Increased chylomicron triglyceride hydrolysis by connective tissue flow in perfused rat hindlimb: implications for lipid storage

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Abstract **Skeletal muscle has two circulatory routes, nutritive (in contact with muscle) and non-nutritive (part of which is located in the connective tissue), and the balance of flow between the two is controlled by neural input and circulating vasomodulators. The purpose of this study was to assess muscle triglyceride hydrolysis given that the two circuits may have a differing vascular distribution of hydrolytic activity. The isolated rat hindlimb was perfused with 6% Ficoll® and a radiolabeled chylomicron–lipid emulsion containing apolipoprotein C-II. Serotonin (0.5–1 μM), a model vasoconstrictor previously shown to preferentially increase connective tissue flow, inhibited hindlimb oxygen uptake (from 16.7 ± 0.6 to 10.2 ± 1.0, mean ± SE, n = 7 ($P < 0.001$)) and stimulated [^{14}C]-labeled fatty acid uptake into muscles (from 184 ± 28 to 602 ± 132, mean ± SE, n = 7 ($P = 0.009$)). These effects were reversed by the vasodilator carbamyl choline. Vasopressin resulted in increased oxygen consumption but no change in triglyceride hydrolysis. Cholesteryl oleate uptake (an indicator of endocytosis of the chylomicron or remnant particle) was unaltered by serotonin. It is concluded that chylomicron triglyceride hydrolysis is enhanced by vasoconstrictors that increase connective tissue flow in the perfused rat hindlimb. Increased hydrolysis appears to be primarily due to an increased access of triglyceride to hydrolytic enzymes, presumably lipoprotein lipase associated with the fat cells commonly observed interlaced amongst bundles of muscle fibers.**—Clerk, L. H., M. E. Smith, S. Rattigan, and M. G. Clark. **Increased chylomicron triglyceride hydrolysis by connective tissue flow in perfused rat hindlimb: implications for lipid storage.** ***J. Lipid Res.*** **2000.** 41: **329–335.**

Supplementary key words lipoprotein lipase • nutritive flow • non-nutritive flow • oxygen consumption • perfusion pressure

The uptake of lipoprotein triglycerides (TG) into target tissues requires initial hydrolysis and this is thought to be facilitated by the enzyme lipoprotein lipase (LPL). LPL is attached to the vascular endothelium by proteoglycans which allow protrusion of the enzyme into the vascular lumen. Here it acts to hydrolyze TG from circulating TG-rich lipoproteins (chylomicrons and very low density lipoproteins) into free fatty acids (FFA) and glycerol. The resulting FFA are taken up by tissues capable of lipid oxidation (e.g., muscle) or storage (e.g., adipose tissue, muscle) (1, 2).

The hydrolysis of TG to FFA and glycerol has been found to be proportional to the active amount of LPL in the vasculature (3) and ultimately may depend on whether circulating TG has access to the active form of LPL or other hydrolytic enzymes. In skeletal muscle an important determinant of muscle metabolism is substrate supply to the myocytes and is controlled by the proportioning of flow between two distinct vascular circuits (4, 5). The first is termed nutritive and describes flow predominantly to the muscle cells. Blood flowing through the second circuit, termed non-nutritive, almost certainly passes through vessels of the connective tissue associated with the muscle (6). Flow through this route results in the physical isolation of nutrients and hormones (including oxygen, glucose, TG, and insulin) from the myocytes (7). As a result there is limited opportunity for muscle nutrient uptake. As TG hydrolysis is dependent upon its exposure to hydrolytic enzymes, it follows that the total hydrolysis of TG entering the muscle will be greater when the predominance of flow is through the circuit in which the majority of the hydrolytic activity is distributed. To date there have been no studies describing the location of skeletal muscle TG hydrolytic activity including LPL, and its relative distribution in muscle nutritive capillaries or in connective tissue vessels (non-nutritive for muscle) (6).

