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Molecular Transducers of Physical Activity Consortium (MoTrPAC): Mapping the Dynamic Responses to Exercise

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Exercise provides a robust physiological stimulus that evokes crosstalk among multiple tissues that when repeated regularly (i.e., training) improves physiological capacity, benefits numerous organ systems, and decreases the risk for premature mortality. However, a gap remains in identifying the detailed molecular signals induced by exercise that benefits health and prevents disease. The Molecular Transducers of Physical Activity Consortium (MoTrPAC) was established to address this gap and generate a molecular map of exercise. Preclinical and clinical studies will examine the systemic effects of endurance and resistance exercise across a range of ages and fitness levels by molecular probing of multiple tissues before and after acute and chronic exercise. From this multiomic and bioinformatic analysis, a molecular map of exercise will be established. Altogether, MoTrPAC will provide a public database that is expected to enhance our understanding of the health benefits of exercise and to provide insight into how physical activity mitigates disease.

INTRODUCTION

Exercise perturbs multiple systems from the whole body to the molecular level in an integrated manner (Hawley et al., 2014). However, in-depth fundamental knowledge into the molecular and cellular mechanisms that are responsible for physical activity's benefits on multiple organ systems and the diseases and disorders that derive from inactivity is incomplete (Booth et al., 2017; Neufer et al., 2015). A better understanding of these biological processes and pathways would allow for the development of targeted exercise interventions and prescriptions and provide a foundation for developing exercise-mimetic pharmacologic interventions.

The Molecular Transducers of Physical Activity Consortium (MoTrPAC) was established to elucidate how exercise improves health and ameliorates diseases by building a map of the molecular responses to acute and chronic exercise. In 2014, a portfolio analysis of National Institutes of Health (NIH) grants revealed that most research regarding physical activity involved disease prevention or treatment. In fact, almost all the grants which employed an exercise intervention only addressed health outcomes and adherence issues. The MoTrPAC initiative provides a much needed comprehensive program to understand the interplay between these biological systems with the goal of improving the design of physical activity interventions. In addition, there is a potential to identify molecular targets that can be manipulated to mimic the effects of exercise in persons unable to do so for a variety of reasons, such as physical disability, coma, or paralysis.

