determine 1-MX, 1-methylurate, and oxyPURINOL concentrations by reverse-phase HPLC, as previously described (27, 28).

Data analysis. All data are expressed as means ± SE. Mean FBF, mean heart rate, and mean arterial blood pressure were calculated from 5-s subsamples of the data, representing 500 flow and pressure measurements every 15 min. Vascular resistance in the hindleg was calculated as mean arterial blood pressure in millimeters of mercury divided by FBF in milliliters per minute and expressed as resistance units. Glucose uptake in the hindlimb was calculated from arteriovenous (a-v) glucose difference and multiplied by FBF and expressed as micromoles per minute. The 1-MX disappearance was calculated from a-v plasma 1-MX difference and multiplied by FBF (corrected for the volume accessible to 1-MX, 0.871, determined from plasma concentrations obtained after additions of standard 1-MX to whole rat blood) and expressed as nanomoles per minute.

Statistics. A two-way analysis of variance was applied to analyze end point data. A Student-Newman-Keuls test was used to detect significant differences. Significance was recognized as P < 0.05. For correlation graphs of FBF, hindleg glucose uptake, and 1-MX metabolism, linear regression analysis was applied to data. All tests were performed using the Sigmas Statistic program (Jandel Software).

RESULTS

Body weight (310 ± 9.1 g, lean; 469.0 ± 7.0 g, obese; P < 0.001) and arterial blood glucose levels (3.85 ± 0.07 mM, lean; 9.55 ± 0.71 mM, obese; P < 0.001) of the obese Zucker rats were significantly higher than those of their lean littermates. The calf mass muscle (soleus, plantaris, red and white gastrocnemius, extensor digitorum longus, and tibialis anterior) of the obese rats was 1.98 ± 0.04 g and was significantly lower (P < 0.05) than that of the lean animals at 2.45 ± 0.15 g, even though it developed tension to the same level (158.4 ± 16.4 g, lean; 187.7 ± 20.4 g, obese) as that of the lean animals.

Hemodynamic effects. Initial FBF before contractions were 0.85 ± 0.15 and 0.76 ± 0.08 ml/min for obese and lean Zucker rats, respectively, and these did not differ significantly. FBF increased within seconds of commencement of the stimulus, and this remained elevated throughout the 1-h experimental period (Fig. 2C). Twitch contraction for 1 h did not change mean arterial pressure in either group of rats over the experimental period (Fig. 2A). Heart rate was increased only in the lean Zucker group compared with its noncontracting control (Fig. 2B). At the end of the 1-h contraction period, the change in vascular resistance was significantly different (P < 0.001) in the contracting obese Zucker compared with its control (Fig. 2D). FBF in both exercising groups was greater than in the noncontracting control. In addition, the contraction-induced FBF in the obese Zucker was greater than that of its lean counterparts, but only at 45 and 60 min (Fig. 2C).
Glucose uptake. Skeletal muscle contraction for 1 h increased 2-deoxyglucose uptake by all individual muscles of the lower leg except the soleus compared with the control, unstimulated state for both lean and obese Zuckers. However, uptake by the plantaris, white gastrocnemius, and extensor digitorum longus was greater by the contracting lean Zucker group than those by the obese group (Fig. 3A). 2-Deoxyglucose uptake by the combined muscles increased significantly (*P < 0.001) relative to the unstimulated state for both Zucker genotypes, although the combined uptake of the contracting lean Zucker group exceeded that of the obese group (Fig. 3B).

Figure 3C shows that glucose uptake for the whole hindleg also increased significantly (*P < 0.001) in both genotypes following contraction, but here the response by lean and obese animals did not differ.

1-MX metabolism. Figure 3D shows data for hindleg 1-MX extraction. Contraction for 1 h resulted in a significant increase (P < 0.001) in 1-MX metabolism in both the lean and obese Zuckers, but there was no difference between the two phenotypes (Fig. 3E).

DISCUSSION

The major finding that emerged from this study was that the obese Zucker rat was found to respond to muscle contraction in terms of both capillary recruitment and glucose uptake. This was in contrast to our previous report, where the obese Zucker was markedly resistant to insulin in terms of both capillary recruitment and glucose uptake (35).

In the present study, contraction-mediated capillary recruitment was equivalent to that achieved by maximal insulin (38) even though only twitch contractions representing low-level exercise were used. We believe this provides further evidence that the pathways by which insulin and exercise lead to capillary recruitment and enhanced glucose uptake by the muscle are different, with the insulin-stimulated pathway able to be circumvented by low-level exercise. It also provides evidence that the mechanisms that activate the nutritive capillary network remain intact in the obese Zucker and are accessible by contraction despite a loss or inhibition of the mechanisms induced by insulin. Furthermore, it may also suggest that insulin resistance in terms of capillary recruitment results from a defect in insulin signaling, as may also be the case for insulin-mediated glucose uptake (5). Recently, the expression and activities of the intracellular proteins involved in the initial stages of the insulin-signaling pathway have been studied in liver and skeletal muscle extracts of the Zucker rat (1). In skeletal muscle, the obese Zucker showed decreased levels of insulin receptor substrate (IRS)-1 and IRS-2 protein expression and impaired phosphorylation of both substrates in response to insulin compared with the lean Zuckers. In addition, there was an impaired amount of the p85 regulatory subunit of phosphatidylinositol 3-kinase associated with the phosphorylated IRS-1 (1). Similar results have been reported in skeletal muscle from obese and diabetic human subjects (5).

