Physical inactivity amplifies the sensitivity of skeletal muscle to the lipid-induced downregulation of lipoprotein lipase activity

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Zderic, Theodore W., and Marc T. Hamilton. Physical inactivity amplifies the sensitivity of skeletal muscle to the lipid-induced downregulation of lipoprotein lipase activity. J Appl Physiol 100: 249–257, 2006. First published September 29, 2005; doi:10.1152/japplphysiol.00925.2005.—Physical inactivity is a risk factor for lipoprotein disorders and the metabolic syndrome. Physical inactivity has a powerful effect on suppressing lipoprotein lipase (LPL) activity in skeletal muscle, the rate-limiting enzyme for hydrolysis of triglyceride (TG)-rich lipoproteins. We tested the ability of several compounds to prevent the decrease in LPL. The present study minimized standing and ordinary light nonexercise movements in rats to compare the effects of inactivity and nonexercise activity thermogenesis (NEAT) on LPL activity. The key new insight was that the typically quick decrease in LPL activity of oxidative muscle caused by physical inactivity was prevented by nicotinic acid (NA), whereas inhibitors of TNF-α, inducible nitric oxide synthase, and NF-κB had no such effect. NA was administered at a dose known to acutely impede the appearance of plasma TG from the liver and free fatty acids from adipose tissue, and it was effective at intentionally lowering plasma lipid concentrations to the same level in active and inactive groups. As measured from heparin-releasable LPL activity, LPL in the microvasculature of the most oxidative muscles was ~90% lower in the inactive group compared with controls, and this suppression was completely blocked by NA. In contrast to inactivity, NA did not raise muscle LPL in ambulatory controls, whereas a large exogenous fat delivery did decrease LPL activity. In vitro control studies revealed that NA did not have a direct effect on skeletal muscle LPL activity. In conclusion, physical inactivity amplifies the ability of plasma lipids to suppress muscle LPL activity. The light ambulatory contractions responsible for NEAT are sufficient for mitigating these deleterious effects.

walking; nicotinic acid; triglyceride; nonexercise activity thermogenesis; metabolic syndrome

The most recent scientific consensus statement by the American Heart Association and the National Heart, Lung, and Blood Institute on the diagnosis and management of metabolic syndrome defined physical inactivity, along with obesity and a high-fat atherogenic diet, as the three primary lifestyle risk factors (14). They said, for patients with the metabolic syndrome, that lifestyle intervention should be the prime emphasis for therapy. In addition to formalized exercise programs, the scientific panel recommended supplementing daily lifestyle activities with increased nonexercise physical activity (e.g., pedometer step tracking, gardening, housework, etc.) and avoiding activities that are associated with physical inactivity (e.g., sitting, television watching, and computer games). However, the mechanisms by which physical inactivity negatively impacts enzymes controlling atherosclerotic risk factors has only recently begun to emerge in the literature (18), and there is little direct evidence that nonexercise physical activity is sufficient to prevent those processes (7).

Lipoprotein lipase (LPL), the rate-limiting enzyme for the catabolism of triglyceride (TG)-rich lipoproteins, has a central role in determining at least two of the five diagnostic criteria for the metabolic syndrome (plasma TG and HDL cholesterol). In addition to systemic effects on plasma lipoprotein concentrations, the local regulation of LPL activity in individual microvascular beds is a means to generate a concentrated source of fatty acids and other lipoprotein-derived lipids (31). Furthermore, an understanding of the regulation of LPL activity during physical inactivity may also be important for other therapeutic targets of the metabolic syndrome such as a proinflammatory state (14), because LPL activity is apparently also involved in limiting the expression of genes regulating inflammation and atherosclerotic signals in the vasculature (55).

Loss of LPL in skeletal muscles after relatively short periods of reduced energy demand in the legs can lead to depressed uptake of TG-derived fatty acids locally in skeletal muscle, and reduced plasma HDL cholesterol concentrations, without a generalized decrease in LPL throughout the body (7). Local contractile activity and/or local inactivity is the major physiological variable regulating the LPL activity in the vasculature of skeletal muscle, as revealed by studies stimulating motor nerves in single muscles (17), comparing muscles that are recruited during exercise with muscles that are not recruited (17, 24), unilateral denervation or tenotomy (7, 48), and experiments reducing weight-bearing activity in one leg and comparing the LPL in the contralateral leg muscles (7). Most impressively, compared with normal control rats, reduced activity in the hindlimbs caused up to 10- to 20-fold decreases in the heparin-releasable (HR) LPL activity and protein (the pool of LPL predominantly associated with the endothelial surface of the microvasculature) in the most oxidative regions of the leg muscles (7). This decrease was reversed in several hours after resuming normal weight-bearing activity and very slow ambulatory contractions. Thus a localized reduction in contractile activity is clearly the most potent physiological factor determining LPL activity in the microcirculation of skeletal muscle, and an understanding of this phenomenon is of high significance.

