Metabolic and molecular changes associated with the increased skeletal muscle insulin action 24 to 48 hours after exercise in young and old humans

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Abstract

The molecular and metabolic mechanisms underlying the increase in insulin sensitivity (i.e. increased insulin-stimulated skeletal muscle glucose uptake, phosphorylation and storage as glycogen) observed from 12 to 48 h following a single bout of exercise in humans remain unresolved. Moreover, whether these mechanisms differ with age is unclear. It is well established that a single bout of exercise increases the translocation of the glucose transporter, GLUT4, to the plasma membrane. Previous research using unilateral limb muscle contraction models in combination with hyperinsulinaemia has demonstrated that the increase in insulin sensitivity and glycogen synthesis 24 h after exercise is also associated with an increase in hexokinase (HKII) mRNA and protein content, suggesting an increase in the capacity of the muscle to phosphorylate glucose and divert it towards glycogen synthesis. Interestingly, this response is altered in older individuals for up to 48 h post exercise and is associated with molecular changes in skeletal muscle tissue that are indicative of reduced lipid oxidation, increased lipogenesis, increased inflammation, and a relative inflexibility of changes in Intramyocellular lipid (IMCL) content. Reduced insulin sensitivity (insulin resistance) is generally related to IMCL content, particularly in the subsarcolemmal (SSL) region, and both are associated with increasing age. Recent research has demonstrated that ageing *per se* appears to cause an exacerbated lipolytic response to exercise that may result in SSL IMCL accumulation. Further research is required to determine if increased IMCL content affects HKII expression in the days after exercise in older individuals, and the effect of this on skeletal muscle insulin action.

Introduction - Effect of acute exercise on insulin action in human skeletal muscle

Elevated pre-exercise muscle glycogen content is essential for optimal exercise performance (1), and nutritional strategies to increase muscle glycogen content are widespread. Administering carbohydrate immediately post-exercise, when muscle glycogen content is <150 mmol·(kg dm)$^{-1}$, has been reported to increase muscle glycogen synthesis, often supercompensating to far above basal levels, in a biphasic manner (2-7). During the initial period of recovery there is a rapid, insulin-independent, increase in muscle glycogen resynthesis lasting around 30 to 60 min, followed by a secondary, slower insulin-dependent phase, lasting several hours to days (6, 8). The rapid phase is most likely attributed to an exercise-induced increase in plasma membrane GLUT4 content (the protein responsible for the facilitated transport of glucose into skeletal muscle; 9, 10), and an increase in glycogen synthase (GS) activity induced by a low muscle glycogen concentration (which appears to be a far more potent regulator of glycogen synthase activity than insulin or muscle contraction; 2, 11-13). Insulin is thought not to play a significant role in this rapid phase, as post-exercise somatostatin infusion designed to suppress the endogenous secretion of insulin, has no effect glycogen synthesis during this period (6). On the other hand, the slow phase of glycogen synthesis is inhibited by somatostatin infusion (6) and, therefore, is most likely due to a marked increase in sensitivity of glucose uptake and glycogen synthesis to insulin (14).

This sustained increase in skeletal muscle insulin action following exercise is not only important for glycogen loading in athletes prior to competition or during intense periods of training, but also beneficial in the treatment of type 2 diabetes (T2D), as even a single bout of exercise can increase insulin sensitivity in insulin resistant individuals by reversing a defect in insulin stimulated glucose transport and phosphorylation (15). However, despite a plethora of studies in this area, the exact cellular mechanisms underlying the well documented increase in insulin stimulated skeletal muscle glucose uptake and glycogen synthesis observed up to 48 hours following a single bout of exercise (16, 17) remain unresolved. Surprisingly, little attention has been given to differences with age, despite the global prevalence of T2D being most apparent in older people (18), and the estimation in 2005 that the number of people over 65 years of age with diabetes will have increased 4.5 fold by 2050 (19).

The aim of this review is to focus on potential intracellular mechanisms leading to the increased skeletal muscle insulin sensitivity following an acute bout of exercise, and whether these differ with age. It is beyond the scope of this review to consider all intracellular mechanisms, and we refer the reader to several excellent reviews that detail molecular mechanisms in the first few hours after

exercise in both animal and human models (20, 21). We will also not focus on extracellular mechanisms such as the role of blood flow post-exercise as not much work has been performed in the area, although it is important to note recent reports suggesting a large contribution (around 50%) to improved insulin sensitivity from increased microvascular perfusion in the first few hours after exercise (22). Instead we focus on human studies that have investigated the later phase of enhanced insulin stimulated glucose uptake following exercise (>12 hours) and the premise that transcriptional events during this time period may contribute to the sustained increase in insulin-stimulated glucose transport and glycogen resynthesis.

Do changes in insulin signaling modulate improved post-exercise insulin action?

