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Exosomes as Mediators of the Systemic Adaptations to Endurance Exercise

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Habitual endurance exercise training is associated with multisystemic metabolic adaptations that lower the risk of inactivity-associated disorders such as obesity and type 2 diabetes mellitus (T2DM). Identification of complex systemic signaling networks responsible for these benefits are of great interest because of their therapeutic potential in metabolic diseases; however, specific signals that modulate the multisystemic benefits of exercise in multiple tissues and organs are only recently being discovered. Accumulated evidence suggests that muscle and other tissues have an endocrine function and release peptides and nucleic acids into the circulation in response to acute endurance exercise to mediate the multisystemic adaptations. Factors released from skeletal muscle have been termed myokines and we propose that the total of all factors released in response to endurance exercise (including peptides, nucleic acids, and metabolites) be termed, "exerkines." We propose that many of the exerkines are released within extracellular vesicles called exosomes, which regulate peripheral organ cross talk. Exosomes (30–140 nm) and larger microvesicles [MVs] (100–1000 nm) are subcategories of extracellular vesicles that are released into the circulation. Exosomes contain peptides and several nucleic acids (microRNA [miRNA], messenger RNA [mRNA], mitochondrial DNA [mtDNA]) and are involved in intercellular/tissue exchange of their contents. An acute bout of endurance exercise increases circulating exosomes that are hypothesized to mediate organ cross talk to promote systemic adaptation to endurance exercise. Further support for the role of exosomes (and possibly MVs) in mediating the systemic benefits of exercise comes from the fact that the majority of the previously reported myokines/exerkines are found in extracellular vesicles databases (Vesiclepedia and ExoCarta). We propose that exosomes isolated from athletes following exercise or exosomes bioengineered to incorporate one or many of known exerkines will be therapeutically useful in the treatment of obesity, T2DM, and other aging-associated metabolic disorders.

Habitual physical activity (exercise) is a fundamental component of human health. There is strong evidence that regular physical activity reduces the risk of chronic diseases and physical disability in later life (health span), and even extends life span (Chakravarty et al. 2008; Balbuena and Casson 2009; Brenner 2009; Bronas 2009; Buchner 2009; Di Francescomarino et al. 2009; Kruk 2009; Nader and Lundberg 2009; Parsons and King-Vanvlack

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2009; Pedersen 2009a; Stuart et al. 2009; Bohm et al. 2010; Booth and Laye 2010; Herring et al. 2010; Kosmadakis et al. 2010; Warburton et al. 2010; Woodcock et al. 2011). In contrast, physical inactivity is a major threat to public health by increasing the risk of chronic metabolic diseases and their resultant impact on health-care budgets (Katzmarzyk et al. 2000; Katzmarzyk and Janssen 2004; Warburton et al. 2010). Importantly, physical activity is a modifiable risk factor for metabolic diseases (type 2 diabetes mellitus [T2DM] and obesity) and other chronic diseases, including cardiovascular disease, cancer, osteoporosis, osteoarthritis, and neuromuscular disorders (Balbuena and Casson 2009; Brenner 2009; Buchner 2009; Di Francescomarino et al. 2009; Kruk 2009; Nader and Lundberg 2009; Parsons and King-Vanvlack 2009; Stuart et al. 2009; ten Hacken 2009; Bohm et al. 2010; Booth and Laye 2010). The importance of developing effective countermeasures for the common metabolic diseases (T2DM and obesity) is evident in that an estimated 38 million lives are lost yearly from these disorders (T2DM and obesity) and nearly half of these occurred in those under 70 years of age (Mendis et al. 2015). Consequently, the World Health Organization has mandated that improving physical activity within the population is important to lower the incidence of common metabolic diseases (Mendis et al. 2015).

Most of the evidence for the benefits of exercise on the risk for metabolic disease has come from studies retrospectively evaluating the effects of habitual endurance exercise or prospective studies with an endurance exercise intervention. Retrospective epidemiological studies have shown that the incidence of T2DM is lower in those with higher levels of physical activity (Helmrich et al. 1991). This relationship is dose-dependent with the most active men and women showing a 35%–55% reduction in the incidence of T2DM and cardiovascular disease (American Association of Diabetes Education 2012). Prospective lifestyle interventions that include endurance exercise have been shown to improve glycemic control and insulin sensitivity in patients with both T2DM and dysglycemic obesity (Avery et al. 2012). A large prospective randomized controlled trial (Diabetes Prevention Program) found that a lifestyle intervention with modest nutritional modification and 150 min/wk moderate-intensity endurance exercise was more effective than the drug metformin at preventing T2DM development in those with dysglycemia over an ~3 year intervention period (Knowler et al 2002). Importantly, these lifestyle modification benefits were still evident 10 years after the original intervention (Diabetes Prevention Program Research Group et al. 2009). Similar intensive lifestyle interventions that include endurance exercise (Look AHEAD trial) have also been shown to improve quality of life (Zhang et al. 2016) and lower the risk of depression (Rubin et al. 2014) and kidney disease (Look AHEAD Research Group 2014). Somewhat surprisingly, the latter study did not show a positive effect on cardiovascular outcomes (Look AHEAD Research Group et al. 2013); however, that may be because of the high burden of cardiovascular disease at baseline and the fact that the patients already had T2DM and not just dysglycemia (Wing et al. 2013). Nevertheless, the economic benefits of the exercise-focused lifestyle intervention led to a health-care cost savings of $5280/person/10 years (Espeland et al. 2014).

Collectively, the human epidemiology data presented above does show that long-term habitual endurance exercise is associated with a reduction in all-cause mortality and that the derived benefits are not just in tissues and organs that would be traditionally associated with exercise benefits (muscle, heart, metabolic disease-associated tissues), but also extend to other tissues/organs less often associated with exercise benefits such as brain, breast, and colon cancer risk, kidney, and even eye health (Zheng Selin et al. 2015). The multisystemic benefits of long-term endurance exercise were apparent in a prospective cohort study that followed health outcomes in runners and age-matched sedentary controls over a 21-year period (Chakravarty et al. 2008). The latter study reported lower disability scores and all-cause mortality in the runners with fewer deaths because of cancer, infections, stroke, and coronary artery disease (Chakravarty et al. 2008). The benefits of run-

ning on health appear to be long-lasting in that many of the benefits were apparent in the subgroup of runners who had run for as short as 1 month and stopped (Chakravarty et al. 2008). In addition to the epidemiological evidence for multisystemic benefits of running/endurance exercise, there is also teleological evidence that running was likely a factor in the departure of *Homo sapiens* from other primates (Bramble and Lieberman 2004).

It is currently not clear how exercise can confer such multisystemic benefits but it is likely that there are many overlapping mechanisms. For running/endurance exercise to allow for successful adaptation and evolutionary selection, it is likely that exerkines coevolved endocrine-like signaling pathways such that multiple tissues derived benefits from exercise and not just skeletal muscle per se. The concept of exercise and, in particular, muscle having an endocrine-like effect on distant tissues was first proposed by Bente Petersen and colleagues whereby the term "myokine" was used to denote such substances derived from skeletal muscle (Pedersen and Fischer 2007a; Pedersen and Febbraio 2012). The myokine concept has survived the test of time and there are currently many myokines that have since been discovered (Pedersen and Hojman 2012; Seldin et al. 2012; Lee et al. 2014; Rao et al. 2014; Crane et al. 2015; Covington et al. 2016; Neidert et al. 2016). Given the fact that there are clearly peptides that are released from nonmuscle tissue in response to acute exercise or exercise training (Hansen et al. 2015; Stanford et al. 2015), we have coined the term "exerkine" as a more general term referring to humoral factors (peptides, metabolites [Pedersen and Febbraio 2012], and RNA [microRNA, miRNA; messenger RNA, mRNA]) that are produced and secreted into circulation by any tissue/organ in response to exercise.

