CALL FOR PAPERS | Insulin Resistance and the Cardiometabolic Syndrome: Adipose Tissue and Skeletal Muscle Factors

Setting the stage: possible mechanisms by which acute contraction restores insulin sensitivity in muscle

John P. Thyfault

Research Service, Harry S. Truman Memorial Veterans Affairs Hospital, Department of Nutritional Sciences and Internal Medicine, University of Missouri, Columbia, Missouri

Thyfault JP. Setting the stage: possible mechanisms by which acute contraction restores insulin sensitivity in muscle. Am J Physiol Regul Integr Comp Physiol 294: R1103–R1110, 2008. First published January 23, 2008; doi:10.1152/ajpregu.00924.2007.—It has long been known that acute exercise can dramatically improve insulin sensitivity in previously insulin-resistant muscle; however, the precise mechanisms underlying this clinically significant interaction remain unknown. Using hindlimb perfusions in obese Zucker rats, our group found that acute muscle contraction synergistically improved insulin-stimulated glucose transport in skeletal muscle, but contrary to our hypothesis, these findings were not associated with either improved insulin signaling or decreased intramuscular lipid metabolites. A further analysis revealed that the improved insulin sensitivity was associated with a robust increase in mitochondrial energy flux. These findings and reports from other labs suggest that mitochondrial energy flux and mitochondrial oxidative capacity may govern insulin sensitivity and override insulin signaling defects associated with obesity. This review will discuss the effects of acute exercise to enhance insulin sensitivity in previously insulin-resistant muscle and present possible novel mechanisms by which alterations in mitochondrial energy metabolism may play a regulatory role.

skeletal muscle; signaling; glucose transport; lipids; contraction; exercise

The incidence of type 2 diabetes has skyrocketed in the last two to three decades and continues to increase (15, 98), directly leading to cardiovascular disease and other morbidities (71), causing a decreased quality of life in its victims (98), and costing the health care system billions of dollars (33). Insulin resistance of skeletal muscle, an impaired ability of insulin to stimulate glucose disposal into skeletal muscle, is the first progressive step toward overt type 2 diabetes. Insulin resistance is counteracted by hyperinsulinemia or increased insulin production by the pancreas. Type 2 diabetes is then diagnosed after hepatic dysregulation and pancreatic β-cell failure lead to chronic hyperglycemia.

As with other chronic health disorders, national health organizations and pharmaceutical companies are working to implement programs and develop pharmacological agents that can prevent or treat insulin resistance and type 2 diabetes. While this is occurring, both biological and epidemiological scientific evidence demonstrates that a shift in our human lifestyle (amount of physical activity vs. inactivity and dietary intake) is the primary cause behind the sudden increase in type 2 diabetes (5, 25, 39–41, 46, 53). In agreement with these findings, a large amount of evidence shows that exercise provides the best prevention and treatment for insulin resistance and type 2 diabetes (22, 25, 26, 29, 36, 53, 81, 82). It has been known for many years that acute exercise (muscle contraction) increases insulin-stimulated glucose disposal in both healthy and insulin-resistant skeletal muscles (16, 17, 43, 72). The consistency of the interactions between exercise and insulin sensitivity resulted in the following comment from DeFronzo in 1981, “These observations raise the possibility that exercise and insulin may act in concert to enhance cellular uptake of glucose” (16).

Although exercise is clearly the “best” absolute medicine for treating skeletal muscle insulin resistance, the precise mechanisms by which contraction enhances insulin sensitivity in muscle have yet to be identified.

Interaction of Acute Muscle Contraction and Insulin-Stimulated Glucose Transport

Contraction and insulin robustly activate glucose transport into skeletal muscle by independently activating the translocation of the glucose transporter Glut4 from an intracellular location to the plasma membrane, an obligatory step for increased rates of glucose transport into skeletal muscle. In insulin-sensitive muscle, contraction immediately followed by insulin stimulation causes an additive increase in glucose transport, i.e., the total glucose transport rate with both stimuli...
equals the transport rate that could be predicted by adding the individual effects of contraction and insulin. After the acute effects of contraction upon Glut4 translocation are gone, insulin sensitivity remains elevated for many hours following a single bout of exercise (10, 55, 95). Many different labs have examined the interaction between muscle contraction or acute exercise and insulin sensitivity (30, 35, 95, 96), and although several key findings have been made, much remains unknown. Despite the current lack of understanding, the interactions between exercise and insulin sensitivity are of obvious clinical importance in the prevention and treatment of skeletal muscle insulin resistance.