It appears that augmentation of the classical insulin signalling cascade may not be involved in the positive effect of exercise on post-exercise insulin action and glycogen synthesis, as many studies have demonstrated that a single bout of exercise does not increase IRS-1 tyrosine phosphorylation, IRS-1 associated PI3K activity, serine phosphorylation of Akt and glycogen synthase kinase 3 (GSK3) in response to insulin for up to one day after exercise (23-28). An overview of these molecular events can be found in several excellent reviews (e.g. 21) and is beyond the scope of the present review. However, it is worth mentioning that immediately after acute exercise, during the insulin independent phase of muscle glucose uptake and glycogen resynthesis, the phosphorylation (activation) of TBC1D4/AS160 (a downstream target of Akt) was increased in rat (29-31) and human muscle in the absence of insulin (22, 32). This suggests that TBC1D4/AS160 phosphorylation could play a potential role in increased insulin sensitivity when muscle is subsequently stimulated by insulin several hours later. Indeed, Treebak *et al*. (32) demonstrated the increased phosphorylation of TBC1D4/AS160 was associated with increase glucose uptake and glycogen resynthesis in response to insulin 4 h following a single bout of one-legged exercise compared to the non-exercised leg. Moreover, TBC1D4/AS160 was still phosphorylated 27 h after a bout of exercise in rats when insulin sensitivity remained enhanced (30), although the rats had been deprived of food for this time and had low muscle glycogen content, which may have also contributed to the improved insulin action (14).

Why is glucose uptake, phosphorylation, and storage enhanced by insulin post-exercise?

It is well established that a single bout of exercise increases the transcription (32) and plasma membrane translocation and content (33-35) of GLUT4. A single bout of exercise also increases skeletal muscle hexokinase II (HKII) activity, transcription and protein content for a number of hours after the end of exercise (28, 36-38). HKII is the predominant hexokinase isoform in skeletal muscle,

where it phosphorylates internalized glucose thus ensuring a concentration gradient across the plasma membrane and sustained glucose transport into muscle and substrate (glucose-6-phosphate; G6P) for glycogen synthesis or glycolysis. As mentioned above, low muscle glycogen content increases GS activity post exercise (22, 27), and may independently also contribute to increased glucose uptake as low muscle glycogen concentration has been observed in most studies in humans reporting enhanced insulin action following exercise (15, 27, 39). However, it is interesting to note that recent observations have demonstrated enhanced skeletal muscle insulin action the day following a single bout of exercise when muscle glycogen content had returned to pre-exercise levels. For example, a recent study by our group performed euglycaemic hyperinsulinaemic clamps 22 hours after 90 min of one-legged cycling exercise at 60% VO_2max in healthy human participants to ensure that muscle glycogen in the exercised leg had returned to a similar content of the non-exercised leg (28). A major strength of the one-legged exercise protocol is that it also ensures both limbs are exposed to the same circulating metabolic milieu and thus, any differences in the molecular adaptations observed between the exercised and non-exercised legs can be attributed to contraction *per se*. Skeletal muscle glycogen content measured biochemically in muscle biopsy samples was similar in the exercised and non-exercised legs before the clamp (471 ± 30 vs. 463 ± 50 mmol·(kg dm)$^{-1}$, respectively), but increased during the clamp in in the exercise leg, such that it was 17% greater than the non-exercised leg (527 ± 20 mmol·(kg dm)$^{-1}$ vs. 449 ± 35 mmol·(kg dm)$^{-1}$). This clearly demonstrated improved insulin action in the absence of glycogen depletion. Prior exercise was associated with increased basal HKII mRNA expression and protein content at 22 hours, but not GLUT4 mRNA, suggesting an increased capacity (through upregulation of HKII content) of muscle to phosphorylate and divert glucose towards glycogen storage is an important contribution to the insulin sensitive phase of skeletal muscle glycogen synthesis the day after a bout of exercise. In support of this finding, HKII, but not GLUT4, overexpression in mice increased insulin-stimulated whole body and skeletal muscle glucose uptake (40). However, it is important to note that inhibition of protein synthesis in rats using cyclohexamide for 3.5 h after a bout of exercise does not affect insulin-stimulated glucose uptake, suggesting that an increase in HKII protein content does not play a role in insulin action the immediate hours post-exercise (41).

Insight in to the role of HKII in skeletal muscle insulin sensitivity the days after a bout of exercise can also be gleaned from studies using the glucose analogue 3-O-methylglucose, which is taken up by muscle but not further metabolized and therefore independent of HKII, GS, and presumably glycogen content. For example, Cartee et al (14) demonstrated increased insulin-stimulated transport of 3-O-methylglucose 18 and 48 h after exercise in rat muscle in the glycogen depleted

state, but not after 18 h in the glycogen supercompensated state, despite the increased glycogen synthesis. This latter observation would suggest that glycogen supercompensation is not reflected by insulin stimulated 3-O-methylglucose uptake and is dependent on glucose metabolism, perhaps by HKII. Collectively, these studies suggest that increased insulin stimulated glucose uptake the day(s) after a single bout of exercise is mediated by enhanced capacity for glucose phosphorylation and storage, that is independent of the prevailing muscle glycogen content. Although this would suggest that muscle glycogen content *per se* is not important during the insulin-sensitive period post exercise, it does not rule out the possibility that glycogen depletion could trigger adaptations within the muscle that sustain the improvement in insulin sensitivity for several days. Indeed, glycogen depletion has been suggested to affect metabolic gene expression (42, 43) and activation of key insulin signalling proteins (44). Although the molecular mechanisms responsible for the upregulation of HKII content following an acute bout of exercise are unclear, possible candidates include the activation of transcription factors such as the peroxisome proliferator-activated receptor-γ (PPARγ) coactivator 1α (PGC1α (45), sterol regulatory binding protein 1c (SREBP1c) (46, 47) and peroxisome proliferator-activated receptor- α (PPARα) (48).