To address the gaps in knowledge about how exercise enhances health and ameliorates disease, multiple agencies at the NIH—including the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), the National Institute on Aging (NIA), and other institutes and centers who participated in the trans-NIH Exercise Interest Group—proposed the Common Fund program supporting MoTrPAC. To create a substantive complex map of molecular transducers in diverse populations across the lifespan, MoTrPAC established a multisite collaboration across the United States encompassing various scientific disciplines: preclinical animal study sites and human clinical exercise sites to perform the exercise testing, exercise interventions, and biospecimen collection; a consortium coordinating center to manage sample collection, distribution of samples, and consortium logistics; chemical analysis sites to perform 'omics analysis from the samples collected; and a bioinformatics center to collaboratively facilitate data quality control, bioinformatics analysis, and dissemination to make the data and related resources available to the broad scientific community (Figure 1). The animal studies will enable analysis of the effects of exercise on many different tissues that are not readily obtainable in humans, thereby enabling a broad view of the systemic effects of exercise. The collection of human specimens (blood, muscle, and adipose) will permit the analysis of these critical systems, which are central to the energetics of exercise and appear to interact in a coordinated manner to improve overall metabolic health (Pedersen and Febbraio, 2012; Romijn et al., 1993; Stanford and Goodyear, 2018). In addition to providing information concerning the effects of exercise at different physiological and molecular levels, the large scope of this study (humans: N = ~2,600; rats: N = 820) will create a complex, integrative dataset that will be available to the scientific community. This dataset and some associated biospecimens can be leveraged by other groups by proposing ancillary studies to MoTrPAC.
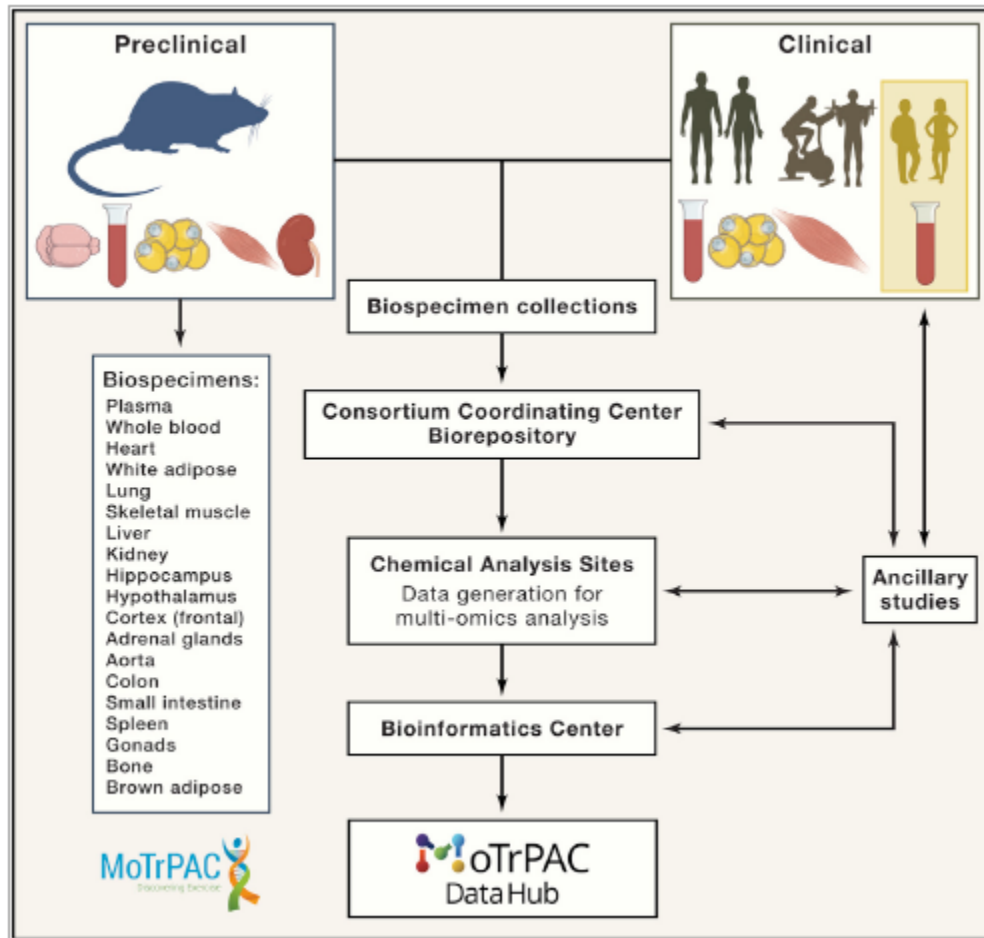
Figure 1. General Overview of MoTrPAC Preclinical Animal Study Sites (PASSs) (rats) and Human Clinical Exercise Sites will collect biospecimen samples after acute and chronic exercise. The biospecimen samples will be sent to a central biorepository where they will be logged, processed, and distributed to various Chemical Analysis Sites (CASs) for 'omics analysis. A portion of the biospecimen samples will be kept at the biorepository for future ancillary study opportunities by the scientific community. Data generated from the CASs will be transferred to the Bioinformatics Center (BIC) for a multiomics, multispecies, multi-tissue, and multi-time point integration of the data with the goal of generating a molecular map of exercise. The data will be available to the scientific community via the MoTrPAC Data Hub: https://motrpacdata.org. Figure design by Jill K. Gregory, Mount Sinai.