The concept of a dual vascular system in skeletal muscle was proposed as early as the 1940s. Experiments done by Pappenheimer (8) showed that norepinephrine administration to the gastrocnemius muscle of dogs resulted in an increase in oxygen consumption while stimulation of vaso-

Abbreviations: 5-HT, serotonin; apoC-II, apolipoprotein C-II; CO, cholesteryl oleate; LPL, lipoprotein lipase; HIRS, heat-inactivated rat serum; $\dot{V}O_2$, oxygen consumption; PP, perfusion pressure; CLE, chylomicron lipid emulsion; TO, triolein; FFA, free fatty acid (unesterified); TG, triglyceride; BSA, bovine serum albumin; HEPES, N-[2-hydroxyethyl]piperazine-N′ [2-ethanesulfonic acid]; CCh, carbamyl choline.

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constrictor nerves resulted in decreased metabolism. These observations did not correlate with changes in total blood flow to the muscle. Other researchers noted that radioisotopes injected either intramuscularly or directly into the circulation displayed two differing venous washout curves (9, 10). The ability for alternative metabolic effects in a muscle with constant total flow led Renkin (9) to propose two metabolically and spatially distinct areas of the vasculature within skeletal muscle. Failure to produce convincing evidence for the existence of arteriovenous anastamoses in skeletal muscle (11, 12) led to the search for other possible anatomical candidates to encompass the "non-nutritive" circulation. Barlow, Haigh, and Walder (13) recorded an increase in the clearance of ^{24}Na injected into the muscle bed during simultaneous infusion of intravenous epinephrine. The opposite response occurred with epinephrine infusion when the ^{24}Na was injected into muscle septa and tendons. Lindbom and Arfors (14) noted that feeding arterioles of the tenuissimus muscle often supplied both vessels of neighboring connective tissue in addition to muscle capillaries. It is now believed that nutritive circulation is through capillaries in close contact with the muscle fibers and as a result the clearance of ions and oxygen is accelerated. When flow is largely through the non-nutritive pathway, the flow is thought to pass through muscle connective tissue; in this state the clearance of ions and oxygen is decreased. Vasoconstrictor action to constrict vessels leading to connective tissue vessels (type A vasoconstrictors) will therefore re-divert flow into the muscle capillaries (nutritive) and consequently increase metabolism. Alternatively, vasoconstrictors impeding flow to muscle capillaries (type B) will increase connective tissue (non-nutritive) flow.

Thus the present study addresses the issue of whether vasoconstrictors (including serotonin (5-HT, type B) and vasopressin (type A) that specifically alter the proportion of non-nutritive and nutritive flow in the constant flow perfused rat hindlimb (5, 7), alter the rate of hydrolysis of circulating TG. The data show that hydrolysis of chylomicron TG is markedly increased with the addition of 5-HT. As this parallels an increase in the extent of non-nutritive flow (without changes in LPL activity), it is likely that the hydrolysis is due to a flow-mediated increase in the exposure of substrate (TG) to TG hydrolytic activity, presumably LPL.

MATERIALS AND METHODS

Animals

All rats 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 Committee on the Ethical Aspects of Research Involving Animals 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 h light/dark cycle and allowed free access to water and a commercial rat chow (Gibsons, Hobart) 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 pentobarbitone sodium (5–6 mg 100 g^{-1} body wt) before all surgical procedures.

Triglyceride emulsion

Chylomicron lipid emulsion (CLE) was prepared essentially as described by Redgrave and Callow (15) and each ml contained 7.5 mg triolein, 0.33 mg cholesteryl oleate, 0.22 mg cholesteryl acetate, 2.6 mg phosphatidyl choline, 10 μmol N-[2-hydroxyethyl]piperazine-N′[2-ethanesulfonic acid] (HEPES, pH 7.4), 150 μmol NaCl, and 0.25 ml heat-inactivated rat serum (HIRS) as a source of apoC-II. CLE contained either 1.25 μCi [^{3}H]cholesteryl oleate (CO, Amersham, [cholesteryl-1,2,6,7-3H(N)]-cholesteryl oleate) or 0.42 μCi [^{14}C]triolein (TO, Amersham, [carboxyl-^{14}C]triolein) and hydrolysis/uptake of each measured separately. The mixture was sonicated at an output frequency of 23 KHz, for two rounds of 1 min each. All emulsions were used on the day of preparation (using fresh HIRS).

