Nonnutritive flow impairs uptake of fatty acid by white muscles of the perfused rat hindlimb

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Clerk, L. H., M. E. Smith, S. Rattigan, and M. G. Clark. Nonnutritive flow impairs uptake of fatty acid by white muscles of the perfused rat hindlimb. Am J Physiol Endocrinol Metab 284: E611–E617, 2003. First published November 26, 2002; 10.1152/ajpendo.00153.2002.—Triglyceride hydrolysis by the perfused rat hindlimb is enhanced by serotonin-induced nonnutritive flow (NNF) and may be due to the presence of nonnutritive route-associated connective tissue fat cells. Here, we assess whether NNF influences muscle uptake of 0.55 mM palmitate in the perfused hindlimb. Comparisons were made with insulin-mediated glucose uptake. NNF induced during 60 nM insulin infusion inhibited hindlimb oxygen uptake from 22.0 ± 0.5 to 9.7 ± 0.8 mmol·g⁻¹·h⁻¹ (P < 0.001), 1-methylxanthine metabolism (indicator of nutritive flow) from 5.8 ± 0.4 to 3.8 ± 0.4 mmol·min⁻¹·g⁻¹ (P = 0.004), glucose uptake from 29.2 ± 1.7 to 23.1 ± 1.8 mmol·g⁻¹·h⁻¹ (P = 0.005) and muscle 2-deoxyglucose uptake from 82.1 ± 4.6 to 41.6 ± 6.7 mmol·g⁻¹·h⁻¹ (P < 0.001). Palmitate uptake, unaffected by insulin alone, was inhibited by NNF in extensor digitorum longus, white gastrocnemius, and tibialis anterior muscles; average inhibition was from 13.9 ± 1.2 to 6.9 ± 1.4 mmol·g⁻¹·h⁻¹ (P = 0.02). Thus NNF impairs both fatty acid and glucose uptake by muscle by restricting flow to myocytes but, as shown previously, favors triglyceride hydrolysis and uptake into nearby connective tissue fat cells. The findings have implications for lipid partitioning in limb muscles between myocytes and attendant adipocytes.

acid uptake, nutritive flow, oxygen consumption, perfusion pressure


Reports by a number of laboratories that vasoconstrictors substantially influence metabolism of the constant-flow perfused rat hindlimb (12, 17, 30, 34) have drawn attention to the possibility that flow redistribution may be responsible for these effects. Consistent with this, a number of our studies (see review in Ref. 6) and those of others (24, 32) have shown that oxygen uptake by the hindlimb is markedly affected by the proportion of nutritive to nonnutritive blood flow. The balance of flow between the two routes, nutritive and nonnutritive, is controlled by vasoconstrictors that act at different sites in the vascular tree to regulate relative flow distribution (6, 7). Vasoconstrictors that mediate such redistribution have been categorized into those that recruit nutritive flow (e.g., low-dose norepinephrine; angiotensins I, II, and III; and vasopressin) and those that recruit nonnutritive flow (e.g., serotonin) in a constant total flow pump-perfused preparation (6).

Physical evidence for the concept of two vascular routes in muscle comes from a number of studies that we have undertaken and includes vascular casting (21), FITC-dextran entrapment as a marker for recruited and deroofed vascular space (21), surface fluorescence measurement of tendon vessels when perfused with a fluorescent vascular marker (22), microdialysis out-to-in ratio of 3H₂O and [¹⁴C]ethanol (23), laser Doppler flowmetry (5), and microsphere embolism with use of latex microspheres of different sizes (38). However, the precise anatomic nature of the nonnutritive route of muscle is still unresolved, despite knowledge of its presence for 70 years (24). There is evidence that it has access to connective tissue of tendons (1, 15), and a recent laser Doppler flowmetry study that used randomly placed microprobes into various muscles suggests that the nonnutritive route is homogeneously distributed within each muscle (5).

In a previous study (9), we showed that, when flow was predominantly nonnutritive during a state of high vascular resistance induced by serotonin, overall chylomicron triglyceride hydrolysis by the perfused rat hindlimb was increased. This may be due to flow being directed to lipoprotein lipase-rich fat cells located on the connective tissue vessels that constitute some of the nonnutritive route of muscle. This implies that fat cells distributed on the interfibrillar connective tissue of the endomysium, perimysium, and epimysium receive nutrient from the nonnutritive route of skeletal muscle, even though their metabolic activity is small compared with muscle. It follows that, when the proportion of nutritive to nonnutritive flow is altered, fuel and hormones are differently partitioned. Thus, in the present study, we examine whether fatty acid and glucose uptake by the constant-flow perfused rat hindlimb in the presence of insulin is influenced by a decrease in the nutritive-to-nonnutritive ratio.