Is glucose diverted from oxidation to storage?

The pyruvate dehydrogenase complex (PDC) plays a central role in the oxidation and, therefore, fate of disposed muscle glucose. The PDC is covalently regulated by two competing enzymes, a Ca^{2+}-dependent phosphatase (PDP), which dephosphorylates the pyruvate dehydrogenase (PDH) component of the complex and transforms the enzyme to the active form, and a kinase (PDK), which catalyses the ATP-dependent phosphorylation of PDH and inactivates the enzyme complex (49). The magnitude of PDC activation (PDCa) is central to the control of acetyl-CoA delivery to the TCA cycle and, thus, carbohydrate oxidation in skeletal muscle. We have previously demonstrated that inhibition of PDC activation (via a high fat diet (50) or intravenous L-carnitine infusion (51)) under insulin stimulated conditions results in a diversion of disposed glucose from oxidation to storage, and is associated with a selective up-regulation of PDK4, but not PDK2, content (the two predominant PDH kinase isoforms in skeletal muscle; 50, 52). Interestingly, an increase in PDK4 mRNA expression has been observed in human skeletal muscle for up to 12 hours after prolonged exercise (although PDK4 protein content was not measured; 53-55). One potential explanation is that a post-exercise induced upregulation of PDK4 would inhibit PDC, which would facilitate a diversion of glucose uptake to storage under insulin-stimulated conditions and, thus, partly explain the routinely observed post-exercise increase in insulin stimulated glycogen synthesis. As the regulation of PDK4 expression occurs primarily at the level of transcription (56) and generally

changes in mRNA are fairly rapid and precede changes in its protein content by several hours (52, 53), it is likely that any functional impact of changes in PDK4 protein on muscle PDCa activity and insulin-stimulated glycogen storage would occur the day after an acute bout of exercise. We have tested this hypothesis using the one-legged exercise protocol described previously (28) and found that neither PDK4 protein content nor activation of the PDCa by insulin were affected by exercise performed the previous day, suggesting that the PDC may not play an important role in facilitating the post-exercise increase in insulin-stimulated glucose uptake and glycogen synthesis. Nevertheless, further research in this area is required.

Do these responses change as we age?

Despite a plethora of studies investigating mechanisms underpinning the increase in insulin stimulated skeletal muscle glucose uptake and glycogen synthesis observed up to 48 hours following a single bout of exercise, surprising little attention has been given to differences with age. Age *per se* does not appear to cause insulin resistance (57-59), but older individuals are more likely to be associated with insulin resistance due to increased abdominal adiposity and reduced physical activity (57, 58), along with declines in muscle mass (60, 61). Of note, intramyocellular lipid (IMCL) is often higher in older individuals, particularly in subsarcolemmal (SSL) regions (62, 63), and has been strongly associated with insulin resistance (64-67). It would appear that the SSL pool may play a role in buffering/trafficking of FFA influx (68), and its juxtaposition with the site of insulin signaling at the and glucose uptake at the sarcolemma would also support a role for perturbed SSL buffering/trafficking impairing insulin action. Although this is yet to be supported experimentally, an improvement in insulin sensitivity has been previously observed with reduced SSL but not intramyofibrillar (IMF) IMCL after 10–12 weeks of exercise training (64, 65), which may also provide insight to the athletes paradox where trained individuals have high IMCL content (69). We (63) and others (70) have recently demonstrated that age *per se* results in an excessive rate of appearance of plasma fatty acids during exercise. Whether this resulted in impaired insulin action post exercise was not investigated, but SSL IMCL accumulated during exercise in overweight insulin-resistant older individuals (63). Indeed, Pehmøller et al (71) provided mechanistic evidence, albeit in young individuals, that the insulin resistance associated with 7 hours of lipid infusion (to provide excessive fatty acids) in a non-exercised leg was not fully reversed by a single bout of prior exercise in the contralateral leg. Nevertheless, despite recent research also highlighting that the microvascular response to exercise may be blunted in older individuals (72), it remains unknown if post-exercise insulin action in human skeletal muscle is impaired by age *per se*.