Heat-inactivated rat serum

Donor animals were anesthetized and blood was collected by an intracardiac puncture. Blood was slowly withdrawn using a 21-gauge butterfly needle attached to a 1-ml syringe containing 0.1 ml citrate. The syringe was changed several times during one collection. This procedure reduced the amount of suction and therefore the amount of platelet activation. Serum collected in this way was devoid of any platelet-derived 5-HT. Serum platelets were eliminated by clotting with thrombin (30 μl/ml). The remaining serum was heated at 56°C for 30 min to inactivate endogenous lipases (16).

Hindlimb perfusions

Hindlimb surgery was essentially as described by others (17), with additional details as given previously (18). String ligatures were tied around the tail and left (perfused) tarsus to prevent extraneous flow. A tight tie was also positioned around the entire animal at the approximate level of the L3 vertebrae. Heparin was not used as an anticoagulant, as this has the potential to displace LPL from the vascular endothelium and distribute it throughout the perfusate (2).

The left hindlimb of the rat was perfused in a recirculating mode at 37°C with 100 ml of perfusion medium comprising Krebs-Ringer bicarbonate buffer containing 6% (wt/vol) Ficoll® (Pharmacia Biotech) (unless indicated otherwise) with 8.3 mm glucose and 1.27 mm $CaCl_2$. The buffer was continuously gassed with 95% O_2:5% CO_2 via a silastic tube oxygenator while maintained at 37°C in a heat exchanger coil. Constant flow perfusions were conducted at 8 ml/min; constant pressure perfusions at 85 mm Hg. Venous effluent oxygen tension was monitored by an in-line temperature-regulated (37°C) Clark-type electrode. Oxygen uptake ($\dot{V}O_2$) was calculated as described previously using the appropriate Bunsen coefficient (19). Perfusion pressure (PP) was constantly monitored via a side arm proximal to the aorta. After a 40-min equilibration period, the buffer reservoir was changed to one containing 125 ml of the 6% Ficoll® and 5 ml of CLE.

Modulation of connective tissue flow

5-HT is one of a number of vasoconstrictors previously shown to increase connective tissue flow at the expense of muscle nutritive flow in the constant flow perfused rat hindlimb (5, 7). Although other members of this group such as high dose norepinephrine, high frequency SNS stimulation, and high dose vanilloids give similar results in terms of decreasing non-nutritive flow, 5-HT is the least complicated (produces a mono-component dose–response curve (20)) and was the model vasoconstrictor of

choice. Thus 5-HT (Sigma) was added as a bolus into the buffer reservoir of the appropriate perfusions to give a final concentration of 0.5–1 μm (to achieve a peak perfusion pressure of approximately 100 mm Hg above basal). After discarding the first 30 ml of perfusate (of the 130 ml perfusion medium containing CLE) the remaining 100 ml was recirculated through the hindlimb for 1 h. Samples (2.5 ml) were withdrawn from the venous line every 15 min.

In some experiments the vasodilator CCh was infused at a final concentration of 100 μm to reverse the effects of 5-HT on PP and $\dot{V}O_2$. CCh infusion commenced before buffer was recirculated. This was designed to allow the PP and $\dot{V}O_2$ to return to near-basal states before TG recirculation and prevent perfusion under predominantly non-nutritive conditions. Infusion was continued for a further 12.5 min after recirculation.

The type A vasoconstrictor vasopressin was added as a bolus dose (0.5 nm) to the reservoir of some perfusions to investigate the effects of increased $\dot{V}O_2$ and PP on chylomicron TG hydrolysis.

Triglyceride hydrolysis

Perfusate samples (1 ml) from perfusions using [^{14}C]TO were added to 4 ml methanol–chloroform 2:1 in 10-ml glass tubes with screw caps. The tubes were vortexed (3 × 30 s) and maintained at room temperature (22°C) before centrifuging at 2500 *g* for 10 min. The entire lower layer was removed with a glass pipette into a 5-ml test tube and evaporated to dryness in a water bath at 40°C under a stream of nitrogen. The residue was reconstituted into 100 μl of the chloroform–methanol mixture and 15 μl was immediately applied to a Merck silica gel 60 F_{254} aluminium sheet (Merck). Standards (10 μl of 10 mg/ml) of TO, CO, and oleic acid were also applied as spots. The plates were run using a mixture of n-heptane–diethyl ether–glacial acetic acid 80:20:1. Plates were visualized in an iodine tank and TO and oleic acid spots were scraped into separate plastic tubes and counted with 4 ml of Amersham Biodegradable Counting Scintillant. Recovery of counts after thin-layer chromatography was periodically checked by comparing the total radioactivity scraped from one lane of the silica plate (a lane was designated for each perfusate sample) with the known amount of radioactivity of the corresponding perfusate sample before solvent extraction. The recovery was between 90 and 110%.