LPL activity is frequently reported to be correlated inversely to plasma TG concentration (49). This relationship is largely
because LPL is the key enzyme determining TG catabolism, but the correlation could in theory also be due in part to the ability of TG and fatty acids to acutely reduce LPL. Some studies support the belief that quickly raising the amount of fat in the blood above normal can lower muscle LPL below normal (10, 22, 38). Thus the authors reasonably suggested that an oversupply of exogenous plasma TG substrate and fatty acid products relative to the rate of fatty acid utilization lowers LPL because of negative feedback at the microvascular sites where lipolysis occurs (38).

However, the key question left unresolved is whether or not the more normal endogenous concentration of plasma lipid substrates downregulates LPL in muscle during conditions of low energy demand. The most definitive test of this would come from lowering plasma TG and fatty acids over the acute time frame wherein physical inactivity is induced. The earliest suggestion that plasma lipid substrates might play a permissive role in the acute regulation of LPL activity came from the little known observation over three decades ago that reducing plasma TG and free fatty acid (FFA) concentration with nicotinic acid could cause an accelerated plasma TG clearance in resting humans (8, 33). Although those studies did not directly measure tissue LPL activity and studied highly variable plasma TG levels, it raised our awareness that lipids might regulate LPL function in some conditions, such as the common behavior of being sedentary.

The primary purpose of the present study was to test the hypothesis that nicotinic acid would attenuate the acute decrease in skeletal muscle LPL activity that typically occurs during physical inactivity. As an alternative to this hypothesis, we also determined whether the suppression of muscle LPL activity during physical inactivity could be prevented by pharmacological compounds known to prevent signals reducing LPL activity in other conditions or nonmuscle tissue (40, 54). As described above, we knew from prior work that a reduction in local contractile activity is required for the suppression of muscle LPL during physical inactivity, and systemic signals are not a sufficient explanation (7). Second, the decrease in muscle LPL after reduced weight-bearing contractile activity, especially the HR-LPL activity in the microvasculature, is a very rapid process that can occur within hours under conditions where fasting hypertriglyceridemia is not yet evident, and the development of obesity is impossible (7). Thus we intentionally studied short-term inactivity to eliminate those confounding effects.

The present study administered nicotinic acid at a pharmacological dose to acutely impede the appearance of plasma TG and FFA (52). Thus the concentration of these plasma lipid substrates was matched at similar moderate concentrations in active and inactive rats to test for a differential responsiveness to plasma lipids. The physical inactivity was induced by preventing rats from standing or walking on the hindlimbs, and the active control group consisted of normally caged rats without ever exercising. Thus all of the muscular movements in the control group were related to normal low-grade nonexercise physical activity, or nonexercise activity thermogenesis (NEAT) (27, 28). For these reasons, this study was also able to address a concept about inactivity physiology, in which it was recently postulated that some potent stimuli regulating disease-related proteins like LPL during sedentary behaviors could be masked by moderate physical activity (18). With each of those pieces of the puzzle in hand, we reasoned that an amplified sensitivity to a nonhyperlipidemic concentration of plasma lipids could possibly develop in skeletal muscles during reduced contractile activity caused by the absence of standing and intermittent ambulatory movements.

METHODS

Animal procedures were approved by the University of Missouri Animal Care and Use Committee and performed in respect of the American Physiology Society principles for research on animals. Sprague-Dawley rats (55 females weighing ~175–220 g) were maintained in a temperature- and light-controlled environment (12:12-h light-dark cycle) and fed a standard rat chow diet. During all experimental treatments, rats were housed individually in cages.