Interestingly, it appears that the HKII transcriptional response is altered in older individuals for up to 48 hours post exercise. For example, we have recently demonstrated that HKII mRNA expression gradually increases at 12, 24, and 48 hours following 45 min of acute resistance type exercise in healthy young men (73). However, despite a rapid increase in expression after 12 hours, HKII mRNA was lower 24 and 48 hours after exercise in healthy older individuals, despite performing the same relative amount of work. In accordance with previous studies (62, 63, 74), the IMCL content was ~2-fold higher at rest (before exercise) in old vs. young but remained unchanged during 12 to 48 hours of the recovery period in the former group, indicating a relative inflexibility in its turnover that is often observed in insulin resistant individuals (75). In contrast, and in line with other studies (76-79) the IMCL content gradually increased during recovery in the young subjects such that the pre-exercise differences between groups were no longer present after 48 hours of post-exercise recovery. It is important to note that HKII protein content and/or activity were not measured in the study of Tsintzas et al (73), and further research is required to determine any role in post-exercise insulin action in older individuals. Furthermore, post-exercise insulin sensitivity was also not measured. However, the older individuals presented with a marked transcriptional response (highlighted in Figure 1) that was consistent with insulin resistance. For example, acute resistance exercise performed by healthy old individuals, when compared to young, led to molecular changes in skeletal muscle during the recovery period favouring reduced lipid oxidation (characterised by reduced LPL, ACAT1 and PPARa), increased lipogenesis (characterised by reduced ATGL and increased FAS and PPARg), and impaired insulin signalling (characterised by increased PI3KR1 and reduced Akt2). Moreover, these changes appeared to be preceded by an exaggerated inflammatory response at 12 hours (characterised by increased expression of COX2, IL6, IkBalpha and CREB1), that is often linked to impaired skeletal muscle lipid metabolism (80) and the development of insulin resistance (81).

Conclusion

The molecular and metabolic mechanisms underlying the increase in insulin sensitivity (i.e. increased insulin-stimulated skeletal muscle glucose uptake, phosphorylation and storage as glycogen) observed for up to several days following a single bout of exercise in humans are clearly multifactorial. It is well established that a single bout of exercise increases the translocation of the glucose transporter, GLUT4, to the plasma membrane. It would also appear that glycogen depletion and enhanced GS activity predominate in the first few insulin-insensitive hours post exercise, followed by activation of distal insulin signaling pathways, and subsequent molecular adaptations after ~24 hours to increase glucose phosphorylation via enhanced protein content of HKII. This latter

phase remains an underexplored area of research and, based on transcriptional events, appears be impaired in older individuals who present with insulin resistance and an inflexibility in IMCL turnover. It remains to be investigated to what extent regular resistance exercise can improve IMCL turnover in skeletal muscle from older individuals and modify this unfavourable molecular signature.

2451 words

References

1. Bergstrom J, Hermansen L, Hultman E, Saltin B. Diet, muscle glycogen and physical performance. *Acta Physiol Scand.* 1967;71: 140-50.

2. Bergstrom J, Hultman E. Muscle glycogen synthesis after exercise: an enhancing factor localized to the muscle cells in man. *Nature.* 1966;210: 309-310.

3. Maehlum S, Hostmark AT, Hermansen L. Synthesis of muscle glycogen during recovery after prolonged severe exercise in diabetic and non-diabetic subjects. *Scand J Clin Lab Invest.* 1977;37, 309-16.

4. Blom PC, Hostmark AT, Vaage O, Kardel KR, Maehlum S. Effect of different post-exercise sugar diets on the rate of muscle glycogen synthesis. *Med Sci Sports Exerc.* 1987;19: 491-6.

5. Ivy JL, Katz AL, Cutler CL, Sherman WM, Coyle EF. Muscle glycogen synthesis after exercise: effect of time of carbohydrate ingestion. *J Appl Physiol.* 1988;64: 1480-5.

6. Price TB, Rothman DL, Taylor R, Avison MJ, Shulman GI, Shulman RG. Human muscle glycogen resynthesis after exercise: insulin-dependent and -independent phases. *J Appl Physiol*. 1994;76: 104-11.

7. Piehl Aulin K, Soderlund K, Hultman E. Muscle glycogen resynthesis rate in humans after supplementation of drinks containing carbohydrates with low and high molecular masses. *Eur J Appl Physiol.* 2000;81: 346-51.

8. Ivy JL. Muscle glycogen synthesis before and after exercise. *Sports Med.* 1991;11: 6-19.

9. McCoy M, Proietto J, Hargreaves M. Skeletal muscle GLUT-4 and postexercise muscle glycogen storage in humans. *J Appl Physiol.* 1996;80: 411-5.

10. Richter EA, Wojtaszewski JF, Kristiansen S, Daugaard JR, Nielsen JN, Derave W, et al. Regulation of muscle glucose transport during exercise. *Int J Sport Nutr Exerc Metab.* 2001;11: S71-7.

11. Zachwieja JJ, Costill DL, Pascoe DD, Robergs RA, Fink WJ. Influence of muscle glycogen depletion on the rate of resynthesis. *Med Sci Sports Exerc.* 1991;23: 44-8.

12. Yan Z, Spencer MK, Katz A. Effect of low glycogen on glycogen synthase in human muscle during and after exercise. *Acta Physiol Scand.* 1992;145: 345-52.

13. Nielsen JN, Derave W, Kristiansen S, Ralston E, Ploug T, Richter EA. Glycogen synthase localization and activity in rat skeletal muscle is strongly dependent on glycogen content. *J Physiol.* 2001;531: 757-69.

14. Cartee GD, Young DA, Sleeper MD, Zierath J, Wallberg-Henriksson H, Holloszy JO. Prolonged increase in insulin-stimulated glucose transport in muscle after exercise. *Am J Physiol.* 1989;256: E494-9.