**Contraction Prior to Insulin Stimulation in Insulin-Resistant Skeletal Muscle**

Although many labs continue to study the interaction between contraction and insulin sensitivity in healthy skeletal muscle, our research group has specifically studied the interaction of muscle contraction and insulin sensitivity in insulin-resistant muscle from obese models. Early studies from our group examined the interactions of acute contraction and insulin sensitivity using hindlimb perfusions in obese and lean Zucker rats (17). In these experiments, glucose transport was measured in the following conditions: basal or resting, contraction, insulin, and contraction prior to insulin. As expected in the lean Zucker rats, acute contraction followed by an insulin stimulus increased glucose transport in additive fashion in both red and white portions of the gastrocnemius. The muscles of the obese Zucker rats had almost no increase in glucose transport during insulin stimulation showing extreme insulin resistance. However, during the contraction prior to insulin stimulus, the muscles (both red and white gastrocnemius and extensor digitorum longus) of the obese Zucker rats showed a large increase in the rate of glucose transport that was synergistic (glucose transport was greater than what was predicted from adding the individual responses of insulin and contraction). Similar synergistic interactions between contraction and insulin action have been witnessed in humans with type 2 diabetes stressing the clinical relevance of these findings (16). Because there was a limited knowledge of the insulin signaling pathway and of the biochemical and molecular alterations causing insulin resistance, it was difficult to surmise a likely mechanism for these early findings. As more information evolved detailing the impact of obesity and a lipid surplus on skeletal muscle insulin resistance, our group formed a new hypothesis to explain the interaction. We hypothesized that contraction removes a factor that inhibits the insulin signaling pathway, thus priming the muscle for enhanced insulin sensitivity. We further predicted that the removable inhibitory factor was elevated intramuscular triglycerides (TAG), diacylglycerol (DAG), and long-chain fatty acyl CoAs (LCACoA), metabolites strongly linked to impaired insulin sensitivity. Thus, our primary aim was to associate contraction-induced alterations of muscle lipids with changes in insulin-signaling and glucose transport.

**Obesity and Insulin Resistance in Skeletal Muscle: Role of Increased Intramuscular Lipids Stores**

Insulin resistance is strongly associated with an increased storage of TAG in myocytes (28, 51, 64, 83). However, inert TAG do not likely participate as direct mediators of insulin resistance, but probably contribute to the accumulation of DAG and ceramides, molecules known to participate in cell signaling pathways that inhibit insulin signaling. Evidence suggests that intramuscular DAGs act as ligands for serine kinases, including protein kinase C, IκB kinase catalytic subunit-β, and c-Jun NH2-terminal kinase, which phosphorylate specific serine sites on the insulin receptor and/or insulin receptor substrate-1 (IRS-1) (32, 44, 45, 66, 77, 97). Serine phosphorylation of the insulin receptor or IRS-1 blocks insulin-stimulated tyrosine phosphorylation at IRS-1 and reduces downstream insulin signaling resulting in reduced Glut4 translocation and insulin resistance (2) Ceramides block distal portions of insulin signaling. Specifically, ceramides appear to block insulin-stimulated phosphorylation of Akt and glycogen synthase kinase, while having no effect on IRS-1 signaling (80). An accumulation of LCACoA in muscle, although there is less mechanistic information detailing its deleterious intracellular effects, has also been strongly associated with insulin resistance (14, 37, 59). The support for lipid oversupply and ectopic lipid storage in muscle causing insulin resistance comes from cross-sectional evaluations of obesity in both rodents and humans (18, 37, 42, 70, 88) and from prospective studies using high-fat feedings and lipid infusions to elevate intramuscular lipids and cause insulin resistance (44, 59, 77, 97). The intramuscular lipid-insulin resistance association is further strengthened by studies in which the lipid oversupply is reduced or removed and insulin sensitivity in skeletal muscle is quickly restored (37, 59, 97).