Muscle radioactivity uptake

After perfusion, the soleus, plantaris, gastrocnemius white and red, tibialis and extensor digitorum longus muscles of the perfused hindlimb were removed. Within the context of this study it is important to note that interfibrillar connective tissue adipocytes are contained within each muscle. Excised muscles were freeze-dried overnight to obtain dry weight and later re-hydrated with 1 ml of water and 1 ml of Soluene® (tissue solubiliser; Packard). When digestion was complete, 100 μl of acetic acid was added together with 14 ml of Amersham Biodegradable Counting Scintillant.

Statistical analysis

The statistical significance of differences between groups of data was assessed by unpaired, two-tailed Student's *t*-test. Significant differences were recognized at $P < 0.05$.

RESULTS

Clearance of chylomicron TG by perfused muscle has not previously been studied and it was therefore necessary to conduct a number of preliminary experiments to determine an optimal procedure. The commonly used albumin-containing perfusion medium was not entirely satisfactory as FFA released by LPL were subsequently bound by the albumin and little was taken up by the hindlimb. A second difficulty encountered was the high contamination of serum albumin by lipases. Thus lipase substrates such as *p*-nitrophenyl palmitate were rapidly hydrolyzed (data not shown) causing basal rates of hydrolysis to be largely attributable to these contaminants when using albumin-containing perfusion medium. Accordingly, we chose to use Ficoll®-containing perfusion medium. With this medium there was no hydrolysis due to the perfusion medium alone and the uptake of released FFA occurred so that muscle-specific uptake could be compared at the completion of each perfusion.

Figure 1 shows the time course for the effects of 0.5–1 μmol 5-HT, 0.5-1 μm 5-HT with 100 μm CCh or 0.5 nm vasopressin on changes in $\dot{V}O_2$ and PP in the constant flow, Ficoll® perfused rat hindlimb. Changes in $\dot{V}O_2$ and PP both reached a maximum at 15 min and then declined as the vasoconstrictors were metabolized by the hindlimb during the recirculating perfusion. However, changes in $\dot{V}O_2$ and PP were significantly different ($P < 0.05$) from controls at all time points for 5-HT and vasopressin. The

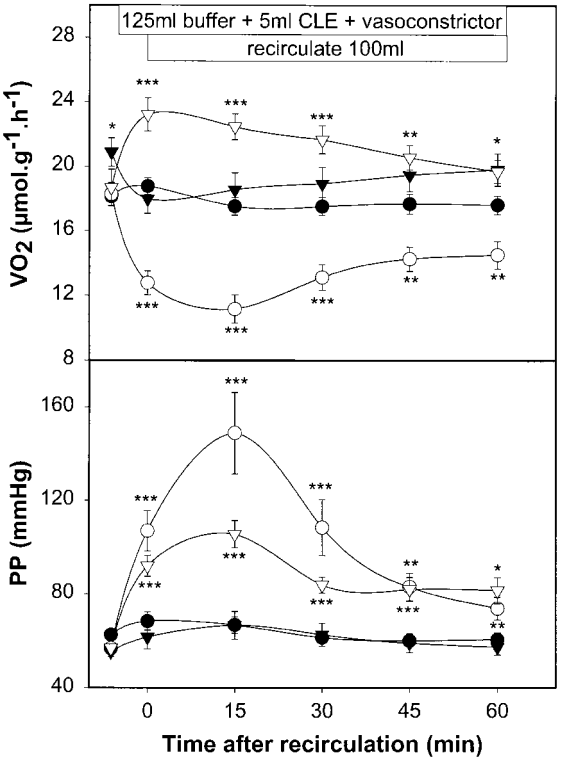

Fig. 1. Time course for the effects of serotonin (5-HT), 5-HT with carbamyl choline (CCh), and vasopressin on oxygen consumption ($\dot{V}O_2$) and perfusion pressure (PP) in the constant flow Ficoll®-perfused rat hindlimb. All perfusions were conducted at constant flow (8 ml/min) using a recirculating mode (total buffer volume = 100 ml). Basal values are at t = −6.25 min. Additions at −6.25 min were vehicle (●), 0.5–1 μm 5-HT (○), 0.5–1 μm 5-HT + 100 μm CCh (▼) or 0.5 nm vasopressin (▽). Values are means ± SE. ** $P < 0.01$; *** $P < 0.001$ for treatment vs. vehicle (n = 10–12).