Physical inactivity model. One-half of the rats were prevented from standing or walking on the hindlimbs to reduce the energy demand of skeletal muscles in the legs (e.g., soleus and gastrocnemius) associated with ordinary low-intensity weight-bearing activity. The hindlimbs were elevated just enough to prevent the rear feet from touching the floor, thus providing a model of reduced contractile activity localized primarily to the two hindlimbs. About 1.5 cm of the tail were wrapped with adhesive tape and connected to a fishing lure swivel secured to an overhanging metal rod to prevent weight-bearing activity. The rats were accustomed to hindlimb unloading for 1–2 h/day for 1–2 days before experiments, and they still had access to move about in the cage with forelimbs for essential activities. This model and duration of unloading the hindlimbs reduces the electromyogram activity (1, 23, 42) and blood flow (13, 30) by ~90% below normal in the soleus muscle. Treatments were administered and muscles were dissected in the dark cycle when rats are most active.

Pharmacological agents administered. Pharmacological agents were administered at doses effective in other studies for antagonizing different stimuli causing LPL activity to decrease. The drugs (or saline control) were administered intraperitoneally in rats during the 8- to 12-h inactivity period using either sterile catheters implanted into the peritoneal cavity or small 27-gauge needles. These drugs included the following: aminoguanidine, an inducible nitric oxide synthase (iNOS) antagonist (50 or 150 mg·kg−1·h−1) that prevented the decreases in muscle LPL activity caused by endotoxin (40); sodium salicylate, an inhibitor for NF-κB signaling in muscle caused by blood lipids when given every 2 h at an average rate of 7 mg·kg−1·h−1 (26); pentoxifylline, an inhibitor of TNF-α signaling that prevented the acute suppression of LPL activity in adipose tissue caused by fasting and lipopolysaccharide when injected every 4 h at a dose of 25 mg·kg−1·h−1 (54); and nicotinic acid, which has been shown to reduce the rate of plasma TG and FFA appearance (52) and attenuate the reduction in adipose tissue LPL activity caused by fasting (35). Because studies revealed a significant preventive effect of nicotinic acid on the profound decrease in LPL activity caused by the physical inactivity, we performed several follow-up studies. In performing the nicotinic acid studies, we experimented with different duration of treatment and muscle type without any major differences in conclusions. The first group of rats received either saline or nicotinic acid every 2 h for 8 h at an average rate of 100 mg·kg−1·h−1. In the 12-h studies, the lowest dosing schedule (injection every 90 min to provide an average rate of 33 mg·kg−1·h−1) did not have significantly different effects on plasma lipids and LPL activities compared with the larger dose we initially used (injection every 30 min to provide 100 mg·kg−1·h−1), and thus results for these groups were pooled.

To focus on the goal of better understanding the role of local contractile activity on LPL regulation, food was acutely removed for the 8- or 12-h duration of pharmacological and physical activity treatments. This would also ensure that we could control against potentially variable rates of food intake, avoid the transient postprandial hypertriglyceridemia sometimes associated with changes in physical activity (16, 19), and control for other dynamic nutritional...
influences such as hyperinsulinemia (24) that may sometimes acutely lower skeletal muscle LPL activity independent of contractile activity. Tissue removal. Muscles were removed at the end of the experimental treatments after anesthetizing the rats with a mixture of ketamine (54 mg/ml), xylazine (2.2 mg/ml), and acepromazine (3.5 mg/ml) given in a volume of 1.4 ml/kg ip. Plasma was obtained from blood sampled from a cardiac puncture and placed into tubes containing EDTA for subsequent triglyceride and FFA assays. Soleus and gastrocnemius muscles were rapidly dissected, and fresh pieces of muscle weighing ~25 mg were minced into 5- to 10-mg pieces and immediately incubated in PBS buffer containing 20 U/ml heparin (Sigma, St. Louis) for 30 min at 37°C (HR-LPL activity). The heparin eluate was then frozen in liquid N₂ and stored at ~80°C. The remaining tissue containing the pool of LPL (non-HR) that was not released by heparin incubation (predominantly cellular) was also quickly frozen in liquid N₂ and stored at ~80°C. The anesthetized rats were killed by removing the heart.

In vitro test of nicotinic acid. A control experiment was performed to test whether nicotinic acid has a direct effect on the in vitro measurement of LPL activity of isolated muscle (n = 36). We tested for the effects of adding nicotinic acid (75 μg/ml) to the PBS-heparin buffer of ~25 mg muscle sections obtained from soleus and quadriceps muscles from active and inactive rats. The heparin (20 U/ml) eluates were frozen in liquid N₂ and stored at ~80°C.