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MATERIALS AND METHODS

Animals. Animals were cared for in accordance with the principles of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (1990, Australian Government Publishing Service, Canberra). Experimental procedures were approved by the Animal Ethics Committee of the University of Tasmania. Males of a local strain of hooded Wistar rats (140–160 g) were housed at 22°C on a 12:12-h light-dark cycle and allowed free access to water and a commercial rat chow (Gibsons, Hobart, Australia) containing 21.4% protein, 4.6% lipid, 68% carbohydrate, and 6% crude fiber with added vitamins and minerals. Rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (5–6 mg/100 g body wt) before all surgical procedures.

Free fatty acid solution. Palmitic acid (369.2 mg), 1.4 ml of 1 M NaOH, 28 ml of distilled H2O, and, when required, 80 μCi [14C]palmitic acid (55 mCi/mmol, Amersham) were heated at 75–90°C for 20 min or until saponified. The solution was then cooled to 50–60°C before the addition of Krebs-Ringer bicarbonate buffer (90.6 ml) containing 6% (wt/vol) BSA. Finally, the mixture was passed through a 1.2-μm filter and stored at −20°C until used. [3H]mannitol was used as a marker for the extracellular space for the palmitate uptake perfusions. A stock solution of 65 mM mannitol and 40 μCi [3H]mannitol (19.7 Ci/mmol, NEN) was prepared. Analysis of the filtered palmitate solution indicated it to be −4.9 mM, with a fatty acid-to-albumin ratio of 7.2.

2-Deoxy-[3H]glucose solution. Although the arteriovenous difference in glucose may be used to calculate hindleg glucose uptake, this measurement does not exclude the amounts taken up by bone, skin, fat, and other tissues present in minimal amounts in the preparation. Similarly, the uptake of 2-deoxyglucose measures glucose uptake into the excised hindlimb muscles, which can include uptake by attendant fat cells, even though this may be relatively trivial because of the mass difference favoring muscle. To measure 2-deoxyglucose uptake, a solution of 90 mM mannitol and 40 μCi [3H]mannitol (19.7 Ci/mmol, NEN) was prepared. Analysis of the filtered palmitate solution indicated it to be −4.9 mM, with a fatty acid-to-albumin ratio of 7.2.

Hindlimb perfusions. Hindlimb surgery was essentially as described by others (33), with additional details given previously (11). The left hindlimb was perfused in a nonrecirculating mode with 6% (wt/vol) Ficoll (Amersham Pharmacia Biosciences) or vehicle were then commenced and maintained for the entire experiment (Fig. 1A). The actual insulin concentration as determined by ELISA (Merckodia, Uppsala, Sweden) was 60 nM and deliberately chosen to achieve maximal response. The infusion of serotonin (5-HT; 3 μM final concentration) or vehicle was commenced at 50 min and continued for the entire experiment. At 60 min, the buffer reservoir was changed and then, while the infusion of insulin and 5-HT or vehicle was maintained, the rat hindlimb was perfused with medium containing 6% Ficoll as an oncotic agent. Perfusion medium contained 2-deoxy-[3H]glucose and [14C]sucrose (A) or [14C]palmitate and [3H]mannitol (B). All perfusions were conducted with medium containing 6% Ficoll as an oncotic agent, 0.55 mM palmitate, and 1-methylxanthine (1-MX) to measure nutrient flow. Infusions were either vehicle, 60 nM insulin, 3 μM serotonin (5-HT), or 60 nM insulin + 3 μM 5-HT. Time 0 is the beginning of hindlimb perfusion; all other additions are indicated.