15. Perseghin G, Price TB, Petersen KF, Roden M, Cline GW, Gerow K, et al. Increased glucose transport-phosphorylation and muscle glycogen synthesis after exercise training in insulin-resistant subjects. *N Engl J Med.* 1996;335: 1357–1362,

16. Mikines KJ, Sonne B, Farrell PA, Tronier B, Galbo H. Effect of physical exercise on sensitivity and responsiveness to insulin in humans. *Am J Physiol Endocrinol Metab.* 1988;254: E248-E259.

17. Dela F, Mikines KJ, Sonne B, Galbo H. Effect of training on interaction between insulin and exercise in human muscle. *J Appl Physiol.* 1994;76: 2386-2393.

18. Wild SG, Roglic G, Green A, Sicree R. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care*. 2004;27: 1047-1053.

19. Narayan KM, Boyle JP, Geiss LS, Saaddine JB, Thompson TJ. Impact of recent increase in incidence on future diabetes burden: U.S., 2005-2050. *Diabetes Care*. 2006;29: 2114–2116.

20. Cartee GD. Mechanisms for greater insulin-stimulated glucose uptake in normal and insulin-resistant skeletal muscle after acute exercise. Am J Physiol Endocrinol Metab. 2015;309: E949-59

21. Maarbjerg SJ, Sylow L, Richter EA. Current understanding of increased insulin sensitivity after exercise - emerging candidates. *Acta Physiol (Oxf)*. 2011;202: 323-35.

22. Sjøberg KA, Frøsig C, Kjøbsted R, Sylow L, Kleinert M, Betik AC, Shaw CS, Kiens B, Wojtaszewski JFP, Rattigan S, Richter EA, McConell GK. Exercise Increases Human Skeletal Muscle Insulin Sensitivity via Coordinated Increases in Microvascular Perfusion and Molecular Signaling. *Diabetes*. 2017;66: 1501-1510.

23. Frosig C, Roepstorff C, Brandt N, Maarberg SJ, Birk JB, Wojtaszewski et al. Reduced malonyl-CoA content in recovery from exercise correlates with improved insulin-stimulated glucose uptake in human skeletal muscle. *Am J Physiol Endocrinol Metab*. 2009;296: E787-795.

24. Goodyear LJ, Giorgino F, Balon TW, Condorelli G, Smith RJ. Effects of contractile activity on tyrosine phosphoproteins and PI 3-kinase activity in rat skeletal muscle. *Am J Physiol*. 1995;268: E987-995.

25. Hansen PA, Nolte LA, Chen MM, Holloszy JO. Increased GLUT-4 translocation mediates enhanced insulin sensitivity of muscle glucose transport after exercise. *J Appl Physiol*. 1998;85: 1218-1222.

26. Wojtaszewski JF, Hansen BF, Gade, Kiens B, Markuns JF, Goodyear, et al. Insulin signaling and insulin sensitivity after exercise in human skeletal muscle. *Diabetes*. 2000;49: 325-331.

27. Wojtaszewski JF, Hansen BF, Kiens B, Richter EA. Insulin signaling in human skeletal muscle: time course and effect of exercise. *Diabetes*. 1997;46: 1775-1781.

28. Stephens FB, Norton L, Jewell K, Chokkalingam K, Parr T, Tsintzas K. Basal and insulin-stimulated pyruvate dehydrogenase complex activation, glycogen synthesis and metabolic gene expression in human skeletal muscle the day after a single bout of exercise. *Exp Physiol*. 2010;95: 808-818.

29. Castorena CM, Arias EB, Sharma N, Cartee GD. Postexercise improvement in insulin-stimulated glucose uptake occurs concomitant with greater AS160 phosphorylation in muscle from normal and insulin-resistant rats. *Diabetes.* 2014;63: 2297–2308.

30. Funai K, Schweitzer GG, Sharma N, Kanzaki M, Cartee GD. Increased AS160 phosphorylation, but not TBC1D1 phosphorylation, with increased postexercise insulin sensitivity in rat skeletal muscle. *Am J Physiol Endocrinol Metab*. 2009;297: E242–E251.

31. Schweitzer GG, Arias EB, Cartee GD. Sustained postexercise increases in AS160 Thr642 and Ser588 phosphorylation in skeletal muscle without sustained increases in kinase phosphorylation. *J Appl Physiol*. 2012;113: 1852–1861.

32. Treebak JT, Pehmoller C, Kristensen JM, Kjobsted R, Birk JB, Schjerling P, Richter EA, Goodyear LJ, Wojtaszewski JF. Acute exercise and physiological insulin induce distinct phosphorylation signatures on TBC1D1 and TBC1D4 proteins in human skeletal muscle. *J Physiol*. 2014;592: 351–375.

33. Kraniou GN, Cameron-Smith D, Hargreaves M. Acute exercise and GLUT4 expression in human skeletal muscle: influence of exercise intensity. *J Appl Physiol*. 2006;101: 934-937.

34. Ren JM, Semenkovich CF, Gulve EA, Gao J, Holloszy JO. Exercise induces rapid increases in GLUT4 expression, glucose transport capacity, and insulin-stimulated glycogen storage in muscle. *J Biol Chem*. 1994;269: 14396-14401.