LPL enzyme activity. LPL enzyme activity was assayed using a [³H]triolein substrate our laboratory has used previously (6, 7, 17). The non-HR (cellular) muscle samples were homogenized in a buffer containing a Tris buffer, heparin, and several protease inhibitors as described previously (17). Each sample was measured in duplicate and at two different concentrations to ensure reproducibility and linearity. To ensure that fatty acids produced by the hydrolysis of triolein or in the samples did not affect the measurement of LPL activity, essentially fatty acid-free albumin was included in the reaction mixture, tissue samples were diluted 50- to 100-fold in buffers before being assayed, and we confirmed the linearity of the assay with respect to sample volumes. LPL activity is reported in units of nanomoles of fatty acids hydrolyzed per gram of muscle per minute. Plasma TG and FFA concentrations. Plasma was isolated by centrifugation (1,000 g for 10 min at 4°C) and frozen at ~80°C until analysis. Plasma TG concentrations were measured using the Infinity TG (Thermostat, Louisville, CO). Plasma FFA concentrations were measured using the Wako FFA colorimetric assay kit (Wako Chemicals, Richmond, VA).

Statistics. Dependent variables from animal and in vitro experiments were examined with a three-way ANOVA (fiber type × physical activity × drug) to test for significant main effects and interactions. If there was a significant main effect or interaction, the ANOVA was followed by a Newman-Keuls post hoc test. Results were considered significant at P < 0.05. Data are presented as means with their corresponding SE.

RESULTS

LPL activity in response to pharmacological treatments. In the first set of experiments, our goal was to identify a candidate mechanism for the marked suppression of muscle HR-LPL activity caused by physical inactivity. Consistent with our laboratory’s previous findings (7), several hours of physical inactivity caused a progressive decrease in soleus HR-LPL activity in rats infused with saline vehicle (Fig. 1). By 8 and 12 h of physical inactivity, soleus HR-LPL activity in saline vehicle-infused rats was reduced to 47 ± 8% (P < 0.05) and 13 ± 3% (P < 0.01) of ambulatory controls levels, respectively. The intraperitoneal administration of aminoguanidine, pentoxifylline, and salicylate had no effect on the decline in soleus HR-LPL activity caused by physical inactivity (Fig. 1).

Nicotinic acid completely prevented the large decrease in soleus HR-LPL activity by maintaining it at 104 ± 14% of standing/ambulatory control rats (Fig. 1). As expected, nicotinic acid was effective in significantly lowering both plasma TG and FFA concentrations from normal values as indicated by a significant main effect for both plasma substrates (Table 1). Importantly, there was not a significant difference in the concentration of either plasma TG or FFA between the inactive and control groups in this study (Table 1). The key finding of this study is that despite the ability of nicotinic acid to prevent the almost 90% decrease in HR-LPL activity in the inactive rats (open bars of Fig. 2A), nicotinic acid did not have an effect on soleus HR-LPL activity (109 ± 8 vs. 100 ± 12%, solid bars of Fig. 2A) of the rats with normal standing/ambulatory activity. Thus the statistical interaction between nicotinic acid treatment and physical activity treatment was highly significant (P < 0.01).

To further investigate the effect of lipid lowering in another muscle, the fast oxidative glycolytic section of the gastrocnemius muscle was studied. There was not a significant interaction between fiber type and drug, nor was there a significant fiber type × physical activity × drug interaction. These results indicate that both muscles were responding similarly to inactivity and nicotinic acid. Similar to the response of the soleus muscle in the inactive group (Fig. 2A), nicotinic acid increased gastrocnemius HR-LPL activity markedly (open bars of Fig. 2B). In the fast-twitch gastrocnemius, the HR-LPL (microvascular fraction) was on average 42% greater (P = 0.12) in the active animals treated with nicotinic acid than those treated with saline vehicle (solid bars of Fig. 2B) with no trend in the non-HR-LPL activity (cellular fraction; Fig. 3B). For both muscle types, the relative effects of inactivity and nicotinic acid were greater in the microvascular LPL (Fig. 2) than in the cellular LPL fraction (Fig. 3). In addition, the calculated total
(HR fraction plus non-HR fraction) LPL activity was significantly reduced by physical inactivity and restored by nicotinic acid.