Preclinical Animal Study Sites

A primary goal of the Preclinical Animal Study Sites (PASSs) investigations is to enable the analysis of the systemic effects of exercise across many different organs and blood, most of which cannot be collected in humans. The first phase of the PASS studies is being conducted across three separate sites, with each collecting numerous biospecimens after acute (i.e., single bout) treadmill exercise or chronic treadmill training of male and female F344 rats at 6 and 18 months of age (Figure 2). For both acute and chronic exercise studies, the same biospecimens are being collected from nonexercised, control animals; for the acute studies this includes groups accounting for potential effects of time of day and time of feeding. This rat strain was selected for MoTrPAC because there is a large body of previous exercise

research utilizing this strain, and rats provide larger amounts of tissue in contrast to mice. Larger tissue samples allow for multiple assays to be performed on the same sample, which supports bioinformatics analysis for the development of the molecular map of exercise. Also, larger tissues will provide additional material for ancillary studies. To control variation across the three PASSs, the rats are being provided from the same NIA colony, and the housing and feeding conditions are standardized across the sites. Furthermore, because rats are most active during the ''dark'' light phase, all rats are first being acclimated to a reverse light cycle for a minimum of 10 days, and with all exercise bouts conducted during the dark cycle.

The PASS study design for the acute response to exercise entails a single 30min bout of treadmill running (intensity: ~80%–90% VO_2max; incline: 5°; speed: 6 months: male – 16.8 m/min, female – 18.0 m/min; 18 months: male – 12.0 m/min, female –13.8 m/min), with tissue collections occurring immediately post-exercise and at six additional times up to 48 h after the exercise bout. This sampling series is weighted toward early time points (0, 0.5, 1, and 4 h post-exercise) to capture the temporal dynamics of the molecular responses, but it also includes later time points (7, 24, and 48 h post-exercise) to capture long-duration primary responses as well as secondary molecular events (detailed protocols are available at https://motrpac.org/protocols.cfm). To study the biological events that occur during the early, intermediate, and later stages of endurance training, the PASS study design for the chronic response to exercise, which has been completed, entailed up to 8 weeks of treadmill training (5 days per week at ~70% VO_2max), with tissues collected 48 h after 1, 2, 4, and 8 weeks of training; incline, duration, and speed of exercise progressively increased on a daily to weekly basis during the initial 6 weeks of training. Sex differences in both the acute exercise response and the training response are being investigated along with other study aims.

The most powerful aspect of the PASS design is the breadth of tissues collected. In addition to being studied in the context of MoTrPAC, these will serve as a data resource for generating hypotheses for future studies. For both the acute exercise and chronic exercise training studies (including the non-exercise controls), as many as 27 biospecimens per rat are being collected for potential analysis. In addition to biospecimen collection, other phenotypic outcomes are being collected, including blood lactate concentration, maximal oxygen consumption (VO_2max), and body composition. At Chemical Analysis Sites, initial biospecimens of focus will include those that overlap with the human studies (i.e., plasma, skeletal muscle, white adipose), as well as liver, heart, kidney, lungs, brain, and brown adipose. It is expected that nucleic acid, proteomic, and targeted metabolomic assays will be performed only on tissues where the amount is nonlimiting, whereas transcriptomics, nontargeted metabolomics, and nontargeted lipidomics will be performed on all tissues. Together, these assays are expected to provide molecular and physiological insights about the effect of exercise on many different organs. Ultimately, MoTrPAC should begin to explain how molecular transducers function across an entire mammal (Pedersen and Febbraio, 2012).

