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Current advances in our understanding of exercise as medicine in metabolic disease

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Exercise is a powerful means to maintain health, prevent disease, and even act as medicine for a wide range of non-communicable diseases. The key effects by which exercise benefits our metabolic health include (i) events that occur during exercise and in the hours to days following exercise, and (ii) the adaptations that occur following long-term repeated exercise training. Here, we provide a contemporary overview of recent significant advances in our knowledge of exercise as medicine in metabolic disease with a focus on muscle glucose metabolism.

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What is old is new again

"If we could give every individual the right amount of nourishment and exercise, not too little and not too much, we would have found the safest way to health" Hippocrates said. Even before that, physicians such as Susruta (600 y before Common Era) would prescribe exercise for their patients. It is thus very old news that exercise has tremendous beneficial effects on our health. However, it is only recently that we are beginning to understand the molecular mechanisms underlying these benefits. This review will describe the most recent advances in our understanding of aerobic exercise as medicine in metabolic disease with focus on muscle due to its prominent role in metabolic control.

The power of exercise – the mechanisms unraveling

It is only relatively recently that the beneficial effects of exercise within specific metabolic conditions have been experimentally proven and, on a population basis, the results are clear: If everybody exercised regularly, obesity and metabolic diseases such as type 2 diabetes (T2D) and cardiovascular disease would be minor problems [1,2].

It has been convincingly documented that regular exercise improves glycemic control and insulin action among both obese and type 2 diabetic patients [3–5] and that this effect can be superior to those exerted by drugs or insulin therapy [6,7]. As such, the problem of lifestyle-related metabolic disease should in theory be highly solvable. However, the majority of adults do not achieve exercise levels according to the guidelines [8,9] and physical activity levels are declining and sedentary behavior is increasing [10]. Thus, it is more than ever relevant to elucidate the molecular mechanisms underlying exercise's beneficial effects in order to develop efficient strategies and pharmaceutical principles to treat conditions related to physical inactivity.

The beneficial effects of exercise are attributed to two main events: Firstly, the effects during and immediately following one acute bout of exercise (phases 1 and 2, respectively depicted in Figure 1). Secondly, the molecular adaptations that occur following continuous repeated exercise training. Importantly, regular exercise increases the amount of total time spent in phases 1 and 2 further enhancing the metabolic benefits of regular exercise training as depicted in Figure 1. In this review, we focus mainly on skeletal muscle-mediated effects due to muscle's major role in whole-body metabolic control [11,12] and we discuss the current evidence for the molecular mechanisms underlying metabolic benefits of exercise.