Direct effect of nicotinic acid on LPL activity. To test whether nicotinic acid directly affects the release of LPL activity during muscle incubations or affects LPL activity in vitro, muscle sections from a slow oxidative muscle (soleus) and a fast oxidative glycolytic muscle (deep red quadriceps) were incubated with either heparin only (20 U/ml) or heparin and a pharmacological concentration of nicotinic acid (75 μM) (Table 2). The addition of nicotinic acid did not affect LPL in vitro when the source of LPL was from either inactive or active muscles. These results suggest that the greater LPL activity in the microvasculature during in vivo nicotinic acid treatment was not due to a direct effect of nicotinic acid promoting the release of LPL activity by heparin or increasing in vitro LPL activity, but it is likely due to the plasma lipid lowering effect of nicotinic acid.

Sensitivity of skeletal muscle LPL to plasma lipid delivery. We confirmed the indication by some earlier studies (10, 22, 38) that a very high intake of fat (~200 kcal/kg) could acutely lower muscle LPL activity of ambulatory control animals (P < 0.05) (solid bars of Fig. 4). Plasma TG concentrations in these rats were increased 83% (P < 0.01) at the 12-h point when tissues were obtained (data not shown). This treatment led to a 63% reduction in soleus HR-LPL activity in standing/ambulatory control rats (Fig. 4), indicating that HR-LPL activity in ambulatory control rats can decrease if exposed to enough

![Table 1. Plasma triglyceride and FFA concentrations](https://www.jap.org/)

<table>
<thead>
<tr>
<th>Lipid, mM</th>
<th>Standing/Ambulatory Control</th>
<th>Reduced Contractile Activity</th>
<th>Main Effect</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total TG (mM)</td>
<td>Saline</td>
<td>Nicotinic acid</td>
<td>Saline</td>
<td>Nicotinic acid</td>
</tr>
<tr>
<td></td>
<td>0.37 (0.03)</td>
<td>0.25 (0.04)</td>
<td>0.32 (0.02)</td>
<td>0.21 (0.02)</td>
</tr>
<tr>
<td></td>
<td>0.61 (0.14)</td>
<td>0.46 (0.12)</td>
<td>0.83 (0.13)</td>
<td>0.46 (0.07)</td>
</tr>
</tbody>
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Values are means (SE); n = 5–8 rats for each mean value. Plasma lipid concentrations were assessed from EDTA-treated blood samples taken immediately on the completion of the 12-h treatments. As revealed by a 2-way ANOVA, there was not a significant main effect for physical activity, but there was a significant main effect for drug treatment for both plasma triglyceride (TG) and free fatty acid (FFA) concentrations. There was not a statistically significant contractile activity × drug interaction, indicating that there was not a difference in the reduction of either plasma TG or FFA by nicotinic acid between the ambulatory and inactive rats. NS, not statistically significant.

![Fig. 2. In vivo effect of nicotinic acid on skeletal muscle HR-LPL activity is dependent on the physiological responses to physical inactivity.](https://www.jap.org/)

Nicotinic acid was administered intraperitoneally in saline (n = 15). The normal plasma lipid control group rats were infused with the saline vehicle (n = 13). Standing/ambulatory control rats (n = 12) were not exercised and only performed ordinary leg contractions associated with standing/spontaneous cage movements. In the physically inactive group (n = 16), the local contractile activity and/or energy demand in leg muscles was reduced for 12 h with hindlimb unloading before measurement of LPL activity. The soleus (A) and the gastrocnemius (B) were studied to investigate the 2 muscle types that contain the majority of the skeletal muscle LPL activity (slow oxidative and fast oxidative glycolytic, respectively). Values are means (SE). *P < 0.01 vs. standing/ambulatory control. †P < 0.05 vs. low lipid.
plasma lipid artificially. Whereas moderately active muscles are not sensitive to normal endogenous lipid levels (Figs. 2 and 3), Fig. 4 indicates that high exogenous lipid can reduce LPL activity also in oxidative muscle of moderately active animals. The integration of results of lipid lowering and high-fat feeding are presented in Fig. 4, and they indicate that physical inactivity (open bars of Fig. 4) increases the sensitivity of HR-LPL activity to lipid delivery.