After preliminary characterization of the changes that occur in the initial set of analyses, a second phase of the PASS will include mechanistic studies of exercise-induced molecules that transduce stress resistance and circulating factors that might be implicated in the health benefits of exercise. Additional studies will focus on the adaptation to chronic resistance exercise and the impact of age and sex on these responses, as well as other studies that have yet to be determined.
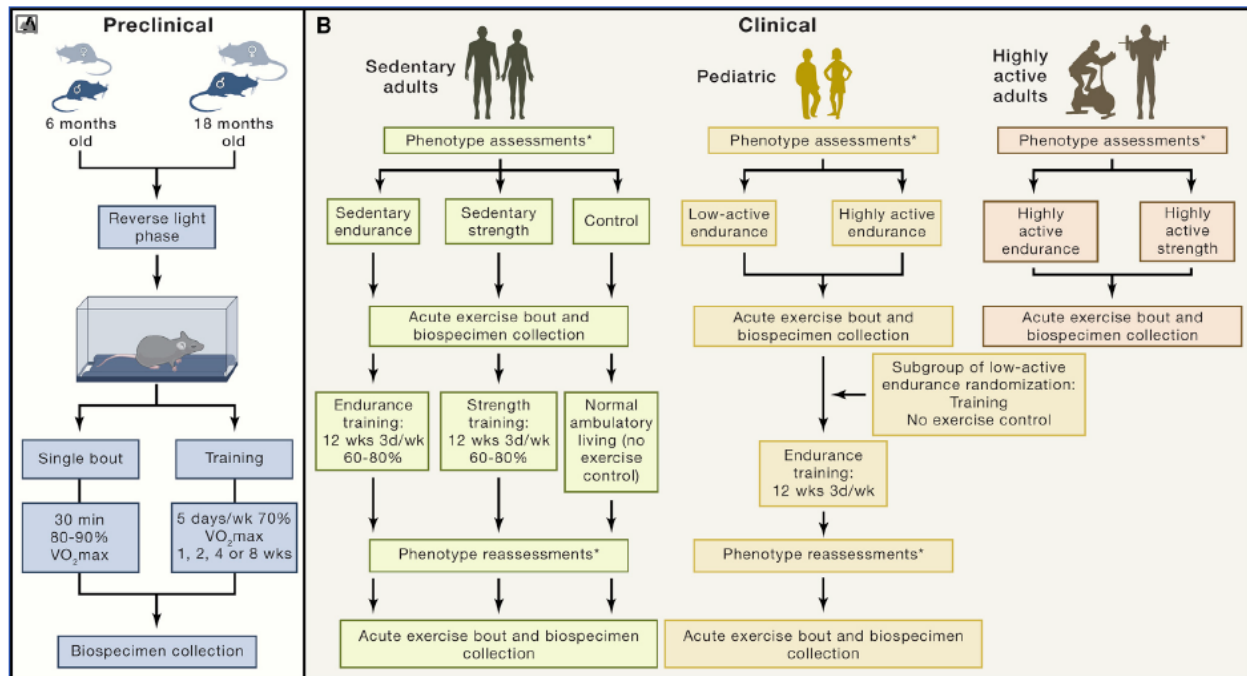
Figure 2. A General Schematic of the Preclinical and Clinical Studies

(A) Biospecimen collection for the rat studies (N = 820, including nonexercised controls) is ongoing and will be completed in Fall 2020. For both the acute (i.e., single bout; n = 526) and chronic phases (i.e., training; n = 294), 6 and 18monthold male and female rats are being studied, and both exercise phases include a cohort of nonexercised controls.

(B) 2,600 healthy individuals will be evaluated physiologically, and biospecimens will be collected to accommodate molecular probing of various tissues before and after acute and chronic endurance or resistance exercise. For the adult cohort, the control participants will rest quietly (i.e., no exercise) during the acute bout with biospecimen collections.

*Assessments include blood profile, body composition, heart health, VO_2max and strength. Figure design by Jill K. Gregory, Mount Sinai.

Human Clinical Exercise Sites

The human component of MoTrPAC is an in-depth study of the effects of two different forms of exercise (endurance and resistance training) across multiple individuals of different ages (including children) and sexes, as well as sedentary and highly active individuals. This large cohort will be used to study the response to exercise at the whole body and cellular levels and attempt to identify the molecular underpinnings that might be responsible for the adaptive process and variation among individuals. Several traditional methods from the field of exercise physiology will be combined with novel biospecimen sampling and high-throughput molecular analytical approaches that will likely yield important insights into the effects of exercise on health. The human study has many unique aspects that are highlighted below and will be conducted as a randomized controlled trial (RCT) with an intent-to-treat design.