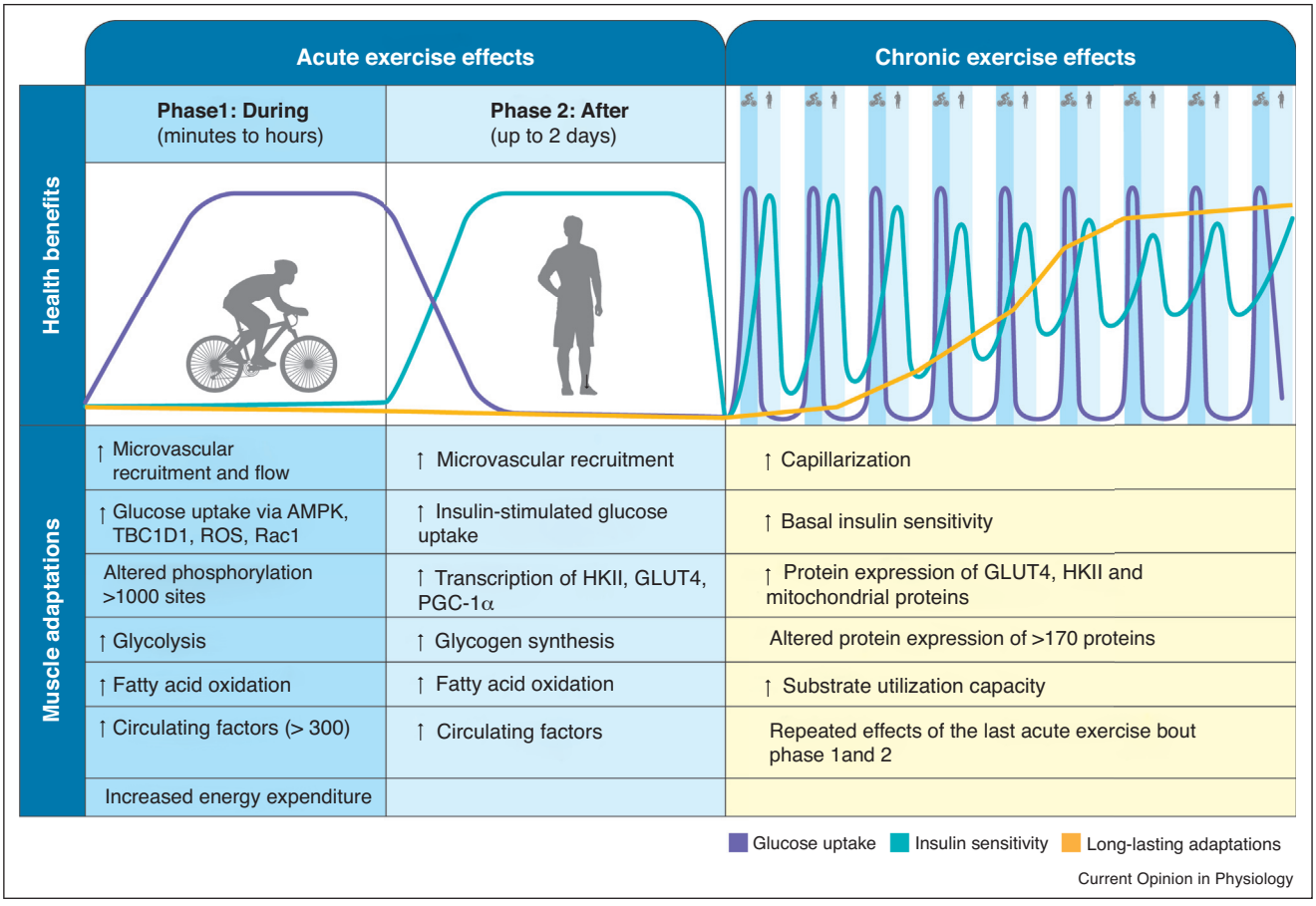
A two-phased metabolic response to an acute exercise bout

Phase 1: during exercise

Physical activity causes a large increase in energy utilization [13,14]. Glucose is a major fuel source for the contracting muscles and glucose uptake acutely increases during exercise [15] as depicted in Figure 1. Because glucose is taken up by the muscles via insulin independent mechanisms [16,17], it is effective in lowering blood glucose in insulin resistant subjects [18].

The molecular mechanisms regulating exercise-induced muscle glucose uptake involve a coordinated and complex regulation of increased glucose delivery to the working muscles, an increased amount of the glucose transporter GLUT4 at the plasma membrane and t-tubules to

Figure 1



A temporal overview of the beneficial effects of exercise in healthy subjects mediated by muscle.
One acute bout of moderate intensity aerobic exercise elicits an increase in glucose uptake by the working muscles as well as an increase in circulating factors and importantly energy expenditure (phase 1). This is followed by a transient increase in insulin sensitivity and circulating factors in the hours and up to two days after that exercise bout (phase 2). Chronic exercise training elicits several molecular adaptations to benefit health, including increased expression of proteins involved in glucose metabolism and increased insulin sensitivity in the rested (basal) state. Furthermore, regular exercise increases the amount of total time spent in phases 1 and 2 further enhancing the metabolic benefits of regular exercise training. We note that resistance exercise training elicits many of the same health benefits as aerobic exercise, however, not covered by this review.

allow transmembrane glucose transport, and accelerated intracellular metabolism of glucose by the muscle (See Ref. [13] for a comprehensive review on exercise-stimulated glucose uptake). Contemporary research has revealed that redundant parallel molecular pathways, involving the metabolic sensor AMPK [19,20•], the Rho GTPase Rac1 [21a], reactive oxygen species [22] and, in fast twitch muscle, the GAP GTPase TBC1D1 [23,24] are activated and seemingly are involved in distinct steps in glucose transport regulation.