Obese Zucker rats possess an excessive storage of lipids in skeletal muscle (18), and because exercise robustly mobilizes endogenous lipids and increases fatty acid oxidation in muscle, we reasoned that elevated lipids were the inhibitory factor removed by contraction. We further postulated that if intramuscular lipids were decreased, the activation of serine kinases would also be reduced, and insulin signaling would be restored. To test these hypotheses, new experiments were designed that again utilized hindlimb perfusions in obese and lean Zucker rats. We first sought to replicate the previous findings of a synergistic interaction between muscle contraction and insulin sensitivity followed by answering two questions: 1) does acute muscle contraction lower intramuscular lipids in the obese Zucker rat, and 2) does this lead to enhanced activation of the classic insulin signaling pathway or enhance other signaling pathways linked to insulin sensitivity?

**Effects of Acute Muscle Contraction on Intramuscular Lipids**

After repeating the hindlimb perfusions, we again witnessed a synergistic effect of contraction and insulin on glucose transport in the gastrocnemius muscle and extensor digitorum longus (data not shown) of the obese Zucker rat, while witnessing slightly less than an additive response between contraction and insulin in the muscle of the lean Zucker rat (Fig. 1). The most striking finding was that contraction prior to insulin stimulation normalized insulin action in the obese Zucker muscle to the level of the lean Zucker controls.

As previously stated, we first wanted to know whether the improved insulin sensitivity was associated with a decrease in intramuscular lipids. Acute muscle contraction significantly lowered intramuscular TAG in the obese Zucker rat, while...
we saw no associated improvements in insulin-stimulated ty-
muscle contraction dramatically improved insulin sensitivity,
sulin signaling in the skeletal muscle of the obese Zucker rat.
mediately followed by insulin stimulation would enhance in-
Effects of Acute Muscle Contraction on Insulin Signaling

causing a nonsignificant decrease in the lean Zucker rat (88).
Unexpectedly, intramuscular DAG was not significantly
changed by contraction in either group. We also witnessed
intramuscular LCACoA levels increase after contraction in
both groups, a likely result of the quick mobilization of
endogenously stored lipids to meet the energy demands of
contraction. Because elevated LCACoA levels are implicated
in the pathogenesis of insulin resistance (14), we were
surprised to see an increase in LCACoA levels at the same time
that insulin action was dramatically improved. Thus, our hy-
thesis that muscle contraction could decrease the lipotoxic
environment was partially held true (decrease TAG in obese
Zucker rat), but was also rejected as muscles from both groups
showed increased LCACoA levels during a period of enhanced
insulin sensitivity.

Other noteworthy disassociations between intramuscular
lipid levels and insulin resistance have been reported; many
such findings have been realized with exercise studies. Exer-

Fig. 1. Rates of glucose transport in whole gastrocnemius from lean (white bars) and obese (black bars) were measured during basal (resting without insulin), contraction (Con), insulin (Ins), or contraction followed by insulin (Con + Ins) stimulation. *Contraction increased glucose transport in both lean and obese animals. Insulin increased glucose transport in lean animals; how-
ever, obese animals showed no insulin stimulation of glucose transport and were †decreased compared to lean. Con + Ins restored insulin-stimulated glucose transport in the obese group. Significance was set at \( P < 0.05 \); Values are means ± SE. (Figure is reprinted from Ref. 88).

Our next hypothesis was that prior muscle contraction im-
mEDIATELY followed by insulin stimulation would enhance in-
sulin signaling in the skeletal muscle of the obese Zucker rat.
Once again our assumptions were not realized. Although prior
muscle contraction dramatically improved insulin sensitivity,
we saw no associated improvements in insulin-stimulated ty-
rosine phosphorylation of the insulin receptor and IRS-1, or
serine\(^{173}\) phosphorylation of Akt in the muscle of either group.
We additionally saw no contraction plus insulin interaction
effect on mitogen-activated protein kinase signaling, a pathway
known to participate in both contraction and insulin-stimulated
glucose transport (76, 85). These results were not totally
surprising as other studies have also shown that acute exercise
does not improve insulin signaling in skeletal muscles of both
humans and rodents (23, 24, 48, 94), while others have re-
ported small improvements in insulin signaling after acute
exercise (12, 74) and more significant improvements after
chronic exercise training in obese Zucker rats (31, 75).