Given the evolutionary importance of both intercellular communication and physical activity to many aspects of human health, the objective of the current review is to describe how these processes are linked and why they are important in mediating the multisystemic benefits of endurance exercise. The main hypothesis put forward is that myokines/exerkines contained within extracellular vesicles (EVs) and in particular exosomes are important mediators of the intercellular communication process occurring in response to exercise in humans.

MYOKINES/EXERKINES AS ENDOCRINE MEDIATORS OF THE SYSTEMIC EFFECTS OF EXERCISE

The first published use of the term, "myokine" for a specific peptide appears from the seminal work of Bente Pedersen in which she proposed that, "... IL-6 and other cytokines, which are produced and released by skeletal muscles, exerting their effects in other organs of the body, should be named 'myokines'" (Pedersen et al. 2003). The term "myokine" also includes other molecules released by skeletal muscle in association with exercise, including miRNA, mRNA, long noncoding RNA (lncRNA), and even metabolites, which exert auto-, para-, or endocrine effects (Little et al. 2011a; Pedersen and Febbraio 2012). Interleukin (IL)-6 was the first of the discovered myokines (Pedersen et al. 2004), and remains the most studied of the myokines (Pedersen et al. 2004; Pedersen and Fischer 2007a,b; Pedersen 2007, 2009b). Acute endurance exercise induces skeletal muscle IL-6 release that has an autocrine/paracrine effect to increase glucose uptake and free fatty acid oxidation in skeletal muscle (Pedersen 2007; Pedersen and Fischer 2007a,b). IL-6 functions also in an endocrine manner by stimulating adipocyte lipolysis and hepatic gluconeogenesis to enhance fuel availability for working muscle (Pedersen 2007; Pedersen and Fischer 2007a,b). Skeletal muscle–derived IL-6 also signals to the gut and pancreatic α cells to release glucagon-like polypeptide that then signals to the pancreatic β cells to induce insulin secretion (Wallenius et al. 2002).

Since the first discovery of IL-6 as a myokine, a number of other peptides have been discovered that are true myokines while others are produced and secreted by fat depots (adipokines), and regulate some adaptations to exercise (Pedersen 2009a,b, 2011; Pedersen and Febbraio 2012; Pedersen and Hojman 2012). Given the muscle, fat, liver (hepatokines), brain (neurokines), kidneys (nephrokines), and other

organs (Pedersen 2009a,b, 2011; Pedersen and Febbraio 2012; Pedersen and Hojman 2012), it is likely that many will be linked to beneficial multisystemic cellular adaptations in the future. For example, myonectin, brain-derived neurotrophic factor (BDNF), and leukemia inhibitory factor (LIF) concentration increase in blood following acute exercise and modulate aspects of free fatty acid metabolism (Florholmen et al. 2006; Matthews et al. 2009; Seldin et al. 2012, 2013). Vascular endothelial growth factor A (VEGFA) is an exercise-induced myokine that functions in a paracrine manner to promote angiogenesis (Pedersen and Febbraio 2012). As predicted, BDNF is likely involved as a systemic myokine promoting neurogenesis in response to endurance exercise (Pedersen and Febbraio 2012). Cross talk between skeletal muscle and bone is likely mediated by the myokines insulin-like growth factor 1 (IGF-1) and fibroblast growth factor (FGF)-2 (Pedersen and Febbraio 2012; Lee et al. 2014).

The other class of molecule that has been best characterized as a myokine/exerkine are the miRNAs. miRNAs are short (~22 nucleotides), nonpeptide-encoding RNA molecules that regulate posttranscriptional gene expression in many physiological processes, including embryonic stem cell development, oncogenesis, myogenesis, and substrate metabolism (Barreiro and Sznajder 2013; Fernandez-Hernando and Baldan 2013; Kuppusamy et al. 2013; Tani et al. 2013; Trounson 2013; Adlakha and Saini 2014; Chen and Verfaillie 2014). It has been estimated that miRNAs regulate up to one-third of the mammalian genome, further supporting a major role in regulating gene expression (Romao et al. 2014). The multiplier effect of miRNAs on biological processes comes from the fact that a single miRNA can target hundreds of mRNAs, and individual mRNAs can be targeted by many different miRNAs, thus providing a powerful, complex, and flexible regulatory potential (Romao et al. 2014). miRNAs are known to be involved in many aspects of the adaptive response to exercise, including mitochondrial biogenesis, myocardial remodeling, skeletal muscle angiogenesis and hypertrophy, contractile force generation, and substrate metabolism (Safdar et al. 2009; Aoi et al. 2010; Nielsen et al. 2010, 2014a; Hoppeler et al. 2011; Timmons 2011; Russell et al. 2013; Mooren et al. 2014; Ooi et al. 2014).

Although initially thought to only act within the cell of production, it is now known that miRNAs are found in plasma and have been called circulating miRNAs (c-miRNAs) (Mooren et al. 2014; Hubal et al. 2016). c-miRNAs can be produced in one cell type and be transported in a paracrine- or endocrine-like fashion to effect distal tissues and influence tissue function (Valadi et al. 2007; Hubal et al. 2016; Muroya et al. 2016). The plasma concentration of c-miRNAs goes up rapidly during exercise as does their intracellular expression (Safdar et al. 2009; Russell et al. 2013; Mooren et al. 2014; Nielsen et al. 2014b). c-miRNAs have been reported to modulate various aspects of cellular metabolism that are regulated by exercise (Mohan et al. 2015; Margolis et al. 2016), and hence we propose c-miRNAs can act as exerkines and play a role in multisystemic adaptive benefits of exercise.

Several papers have reviewed the potential for myokines to mitigate aspects of aging-associated metabolic disorders and cancer (Pedersen and Fischer 2007b; Demontis et al. 2013; Di Raimondo et al. 2016). The next section will review the evidence for exercise to lower the risk of diabetes and obesity, aging-associated pathology, and cancer and review the main myokines/exerkines that mediate the effects.