Once taken up by the working muscle, glucose is utilized to produce ATP. Studies in humans exercising at 50% of VO2 max for 2 hours have shown that glucose oxidation is equal to glucose disappearance indicating that all of the glucose taken up during exercise is oxidized [25]. During most types of exercise with adequate muscle glycogen stores glucose covers between 10 and 20% of oxidative metabolism [26] and uptake of glucose from the blood increases with increasing exercise intensity and duration. However, uptake of glucose can account for up to 40% of oxidative metabolism when exercise is prolonged and muscle glycogen is depleted [15,27,28]. There is a reciprocal relationship between muscle glycogen breakdown and glucose uptake so the higher glycogen breakdown the lower glucose uptake and *vice versa* [29–31].

Because exercise stimulates glucose uptake independently from insulin, it was recently proposed that the timing of the exercise bout in relation to a meal may be an important factor for exercise's optimal blood glucose lowering effect [32]. In support of this, Colberg *et al.*

showed that postprandial walking was better for lowering the glycemic effect of dinner than pre-dinner exercise in individuals with T2D [33]. Thus, there might be therapeutic potential to apply chronology in the training regimen to drain off, in real time, the exogenous glucose entering the bloodstream from the food being digested.

Importantly, in metabolic disease where insulin-stimulated glucose uptake by muscle is often severely impaired, the exercise-stimulated glucose uptake response remains intact [18]. Thus, it has been hypothesized that activating the contraction-induced molecular mechanisms by pharmacological agents could lead to increased glucose uptake in insulin resistant muscle. In agreement, pharmacological activation of AMPK has proven effective in stimulating muscle glucose uptake and reducing blood glucose in T2D mice and non-human primates [34••,35••]. However, in contrast to exercise-induced increase in glucose uptake, pharmacological activation of proteins to increase muscle glucose uptake occurs without increased energy utilization and, therefore, leads to glycogen storage in contrast to exercise that leads to glycogen utilization [29].

Exercise also induces the secretion of circulating factors that can act in an autocrine or a paracrine fashion, the latter providing a mechanism by which exercise may benefit other tissues [36,37•,38••]. Using proteomic analysis Whitham *et al.* detected over 300 secreted proteins released in response to exercise packaged in extracellular vesicles [39••]. A closer look at what was directly secreted from the muscle during exercise (by analyzing femoral arteriovenous difference) identified 35 novel factors, suggesting that we are only just beginning to understand the complexity and magnitude of exercise-induced secretions. Similarly, Parker *et al.* found that exercise rapidly modulated hundreds of bioactive peptides in the circulation [40•]. These peptides underwent large changes during exercise on a time-scale of minutes with many rapidly reversible after exercise. Such unbiased discovery tools will likely be key methods for pinpointing exercise-regulated factors that generate health benefits. Apelin is an example of one such protein. Apelin was recently discovered to be secreted from muscle during exercise where it activated AMPK [41], prevented age-associated sarcopenia in mice [42••], and also increased insulin action in overweight men [43], suggesting that apelin could be involved in mediating the beneficial effects of exercise. Two recent studies have emphasized the role for muscle-crosstalk in regulation of our health. One study found that interleukin-6, which is released from skeletal muscle during exercise, stimulated lipolysis and thereby reduced visceral adipose tissue mass following exercise training in humans [38••]. Likewise, secreted from muscle [44], the protein irisin was found to play an essential role in exercise-mediated befits on Alzheimer's disease [37•]. Importantly, a recent study found that selected known and putative myokines were equally regulated by acute exercise in patients with T2D and weight-matched controls further highlighting the potential for exercise in combatting metabolic diseases [45•]. During the past 10 years, the list of exercise-secreted factors has expanded, and many more are likely to be discovered with recent technological advances. Future research should aim at elucidating their functions and potential to treat diseases that are known to be preventable by regular exercise.

Phase 2: the effects following an acute exercise bout

One bout of exercise not only has major acute metabolic impact, it also elicits tremendous metabolic changes in the hours and even days that follow. As depicted in Figure 1, this second phase of the acute exercise response involves a transient increase in muscle insulin sensitivity for up to 48 hours after exercise in healthy volunteers [46–48] and for $\geq$15 hours in patients with T2D [49]. Increased insulin sensitivity by the exercised muscles can improve whole body post exercise insulin-stimulated glucose disposal [47–49], although not all studies find this insulin sensitizing effect on a whole-body level in already healthy subjects [50,51]. Insulin resistance is a risk factor for many non-communicable diseases including T2D. Therefore, this second phase response to an acute exercise bout, where muscle insulin sensitivity is enhanced [46–48,50–52] is an important step in preventing and treating whole-body insulin resistance.