Although we found that contraction did not restore insulin
activation of the classic signaling cascade (IR, IRS-1, and Akt),
we did witness that acute contraction caused a marked im-
provement in insulin-stimulated phosphorylation of Akt sub-
strate 160 (AS160), a newly studied insulin signaling protein
that is downstream of Akt and integral for Glut4 translocation.
Recent work has shown that AS160 (RabGAP protein) is a
point of convergence for both insulin and contraction-induced
signals (4, 19, 38, 50), which can both phosphorylate and
inhibit AS160 GAP, allowing Rab to be in an active GTP form,
and subsequently permitting Glut4 translocation and glucose
transport (54). Contraction and insulin phosphorylate AS160
independently, and the combined treatments can act synergis-
tically upon AS160 phosphorylation in healthy skeletal muscle
(8). In our hands, contraction followed by insulin also syner-
gistically increased AS160 phosphorylation in the muscles of
the obese and lean Zucker rats (Fig. 2). Furthermore, the prior
contraction of obese Zucker muscle enabled insulin to phos-
phorylate AS160 to levels found in the insulin-stimulated lean
Zucker muscle (no contraction) condition. It is possible that the
partial restoration of AS160 signaling in insulin-resistant mus-

Fig. 2. Akt substrate 160 (AS160) phosphorylation was assessed at basal and
after contraction, insulin, or Con + Ins (basal set to 1 for each treatment).
*AS160 phosphorylation increased after both contraction and insulin. †Insulin
stimulation in obese was lower than that found in lean; however, there was no
significant difference between the groups after Con + Ins. Prior muscle
contraction improved insulin signaling only at the distal signaling protein,
AS160. Significance was set at \( P < 0.05 \); Values are means ± SE. (Figure is reprinted from Ref. 88).
muscle, it is unlikely that enhanced AS160 signaling is the mediator of contraction-induced enhancement of insulin sensitivity. The convergence of contraction and insulin signals upon AS160 may play a more integral role in the additive impact of glucose transport witnessed in healthy skeletal muscle (50), although future studies of AS160 signaling in obesity are warranted.

These signaling results indicated that muscle contraction caused improved insulin sensitivity by sensitizing chronic defects in insulin signaling and lead us to speculate that some other unidentified and novel mechanism was linking acute exercise to enhanced insulin sensitivity.

Contraction Increases Mitochondrial Energy Flux in Skeletal Muscle

To gain additional insight into the contraction-induced improvements in insulin sensitivity, we further examined skeletal muscle acylcarnitine levels in the basal (rested) state or immediately after contraction. Acylcarnitines are generated when accumulating acetyl-CoA and acyl-CoA metabolites are converted to carnitine esters by a family of acyltransferase enzymes that are localized exclusively in subcellular organelles. In skeletal muscle, this process occurs mainly in mitochondria where acylcarnitines are present in equilibrium with their respective acyl-CoAs. Thus, intramuscular acylcarnitine levels can be used as markers of mitochondrial energy metabolism. Furthermore, by evaluating the ratio of long-chain to short-chain acylcarnitines it is possible to infer changes in substrate selection, metabolic flux, and flux control. For example, β-oxidation of fatty acid is accomplished through a recurring sequence of four reactions that result in the cleavage of a two-carbon unit (acetyl-CoA), while also generating a long-, medium-, or short-chain acyl-CoA intermediate. The relative abundance of the corresponding acylcarnitine byproducts provides information with regard to specific enzymatic steps that are flux limiting. Under circumstances in which the supply of LCACoA is rate limiting, an increase in β-oxidative flux would result in a new steady-state condition characterized by a rise in short-chain acylcarnitines relative to their long-chain precursors. Alternatively, when TCA cycle activity is rate limiting, one would expect to observe a disproportionate increase in acetylcarnitine accumulation. This methodology is commonly used to diagnose inborn errors in mitochondrial metabolism (62, 92). In our studies, we applied a similar strategy to assess contraction-mediated adjustments in mitochondrial energetics. The ensuing results suggested that in skeletal muscles of both lean and obese rats, acute contraction caused a robust increase in substrate flux through β-oxidation and the TCA cycle (Table 1). Taken together with previous reports, these findings led us to speculate that changes in mitochondrial energy flux modulate insulin action.