BIOLOGY AND EVOLUTION OF EXTRACELLULAR VESICLES

Eukaryotic cells can communicate through direct juxtacrine signaling or by the release of soluble factors into the interstitial fluid or blood as endocrine signaling. In addition to the endocrine signaling to distant tissues, soluble factors can act on the cell of production (autocrine signaling) or with adjacent cells (paracrine signaling). Although endocrine signaling is classically mediated by peptides and steroid hormones (Thery et al. 2002), a wide variety of other factors can function as myokine factors, including mRNA, cytokines, chemokines, and

miRNA. Peptides that are secreted into the extracellular space usually have a secretory signal sequence (one or more positively charged amino acids followed by a stretch of 6–12 hydrophobic residues) on the amino terminus that directs the growing polypeptide to the endoplasmic reticulum (ER) (Thery et al. 2002). In addition to this classical pathway, proteins that do not contain secretory sequences, pulsatile or stimulus-dependent peptides, and signaling molecules that may be labile within the extracellular environment, can be secreted in EVs called exosomes (Januszyk and Lima 2004; Hagiwara et al. 2014; Choi et al. 2015). EVs are traditionally characterized by size into (1) exosomes (20–140 nm), (2) microvesicles [MVs] (100–1000 nm), and (3) apoptotic bodies (500–5000 nm) (Fig. 1). A challenge to characterization by size alone is that the larger exosomes overlap with the smaller MVs. Consequently, biochemical characterization of the proteins and lipids in the vesicles can aid in their

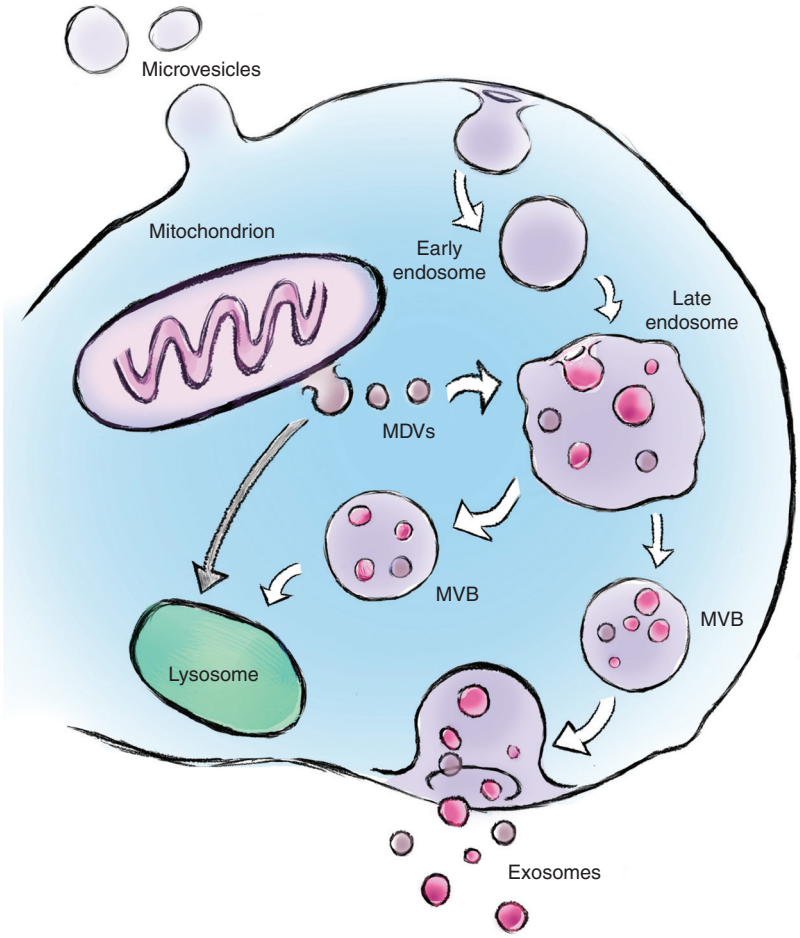

Figure 1. Biogenesis and secretion of extracellular vesicles. The maturation of early endosomes into late endosomes involves the formation of intraluminal vesicles derived from inward budding of the limiting membrane producing multivesicular bodies (MVBs). MVBs can translocate to the lysosome for degradation of vesicles and/or fuse with the plasma membrane to release exosomes (size 30–140 nm) into the extracellular milieu. Microvesicles (size 100–1000 nm) are formed by the outward budding of the plasma membrane. Mitochondria also release exosome-like vesicles called mitochondrial-derived vesicles (MDVs) (70–150 nm). MDVs can traffic to peroxisomes or to late endosomes/lysosomes.

classification. Exosomes are enriched in specific proteins, including biogenesis markers (ALIX, TSG101), integrins and tetraspanins (CD9, CD63, CD81, CD82), heat shock proteins (HSP60, HSP70, HSP90), and transport and fusion proteins (ESCRT proteins, LAMP1/2, flotillin, Rab GTPases, annexins [I, II, III, IV]) (Mathivanan et al. 2010). Exosomes also contain an enrichment of various lipid species, including lysobisphosphatidic acid (LBPA), sphingolipids, cholesterol, ceramides, and glycerophospholipids (Mathivanan et al. 2010). In contrast, MVs are enriched in integrins, selectins (CD62, P-, and E-selectin), CD40 ligand, and MHCI/II, and the lipid component is higher in phosphatidlyserine and does contain cholesterol. Given that apoptotic bodies are fragments of cells, they do contain fragmented DNA, mitochondrial proteins, and ER markers. Further complicating the definition of exosomes versus MVs is the fact that vesicles containing classical exosomal markers and in the size range of exosomes (50–100 nm) have been shown to bud from the plasma membrane (Booth et al. 2006). Exosomes and MVs also contain a large number of proteins and other molecules such as RNA species (miRNA, mRNA, lncRNA), DNA, and mitochondrial DNA (mtDNA). The proteomic profile of exosomes and MVs can be found in public databases, including Vesiclepedia (www.microvesicles.org), EVpedia (www.evpedia.info), and ExoCarta (www.exocarta.org). It is important to note that the challenges in separating the exosomes from the smaller MVs have led to some uncertainty as to the exact origin of the proteins found in such databases.

It is interesting that even down to prokaryotes there are methods of intercellular communication termed quorum sensing. Quorum sensing allows bacteria to coordinate their activities by sensing alterations in the environment and releasing proteins and lipids (including lipopolysaccharide [LPS]) that influence gene expression of adjacent bacteria (Deatherage and Cookson 2012). These intercellular communication molecules appear to be released within microbial membrane vesicles (MMVs) derived from the outer cell membrane. Gram-positive and Gram-negative bacteria release MMVs that range from 10 to 300 nm in diameter and contain LPS, peptides, lipoproteins, phospholipids, and toxins (McBroom et al. 2006; Lee et al. 2009; Kulp and Kuehn 2010; Rivera et al. 2010). The cell surface origin of the prokaryote MMVs is very similar to eukaryotic MVs as described below. Given that mitochondria have coevolved a symbiotic relationship with eukaryotic cells after their origins as purple photosynthetic bacteria (Gray 1989), it is not surprising that mitochondria retain a vesicle communication system named mitochondrial-derived vesicles (MDVs) (70–150 nm diameter) (Sugiura et al. 2014). MDVs that can shuttle to peroxisomes have been shown to transport cargo to peroxisomes (Andrade-Navarro et al. 2009; Soubannier et al. 2012) and MVBs/lysosomes (McLelland et al. 2014). The transport of MDVs from mitochondria to the lysosomes is activated by oxidative stress (Soubannier et al. 2012) before PINK/Parkin-dependent mitophagy (McLelland et al. 2014). It is speculative that the release of vesicles containing mtDNA from muscle and nerve for horizontal mtDNA transfer (Esquilin et al. 2012), could be derived from an MDV>endosome>exosome pathway. The latter would provide mechanistic support for the observed improvement in mtDNA mutational load in multiple tissues in the endurance-trained POLG1 mouse (Safdar et al. 2011a, 2015).