2-DG uptake. After completion of the surgical procedure, the rat hindlimb was perfused for 40 min with 6% Ficoll-Krebs buffer, pH 7.4, containing 8.3 mM glucose as well as other components to be described. Insulin (Humulin, Aza Research) or vehicle infusions were then commenced and maintained for the entire experiment (Fig. 1A). The actual insulin concentration as determined by ELISA (Merckodia, Uppsala, Sweden) was 60 nM and deliberately chosen to achieve maximal response. The infusion of serotonin (5-HT; 3 μM final concentration) or vehicle was commenced at 50 min and continued for the entire experiment. At 60 min, the buffer reservoir was changed and then, while the infusion of insulin and 5-HT or vehicle was maintained, the rat hindlimb was perfused with medium containing 6% Ficoll as an oncotic agent. Perfusion medium contained 2-deoxy-[3H]glucose and [14C]sucrose (A) or [14C]palmitate and [3H]mannitol (B). All perfusions were conducted with medium containing 6% Ficoll as an oncotic agent, 0.55 mM palmitate, and 1-methylxanthine (1-MX) to measure nutrient flow. Infusions were either vehicle, 60 nM insulin, 3 μM serotonin (5-HT), or 60 nM insulin + 3 μM 5-HT. Time 0 is the beginning of hindlimb perfusion; all other additions are indicated.

Free fatty acid uptake. Perfusion details were essentially as for 2-DG uptake. After completion of the surgical procedure, the rat hindlimb was perfused for 40 min with 6% Ficoll-Krebs buffer. Insulin or vehicle infusions were commenced and maintained for the entire experiment (90 min). 5-HT or vehicle infusion was commenced at 50 min, and at 60 min (while the infusion of insulin and 5-HT or vehicle was maintained), the buffer reservoir was changed to one containing 26 ml [14C]palmitic acid solution (0.55 mM

Fig. 1. Perfusion protocols for determining uptake of 2-deoxy-[3H]glucose (2-DG) or palmitate by perfused rat hindlimb muscle. Perfusion medium contained 2-deoxy-[3H]glucose and [14C]sucrose (A) or [14C]palmitate and [3H]mannitol (B). All perfusions were conducted with medium containing 6% Ficoll as an oncotic agent, 0.55 mM palmitate, and 1-methylxanthine (1-MX) to measure nutrient flow. Infusions were either vehicle, 60 nM insulin, 3 μM serotonin (5-HT), or 60 nM insulin + 3 μM 5-HT. Time 0 is the beginning of hindlimb perfusion; all other additions are indicated.
were made using the Student-Newman-Keuls multiple comparisons test. One, two, or three symbols (* to show significance from control) were added to denote statistically significant effects of 5-HT, with or without insulin, occurred soon after addition, with VO₂ decreasing from 22.0 ± 0.5 to 9.7 ± 0.8 μmol·g⁻¹·h⁻¹ (P < 0.001) at the maximum. This was accompanied by an increase in PP from 41.6 ± 1.3 to 167.7 ± 13 mmHg (P < 0.001) at the same time point (10 min). At subsequent time points, there was a gradual decline so that, at 90 min, only approximately one-half of the maximal response remained.

The metabolism of 1-MX, by the capillary endothelial enzyme xanthine oxidase, has been used previously by us as an indicator of nutritive flow (27, 41). Figure 3 shows the effect of insulin, 5-HT, and the combination of insulin + 5-HT on 1-MX metabolism, determined at the 80-min time point, when steady-state conditions have been attained (27). Whereas insulin had no effect
The increase was greater than sixfold (from 13.6 to 82.1 \text{ mmol min}^{-1} \text{g}^{-1} \text{ww}, P < 0.001) with 5-HT alone and to 3.8 \pm 0.4 \text{ mmol min}^{-1} \text{g}^{-1} \text{ww} (P = 0.004) with 5-HT + insulin. The trend for insulin to further increase the inhibitory effect of 5-HT was not significant (Fig. 3).