As indicated above, previous experiments with the obese Zucker in vivo showed no response to a high concentration insulin infusion. Insulin-mediated hemodynamic changes, including FBF and capillary recruitment, were absent, and there was no response to insulin in terms of an increase in glucose flux into the cell (35). Similarly, in obese humans, insulin failed to increase flow, and glucose extraction was reduced (24). Even so, there is no question that the muscle itself is insulin resistant, and this was convincingly demonstrated over 20 years ago by incubation of excised muscles of the Zucker obese and lean rats with various doses of insulin (10). The failure of muscle to respond to insulin in vivo may be due to an
inability to produce a metabolic vasodilator following the poor metabolic response to insulin in the muscle itself. Alternatively, because the insulin-mediated increases in total flow (31) and capillary recruitment (33) are each dependent on nitric oxide, a failure to respond hemodynamically to insulin in vivo may be due to a defect in insulin signaling in the vasculature, which then fails to activate nitric oxide synthase and generate nitric oxide. There is also the possibility that the bioavailability of nitric oxide is reduced due to increased scavenging by oxidative free radicals. For example, treatment of cremaster muscle arterioles from the obese Zucker rat with polyethylene glycol-superoxide dismutase and catalase decreased the concentration of superoxide. In response, arteriolar dilation improved to the same level as that in the lean animals, suggesting that the obese Zucker’s impaired response to insulin may be due, at least in part, to decreased nitric oxide availability (13).

Although the obese Zucker does not respond to insulin, this study shows a hemodynamic response to a single bout of contraction as evidenced by changes in FBF and vascular resistance. This was accompanied by increased 2-deoxyglucose uptake by all muscles except the primarily red fiber soleus, although in the plantaris, white gastrocnemius, extensor digitorum longus, and combined muscles, this uptake remained significantly lower than that of the lean Zucker. Increased glucose metabolism following a single bout of exercise due to an upregulation in GLUT4 translocation to the plasma membrane has already been established by others in this rat model as well as in normal and diabetic humans (20, 22). The novel...
finding of this project is its association with the recruitment of the nutritive capillary network evidenced by an increase in the metabolism of 1-MX and, therefore, an increase in endothelial surface area available to participate in glucose flux into the muscle cell.

Of particular interest to this study was the positive correlation between FBF and 1-MX metabolism for the combined obese and lean animals (Fig. 4) as well as for obese (Figure 4 inset) and lean individuals (Figure 4 inset) separately (data not shown), suggesting that as capillary perfusion increases so too does total blood flow. Alternatively, an increase in 1-MX metabolism could be regarded as resulting from increased flow, but this generally is not the case (see Ref. 27). Thus, when a vasodilator such as epinephrine is infused systemically to induce an increase in FBF similar to that of a high dose of insulin, there was no increase in capillary recruitment (27).

The positive correlation between capillary recruitment and FBF, although not revealing whether one is caused by the other, suggests that the control of both may be mediated by the same mechanism, particularly under the stimulation conditions used. Possible mechanisms include the release of vasodilator substances such as adenosine, K+ and nitric oxide. Also, there is the possibility of a common mechanism of vasodilatation involving a radiating hyperpolarization initiated by electrical stimulation (12). Of these various possibilities there is recent evidence that nitric oxide release is responsible for the sympatholysis of reactive hyperemia (7). Thus it would seem likely that vasodilatation of both terminal arterioles controlling capillary recruitment and resistance arteries controlling bulk flow are regulated by the same mechanism during contraction, so that the FBF increase and capillary recruitment are increased in unison. However, one must be mindful that the relationship between capillary recruitment and FBF is dependent on the intensity of the stimuli for muscle contraction. Thus Honig et al. (17) showed that, at low frequencies (e.g., 0.07 Hz), it was possible to increase muscle capillary recruitment independently of limb blood flow.

It is interesting that not all studies of obese Zucker rats have documented normal contraction-stimulated glucose uptake in skeletal muscle. Henriksen and Jacob (16) showed a 35% reduction in contraction-stimulated 2-deoxyglucose uptake in isolated epitrochlearis muscles from the obese Zucker rat compared with lean muscle. Because there is no influence of capillary recruitment in this in vitro contraction set-up, the capillary recruitment in the intact hindlimb of the obese Zucker rat (shown in the present study) can make up for any local impairment of glucose transport activity, thus explaining why most studies using intact hindlimb perfusions have not demonstrated significant deficits in contraction-mediated glucose uptake.

In conclusion, the obese Zucker rat, which is markedly resistant to insulin in both hemodynamic and metabolic responses in vivo, responds well to twitch contraction compared with its sedentary controls and its lean littermates. Not only did capillary recruitment and FBF increase in response to contractions, but whole body glucose turnover and 2-deoxyglucose uptake by individual muscles were increased, although these parameters were sometimes significantly lower than the findings in their lean counterparts. Overall, the data suggest that contraction-mediated control of microvascular perfusion is intact and capable of increasing the endothelial surface area available to participate in nutrient exchange.

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