The initial concept of exosomes as mediators of biological processes in eukaryotic cells came from work on transferrin receptors and iron handing in reticulocytes published in 1983 by Clifford Harding and colleagues (Harding et al. 1983), and Rose Johnstone and colleagues (Pan and Johnstone 1983). Exosomes are secreted by many cell lines in culture and many cell types in vivo (Hwang 2013; Kalani et al. 2014; Record et al. 2014). Exosomes are primarily single membrane EVs, although rare double membrane exosomes are seen less commonly. Exosomes are formed by inward budding of the limiting membrane of late endosomes, leading to the formation of intraluminal vesicles within multivesicular bodies (MVBs) (Thery et al. 2002). MVBs can either fuse with lysosomes for

proteolytic degradation, or they can fuse with the plasma membrane and release exosomes into extracellular fluid/blood (Fig. 1) (Thery et al. 2002). The fate of MVBs within a cell and the released MVs and exosomes are likely determined by their cargo (peptides, RNA, and DNA) and composition (surface proteins and lipid composition) (Raposo and Stoorvogel 2013).

The formation of lysosomal and secreted exosomes with the MVB is partially dependent on a series of evolutionarily conserved endosomal sorting complexes required for transport (ESCRT) proteins (ESCRT-0, I, II, and III) (Raposo and Stoorvogel 2013; Urbanelli et al. 2013; Yáñez-Mó et al. 2015). These proteins bind to the outer (cytosolic) membrane of the endosome (cytosolic face), and are involved in exosomal formation through interactions forming an ALIX/TSG-101/ESCRT-I complex, which activates ESCRT-II oligomerization and formation of an ESCRT-III complex (Raposo and Stoorvogel 2013; Urbanelli et al. 2013; Yáñez-Mó et al. 2015). There are also ESCRT-independent MVB/exosome formation pathways (Stuffers et al. 2009; van Niel et al. 2011) that involve ceramide (Trajkovic et al. 2008). Sphingomyelinase is also involved in exosomal formation by liberating ceramide that helps with membrane invagination (Bianco et al. 2009). The fusion and release of exosomes to the extracellular fluid environment involves the soluble NSF attachment protein receptor (SNARE) pathway analogous to the pathways involved in neurotransmitter release and glucose transporter type 4 (GLUT-4) migration to the sarcolemma in response to exercise or insulin (Raposo and Stoorvogel 2013; Urbanelli et al. 2013; Yáñez-Mó et al. 2015). The membrane fusion between the MVBs and the plasma membrane is mediated by GTPases (Rab11, Rab27) that facilitate vesicular SNARE (on MVBs)/target SNARE (on plasma membrane) interaction (Raposo and Stoorvogel 2013; Urbanelli et al. 2013). The mechanism(s) triggering exosome release in response to acute exercise or exercise training in skeletal muscle and other tissues is not currently known; however, some of the classical pathways activated by acute exercise are likely important. A variety of stimuli including glutamate and K^+ activation of neurons, intracellular Ca^{2+} changes, ATP-mediated dendritic cell activation, and phosphatidic acid have all been shown to influence exosomal secretion/release (Skokos et al. 2003; Savina et al. 2005; Lachenal et al. 2011; Urbanelli et al. 2013). The Ca^{2+}-mediated exosomal release is of particular interest in skeletal muscle given that the major Ca^{2+} transients that occur in the cytosol with contraction are known to signal many important processes through calcineurin, calcium/calmodulin-dependent protein kinases (CaMKs), and p38MAP kinase (Hood 2001).

Once released, exosomes interact with and modulate the metabolic behavior of the target cells in a variety of ways, which is hypothesized to be dependent on the cell type and material cargoed within the exosomes. Exosomes can (1) activate downstream signaling in the target cell via interaction of exosomal surface protein with receptors on the plasma membrane of the target cell; (2) fuse with the plasma membrane of the target cells, thus delivering their cargo into the cytosol of the target cell; and (3) be phagocytosed or endocytosed by the target cells, which can then be delivered to a specific organelle in the target cells (Mulcahy et al. 2014). The exact mechanism(s) by which exosomes are targeted to specific tissues is unclear; however, recent evidence in tumor cells show that specific integrins on the exosome surface are important in tissue-homing specificity (Hoshino et al. 2015).

Exosome biology is an emerging discipline in the past decade with most research focusing on the role of exosomes in various pathologies and in disease biomarker discovery (Wolfers et al. 2001; André et al. 2002; Thery et al. 2002; Aucher et al. 2013; Pope and Lässer 2013; Ramakrishnaiah et al. 2013). In contrast, the potential role for exosomes in exercise physiology/medicine is embryonic in development with only 17 references appearing in a PubMed (www.ncbi.nlm.nih.gov/pubmed) search on August 19, 2016. Conceptually, however, the role of exsosomes as mediators of the multisystemic benefits of exercise is attractive given that exosomes are known to function in an intercellular communication "signalosome" manner (Thery et al. 2002). Exosomes influence self- (autocrine), adjacent (paracrine), or distant

(endocrine) cells through juxtacrine signaling, direct activation of cell-surface receptors with exosome protein ± lipid ligand interactions (receptor-mediated endocytosis), or by fusing the recipient cell plasma membrane (Thery et al. 2002). Exosomes are ubiquitously expressed and have been isolated from cell culture media, plasma, serum, saliva, amniotic fluid, breast milk, urine, ascites, and cerebrospinal fluid (Mathivanan and Simpson 2009; Urbanelli et al. 2013; Yáñez-Mó et al. 2015). There is currently no consensus on the "gold standard" for the isolation of exosomes or MVs; however, the most common methods use ultracentrifugation with and without a density gradient, polyethylene glycol, ultrafiltration, alone or a combination thereof (Baranyai et al. 2015; Witwer et al. 2015; Rider et al. 2016; Weng et al. 2016). Irrespective of the isolation method used, it is important for future studies to carefully characterize the EVs from any source and we recommend that the reader consider and follow the guidelines set forth by the International Society for Extracellular Vesicles regarding the minimal requirements for EV categorization (Lötvall et al. 2014).

PROMETABOLIC EXERKINES MITIGATE OBESITY AND TYPE 2 DIABETES

Studies in the last four decades have determined that development of obesity and T2DM are mechanistically linked with skeletal muscle mitochondrial dysfunction (Simoneau et al. 1995; Morino et al. 2005, 2006; Ritov et al. 2010). Mitochondrial dysfunction (including attenuated mitochondrial biogenesis, reduced mitochondrial content, and/or lower oxidative capacity) in skeletal muscle contributes to various aspects of metabolic syndrome, likely because of the pathological accumulation of intracellular lipids and lipid intermediates that inhibit insulin signaling (Lowell and Shulman 2005; Morino et al. 2006; Petersen and Shulman 2006). Endurance exercise is touted as the gold-standard therapy for obesity and T2DM, and one of the mechanisms by which exercise circumvents insulin resistance in obesity and T2DM is by increasing skeletal muscle mitochondrial biogenesis and an overall improvement in mitochondrial bioenergetic capacity (Hawley 2004; Morino et al. 2006; Joseph and Hood 2014). Additionally, endurance exercise training promotes insulin signaling that results in both a greater abundance in GLUT4 and modulation of intracellular localization of GLUT4 to efficiently clear excess glucose from circulation, which may positively affect whole-body glycemic control (Dela et al. 1994; Ren et al. 1994; Gulve and Spina 1995; Phillips et al. 1996; Greiwe et al. 2000). Recently, endurance exercise has been shown to ameliorate adiposity and insulin resistance via promoting skeletal muscle-immune system-adipose tissue cross talk that leads to an induction of a thermogenic gene network resulting in browning of white adipose tissue (WAT) (Langin 2010; Boström et al. 2012; Lo and Sun 2013). Browning of WAT in response to endurance training, characterized by the upregulation of the uncoupling protein 1 (UCP1) content in subcutaneous WAT (scWAT), renders WAT to be metabolically active in dissipating excess energy as heat and is intimately linked with improved metabolic status in high-fat-fed diet murine model of obesity and T2DM and is thus an attractive therapeutic target (Kajimura et al. 2015).