DISCUSSION

This study adds to our understanding of skeletal muscle LPL regulation during physical inactivity. There is a strong interaction between the level of physical activity and the effect of lowering plasma lipids with nicotinic acid (Figs. 2 and 3). The present experimental design was effective at intentionally avoiding hypertriglyceridemia during physical inactivity and reducing plasma TG and FFA concentrations to the same level in both the physically inactive and standing/ambulatory groups (Table 1). Although there was not a difference in plasma TG and FFA concentrations between the two levels of physical activity (Table 1), the effect of lowering lipids was only evident when sedentary (Figs. 2 and 3). Thus these findings indicate that the sensitivity of LPL activity to circulating plasma lipids was heightened by low contractile activity (Fig. 4).

Several compounds were utilized that have previously been shown to prevent a lowering of LPL (Fig. 1). Nicotinic acid was the one drug that was effective. It has been used many times before to artificially repress the total fatty acid delivery to tissues (plasma TG and FFA) vis-à-vis inhibition of hepatic VLDL secretion (52) and hormone-sensitive lipase activity in
Experimental Lipid Delivery

Fig. 4. Contrast of the sensitivity of skeletal muscle HR-LPL activity during physical inactivity and nonexercise physical activity to 3 levels of plasma lipid delivery. A: the sensitivity of soleus microvascular LPL activity to suppression by plasma lipid was heightened by physical inactivity. Low lipid delivery refers to the conditions with low triglyceride (TG) and free fatty acids (FFA) elicited by nicotinic acid (Table 1). Normal (saline) lipid delivery refers to the standing/ambulatory control group with the saline vehicle. An excessive plasma lipid delivery (high lipid delivery) was achieved by the consumption of a large fat load over a 12-h period (80% fat diet to supply ~200 kcal/kg). Open bars, physical inactivity; solid bars, standing/ambulatory activity. Values are means (SE). †P < 0.05 vs. low plasma lipid (nicotinic acid). *P < 0.05 vs. control rats with low-grade standing/ambulatory activity.

adipose tissue (53). The acute nicotinic acid treatment for 8–12 h was capable of preventing the typical suppression of HR-LPL activity caused by reduced local energy demand (7). The effects of inactivity are most striking for the HR-LPL activity, with up to 10-fold differences in the most oxidative muscle regions (Ref. 7, Fig. 2). Previous studies using this model of physical inactivity showed that the HR (microvascular) pool of LPL protein decreases hours before the total muscle LPL protein decreases and to a greater degree than the total LPL protein (7). In addition, LPL mRNA is not decreased after either 1 or 11 days of inactivity as shown with both real-time PCR (5) and Northern blot analyses (7). It is self-evident that before plasma lipids can be taken up by muscle cells as a source of fuel they first must pass through the endothelium.

Given that fact and other observations that the endothelium is a site of LPL regulation (22, 38, 47), the emerging perspective (Fig. 5) is that the exposure of inactive skeletal muscle tissue to even moderate concentrations of plasma lipids can exert a negative effect on HR-LPL activity and thereby minimize the uptake of TG-derived fatty acids during periods of low fatty acid utilization (7). Interestingly, elevated plasma and muscle lipids have also been often reported to be key players in muscle insulin resistance, a component of the metabolic syndrome (3, 46). Thus the uptake of both plasma glucose and plasma TG-rich lipoproteins by muscle can be reduced by the cellular effects of plasma lipids. However, unlike the novel finding in the present study where lipid lowering raises LPL activity caused specifically by physical inactivity, we are unaware of any studies that have yet to determine whether lipid lowering reverses the insulin resistance caused by physical inactivity. Thus the present findings raise the novel hypothesis that components of the metabolic syndrome may be related to an increased sensitivity to plasma lipids during physical inactivity.

Contrary to expectations for our alternative hypotheses, pharmacological studies did not reveal a role of signaling by TNF-α, NF-κB, or iNOS in the downregulation of muscle LPL during physical inactivity. Previously, iNOS inhibition with aminoguanidine prevented the decrease in gastrocnemius LPL activity caused by endotoxemia (40). Wu et al. (54) demonstrated that the decrease in LPL activity in adipose tissue caused by lipopolysaccharide and fasting was completely prevented by the intraperitoneal administration of pentoxifylline, an inhibitor of TNF-α expression, and partially by an NF-κB signaling inhibitor. A high dose of salicylate, a known inhibitor of NF-κB, also prevented skeletal muscle insulin resistance caused by the infusion of TG into plasma (26). These treatments were completely ineffective against the decrease in soleus HR-LPL activity caused by physical inactivity.