Mitochondrial Function, Cellular Energy Flux, and Links to Insulin Sensitivity

A plethora of new evidence indicates that skeletal muscle insulin sensitivity is tightly linked to mitochondrial energy metabolism. Mootha et al. (56) found that a coordinated set of oxidative phosphorylation genes and peroxisome proliferator-activated receptor coactivator-1α (PGC-1α), a potent transcriptional regulator of mitochondrial function, is reduced in the skeletal muscle of type 2 diabetic subjects. Petersen and colleagues found that insulin-resistant offspring of type 2 diabetics display reduced mitochondrial fatty acid oxidation compared with age and body weight matched controls (68) and that mitochondrial dysfunction or reduced mitochondrial oxidative capacity plays an integral role in the insulin resistance witnessed in elderly subjects (67). These and a number of other studies demonstrate a consistent link between mitochondrial dysfunction and insulin resistance (47, 73, 84, 86). Furthermore, chronic exercise studies have found that exercise-induced improvements in skeletal muscle mitochondrial number and content or expression of genes known to regulate mitochondria function like PGC-1α, are tightly correlated to improvements in whole body insulin sensitivity in obesity (63, 91) and type 2 diabetes (90).

Even more convincing is evidence that skeletal muscle mitochondrial fatty acid oxidation, whole-body fatty acid oxidation, and whole-body aerobic capacity are better predictors and correlates of insulin sensitivity than intramuscular lipid levels (7, 20, 22, 69). In particular, our study in obese Zucker rats (88) and a study from Bruce et al. (7) showed that enhanced insulin sensitivity was paired with increased and not decreased LCACoA levels. In support of this concept, there is additional evidence showing that elevated rates of mitochondrial oxidative capacity and fatty acid oxidation in muscle are associated with increased insulin sensitivity (1, 3, 11, 49, 58, 65).

In nontransgenic models, enhanced mitochondrial oxidative capacity or fatty acid oxidation is commonly brought about by chronic exercise training or repeated daily muscle contractions. This brings forth the question of whether chronic exercise-induced changes in mitochondrial function are the main link to enhanced insulin sensitivity; or as shown in our study with obese Zucker rats, whether it is the acute effect of muscle contraction bringing about increased energy flux through the mitochondria and the subsequent demand for ATP production that regulate insulin sensitivity. Although in many chronic exercise studies, insulin sensitivity is measured 48 h or longer, after the last bout it is plausible that the acute effects of muscle contraction are the primary mechanism improving insulin sensitivity. As previously reviewed by Schenk and Horowitz (78), chronic exercise-induced increases in resting fatty acid oxidation rates are not robust. Furthermore, both weight loss and chronic exercise training improve insulin sensitivity despite...
weight loss having no significant impact on skeletal muscle fatty acid oxidation (79, 89). In addition, while we and others have found that one bout of exercise dramatically improves insulin sensitivity in insulin-resistant muscle, it is commonly known that successive exercise sessions are needed to increase mitochondrial proteins and enzyme activities, providing evidence that exercise-induced improvements in insulin sensitivity do not rely on chronic mitochondrial alterations. Finally, much like our insulin signaling results, both 8 wk of daily exercise in obese Zucker rats (13) and 7 days of exercise in insulin-resistant humans (87) failed to significantly improve insulin signaling despite improved insulin sensitivity, further suggesting that exercise enhances energy flux and insulin sensitivity by overriding insulin signaling perturbations. All of these factors indicate that contraction-induced energy flux and a sudden depletion and demand for ATP synthesis are likely linked to insulin sensitivity. In a recent exercise-training study, the amount of energy expenditure during each exercise bout (measure of energy flux) was a robust predictor of the improved insulin sensitivity (measured 48 h after the last exercise bout) witnessed at the end of the study (90). The mean daily energy expenditure accounted for nearly three-quarters of the variance in improved insulin sensitivity, suggesting that energy flux and expenditure in skeletal muscle governs insulin sensitivity. The link is further supported by transgenic models like the ACC2 knockout mouse, which displays both elevated energy expenditure and increased insulin sensitivity during both normal chow and high-fat diets (11).