TABLE 1. Effect of 5-HT, 5-HT + CCh, and vasopressin on TO hydrolysis, $\dot{V}O_2$ and PP by the perfused rat hindlimb at constant flow

	Constant Flow Ficoll® Perfusions			
	Control	5-HT	5-HT + CCh	Vasopressin
TG hydrolysis (nmolFFA·h^{-1}·g^{-1})	184 ± 28	602 ± 132[a]	231 ± 24	255 ± 68
$\dot{V}O_2$ (μmol·g^{-1}·h^{-1})	16.7 ± 0.6	10.2 ± 1[b]	19.2 ± 2.1	21.3 ± 0.9[b]
PP (mm Hg)	70.6 ± 5	170 ± 27[a]	62.4 ± 7	99.0 ± 8[a]
Muscle FFA (nmol·g^{-1}·h^{-1})	161 ± 25	508 ± 133[a]	200 ± 6.3	169 ± 55
Perfusate FFA (nmol·g^{-1}·h^{-1})	23.5 ± 12	93.1 ± 15[a]	30.7 ± 19	86.3 ± 22[a]

The TO substrate was a chylomicron–lipid emulsion (8). Perfusions were constant flow, recirculating, and set at 8 ml/min. TO and oleic acid radioactivity analyses were conducted on perfusate samples taken at intervals of 15 min throughout perfusions of 60 min duration (Fig. 1). Average rates of FFA formation (perfusate FFA) over 60 min were measured by thin-layer chromatography. Muscle [^{14}C]oleic acid uptake into the hindlimb were at 60 min. Values for maximal changes in $\dot{V}O_2$ and PP were taken 15 min after recirculation. Values are means ± SE. [a] $P < 0.05$; [b] $P < 0.001$ for 5-HT, 5-HT + CCh and vasopressin vs. control.

addition of CCh blocked the pressor and oxygen effects by 5-HT. Mean values for muscle ^{14}C-labeled fatty acid uptake (60 min), oxygen uptake (15 min), and perfusion pressure (15 min), are shown in **Table 1**. $\dot{V}O_2$ was inhibited by approximately 38.9% and muscle ^{14}C-fatty acid uptake increased by approximately 227.2% by 5-HT (Table 1). The increase in TG hydrolysis and the decrease in oxygen uptake due to 5-HT were each significant ($P < 0.05$ and $P < 0.001$, respectively). FFA found in the perfusate of 5-HT perfusions were also significantly higher than the controls ($P < 0.05$). These high hydrolytic rates were reversed when 5-HT effects were blocked by CCh, thereby supporting the view that the effects were indicative of flow redistribution and not due to 5-HT receptor-mediated effects on muscle cells.

Addition of vasopressin to the buffer reservoir produced no significant decline in chylomicron TG hydrolysis (Table 1), despite an increase in $\dot{V}O_2$ (Table 1). However, the perfusate FFA with vasopressin were significantly higher than those for the control perfusions (Table 1).

Constant pressure perfusions were also conducted. These confirmed the high hydrolytic rates with 5-HT (**Table 2**). As these perfusions deliberately used bovine serum albumin (BSA) rather than Ficoll®, FFA released from TO became bound to the BSA and allowed measurement of its appearance in perfusate samples. Corrections due to endocytosis were not necessary, however basal rates of TO hydrolysis in control perfusions may have been affected by contamination of the serum albumin by lipases (as confirmed by the high activity of serum albumin perfusate to hydrolyze *p*-nitrophenyl palmitate). As with the Ficoll® perfusions, TO hydrolysis was, however, also increased with 5-HT in these constant pressure perfusions. Increases in TO hydrolysis were not a result of 5-HT activating the enzyme (**Table 3**).