Although much is known about how nicotinic acid administration affects plasma lipids, we are unaware of any evidence that nicotinic acid has a direct effect on either muscle or endothelial lipid metabolism. From the in vitro studies (Table 2), we observed that nicotinic acid did not directly increase

Fig. 5. Model of LPL response to physical inactivity and plasma lipid in the skeletal muscle microvasculature. Left: vascular endothelial cells are at the interface with plasma and directly exposed to plasma TG and fatty acids bound to albumin (not drawn). There is a high amount of HR-LPL activity associated with the microvascular endothelial cells in standing/ambulatory rats. This high HR-LPL activity hydrolyzes plasma TG to locally generate a high concentration of fatty acids. Middle: removal of normal local energy demand by physical inactivity under prevailing normal TG/FFA concentrations leads to a marked reduction in HR-LPL activity. Right: experimentally reducing circulating plasma TG and FFA bound to albumin during physical inactivity by nicotinic acid lowers the exposure of muscle tissue to these lipids, and this maintains HR-LPL activity.
HR-LPL activity. Others reported that the PUMA-G/HM74 receptor for nicotinic acid is apparently not expressed in skeletal muscle (50), supporting the early evidence that there was not a significant uptake of plasma nicotinic acid into skeletal muscle (11). In contrast, adipose tissue is rich in this receptor that modulates the responses of nicotinic acid (50), and adipose tissue accumulates tritiated nicotinic acid (11). Also, unlike adipose tissue, the hormone sensitive lipase activity in skeletal muscle is not reduced by nicotinic acid (53). At pharmacological doses, nicotinic acid suppresses hormone-sensitive lipase activity in adipose tissue by \( \sim 30-40\% \) (53), and it is commonly used as a means to attenuate release of FFA from adipose tissue (52). A previous study used nicotinic acid to reduce FFA in the local extracellular environment of adipose tissue and made the compelling argument that this could blunt the decrease in adipose tissue LPL activity caused by fasting (35, 37). Nicotinic acid is also used clinically to lower plasma TG concentrations, and this is thought to be due in large part to a reduction in hepatic TG production (52). Therefore, this well-known effect of reducing total fatty acid delivery (TG and albumin-bound FFA) has been used to determine the role of plasma lipid substrates on tissue metabolism. It should not be assumed that dietary amounts of niacin have any effect on LPL because only pharmacological doses of nicotinic acid repeated at frequent intervals cause a significant or sustained lowering of plasma lipids.

Other studies have also examined whether plasma lipids can decrease LPL activity. Cell culture studies have reported that the LPL activity of quiescent noncontracting cardiomyocytes (2, 12) and endothelial cells (47) can be reduced by fatty acids (2, 12, 47) or plasma triglycerides (47). Some (22, 38) but not all (39, 43) studies have indirectly suggested that raising plasma lipids may quickly decrease LPL activity, as shown in Fig. 4. A lifetime of complete ablation of hormone sensitive lipase expression increased skeletal muscle LPL in association with a significantly lower plasma TG and FFA concentration (15). These important previous findings opened the door to our hypothesis that in contrast to leg muscles in conscious freely moving rats, muscles with low contractile activity would experience a greater response to the lowering of endogenous delivery of plasma lipids.