The collection of exerkines, including irisin, meteorin-like (METRNL), and FGF-21, have emerged as important regulators of adipose tissue browning released systemically by skeletal muscle (irisin and METRNL), adipose tissue (METRNL), liver (FGF-21), and cells of the immune system (METRNL) (Boström et al. 2012; Fisher et al. 2012; Lee et al. 2014; Rao et al. 2014). The discovery of these exerkines has garnered much support for that notion that exerkines promote organ cross talk and mediate the endocrine effects on peripheral tissues to mitigate various aspects of metabolic syndrome, including fatty liver, dysglycemia, insulin resistance, increased adiposity, and exercise intolerance (Boström et al. 2012; Fisher et al. 2012; Lee et al. 2014; Rao et al. 2014). Endurance exercise is also known to potentially affect pancreatic physiology, including increase in pancreatic β-cell proliferation through insulin/IGF-1-mediated signaling (Choi et al. 2006). Studies have

shown that circulatory levels of secreted peptide IGF-1 increase after both an acute bout of endurance exercise and exercise training in patients with T2DM and are directly related to an overall improvement in exercise-mediated metabolic homeostasis (Gregory et al. 2013; Mohajeri Tehrani et al. 2015). The hunt for exerkines like irisin, METRNL, FGF-21, IGF-1, and other humoral factors is concluded as instrumental in designing improved therapies for obesity, T2DM, and other aging-associated metabolic diseases and immediately necessitates future studies to unravel the identity of such exerkines.

In addition to peptides, altered levels of miRNA species both in tissues and circulation have been shown to play an important role in mediating metabolic diseases including obesity and T2DM (Sethupathy 2016). Impaired insulin signaling and dysglycemia in T2DM is linked with an impairment in the expression of the muscle-specific miRNAs (myo-miRs), miR-1 and miR133a (Granjon et al. 2009). miRNAs (miR-192, miR-193b) are reported to be higher in the plasma of prediabetic mice and humans (Párrizas et al. 2015.). miRNAs have also been implicated in the control of pancreatic β-cell function and fate (Filios and Shalev 2015). Incidentally, acute endurance exercise increases miR-1 and miR-133a in skeletal muscle, and miR-132 and miR-338-3p in the pancreas (Nielsen et al. 2010, 2014b; Radom-Aizik et al. 2012; Russell et al. 2013). Additionally, endurance exercise training normalized expression of miR-192 and miR-193b in prediabetic mice and humans (Párrizas et al. 2015). We and others have shown that an acute bout of endurance exercise influences the expression of miRNA species that are involved in regulating various aspects of skeletal muscle metabolism, including regulating peroxisome proliferator-activated receptor γ coactivator 1α and β (PGC-1α/β) expression, a transcription coactivator and master regulator of muscle mitochondrial biogenesis in response to endurance exercise (Pilegaard et al. 2003; Safdar et al. 2009, 2011b; McLean et al. 2015). PGC-1α and its responsive downstream mitochondrial genes network are significantly down-regulated in skeletal muscle of patients with T2DM (Mootha et al. 2003). Interestingly, muscle-specific overexpression of PGC-1α is associated with increased mitochondrial biogenesis, improvements in insulin sensitivity and glucose homeostasis, and browning of WAT (Puigserver et al. 1998; Lin et al. 2003), as well as improvements in vascular function in T2DM (Sawada et al. 2014). Together, the above data suggests that obesity and T2DM alter the expression of several species of muscle miRNA and c-miRNA. Since endurance exercise normalizes the altered expression of these miRNA species in some metabolic disorders, we propose that these miRNA species may act as prometabolic exerkines. Certainly, there is a crucial need for mechanistic (loss- and gain-of-function) studies to evaluate the role of exercise-responsive miRNA species in affecting overall cellular metabolism.

Chronic low-grade systemic inflammation is another of the hallmarks of obesity and T2DM (Sell and Eckel 2009; Gregor and Hotamisligil 2011; Cipolletta et al. 2012). Both in vitro and in vivo studies have shown that visceral adipocytes promote systemic inflammation by secreting various cytokines such as IL-6, tumor necrosis factor (TNF)-α, and transforming growth factor (TGF)-β1 that may have proximal and distal proinflammatory and metabolic remodeling effects associated with development of secondary pathologies including nonalcoholic fatty liver diseases, cancer, etc. Recently, adipocytes have been shown to also secrete exosomes (Ferrante et al. 2015; Hubal et al. 2016), and adipocyte-derived exosomes mediate activation of macrophages that secrete TNF-α and IL-6, and promote insulin resistance in high-fat-diet-induced murine model of obesity (Deng et al. 2009). Exosomes derived from visceral adipose tissue promoted hepatic inflammatory and fibrotic signaling pathways that may contribute to nonalcoholic fatty liver disease (Koeck et al. 2014). Since exercise is regarded as a gold-standard therapy to mitigate molecular pathologies associated with obesity and T2DM, we propose that the humoral factors that are touted to have antiobesogenic properties (Boström et al. 2012; Fisher et al. 2012; Lee et al. 2014; Rao et al. 2014) are released systemi-

cally as part of exosomes to induce browning in peripheral adipose tissue depots.

ANTI-TUMOROGENIC EXERKINES

A number of studies have found that exercise is associated with a lower risk of several types of cancer, including breast, esophagus, ovarian, and colon (Chakravarty et al. 2008; Lee et al. 2013; Friedenreich et al. 2016; Gong et al. 2016; Moore et al. 2016). A recent meta-analysis of 1.44 million people found that higher levels of leisure time physical activity were associated with a lower risk of 13 of 26 different types of cancer (Moore et al. 2016). The two cancers whose risk was shown to be increased with higher levels of leisure time activity was melanoma (1.27 relative risk) and prostate (1.05 relative risk) cancer (Moore et al. 2016). The latter observation regarding melanoma risk is likely because of the higher sun exposure in those doing activity outside and illustrates the importance of sun protection in those who do their physical activity outdoors. There was a small increased risk of prostate cancer also in the large study (Moore et al. 2016), and tiny increased risk occurred in another study of cross-country skiers (Hållmarker et al. 2015). The latter study also found a lower risk of cancer across all types, especially in those with lower finishing times in races, presumably reflective of a higher physical fitness level and more hours of training (Hållmarker et al. 2015). In addition to cancer risk, there is also evidence that exercise is associated with health improvements after diagnosis (Fong et al. 2012; Friedenreich et al. 2016), even for prostate cancer (Bourke et al. 2016). In contrast to the benefits of physical fitness, adipose-derived exosomes are shown to promote melanoma progression in both mice and humans (Lazar et al. 2016).