Participants

The goal is to recruit 270 children and adolescents (10–17 years of age) who are low-active in endurance-type exercise and 1,980 healthy sedentary adults (age 18 years or greater) who will be medically screened and randomly assigned to endurance training (170 youth, 840 adults), resistance training (840 adults), or non-exercise control (50 youth, 300 adults) (Figure 2). An additional group of highly active endurance (50 youth, 150 adults) and resistance (150 adults) trained individuals will serve as comparators and will not participate in the MoTrPAC exercise training programs. The recruitment and enrollment approach will be sex balanced and provide participants across a wide range of ages (10–17, 18–39, 40–59 and R60 year age groups) and of different races.

Exercise Training Program: Adults

The sedentary adult participants randomized to endurance or resistance exercise training will perform 12 weeks of supervised exercise, 3 days per week, with progression in both volume and intensity. Each endurance training session will be 1 h in duration and be evenly split between cycling and treadmill (walking/running) exercise with intensity set to 60%–80% of heart rate reserve and monitored in real time during each session. Each resistance training session will target the whole body and consist of eight total exercises (five upper body: chest press, military press, seated row, triceps extension, biceps curl; three lower body: leg press, leg curl, knee extension) at a prescribed plan of 3 sets of 8–12 repetitions at an intensity of 60%–80% of maximum for each exercise. These exercise protocols are well known to improve clinically relevant parameters (i.e., VO_2max and muscular strength and hypertrophy) via alterations in metabolic, biochemical, and molecular signatures (Coggan et al., 1990; Gollnick et al., 1973; Raue et al., 2012; Rönn et al., 2014; Timmons et al., 2010).

Acute Exercise Bout and Biospecimens Collections: Adults

A unique feature of the MoTrPAC adult protocol will be the integration of strategic biospecimen collections (blood, muscle, and adipose) before, during, and after standardized bouts of acute exercise. Participants will perform a 40 to 45min bout of exercise (exercise-mode specific; rest for the non-exercise controls) with biospecimens collected before and after 12 weeks of training. The highly active group will perform the exercise-mode specific bout only once. Compared to resting homeostasis, these types of exercise challenges are expected to dramatically increase metabolic rate ~5- to 10-fold (Coggan et al., 1990; Farinatti and Castinheiras Neto, 2011; Mulla et al., 2000; Romijn et al., 1993), bioenergetic flux >10-fold (Kjaer et al., 1991; Romijn et al., 1993; Steensberg et al., 2000), and large dynamic range in gene expression from small to >100-fold changes (Louis et al., 2007; Radom-Aizik et al., 2013, 2014) and likely enhance crosstalk among many organs (Pedersen and Febbraio, 2012).

Standardized conditions that control for physical activity, time of day, and dietary intake will be implemented prior to the acute exercise bout. On the day of an acute exercise bout with biospecimen collections, volunteers will arrive at a Human Clinical Center in the morning after an overnight fast and rest comfortably for 0.5 h prior to obtaining baseline blood (antecubital vein), skeletal muscle (vastus lateralis), and adipose (periumbilical region) samples. Participants will then perform the standardized acute exercise bout (or rest for the non-exercise control group) with additional biospecimen samples (blood, muscle, adipose) obtained 0.5 h (early), 4h(middle),and 24 h (late) after exercise. These time points were chosen to capture the dynamic changes in the response to exercise as metabolic, posttranslational, and epigenetic modifications can occur quite rapidly (Barr `es et al., 2012; Bolster et al., 2003; Hoffman et al., 2015; Romijn et al., 1993), whereas mRNA induction generally peaks a few hours after exercise (Louis et al., 2007; Yang et al., 2005), and increases in protein synthesis rates are detectable in the hours and days following exercise (Phillips et al., 1997)(Figure 3). Additional blood samples will

be collected during the endurance exercise bout (20and 40min time points) and shortly after (10 min) both endurance and resistance exercise bouts. All participants will have pre-exercise biospecimen collections, but to reduce participant burden in the post-exercise phase, sedentary participants will undergo skeletal muscle and adipose biopsies at one of three time points (early, middle, late). The highly active participants will have muscle biopsies and blood at all time points, whereas adipose biopsies will be collected at the pre and middle-timepoints.