The remarkable increase in the sensitivity of HR-LPL to plasma lipid during inactivity (Fig. 2) may be due to some of the many differences in lipid metabolism between inactive and active muscles. The endothelium is in direct contact with plasma lipids (Fig. 5), and the microvasculature would seem well poised to respond to the local rates of TG hydrolysis (38, 47). Because of obvious technical limitations, little is known about the metabolic responses in microvascular endothelial cells where the functional HR-LPL is located. However, it is well known that, compared with a resting muscle, an active muscle has greater rates of TG-derived fatty acid uptake (7, 29), albumin-bound fatty acid transport (9, 51), fatty acid oxidation (45), and intracellular TG synthesis (45) and has reduced concentration of intramuscular fatty acids (25, 45) and fatty acyl-CoA (36). These characteristics enhance the ability of contracting muscle to act as a sink for clearing lipids out of the surrounding extracellular fluid, which possibly also decreases the exposure of neighboring endothelial cells to an accumulation of plasma fatty acids and triglycerides. HR-LPL activity is frequently reported to be inversely related to plasma TG concentration (49). The prevailing thought is that the reason for this relationship is because LPL is the essential enzyme for TG uptake in the capillary beds of tissues with a high rate of TG utilization. However, in light of these new data (Fig. 2), it is possible that this inverse relationship between plasma TG and LPL may be also in part due to lower TG concentrations, which may lead to elevated muscle LPL activity in resting muscle. A mismatch between TG-derived fatty acid uptake and utilization likely leads to changes in HR-LPL activity to temper imbalances between fatty acid utilization (oxidation and/or storage) and lipid delivery. Interestingly, the reduction in plasma TG concentration caused by exercise was correlated with muscle HR-LPL activity (21).

Although lifestyle interventions are recommended as the basic therapy, the American Heart Association/National Heart, Lung, and Blood Institute scientific panel also recommended that nicotinic acid (also called niacin) be a first-line pharmacological therapy for patients with hypertriglyceridemia (14). The variable sensitivity of LPL to nicotinic acid following periods of modest differences in physical inactivity might provide an answer to clinical investigators who have asked why there are apparently variable responses to nicotinic acid (34). Early studies provided the first indirect suggestion that nicotinic acid might enhance LPL activity in some conditions (35, 37, 41). It has been reported that the removal of TG-rich lipoproteins can be significantly enhanced by acute nicotinic acid administration in some (8) but not all study groups (4, 34). This raises the clinical hypothesis for translational studies that the actions of nicotinic acid in treating hypertriglyceridemia may be partly due to direct effects in reducing hepatic TG secretion, and secondarily due to an increased HR-LPL activity, particularly in inactive skeletal muscle.

Our laboratory recently introduced the concept of inactivity physiology as being important to identify potent physiological regulation of disease-related proteins during periods of inactivity (18). The paradigm of inactivity physiology indicates that novel physiological insights regarding the effects of physical inactivity (such as enhanced sensitivity of LPL to plasma lipids) will be recognized best by directly comparing treatments involving inactivity with very low-intensity physical activity that can occur for many hours in ordinary life. It is possibly a misconception, although understandable, to think that ordinary spontaneous low-grade movements and weight-bearing activity are insufficient to have some specific powerful physiological effects compared with physical inactivity. As far as we know, no treatment is known to alter LPL activity in skeletal muscle more than reducing the normal amount of NEAT that occurs in leg muscles during standing and low-grade movements (7). It will be important for additional research on the regulation of proteins like LPL at the low end of the physical activity continuum because inactivity is an actual cause for mortality (32), and some of the cellular regulatory processes during inactivity are not the mirror image of those occurring during exercise (18). Even people who are classified as “couch potatoes” stand on average 8 h and 43 min per day (28), and there are motor units in rat leg muscles, presumably red oxidative fibers, that are recruited \( \sim 8 \) h per day (20). In contrast to the present interventional type of investigation minimizing standing to study a health-related protein, observational type of clinical studies have correlated body...
composition with interindividual differences in standing duration and other types of mild contractions associated with NEAT (27, 28), energy expenditure of leg muscles during very low-intensity contractile activity (10 W) (44), and spontaneous physical activity (56). Because of the fact that the control rats in the present studies were never exercise conditioned or obese, it is clear that the specific physiological response of reduced LPL during physical inactivity is preventable by the ordinary low-intensity and intermittent type of contractions associated with NEAT. Because of the well-established importance of HR-LPL activity on plasma lipoprotein metabolism, these findings provide an explanation for why the light contractions associated with NEAT may impact health and a rationale for why NEAT should be further investigated as a pragmatic means to attenuate the dyslipidemia and other problems linking inactivity to the metabolic syndrome.

In conclusion, the present study is relatively unique in being one of the first interventional studies to experimentally prevent standing to determine the importance of this type of light contractile activity on regulation of an enzyme controlling plasma lipid metabolism. We learned that a heightened sensitivity to lipid metabolism may be involved in causing the typically very large reduction in HR-LPL activity in vivo during physical inactivity.

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