One candidate myokine/exerkine mediating the benefits of exercise on cancer risk and progression is IL-6 (Pedersen et al. 2016). This study found that acute exercise was associated with an epinephrine and muscle-derived IL-6-mediated redistribution of natural killer cells to the tumor (Pedersen et al. 2016). The study also reported ~60% reduction in tumors across five different models (Pedersen et al. 2016). IL-6, however, is complex in that cancer patients have a chronic elevation of IL-6 that can contribute to cancer cachexia (Mathur and Pedersen 2008; Hetzler et al. 2015). This apparent paradox is explained by the pulsatility of IL-6 that is induced by bouts of exercise that lead to an activation of muscle satellite cells and contribute to muscle hypertrophy and/or maintenance (Toth et al. 2011; Belizario et al. 2016; Joanisse and Parise 2016). Another myokine that could mediate the beneficial effects of exercise on colon cancer is secreted protein acidic and rich in cysteine (SPARC) (Aoi et al. 2013). This study found that acute endurance exercise led to the up-regulation of skeletal muscle SPARC and plasma SPARC protein and that the benefits of exercise on chemically induced colon cancer was not evident in the SPARC-null mice (Aoi et al. 2013). Furthermore, spent media from stretched myoblasts inhibited the proliferation of colon cancer cells in vitro (Aoi et al. 2013).

AGING AND THE MULTISYSTEMIC BENEFITS OF ENDURANCE EXERCISE

Habitual physical activity/endurance exercise and higher levels of physical fitness have been consistently shown to reduce all causes of mortality (Chakravarty et al. 2008; Woodcock et al. 2011; Bouchard et al. 2015; Schnohr et al. 2015; Lin et al. 2016). The age-associated decline of function of many organs and tissues has also been shown to be attenuated in those who regularly perform exercise or are more physically fit (Chakravarty et al. 2008; Balbuena and Casson 2009; Brenner 2009; Bronas 2009; Buchner 2009; Di Francescomarino et al. 2009; Kruk 2009; Nader and Lundberg 2009; Parsons and King-Vanvlack 2009; Pedersen 2009a; Stuart et al. 2009; Bohm et al. 2010; Booth and Laye 2010; Herring et al. 2010; Kosmadakis et al. 2010; Warburton et al. 2010; Woodcock et al. 2011). Aging is a complex phenomenon that clearly has many fundamental causes that may differ among individuals. It has been suggested that there are nine hallmarks of aging, including genomic instability, telomere shortening, epige-

netic alterations, altered proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem-cell exhaustion, and altered intercellular communication (Lopez-Otin et al. 2013). Several of these processes and other important components of aging can be linked to mitochondrial dysfunction, including telomere shortening, oxidative stress, inflammation, and even genomic instability (Safdar et al. 2011a, 2015; Kolesar et al. 2014). Training studies in murine models of aging further support the epidemiological data in showing that exercise training attenuates the age-associated deleterious effects on mitochondria, antioxidant enzyme activity, and oxidative damage in skeletal muscle, adipose tissue, kidney, brain, and liver (Boveris and Navarro 2008; Sutherland et al. 2009; Safdar et al. 2011a; Clark-Matott et al. 2015).

It has previously been shown that the benefits of endurance exercise are multisystemic in nature and that these benefits are associated with improvements in cellular functional, structural, and health outcome metrics (van Praag et al. 1999; Boveris and Navarro 2008; Chakravarty et al. 2008; Safdar et al. 2011a; Cameron et al. 2012; Devries et al. 2013; Dutheil et al. 2013; Samjoo et al. 2013; Moon et al. 2016). Although the phenotypic benefits of habitual physical activity on multiple tissues and organs are well established in rodents (van Praag et al. 1999; Boveris and Navarro 2008) and humans (Chakravarty et al. 2008), the complex array of interorgan and intertissue signaling that mediates and coordinates this phenomenon remains largely unknown (Moon et al. 2016). To develop effective countermeasures for inactivity-associated disorders, such as obesity and T2DM, it is important to understand the biology of activity-induced interorgan communication (Kruk 2009; Stuart et al. 2009; Warburton et al. 2010).

One approach that we have used recently is to study the multisystemic effects of chronic endurance exercise training in a murine model of aging/mitochondrial dysfunction called the POLG1 mutator mouse. Endurance exercise training in this model over most of their life span was associated with a reduction in mitochondrial dysfunction, a dramatic preservation of tissue histology and a prolonged life span (Safdar et al. 2011a). We have also shown that Master's athletes have more youthful skin histology and preserved mitochondrial capacity versus sedentary people (Crane et al. 2015). The combination of both epidemiological studies showing the protective effects of exercise in multiple nonexercised tissues (skin, kidney, brain, eyes) and the dramatic benefits seen in the progeroid POLG1 murine aging/mitochondrial dysfunction model (Safdar et al. 2011a) provide strong support for the concept of myokines/exerkines functioning in an endocrine-like manner to coordinate the multisystemic beneficial response to habitual physical activity (Little et al. 2011a; Safdar et al. 2011a; Pedersen and Febbraio 2012).

ARE EXOSOMES IMPORTANT MEDIATORS OF THE MULTISYSTEMIC BENEFITS OF EXERCISE?

There has been very little attention given to the mechanism(s) of myokine/exerkine release into circulation. It is known that the quintessential myokine, IL-6, is rapidly depleted from muscle during contractions and that the IL-6 was within vesicle structures (Lauritzen et al. 2013), indirectly implying that IL-6 may indeed be released as part of the exosome pathway. Furthermore, HSP70 and HSP72 are reported to be released in exosomes from macrophages in response to acute heat stress (Lancaster and Febbraio 2005a,b). An increase in HSP72 abundance in skeletal muscle mitigates the dysglycemic effects of high-fat feeding in mice and increases mitochondrial abundance and exercise capacity (Henstridge et al. 2014).

Although the above evidence supporting that myokines/exerkines are released in exosomes is circumstantial and rather incomplete, there are several lines of evidence suggesting that exosomes do play a role in mediating the multisystemic effects of endurance exercise. First, the role of exosomes/MVs as mediators of intercellular communication is a highly conserved process throughout evolution and across prokaryotes, plants, and eukaryotes, including yeast and fungi. As described above, exosomes

and possibly MVs can transfer a wide variety of biomolecules (peptides, mRNA, DNA, miRNA, mtDNA, etc.) between cells using receptor-dependent and -independent mechanisms (Lötvall et al. 2014). Strong evidence suggests that skeletal muscle functions as a secretory organ (Pedersen and Febbraio 2012), even in vitro (Forterre et al. 2014). It has also been shown that skeletal muscle releases exosomes that contain hundreds of peptides (Aswad et al. 2014), and myoblast differentiation into myotubes resulted in a change in the secretory pattern involving 437 proteins (Ojima et al. 2014). In the latter study, only 10% of the peptides had a canonical secretory sequence with 43% being nonclassical secreted proteins lacking an amino-terminus secretory sequence and 65.3% of the peptides being bioinformatically linked to exosomes (www.exocarta.org) (Ojima et al. 2014). Although still a preliminary observation, our pilot data showing that the exosome marker, apoptosis-linked gene 2 interacting protein X (ALIX) was present in skeletal muscle and was severely depleted immediately after acute endurance exercise, supports that exosomes are present in skeletal muscle and released in response to endurance exercise (Safdar et al. 2016).