Acute Exercise Bout and Biospecimens Collections: Pediatrics

Children and adolescents undergo critical periods of growth and development, which are distinct from adult physiology. Pediatric studies must also comply with additional ethical considerations (RadomAizik and Cooper, 2016). Consequently, although the pediatric arm of the study will mimic the adult protocol as closely as possible, there are a few notable exceptions: (1) children who are lowactive in endurancetype exercise will be recruited (versus sedentary adults) to account for the fact that children are naturally more active than adults and also participate in mandatory physical education classes; (2) no tissue biopsies (only blood will be collected); (3) an acute bout of endurance exercise with blood collection will be performed in both the training intervention and noexercise control groups; (4) blood samples will be collected in all participants before, 20 and 40 min during exercise, and 10 min, 0.5 h, and 3.5 h into recovery; and (5) for a subgroup of 170 lowactive endurance exercise children and adolescents who will be randomized to receive 12week endurance training (N = 120) or continue their standard practice (N = 50) (Figure 2), the endurance exercise intervention will be modified to provide an exercise intervention that is appropriate to the pediatric participants' age group. For middle and high school students the modes of endurance exercise will include cycling and treadmill and also an option for elliptical and rowing machines. Elementary school students will be trained in a form of circuit training (e.g., endurance activity stations: cycle ergometer, steppers, individual jump rope, pacer, and sliders) to keep the younger participants engaged.