35. Hansen PA, Wang W, Marshall BA, Holloszy JO, Mueckler M. Dissociation of GLUT4 translocation and insulin-stimulated glucose transport in transgenic mice overexpressing GLUT1 in skeletal muscle. *J Biol Chem*. 1998;273: 18173-18179.

36. O'Doherty RM, Bracy DP, Osawa H, Wasserman DH, Granner DK. Rat skeletal muscle hexokinase II mRNA and activity are increased by a single bout of acute exercise. *Am J Physiol.* 1994;266: E171-178.

37. Koval JA, DeFronzo RA, O'Doherty RM, Printz R, Ardehali H, Greanner DK, et al. Regulation of hexokinase II activity and expression in human muscle by moderate exercise. *Am J Physiol*. 1998;274: E304-308.

38. Pilegaard H, Osada T, Andersen LT, Helge JW, Saltin B, Neufer PD. Substrate availability and transcriptional regulation of metabolic genes in human skeletal muscle during recovery from exercise. *Metabolism*. 2005;54: 1048-1055.

39. Bogardus C, Thuillez P, Ravussin E, Vasquez B, Narimiga M, Azhar S. Effect of muscle glycogen depletion on in vivo insulin action in man. *J Clin Invest*. 1983;72: 1605–1610.

40. Fueger PT, Shearer J, Bracy DP, Posey KA, Pencek RR, McGuinness OP, Wasserman DH. Control of muscle glucose uptake: test of the rate-limiting step paradigm in conscious, unrestrained mice. *J Physiol*. 2005;562: 925-35.

41. Fisher JS, Gao J, Han DH, Holloszy JO, Nolte LA. Activation of AMP kinase enhances sensitivity of muscle glucose transport to insulin. *Am J Physiol Endocrinol Metab*. 2002;282: E18-23.

42. Pilegaard H, Keller C, Steensberg A, Helge JW, Pedersen BK, Saltin B, et al. Influence of pre-exercise muscle glycogen content on exercise-induced transcriptional regulation of metabolic genes. *J Physiol.* 2002;541: 261-271.

43. Garcia-Roves PM, Han DH, Song Z, Jones TE, Hucker KA, Holloszy JO. Prevention of glycogen supercompensation prolongs the increase in muscle GLUT4 after exercise. *Am J Physiol Endocrinol Metab.* 2003;285: E729-E736.

44. Creer A, Gallagher P, Slivka D, Jemiolo B, Fink W, Trappe S. Influence of muscle glycogen availability on ERK1/2 and Akt signaling after resistance exercise in human skeletal muscle. *J Appl Physiol*. 2005;99: 950-956.

45. Wende AR, Schaeffer PJ, Parker GJ, Zechner C, Han DH, Chen MM, et al. A role for the transcriptional coactivator PGC-1alpha in muscle refueling. *J Biol Chem*. 2007;282: 36642-36651.

46. Ikeda S, Miyazaki H, Nakatani T, Kai Y, Kamei Y, Miura S, et al. Up-regulation of SREBP-1c and lipogenic genes in skeletal muscles after exercise training. *Biochem Biophys Res Commun*. 2002;296: 395-400.

47. Boonsong T, Norton L, Chokkalingam K, Jewell K, Macdonald I, Bennett A, et al. Effect of exercise and insulin on SREBP-1c expression in human skeletal muscle: potential roles for the ERK1/2 and Akt signalling pathways. *Biochem Soc Trans*. 2007;35: 1310-1311.

48. Burkart EM, Sambandam N, Han X, Gross RW, Courtois M, Gierasch CM, et al. Nuclear receptors PPARbeta/delta and PPARalpha direct distinct metabolic regulatory programs in the mouse heart. *J Clin Invest*. 2007;117: 3930-3939.

49. Wieland OH. The mammalian pyruvate dehydrogenase complex: structure and regulation. *Rev Physiol Biochem Pharmacol.* 1983;96: 123–170.

50. Chokkalingam K, Jewell K, Norton L, Littlewood J, van Loon LJ, Mansell P, et al. High-fat/low-carbohydrate diet reduces insulin-stimulated carbohydrate oxidation but stimulates nonoxidative glucose disposal in humans: An important role for skeletal muscle pyruvate dehydrogenase kinase 4. *J Clin Endocrinol Metab.* 2007;92: 284–292.

51. Stephens FB, Constantin-Teodosiu D, Laithwaite D, Simpson EJ, Greenhaff PL. An acute increase in skeletal muscle carnitine content alters fuel metabolism in resting human skeletal muscle. *J Clin Endocrinol Metab.* 2006;91: 5013–5018.

52. Tsintzas K, Jewell K, Kamran M, Laithwaite D, Boonsong T, Littlewood J, et al. Differential regulation of metabolic genes in skeletal muscle during starvation and refeeding in humans. *J Physiol.* 2006;575: 291–303.

53. Pilegaard H, Ordway GA, Saltin B, Neufer PD. Transcriptional regulation of gene expression in human skeletal muscle during recovery from exercise. *Am J Physiol Endocrinol Metab.* 2000;279: E806-E814.