The effects of insulin, 5-HT, and the combination of insulin + 5-HT on hindlimb glucose uptake were assessed and shown in Fig. 4. Uptake was determined from arteriovenous differences, total flow rate, and the perfused muscle mass. Glucose uptake across the total perfused mass was stimulated approximately threefold (from 12.2 \pm 0.7 to 29.2 \pm 1.7 \text{ mmol g}^{-1} \text{h}^{-1}, P < 0.001). 5-HT reduced the insulin-mediated glucose uptake across the entire hindlimb (from 29.2 \pm 1.7 to 23.1 \pm 1.8 \text{ mmol g}^{-1} \text{h}^{-1}, P = 0.005), and the insulin-mediated increment from 17.0 to 10.9; i.e., 36\% but was without effect on its own compared with control. A subset of experiments using the nonmetabolizable glucose tracer 2-DG was conducted to assess the effect of 5-HT on insulin-mediated glucose uptake by individual hindlimb muscles. Insulin significantly increased 2-DG uptake in all hindlimb muscles tested and was particularly evident in the EDL and tibialis muscles (Fig. 5A). The increase was greater than sixfold (from 13.6 \pm 1.4 to 82.1 \pm 4.6 \text{ mmol g}^{-1} \text{h}^{-1}, P < 0.001) when the uptake into all muscles was averaged (Fig. 5B). The addition of 5-HT with insulin decreased 2-DG uptake in all muscles, and for the average this was reflected by a decrease from 82.1 \pm 4.6 to 41.6 \pm 6.7 \text{ mmol g}^{-1} \text{h}^{-1} (P < 0.001; Fig. 5B). Measuring the uptake of 2-DG into individual muscles is a more accurate indicator of muscle glucose uptake (although a contribution from attendant fat cells cannot be ruled out) than arteriovenous hindlimb differences, because measurements of glucose uptake across the entire hindlimb may also have contributions from other tissues, including fat, bone, and skin.

A second subset of experiments was conducted to assess the effects of insulin and 5-HT on the uptake of [14C]palmitic acid by the hindlimb muscles. Figure 6A shows that, whereas insulin tended to increase the uptake of [14C]palmitic acid, particularly by soleus and G. Red, this was not significant. When all muscles were averaged, there was also no significant effect of 5-HT. However, when insulin was coinfused with 5-HT, there was a significant reduction in fatty acid uptake by the muscles with predominantly white fibers (EDL, G. White, and tibialis). Thus 5-HT decreased insulin-mediated [14C]palmitic acid uptake from 19.9 \pm 1.3 to 5.8 \pm 2.1 \text{ mmol g}^{-1} \text{h}^{-1} in the EDL (P < 0.001), 7.6 \pm 1.1 to 3.1 \pm 0.9 \text{ mmol g}^{-1} \text{h}^{-1} in the G. White (P = 0.036), and 17.5 \pm 0.3 to 5.3 \pm 1.9 \text{ mmol g}^{-1} \text{h}^{-1} in the tibialis (P < 0.001). When the uptake across the selected muscles was averaged (Fig. 6B), the combination of 5-HT and insulin resulted in a significant reduction compared with insulin alone (i.e., from 13.9 \pm 1.2 to 6.9 \pm 1.4 \text{ mmol g}^{-1} \text{h}^{-1}, P = 0.02).
DISCUSSION

The main findings from this study were the reduced muscle fatty acid and insulin-mediated glucose uptake across the hindlimb when nonnutritive flow predominated. This contrasted with our previous study, in which chylomicron triglyceride uptake was enhanced with the muscles and situated between the fibers are likely to have contributed to the enhanced uptake of chylomicron triglyceride previously reported (9). Although LPL is known to be distributed in the skeletal muscle myocytes, Camps et al. (4) also found high levels in the muscle connective tissue, and this was the explanation by us (9) for the enhanced uptake of LPL-associated fatty acid when nonnutritive flow was predominant in red muscles. In the present study, we have

Fig. 5. Hindlimb muscle uptake of 2-DG (R’g). Perfusion with Ficoll-Krebs buffer contained 8.3 mM glucose and 0.55 mM palmitic acid. Additions were vehicle (open bars), 60 nM insulin (solid bars), or 3 μM 5-HT + 60 nM insulin (hatched bars). Data are means ± SE (n = 5–6) and are given in gww for individual (A) and average (B) hindlimb muscles at 90 min. EDL, extensor digitorum longus; G. Red and G. White, gastrocnemius red and white, respectively; Tibialis, tibialis anterior. Significance is denoted by * (from control) and † (from insulin), where 2 or 3 symbols represent P < 0.01 and P ≤ 0.001, respectively.