Molecularly, the occurrence of improved insulin sensitivity is somewhat surprising because insulin and exercise activate distinct proximal signaling pathways. Yet they do converge at several distal signaling proteins such as Rac1 [53] and TBC1D4 [54]. While Rac1 does not seem to play a role in post-exercise enhanced insulin sensitivity [55], AMPKs kinase activity is necessary to increase insulin sensitivity in mice in the hours following muscle contraction [56••]. During exercise, TBC1D4 is activated downstream of AMPK [54], and recent human evidence showed that increased TBC1D4 phosphorylation together with microvascular perfusion participate in increased insulin sensitivity in muscle after exercise [57••,58]. While exercise may be used to increase insulin action in states of insulin resistance [49], the physiological role of increased insulin sensitivity of muscle following exercise is to direct glucose uptake to replenish muscle glycogen stores in the muscles that were active during exercise. Hence, the insulin-sensitizing effect of exercise is primarily a local effect restricted to the muscles that were active during exercise [59].

Thus, one single bout of exercise has major health bearing effects regardless of training status, age, body weight, and insulin sensitivity. It is thus never too late to begin exercising and pharmaceutical strategies that target these

transient exercise-induced cellular mechanisms could be efficient in promoting heath throughout the lifespan.

Exercise training elicits adaptations that improve energy turnover and stress handling by muscle

The long-term adaptations following chronic exercise training improves the muscle glucose handling machinery. High GLUT4 and hexokinase II [60,61] protein content increase the capacity for the muscle to take up glucose, while increased capillarization [62,63] ensures improved delivery of nutrients to the muscle. Interestingly, chronic exercise training actually diminished the transient insulin sensitizing effect of an acute exercise bout due to enhanced chronic insulin sensitivity in the trained leg [64•] as depicted in Figure 1. In insulin resistant T2D subjects, as little as two weeks of high intensity interval training markedly increased insulin sensitivity in the trained muscle [65], showing the remarkable adaptability of these exercise-mediated processes.

Recently, the Hesselink group found that athletes stored intra-muscular lipids in small intramyofibrillar droplets, while diabetics had much larger subsarcolemmal droplets [66]. This implies that athletes store lipids more 'safely' and the intramyofibrillar localization allows for quick access to fuel without lipotoxiticy. Likewise, altered spatial distribution was reported for glycogen with training [67] and together with increased mitochondria function [68,69•] this likely improves muscles substrate utilization capacity during exercise. Holloszy's seminal work [68] showed that the twofold increase in respiration brought about by training was greater than the 60–80% increase in mitochondrial content as recently reviewed elsewhere [70].

Recently, omics tools have markedly increased our information on how muscles adapt to training on a global scale. Here, chronic endurance training upregulates the tricarboxylic acid cycle and oxidative phosphorylation system thus increasing the muscles ability to efficiently increase energy expenditure [71]. Knowing that a fat rich diet induces somewhat similar adaptations as exercise training in terms of improving fatty acid metabolism, we performed proteomic analysis on muscles following an exercise training period in mice fed a normal or fat rich diet. Given that exercise training increased insulin sensitivity in both diet groups, we surmised that the proteins similarly regulated by exercise in both diet groups would have a particularly health-related bearing on skeletal muscle metabolism [72••]. This work and recent investigations by others [73,74] have significantly improved our knowledge of which metabolic adaptations occur in muscle with training and identified novel targets. Recently, an online search tool, geneXX, was released where the authors reanalyzed available transcriptomic exercise data sets stratified by exercise type, training status and sex of the participants and time point after the exercise bout [75•]. This tool provides immediate information regarding the response of a transcript of interest to exercise in skeletal muscle and is useful to formulate novel hypotheses on the complex effects of exercise. However, proteomics leaves out the important aspect of posttranslational modifications. Moving forward, hypothesis-driven research and/or larger scale unbiased phenotyping investigations should focus on determining the functional role (s) of novel exercise-regulated proteins identified in such omics studies as well as their posttranslational regulation.