54. Pilegaard H, Osada T, Andersen LT, Helge JW, Saltin B, Neufer PD. Substrate availability and transcriptional regulation of metabolic genes in human skeletal muscle during recovery from exercise. *Metabolism.* 2005;54: 1048-1055.

55. Coffey VG, Shield A, Canny BJ, Carey KA, Cameron-Smith D, Hawley JA. Interaction of contractile activity and training history on mRNA abundance in skeletal muscle from trained athletes. *Am J Physiol Endocrinol Metab.* 2006;290: E849-E855.

56. Pilegaard H, Neufer PD. Transcriptional regulation of pyruvate dehydrogenase kinase 4 in skeletal muscle during and after exercise. *Proc Nutr Soc.* 2004;63: 221-226.

57. Karakelides H, Irving BA, Short KR, O'Brien P, Nair KS. Age, obesity, and sex effects on insulin sensitivity and skeletal muscle mitochondrial function. *Diabetes.* 2010;59: 89-97.

58. Amati F, Dubé JJ, Coen PM, Stefanovic-Racic M, Toledo FG, Goodpaster BH. Physical inactivity and obesity underlie the insulin resistance of aging. *Diabetes Care*. 2009;32: 1547–1549.

59. Basu R, Breda E, Oberg AL, Powell CC, Dalla Man C, Basu A, et al. Mechanisms of the age-associated deterioration in glucose tolerance: contribution of alterations in insulin secretion, action, and clearance. *Diabetes*. 2003;52: 1738–1748.

60. Park SW, Goodpaster BH, Strotmeyer ES, Kuller LH, Broudeau R, Kammerer C, et al. Accelerated loss of skeletal muscle strength in older adults with type 2 diabetes: The Health, Aging, and Body Composition Study. *Diabetes Care*. 2007;30: 1507–1512.

61. Leenders M, Verdijk LB, van der Hoeven L, Adam JJ, van Kranenburg J, Nilwik R, et al. Patients with type 2 diabetes show a greater decline in muscle mass, muscle strength, and functional capacity with aging. *J Am Med Dir Assoc*. 2013;14: 585-592.

62. Crane JD, Devries MC, Safdar A, Hamadeh MJ, Tarnopolsky MA. The effect of aging on human skeletal muscle mitochondrial and intramyocellular lipid ultrastructure. *J Gerontol A Biol Sci Med Sci*. 2010;65: 119-128.

63. Chee C, Shannon CE, Burns A, Selby AL, Wilkinson D, Smith K, et al. Relative Contribution of Intramyocellular Lipid to Whole-Body Fat Oxidation Is Reduced With Age but Subsarcolemmal Lipid Accumulation and Insulin Resistance Are Only Associated With Overweight Individuals. *Diabetes.* 2016;65: 840-50.

64. Nielsen J, Mogensen M, Vind BF, Sahlin K, Hojlund K, Schroder HD. Increased subsarcolemmal lipids in type 2 diabetes: effect of training on localisation of lipids, mitochondria, and glycogen in sedentary human skeletal muscle. *Am J Physiol Endocrinol Metab.* 2010;298: E706-E713.

65. Li Y, Lee S, Langleite T, Norheim F, Pourteymour S, Jensen J, et al. Subsarcolemmal lipid droplet responses to a combined endurance and strength exercise intervention. *Physiol Rep*. 2014;2: e12187.

66. Pan DA, Lillioja S, Kriketos AD, Milner MR, Baur LA, Bogardus C. Skeletal muscle triglyceride levels are inversely related to insulin action. *Diabetes.* 1997;46: 983-988.

67. Perseghin G, Scifo P, De CF, Pagliato E, Battezzati A, Arcelloni C. Intramyocellular triglyceride content is a determinant of in vivo insulin resistance in humans: a 1H-13C nuclear magnetic resonance spectroscopy assessment in offspring of type 2 diabetic parents. *Diabetes.* 1999;48: 1600-1606.

68. Kanaley JA, Shadid S, Sheehan MT, Guo Z, Jensen MD. Relationship between plasma free fatty acid, intramyocellular triglycerides and long-chain acylcarnitines in resting humans. *J Physiol* 2009;587: 5939–5950

69. Goodpaster BH, He J, Watkins S, Kelley DE. Skeletal muscle lipid content and insulin resistance: evidence for a paradox in endurance trained athletes. *J Clin Endocrinol Metab*. 2001;86: 5755-61.

70. Boon H, Jonkers RA, Koopman R, Blaak EE, Saris WH, Wagenmakers AJ, van Loon LJC. Substrate source use in older, trained males after decades of endurance training. *Med Sci Sports Exerc.* 2007;39: 2160-2170.

71. Pehmøller C, Brandt N, Birk JB, Høeg LD, Sjøberg KA, Goodyear LJ, et al. Exercise alleviates lipid-induced insulin resistance in human skeletal muscle-signaling interaction at the level of TBC1 domain family member 4. *Diabetes*. 2012;61: 2743–2752

72. Hildebrandt W, Schwarzbach H, Pardun A, Hannemann L, Bogs B, König AM, Mahnken AH, Hildebrandt O, Koehler U, Kinscherf R. Age-related differences in skeletal muscle microvascular response to exercise as detected by contrast-enhanced ultrasound (CEUS). *PLoS One*. 2017;12: e0172771.