Collectively, the above data suggests that skeletal muscle releases many peptides in response to cellular stress or physiological processes but the link between acute endurance exercise and exosome release and their role in mediating multisystemic benefits is still very limited. To date, there has been only one study reporting the effects of acute exercise on serum EVabundance in humans (Fruhbeis et al. 2015). The response of EVs was evaluated in response to acute endurance exercise (both cycling and treadmill running) using ultracentrifugation and filtration to quantify small EVs/exosomes and MVs. The found an acute intensity-dependent increase in small EVs/exosomes but not MVs in response to both modes of exercise with a return to resting levels 90 min after cycling and 180 min after running (Fruhbeis et al. 2015). It is unclear what proportion of the small EV/exosome fraction was in fact exosomes for the approximate mean vesicle diameter was $>$140 nm in the representative images from the treadmill data (Fruhbeis et al. 2015), and because this is the upper limit of the size of exosomes (Harding et al. 2013), it is likely that a substantial number of these vesicles were small MVs. The acute exercise study also found that HSP70 content of EVs was slightly elevated post-exercise (Fruhbeis et al. 2015). The same group has also reported that the acute endurance exercise induced increase in cell-free DNA (cfDNA) occurred independent of MVs (Helmig et al. 2015). Although not skeletal muscle derived, one study reported that an acute bout of endurance exercise was associated with a proangiogenic gene expression profile in CD62E+ve and CD34+ve blood cells (Lansford et al. 2016). In the latter study, men showed acute exercise-induced increase in CD62E+ve cell microparticles and women showed higher CD34+ve cell microparticle abundance (Lansford et al. 2016). A study using the db/db murine model of T2DM found that cardiac-derived exosomes containing several miRNAs (miR-455, miR-29b, miR-323-5p, and miR-466) were released in response to acute endurance (Chaturvedi et al. 2015). They speculated that the increase in these specific miRNAs would negatively influence matrix metalloprotease 9 (MMP9) gene expression and lessen cardiac fibrosis (a known pathology with aging and T2DM) (Chaturvedi et al. 2015). To date, there have been no studies looking at MV/EV release in response to other modes of exercise; however, the increase in plasma CD34+ve cells following an acute bout of sprint interval exercise (Harris et al. 2014), combined with the above data indirectly implies that other modes of exercise may lead to the release of exosomes and/or MVs into circulation.

The limited collective data regarding exercise and exosome/MV release and function is very scant but is likely to be expanded upon over the next several years (Safdar et al. 2016). We have previously reported that up to 75% of the reported exerkines/myokines were listed in the annotation of peptides that have been reported to exist in exosomes/MVs (ExoCarta and Vesiclepedia) (Safdar et al. 2016). We have updated this list by including more of the reported myokines/exerkines using a PubMed (www.pubmed.org) search on November 3,

2016 and the latest annotation of the exosome and/or MV listings on the www.exocarta.org and www.microvesicles.org websites. We found that of the 35 myokines/exerkines, 51% were listed in www.exocarta.org and 80% in www .microvesicles.org (Table 1).

Collectively, the above data strongly suggests that many of the reported myokines/exerkines are contained within exosomes and possibly MVs. Furthermore, we propose that the myokines and exerkines cargoed within exosomes are integral in promoting interorgan cross talk that modulate the systemic benefits of endurance exercise (Fig. 2).

FUTURE DIRECTIONS

The first challenge moving forward will be the ability to separate exosomes from MVs and agree on a definition of size. Exosomes are generally assumed to be between 30 and 140 nm; however, this has been based primarily on nanoparticle counting and dynamic light scattering methods that tend to overestimate the size. For example, when measuring the same exosomal fraction with three different methods, investigators reported a size of 30–50 nm using scanning electron microscopy, 110–120 nm using nanoparticle counting, and 210–220 nm using dynamic light scattering methods (Sokolova et al. 2011). Our data agrees with this for we find that exosome size is about 30%–50% smaller using the gold standard of cryoelectron microscopy as compared with dynamic light scattering (unpubl.). Another issue is that particles that bud off the plasma membrane (MVs) have been reported in the size range that overlaps with exosomes (20–120 nm) (Booth et al. 2006). As we also discussed above, several studies that claim to have highly purified exosomal fractions show nanoparticle tracking data with much of the area under the curve above the 140 nm size (El-Andaloussi et al. 2012). In addition, there appears to be some overlap in the peptides that are reported to be vesicle exclusive (Soekmadji et al. 2013). We would recommend that it is important to follow consensus statements regarding experimental rigor such as that released by the International Society for Extracellular Vesicles in 2014 (Lötvall et al. 2014).

Another issue that will require in depth analysis will be exercise-specific questions such as the duration, intensity, and mode of exercise and the relationship to exosomes and MVs. It is known that many of the classical adaptations to endurance exercise also occur in response high intensity interval training (Little et al. 2011b,c; Earnest et al. 2013), and evaluating the exosome and MV characteristics will be revealing. Along these lines, resistance exercise has clearly been shown to be an effective countermeasure for sarcopenia (Tarnopolsky et al. 2007), and characterizing the myokines/exerkines in this mode of exercise will be important. It is also known that unaccustomed lengthening contractions (sometime called eccentric) can damage contractile elements (Stupka et al. 2001), and determining whether this mode of exercise impairs exosome release and/or alters the ratios of exosome/MV release will be low-hanging fruit. There is an impending need to study the effects of aging and chronic metabolic disease on exosome biology and circulating exosomal biomarkers. Exercise is generally regarded as the gold-standard therapy to promote both health span and life span, and we propose that one of the ways that endurance exercise mitigates chronic metabolic diseases is by promoting homeostatic cross talk between organs and tissues. It will also be very important to determine how chronic exercise training affects the basal and acute exercise response of exosomes and MVs. These studies should use a repeated measures design and also covariate age, sex, and comorbidities (i.e., obesity and T2DM). Our preliminary data comparing the serum exosome dynamic light scattering profile between a middle-aged sedentary male and a very well trained endurance athlete showed very distinctive profiles, and proteomics, transcriptomics, and metabolomics characterization of such vesicles should provide important information.