Uptake of [^{14}C]FA and [^{3}H]CO into hindlimb muscles after 1 h of chylomicron recirculation is shown in **Fig. 2**. Panel A illustrates that perfusing the hindlimb with 5-HT increased the uptake of [^{14}C]FA into certain muscles. The increased uptake was significant in the soleus, plantaris and gastrocnemius red muscles. The percentage uptake of [^{3}H]CO into hindlimb muscles from Ficoll® perfusions is shown in Fig. 2, panel B. After TG hydrolysis the chylomicron retains the CO, and therefore the uptake of [^{3}H]CO into muscles may be due to either endocytosis of the chylomicron or chylomicron remnant. Even if the reported CO uptake was of the nascent chylomicron, the uptake of [^{14}C]FA into the muscles of the 5-HT perfusions were still considerably greater than that recorded for endocytosis. Therefore the [^{14}C]FA found in the muscles are likely to be attributable to TG hydrolysis. Alternatively, CO uptake exceeded that of TO in the vasopressin perfusions. This is suggestive of uptake of the chylomicron remnant. If this were the case then the FFA formed from TG in the chylo-

TABLE 2. Effect of 5-HT on TO hydrolysis, $\dot{V}O_2$ and PP by the perfused rat hindlimb at constant pressure

	Constant Pressure BSA Perfusions	
	Control	5-HT
TO hydrolysis (nmolFFA·h^{-1}·g^{-1})	201 ± 35	421 ± 63[a]
$\dot{V}O_2$ (μmol·g^{-1}·h^{-1})	16.2 ± 2.9	6.7 ± 1.8[a]
PP (mm Hg)	84 ± 3	91 ± 8

A set of BSA perfusions were conducted at constant pressure (set at approx. 85 mm Hg). The TO substrate was a chylomicron–lipid emulsion (8). TO and oleic acid radioactivity analyses were conducted on perfusate samples taken at intervals of 30 min throughout perfusions of 120 min duration. Average rates were calculated and means ± SE for $\dot{V}O_2$ (30 min) and PP (30 min) are shown. [a] $P < 0.05$ for 5-HT vs. control.

TABLE 3. Lipoprotein lipase (LPL) activity in muscle homogenates after BSA-perfusion with vehicle or 5-HT

	LPL Activity		
	Gastrocnemius Red	Gastrocnemius White	Plantaris
	nmol·min^{-1}·g^{-1}		
Vehicle	198 ± 52	110 ± 40	150 ± 60
5-HT	180 ± 30	90 ± 30	70 ± 5

To assess whether serotonin activated LPL during perfusion and therefore had a direct effect on the rate of TG hydrolysis, perfusions were conducted in which vehicle or 5-HT was infused for 20 min. Muscles were then excised, homogenized, and assayed for LPL activity using the technique of G. Bengtsson-Olivecrona, and T. Olivecrona (16). No difference was seen in the activity of muscle LPL after perfusion with 5-HT.