Phenotype Measures: Pediatrics and Adults

To complement the molecular map, selected phenotypic measures will be obtained. Participants will be assessed before and after the 12week intervention period, whereas the highly active adult comparator group members will be assessed once. These measurements include maximal oxygen consumption on a cycle ergometer (VO_2max), grip strength, maximal isometric knee extension strength, body composition (DXA, dualenergy Xray absorptiometry), clinical blood profiles, heart rate profiles during the acute endurance and resistance exercise bouts, substrate utilization (carbohydrate and fat) during the acute endurance exercise bout at 65% VO_2max (adult endurance participants only), and upper and lower body strength (onerepetition maximum; adult resistance participants only). Most of the adult participants also will provide information on selfreported health outcomes using PROMIS measures (www.nihpromis.org) that will provide opportunities to investigate the effect of exercise on mood, anxiety, and depression. In a subgroup of adult participants, skeletal muscle and adipose histology (cell type, size, capillarization) and single cell analysis across various 'omics platforms will be conducted. Investigators are exploring the potential for collecting and analyzing microbiome samples from a subgroup of adult participants.
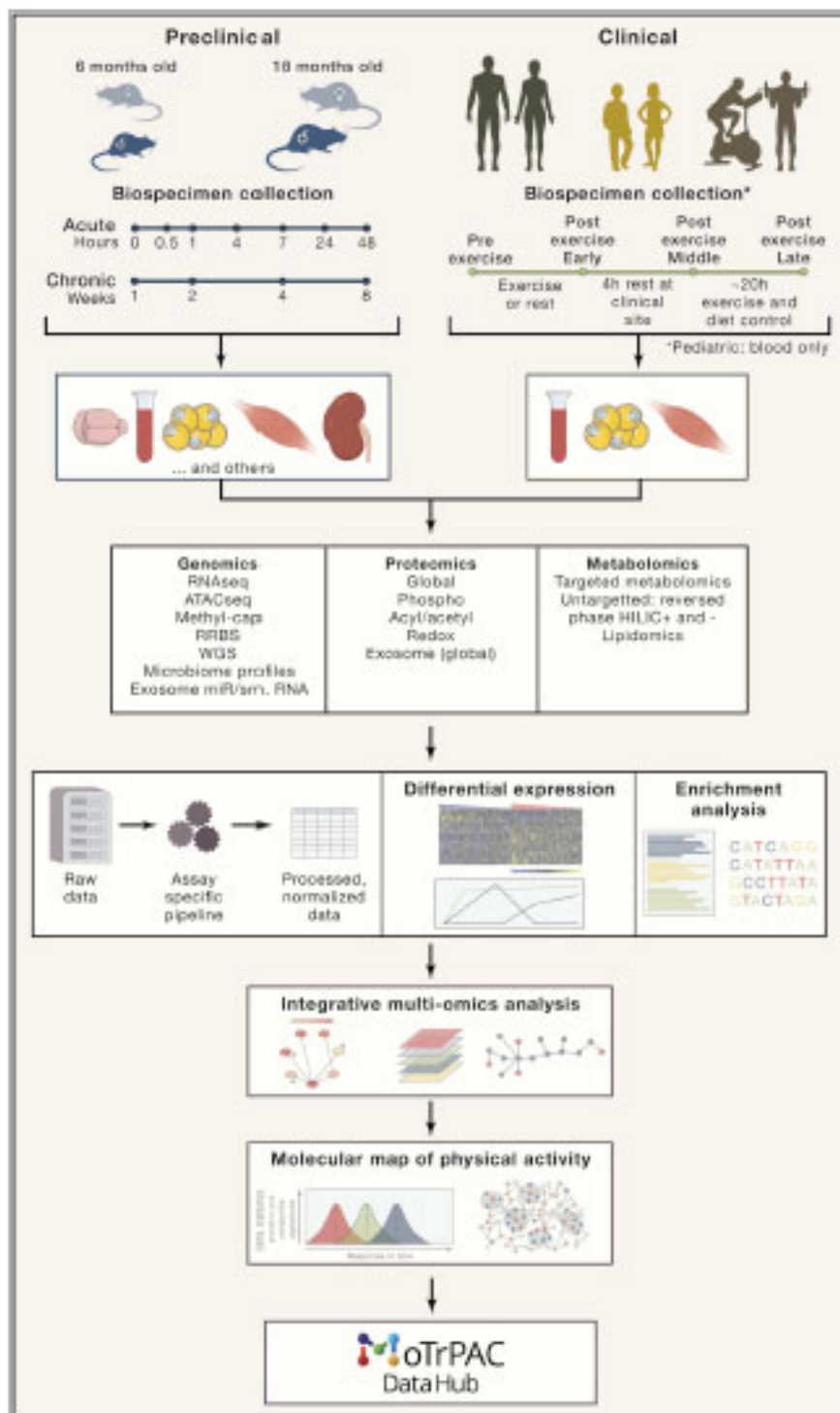
Figure 3. Overview of Biospecimen Collections and Integrated Analysis Plan Preclinical studies: For the acute exercise arm, biospecimens (N = 25 per animal) are being collected 0 (i.e., immediately post), 0.5, 1, 4, 7, 24, or 48 h following exercise. For the chronic exercise training arm, which has been completed, biospecimens (N = 28) were collected 48 h after rats completed 1, 2, 4, or 8 weeks of treadmill training. For both study arms, the same biospecimens are being collected from an unexercised group of rats. Clinical Studies: Human biospecimens (blood, muscle, adipose; blood only for pediatrics) will be obtained before and at 0.5, 4, and 24 h following endurance or resistance exercise. The sedentary participants will perform the acute exercise bout with biospecimen collections twice (before and after 12 weeks of training) whereas the highly active participants will perform this one time (see Human Clinical Sites description in the text for more detail). The 'omics data generated from multiple different genomics, proteomics, and metabolomics assays will be processed in assay-specific pipelines. Omicspecific analyses followed by stateoftheart integrative methods will be applied for a multiomic analysis of the multiple time points and tissues collected in MoTrPAC with the goal of creating a map of the molecular transducers of exercise. All data (pipelines, raw and processed data, results, and integrative analyses) will be made publicly available through the MoTrPAC Data Hub: https://motrpacdata.org. Figure design by Jill K. Gregory, Mount Sinai.