73. Tsintzas K, Stephens FB, Snijders T, Wall BT, Cooper S, Mallinson J, et al. Intramyocellular lipid content and lipogenic gene expression responses following a single bout of resistance type exercise differ between young and older men. *Exp Gerontol*. 2017;93: 36-45.

74. Cree MG, Newcomer BR, Katsanos CS, Sheffield-Moore M, Chinkes D, Aarsland A. Intramuscular and liver triglycerides are increased in the elderly. *J Clin Endocrinol Metab.* 2010;89: 3864-71.

75. Bergman BC, Perreault L, Hunerdosse DM, Koehler MC, Samek AM, Eckel RH. Increased intramuscular lipid synthesis and low saturation relate to insulin sensitivity in endurance-trained athletes. *J Appl Physiol (1985).* 2010;108: 1134-41.

76. Koopman R, Manders RJ, Jonkers RA, Hul GB, Kuipers H, van Loon LJ. Intramyocellular lipid and glycogen content are reduced following resistance exercise in untrained healthy males. *Eur J Appl Physiol.* 2006;96: 525-34.

77. van Loon LJ, Schrauwen-Hinderling VB, Koopman R, Wagenmakers AJ, Hesselink MK, Schaart G, et al. Influence of prolonged endurance cycling and recovery diet on intramuscular triglyceride content in trained males. *Am J Physiol Endocrinol Metab.* 2003;285: E804-11.

78. Decombaz J, Schmitt B, Ith M, Decarli B, Diem P, Kreis R, et al. Postexercise fat intake repletes intramyocellular lipids but no faster in trained than in sedentary subjects. *Am J Physiol Regul Integr Comp Physiol.* 2001;281: R760-9.

79. Schenk S, Horowitz JF. Acute exercise increases triglyceride synthesis in skeletal muscle and prevents fatty acid-induced insulin resistance. *J Clin Invest.* 2007;117: 1690-8.

80. Glass CK. Olefsky JM. Inflammation and lipid signaling in the etiology of insulin resistance. *Cell Metab.* 2012;15: 635-45.

81. Boden G. Fatty acid-induced inflammation and insulin resistance in skeletal muscle and liver. *Curr Diab Rep.* 2006;6: 177-81.

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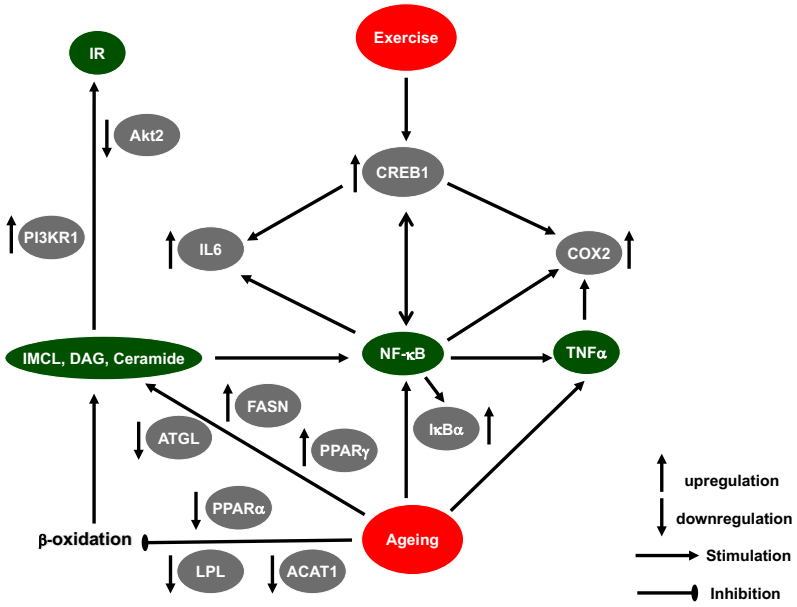

Figure 1. This figure shows the interaction between exercise and ageing (shown in red) and their effects on biological processes [such as intramyocelular lipid (IMCL) accumulation, Insulin Resistance (IR) and proinflammatatory response] often linked to ageing (depicted in green). Genes with significant differential changes in their expression in response to acute resistance exercise in old vs.young subjects are shown in grey.

IR=Insulin Resistance. IMCL=Intramyocellular lipid. DAG=diacylglycerol. ACAT1=Acetyl-CoA acetyltransferase1. FASN= Fatty Acid Synthase. NF-kB = Nuclear Factor kappaB. TNFa = Tumour Necrosis Factor alpha. CREB1=Cyclic AMP responsive element binding protein. IL6=Interleukin 6. COX2=Cyclooxygenase 2. PI3KR1= Phosphatidylinositol 3-kinase, regulatory 1 (p85 alpha). LPL= Lipoprotein lipase. ATGL=Adipose Triglyceride Lipase. PPARa=Peroxisome Proliferator Activated Receptor alpha. PPARg= Peroxisome Proliferator Activated Receptor gamma. IkBalpha=Inhibitor of kappaB kinase alpha. Akt2=Protein kinase B/Akt, isoform 2.