Fig. 6. Hindlimb muscle uptake of [14C]palmitate. Perfusion with Ficoll-Krebs buffer contained 8.3 mM glucose and 0.55 mM palmitic acid. Additions were vehicle (open bars), 60 nM insulin (solid bars), 3 μM 5-HT (gray bars), or 3 μM 5-HT + 60 nM insulin (hatched bars). Data are means ± SE (n = 5–6) and are given in g dry wt (gdw) for individual (A) and average (B) hindlimb muscles at 90 min. Significance is denoted by * (from control) and † (from insulin), where 1 or 3 symbols represent P < 0.05 and P ≤ 0.001, respectively.
focused on the uptake of albumin-bound free fatty acid, which is not dependent on LPL activity and is likely to be more dependent on endothelial surface area and fatty acid transporters (35, 36). Therefore, free fatty acid uptake was expected to decrease with reduced nutritive (capillary) perfusion (5-HT infusion) in all muscles. Although this appears to have occurred only in muscles with predominantly white fibers (40), it is likely to have occurred also in the red muscles. The uptake into the myocytes of the red muscle may be masked by free fatty acid extraction into the associated white fat cells in these muscles. There is the additional possibility that the estimations of 2-DG uptake are to some degree distorted by a background of associated fat cells, which are more concentrated in connective tissue of red muscles (40).

Reduced muscle uptake of palmitate during hemodynamic insulin resistance implies that triglyceride deposits within the myocyte will be reduced. Classically, it is thought that elevated plasma free fatty acids during insulin resistance are reassembled into lipoproteins in the liver for metabolism in the periphery, leading to increased skeletal muscle triglyceride. Consistent with this notion, a correlation between insulin resistance and muscle triglyceride levels (14, 20, 25, 26) and increased lipid oxidation (16) has been recorded. Despite this, there are reports showing that fatty acid uptake is decreased with impaired glucose tolerance (37), type 2 diabetes (3), and in women with visceral obesity (10). In addition, reduced lipid oxidation has been recorded in subjects with non-insulin-dependent diabetes mellitus (19) and visceral obesity (10). The findings reported in this study may in part explain those reported by Turpeinen et al. (37), showing that the uptake of fatty acid was reduced in states of insulin resistance. Therefore, intracellular accumulation of triglyceride may be a result of reduced lipid oxidation rather than increased fatty acid uptake.

Impaired insulin-mediated glucose uptake during nonnutritive blood flow is impaired and, therefore, nutritive flow in the present experiments involving constant-flow perfusions may be attributable to an inhibitory effect of palmitate. There is also the possibility that the hindlimb under basal conditions, with no vasoconstrictor present, is essentially fully dilated (31); thus insulin is unable to vasodilate further and recruit nutritive flow. Within this context, insulin does not dilate the hindlimb vasculature when preconstricted by 5-HT2 agonists either in vivo (28) or in perfusion (29). We have previously shown that 5-HT reduces hindlimb lactate output, possibly due to reduced uptake of glucose, fatty acids, and oxygen, reflecting a state of metabolic hibernation rather than leading to an anaerobic state in which breakdown of glycogen stores is stimulated.

Finally, it is important to note that the vasoconstrictor 5-HT has been used herein as a model substance to induce an acute state of predominantly nonnutritive flow. Estimates from the Vo₂ data of Fig. 2 and 1-MX metabolism of Fig. 3 suggest that, at the dose of 5-HT used, there was a decrease in nutritive flow to 57% of basal initially (Fig. 2 at 60 min) declining to 29% (Fig. 2) or 35% (Fig. 3). We have shown previously that 5-HT has no direct effects on muscle metabolism or contractility independent of its vascular effects. Thus isolated incubated muscles showed no effect of 5-HT addition on insulin-mediated glucose uptake (29) or contractility from field stimulation (13). Although 5-HT-mediated vasoconstriction to induce nonnutritive flow represents only an acute state of insulin resistance with decreased free fatty acid uptake, long-term nonnutritive flow may occur in vivo during extended periods of low physical activity or during stress when sympathetic outflow is increased. There are data to show that high frequency sympathetic nervous system activity mediates a pattern of nonnutritive flow in the constant flow perfused rat hindlimb (18).

In summary, the predominantly nonnutritive flow pattern induced in these experiments by the vasoconstrictor 5-HT resulted in significant reduction in free fatty acid and insulin-mediated glucose uptake across the hindlimb. Thus reduced access for glucose and free fatty acid to myocytes is offset by enhanced access for triglycerides to attendant connective tissue adipocytes. Such findings illustrate a role of nutritive and nonnutritive blood flow in controlling nutrient partitioning. The findings also have particular implications for glucose uptake in insulin-resistant states, such as Intralipid/heparin infusions (8) and the genetically obese Zucker rat (39). In both examples, insulin-mediated capillary recruitment (nutritive flow) is impaired and may contribute to the decrease in insulin-mediated glucose uptake by muscle in vivo.

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REFERENCES