Perhaps unfortunately, the training-induced molecular adaptations and the increase in insulin sensitivity seems relatively short lived [76–78]. Even in healthy, active volunteers, an acute transition from high to low levels of daily physical activity for a three-days period impaired glycemic control [79]. However, epigenetic adaptations in muscle to exercise [80] may last longer. It has recently been found that the ability of muscle to store this epigenetic memory makes it able to 'remember' past resistance exercise (even a single bout) for up to five months [81•,82]. How this translates into metabolic effects or if such changes can be exploited pharmaceutically remains to be explored. Even more remarkably, maternal and paternal exercise or obesity during pregnancy can result in gamete alterations and those can affect the metabolic regulation of the offspring later in life [83,84,85•,86]. Thus, muscular epigenetic changes in response to exercise, as well as gamete alterations passed on by the parent to the offspring, show the relevance of epigenetic changes that we are only beginning to understand let alone pharmacologically apply.

Status on the exercise pill: can we harness the exercise benefits without actually exercising?

As depicted in Figure 1, exercise elicits acute beneficial effects as well as more sustained adaptations to chronic long-term training. Much of the health bearing effects of exercise training are the result of spending considerable amount of time on the acute exercise phases 1 and 2. However, it is becoming clear that there is a substantial individual variation in the metabolic benefits of exercise [87]. Currently large scale studies are attempting to delineate genetic and epigenetic traits that might be related to the individual variation in responses to exercise training in humans [88,89•]. Pharmacologically targeting the molecular processes that impair adaptations to exercise training might be useful for personalized medicine. In addition, pharmacologically activating the molecular pathways that lead to adaptations to training may provide a means to improve metabolic health in individuals who are unable to perform sufficient amounts of physical activity. Good examples of the latter are studies [34••,35••] showing metabolic benefits in mice and non-human primates by treatment with an AMPK activator. Sustained AMPK activation, however, may induce

cardiac hypertrophy and glycogen accumulation [35••,90] highlighting the complexity of activating AMPK [91].

Thus, imitating the exercise-induced intermittent activation of glucose uptake-stimulating proteins in muscle may be a safer and more attractive strategy. Yet, mimicking the effects of exercise in a pill would be an extremely difficult endeavor. Contemporary research is illuminating how extremely complex muscular signaling during exercise is. In a recent collaboration between Sydney and Copenhagen it was discovered that >1000 phosphorylation sites in human skeletal muscle were acutely regulated by exercise, only 10% with already known functions in exercise [92]. Clearly more molecular regulators of muscle glucose uptake are likely yet to be discovered and some of them may be potential targets for glucose lowering therapy independently of insulin. Thus far, the focus has been largely on approaches for increasing muscle glucose uptake to lower blood glucose. Yet, increasing glucose uptake in the absence of increased energy demand and/or glycogen storage capacity might prove problematic. One of the hallmarks of exercise is an increase in energy expenditure. Indeed, many exercise-regulated proteins control energy turnover and substrate storage, rather than glucose uptake regulation [72••,92] and those could be explored to improve energy utilization. In agreement with this, overexpression of skeletal muscle sarcolipin, a protein that decreases the efficiency of the SERCA pump and leads to increased muscle thermogenesis, protects against diet-induced obesity and glucose intolerance [93,94]. Likewise, if the brown adipose tissue protein, uncoupled protein 1 is expressed in muscle, mitochondrial uncoupling and increased muscle thermogenesis occurs. This protects against development of diabetes in diabetes-prone mice [95]. Thus, a good exercise pill also needs to increase energy expenditure. Perhaps this is the most important effect of exercise as it may lead to weight loss if not compensated by increased energy intake, and preventing or treating obesity is in itself a major health benefit (World health organization).

Concluding remarks

Despite great inter-individual variations in the health bearing effects of physical activity, on a global scale there is little doubt that Hippocrates was right that exercise would be " . . . the safest way to health" and contemporary research has proven that this holds true for a wide range of non-communicable diseases and throughout the entire life-span. The past few years have provided us with a much deeper and more comprehensive global understanding of the molecular network regulated by exercise. However, it has also revealed an underappreciated complexity of the molecular signaling activated in various physiological and pathological contexts. There is little doubt that exercise is a powerful means to prevent deterioration of glycemic control and to improve insulin action among both obese and T2D patients. Hopefully, we will soon be able to better understand this complexity to harness the benefits of exercise more efficiently.

Conflict of interest statement

Nothing declared.

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