In relation to chronic exercise training and improved insulin sensitivity, it is likely that acute factors (daily muscle contraction-induced energy flux) and chronic alterations (enhanced mitochondrial oxidative capacity and increased Glut4 protein content) both play a role in enhancing insulin sensitivity. An important tool in understanding the links among these acute and chronic mechanisms and insulin sensitivity is the evidence that the cessation of daily physical activity or exercise lead to rapid (hours to days) and transient declines in skeletal muscle insulin sensitivity in both animals and humans (6, 9, 27, 52) that can nearly be restored with only one bout of exercise that occurs 10 days later (27). Thus, a plausible hypothesis is that the cessation of activity provokes a need to match lowered ATP requirements with lowered glucose entry and utilization in the muscle cell, while conversely, as already hypothesized, acute muscle contraction provokes a need for increased glucose entry and insulin sensitivity due to depleted fuel storage and elevated ATP requirements. The hypothetical association between energy flux and ATP demand is very similar to the already studied mechanism by which elevated muscle glycogen levels reduce insulin sensitivity and nonoxidative disposal of glucose in muscle. If the energy status of the muscle cell is positive and ATP demands are low, it is inherent that the cellular uptake of substrate should be reduced. In contrast, if there is a negative energy balance, the cell requires substrate for energy repletion and processes that control the rate of substrate entry would become more sensitive or work through alternative/unknown pathways. This link in muscle glycogen status was highlighted by Wojtaszewski et al. (95) who showed that the amount of muscle glycogen degraded during an exercise bout predicted insulin sensitivity 4 h later.

Although these links with muscle contraction, mitochondrial energy flux, and insulin sensitivity are plausible, they don’t completely explain the differences in insulin sensitivity found between obese rodent models and lean controls as they both remain caged in a sedentary environment; but the lean controls maintain a higher degree of insulin sensitivity. It may be that a hypercaloric/lipidic environment plus low energy flux (physical inactivity) is required to induce skeletal muscle insulin resistance in obesity. Caloric restriction can improve insulin sensitivity in both human obesity (93) and in rodent obesity models (60, 61) without a major change in weight loss proving that excessive caloric intake is an integral component of the insulin resistance found in obesity. Caloric restriction’s ability to enhance insulin sensitivity provides additional evidence that cellular energy depletion, regardless of whether it is induced by dietary restriction or by muscle contractions, can mediate insulin sensitivity, although there is no evidence to suggest that these mechanisms work through similar mechanisms.

Perspectives and Significance

Acute muscle contraction can dramatically improve or restore insulin sensitivity in insulin-resistant skeletal muscle, but the precise mechanism driving these events remains elusive. Our investigations in obese Zucker rats revealed that muscle contraction just prior to insulin stimulation dramatically improves insulin-stimulated glucose transport, but that it was not associated with improved activation of the classic insulin signaling pathway nor was it associated with decreased intramuscular lipid metabolites implicated in causing insulin resistance. A further analysis of acylcarnitine ratios suggest that enhanced insulin sensitivity following contraction was associated with a robust energy flux through β-oxidation and the TCA cycle. These results combined with other additional evidence suggest that insulin sensitivity may be modulated by changes in contraction-induced mitochondrial energy flux and that these effects can override existing perturbations of the insulin signaling pathway. Regardless of the precise mechanism, the ability of acute muscle contraction to enhance insulin sensitivity is clinically significant and highlights the importance of using increased physical activity and chronic exercise to prevent and treat type 2 diabetes.

ACKNOWLEDGMENTS

The author thank Drs. Lynis Dohm and Deborah Muoio for their mentorship and contribution to the ideas presented.

REFERENCES


65. Pan DA, Lilloioja S, Kriketos AD, Milner MR, Baur LA, Bogardus C, Jenkins AB, Storlien LH. Skeletal muscle triglyceride levels are in-