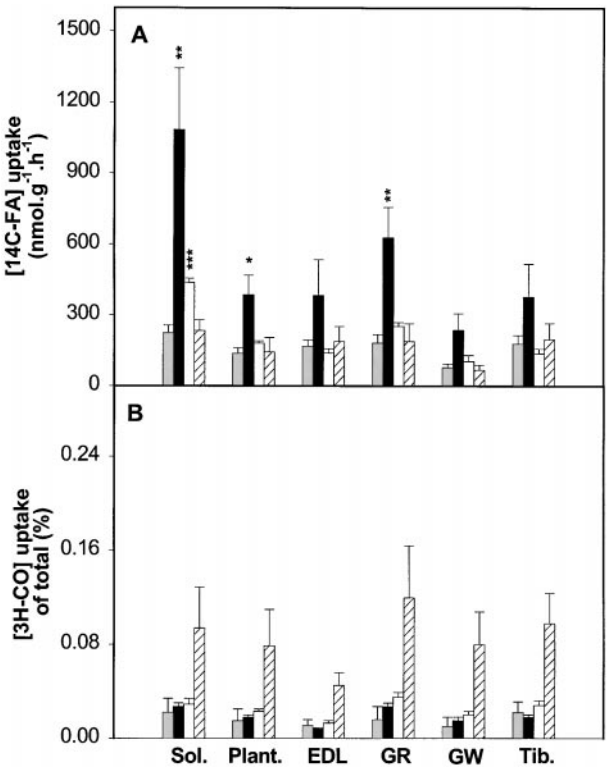

Fig. 2. Uptake of [^{14}C]oleic acid and [^{3}H]cholesteryl oleate ([^{3}H]CO) into hindlimb muscles in constant flow Ficoll®-perfused rat hindlimb using a chylomicron lipid emulsion (CLE) and heat-inactivated rat serum (HIRS) as a source of apolipoprotein CII. Hindlimb muscles dissected include the soleus (Sol.), plantaris (Plant.), extensor digitorum longus (EDL), gastrocnemius red (GR), gastrocnemius white (GW) and tibialis (Tib.). The effects of serotonin (0.5–1 μm, 5-HT, black bar), 5-HT and carbamyl choline (0.5–1 μm 5-HT + 100 μm CCh, white bar) and vasopressin (0.5 nm, hatched bar) were measured for each CLE type and compared to perfusions with no additions (control, grey bar). CLE contained either [^{14}C]triolein ([^{14}C]TO (panel A)) or [^{3}H]CO (panel B). The values in panel B are the percentage of total circulating CO that is taken up by each muscle. Values are means ± SE (n = 4–7) * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ for treatment versus vehicle.

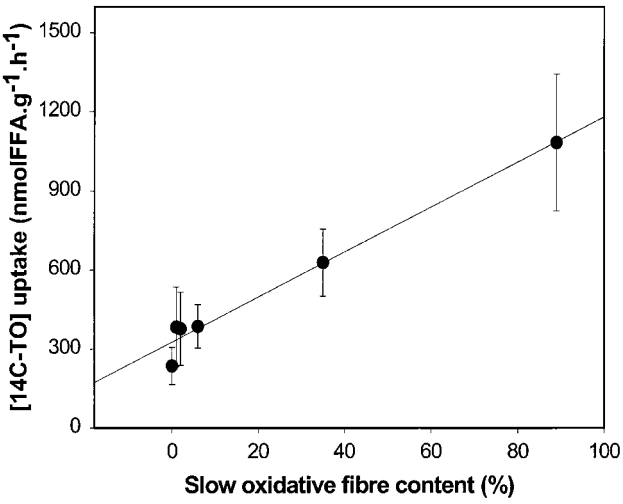

Fig. 3. Uptake of FFA from a synthetic chylomicron emulsion using the mean values for uptake with 0.5–1 μm serotonin (5-HT) for each hindlimb muscle and their corresponding percentage content of slow oxidative fibers. The linear regression produces an $r^2 = 0.975$ ($P < 0.001$). The percentage content of slow oxidative fibres was taken from reports by Ariano, Armstrong, and Edgerton (31) and Armstrong and Laughlin (32).

micron must remain in the perfusate. In fact, the perfusate FFA levels with vasopressin are significantly higher than the control value (Table 1).

Figure 3 is a plot of the percentage content of slow oxidative fibers against the uptake of [^{14}C]FA in a hindlimb preconstricted with 5-HT for each muscle. [^{14}C]FA uptake when flow is predominantly non-nutritive significantly correlates ($r = 0.987$, $P < 0.001$) with the percentage content of slow oxidative fibers.

DISCUSSION

The importance of skeletal muscle in total circulating lipid clearance is often underestimated, and all previous reports have neglected the effect of flow partitioning on TG clearance in muscle, due to the presence of nutritive and non-nutritive routes. Here we report that the hydrolysis of TO was markedly increased in the perfused rat hindlimb when a high proportion of connective tissue flow occurred. Recruitment of connective tissue flow for these experiments was induced by the addition of 5-HT, a representative type B vasoconstrictor, which has previously been reported to decrease oxygen uptake, lactate output, insulin-mediated glucose uptake (21, 22), and tension development of aerobically contracting muscle (23). All of these changes are characteristic of type B vasoconstriction (7) and are representative of decreased nutrient delivery to muscle, and decreased muscle metabolism secondary to increasing the proportion of non-nutritive or connective tissue flow within muscle (5).