CONCLUSIONS

The multisystemic benefits of habitual endurance exercise on multiple organs and tissues and

Table 1. Presence of known exercise-responsive myokines/adipokines (exerkines) within extracellular vesicle compendiums

Protein	www .exocarta.org	www .microvesicles.org	Association
Adiponectin (AdipoQ)	Y	Y	Obesity, T2DM
Apelin (APLN)	Y	Y	Obesity, cancer
Brain-derived neurotrophic factor (BDNF)	Y	Y	Obesity, cancer
Betatrophin (ANGPTL8)	N	N	T2DM
Cathepsin B (CTSB)	Y	Y	T2DM, cognition, cancer
Cbp/p300-interacting transactivator (CITED4)	N	Y	Cancer, muscle hypertrophy
Chemokine ligand 1 (CXCL1)	Y	Y	Lung fibrosis, asthma
Chemokine ligand 2 (CCL2)	Y	Y	Systemic inflammation, cancer
Chitinase 3-like 1 (CHI3L1)	N	Y	Obesity, myocyte proliferation
Colony-stimulating factor 3 (CSF3)	Y	Y	Unknown
Connective tissue growth factor (CTGF)	Y	Y	Connective tissue, ? muscle growth
Dipeptidyl-peptidase 4 (DPP4)	Y	Y	T2DM, ? intestinal function
Fibroblast growth factor (FGF)-2	N	Y	Chondrocyte and muscle regeneration
FGF-21	N	N	Obesity, T2DM, NAFLD
Follistatin-like 1 (FSTL-1)	Y	Y	Endothelial function
Growth differentiation factor 11 (GDF-11)	Y	Y	? Muscle growth
Growth differentiation factor 15 (GDF-15)	N	Y	Skeletal and cardiac muscle, ? neurogenesis
Irisin	N	N	Obesity, T2DM
Insulin-like growth factor 1 (IGF-1)	N	Y	Skeletal muscle growth
Interleukin-1 (IL-1)	N	N	Chronic inflammation
Interleukin-4 (IL-4)	N	Y	Anti-inflammatory response
Interleukin-6 (IL-6)	N	Y	Gluconeogenesis, cancer, chronic inflammation
Interleukin-7 (IL-7)	Y	Y	Chronic inflammation
Interleukin-8 (IL-8)	N	Y	Chronic inflammation
Interleukin-10 (IL-10)	Y	Y	Anti-inflammatory response
Interleukin-15 (IL-15)	N	Y	Muscle growth, skin health
Interleukin-18 (IL-18)	N	Y	Chronic inflammation
Interleukin-15 receptor α (IL-15RA)	Y	Y	Immunity
Leptin (LEP)	N	Y	Obesity
Leukemia inhibitory factor (LIF)	Y	Y	Cancer
Meteorin-like protein (METRNL)	Y	Y	Obesity, T2DM neurogenesis
Myonectin (CTRP15)	N	N	Adipose tissue regulation
Myostatin (GDF-8)	N	N	Muscle growth
Secreted protein acidic and rich in cysteine (SPARC)	Y	Y	Cancer
Vascular endothelial growth factor A (VEGFA)	Y	Y	Angiogenesis

The public databases www.exocarta.org and www.microvesicles.org were searched on November 3, 2016. Eighteen (51%) of the 35 reported exerkines were listed in www.exocarta.org, and 28 (80%) of the 35 reported exerkines were listed in www .microvesicles.org. Association, Association with a condition or disease in relation to exercise; T2DM, type 2 diabetes mellitus; NAFLD, nonalcoholic fatty liver disease.

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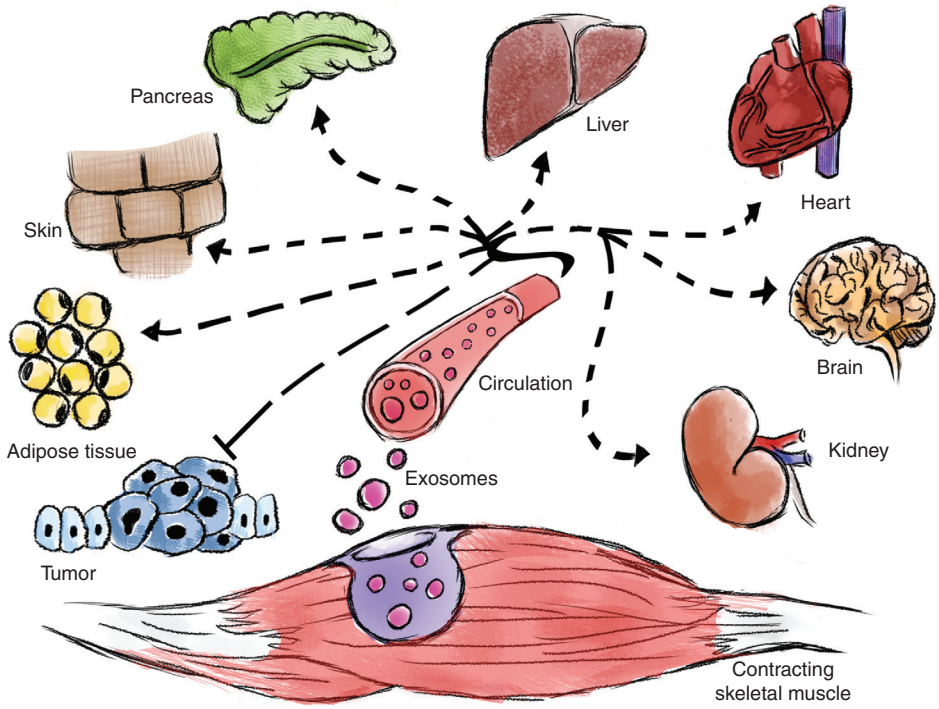

Figure 2. Exosomes promote interorgan cross talk that modulates the systemic benefits of endurance exercise. We hypothesize that the contraction of skeletal muscle during exercise induces the release of *exersomes* with specific exerkines (peptides, nucleic acids, and lipids) into the blood for interorgan cross talk and mediates the systemic adaptations to endurance exercise.

all-cause mortality have been well established, especially in the areas of obesity, T2DM, cancer, and cardiovascular disease. The support that acute exercise leads to the release of factors from muscle (myokines) and other tissues (exerkines) to orchestrate the multisystemic benefits of exercise are also strongly supported by the literature. The role of exosomes and possibly MVs as intercellular communicators is also very well established in the literature. We have previously hypothesized that exosomes (and possibly MVs) are mediating the interorgan exchange of endocrine-like factors (myokines and exerkines) and represent the true "exercise factors" (Safdar et al. 2016). The scant current data suggests that exosomes and small MVs are released into the circulation in response to acute endurance exercise in an intensity-dependent manner. Many of the currently reported myokines/exerkines have been reported to exist in exosomes/MVs. Finally, exosomes within skeletal muscle are depleted in response to an acute bout of endurance exercise. Although compelling, the above hypothesis is far from axiomatic or well established, and a number of experiments will be required to conclusively understand the role of exosome and MVs in the acute and chronic response to different modes of exercise and how these relate to the multisystemic benefits of exercise.

There is no doubt that there is a growing interest in the role of exosomes and MVs in biology and medicine, and the application to exercise, although embryonic, is garnering the attention of funding agencies as reflected in the "Molecular Transducers of Physical Activity in Humans" RFA (request for application) issued by the National Institutes of Health (NIH) Common fund. In addition to better understanding the biology of exosomes, MVs, exercise physiology, aging, and the systemic benefits of exercise, it is likely that this area of research will lead to

therapeutic discovery. Although allogenic exosome transfusions are not likely to be logistically or immunologically viable, it is very possible that combinations of exerkines that are reflective of the molecular patterns associated with the multisystemic benefits of exercise could be given with intermittent subcutaneous injections that mimic the effects of exercise and be independently or adjunctively (e.g., concomitant with insulin) used for patients with T2DM and other morbidities such as obesity, aging-associated sarcopenia, muscular dystrophy, T1DM, and mitochondrial diseases.

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