When connective tissue flow was increased by addition of the vasoconstrictor, 5-HT, there was a marked increase in TG hydrolysis (indicated by [^{14}C]FA uptake) in the soleus, plantaris and gastrocnemius red muscles. Uptake of [^{14}C]FA greatly exceeded that of the CO with serotonin. This implies that uptake due to endocytosis of the chylomicron (causing TO radioactivity to be found in the muscle without any detectable amounts of hydrolysis) or of the chylomicron remnant (where all of the [^{14}C]oleic acid or [^{14}C]TO in the muscle is due to hydrolysis) could not account for the observed increase. It is important to note that the reported increase in TG hydrolysis occurred without any stimulation of LPL activity by 5-HT. This indicates that the TG hydrolysis due to 5-HT is likely to be the result of a vascular effect whereby the exposure of TG to TG hydrolytic activity, presumably LPL, is increased. From this, it would also seem likely that the distribution of TG hydrolytic activity is greater along the non-nutritive or connective tissue circuit that nourishes interlacing adipocytes than along the nutritive route supplying muscle cells.

Evidence that the vessels of non-nutritive flow are those

of the connective tissue associated with muscle has been supported by direct visualization during various pharmacological interventions (6, 24). The increased TG hydrolytic activity associated with flow through this region implies access to LPL associated with an active population of interfibrillar adipocytes. This population may correspond to the interfasicular fat cells responsible for the marbling effect of meat. The amount of muscle marbling is reported to be highly variable (25) which may contribute to the large error bars for the 5-HT data. In addition, the amount of muscle marbling can be used as a marker for red oxidative (type 1) muscle fibers (26). From the present study, the uptake of [^{14}C]FA from TG hydrolysis correlated with the percentage of slow oxidative fibers when flow was predominantly non-nutritive (i.e., with 5-HT, Fig. 3). Thus increases in connective tissue blood flow proposed to occur with age, hypertension and diabetes (5, 6) may therefore allow increased fat deposition and perimysial marbling within the muscle.

Reports of fat cells being nourished by connective tissue vessels have been implied from anatomical studies by Eriksson and Myrhage (27) and Lindbom and Arfors (14). In addition, Camps et al. (28) reported diffuse LPL mRNA levels in the connective tissue of skeletal muscle. The location of adipocytes in this region would be consistent with the findings herein of increased TG hydrolysis where flow is significantly redirected through these vessels. From this it follows that the non-nutritive vessels supply the connective tissue and the closely associated adipocytes.

It therefore appears likely that the vascular system plays a significant role in partitioning lipid and carbohydrate between muscle and connective tissue fat cells. This type of fuel partitioning is likely to be under the control of the local release of vasomodulators. Whereas predominantly nutritive flow (such as in exercise) allows delivery of glucose and FFA to muscle cells, non-nutritive flow to connective tissue adipocytes could promote either TG hydrolysis or the deposition of FFA to form adipocyte TG. The presence of insulin and glucose in this latter circuit is likely to further nourish and stimulate adipocyte TG enlargement by providing glycolytically derived glycerol phosphate. As the two vascular networks are in parallel, FFA and glycerol hydrolyzed from TG by the LPL situated along the non-nutritive circuit must first enter the venous circulation and redistribute throughout the body before it is presented to the myocytes. This implies a mechanism evolved for the storage of excess fat rather than for use by neighboring myocytes. van der Vusse (29) has suggested that muscle connective tissue fat cells may be an important store of TG.

Many reports on muscle TG hydrolytic activity do not discern between myocytes, connective tissue, and fat cells. Any measure of such activity, including LPL from a whole muscle sample, will contain all of these components. If the TG hydrolytic activity described in this study is of non-nutritive origin, then data from whole muscle samples will be more dramatically affected than has previously been recognized (30). Therefore, assays of muscle homogenate that are significantly contaminated by connective tissue adipocytes may overestimate muscle TG hydrolytic activity, including that of LPL. Similarly, no discrimination is made between the two flow regions possible in skeletal muscle. A combination of these factors poses further difficulty when interpreting the data of any muscle preparation that receives nutrient by the vascular route.

In conclusion, TG hydrolysis was enhanced in the perfused rat hindlimb by the addition of the model vasoconstrictor 5-HT. 5-HT had no direct effect on LPL activity and thus alterations in TG hydrolysis are likely to be due to the ability of 5-HT to alter flow patterns within muscle. Increases in non-nutritive flow (possibly with hypertension and insulin resistance) may therefore enhance perimysial, endomysial, and tendon fat deposition, leading to increases in muscle adiposity.

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