Implementation

To optimize this complex protocol, the adult component of the study will be implemented in two phases. The first phase will involve 150 adult participants and will require 4–6 months, enabling assessment of participant and clinical burden and feasibility, as well as allowing for refinement of the MoTrPAC protocol. In phase two, the remainder of the project with a target of over 2,000 participants will be implemented.

Consortium Coordinating Center

The Consortium Coordinating Center (CCC) is composed of four parts: an Administrative Coordinating Center (ACC), a Data Management, Analysis and Quality Control (DMAQC) Core, an Exercise Intervention Core (EIC), and a central Biorepository. The role of the ACC is to enable the organization and governance of MoTrPAC by facilitating key processes such as meeting logistics, IRB submission, and preparation of Manuals of Operations.

The Biorepository, working with the preclinical and clinical sites, the DMAQC, and the Chemical Analysis Sites, oversees sample collection, shipping, archiving, and distribution of human and animal samples. This includes ensuring that homogeneous cryo-pulverization of tissue samples occurs prior to distribution of aliquots to the various Chemical Analysis Sites. Uniform sample processing is important to ensure that diverse data types can be directly compared. Each tissue sample will also be stored for future use by MoTrPAC and non-MoTrPAC investigators. Samples include serum, EDTA plasma, PAXgene-protected whole blood and peripheral blood mononuclear cells, and vastus lateralis skeletal muscle and subcutaneous abdominal adipose tissue from humans and >20 different tissues from the preclinical animals. Each sample will be analyzed by the Chemical Analysis Sites, and additional material will be archived for future use. The Biorepository inventory system interacts with the DMAQC to enable sample tracking, quality control, and other process support systems.

Chemical Analysis Sites

To understand the exercise response in detail, an in-depth analysis of molecular and 'omic assays will be performed using state-of-the-art laboratory techniques. Technologies include genomics, transcriptomics, DNA methylomics, targeted and untargeted proteomics, and targeted and untargeted metabolomics. Genomic, Transcriptomic, and Regulatory Analyses Evidence has shown, through more than 150 small cohort studies (typically with under 50 participants analyzed) (Bouchard et al., 2011; Pacheco et al., 2018), that exercise is accompanied with massive changes at both the transcriptional and epigenomic levels in muscle, adipose, and most other tissue systems (Lindholm et al., 2014; Ling and Rönn, 2014; Rönn and Ling, 2013) with the poorly understood influence of the underlying human genetic/environmental variation that exists between and within populations (Leońska-Duniec et al., 2016). Therefore, recent scientific studies have been conducted generating data reflecting some of the underlying genetic and epigenetic basis for responses to exercise, physical activity, and training linking specific targets to benefits (Carter et al., 2017). Although several studies (for review see Loos et al., 2015; Warburton et al., 2006) have provided a rich source of information to develop a foundation for larger and more comprehensive genomic, epigenomic, and transcriptomic (GET) analyses, sufficiently powered studies with the complementary detailed study design to make accurate predictions as machine-learned models remain underdeveloped. Moreover, the information needed to understand the role genetic variation plays in the response of individuals to acute and chronic exercise remains limited. MoTrPAC, although predicted to be statistically underpowered for a genome-wide association study (GWAS), should

be able to be statistically organized and prioritized (Cantor et al., 2010) so that there is potential benefit from the orthogonal measurements assessed through other 'omes, leading to improved mechanistic insight. Such information can begin as a knowledge base for enabling better treatment considerations for a variety of diseases (whether acute or chronic) through recognizing potential genetic and epigenetic differences in responses to exercise and training. This could be accomplished through identifying novel gene/genetic network involvement, their corresponding changes in RNA transcripts and how such genes are regulated at the epigenetic level from adult and adolescent and between athletic and sedentary individuals, and associated sex differences in response to acute and chronic exercise.

The goal of the GET assays are to map and measure changes in the (1) RNA transcriptome and transcript isoforms including small and micro RNA using RNA sequencing, (2) DNA methylation and chromatin accessibility from rat and human tissues using reduced representation bisulfite (RRBS) for rat or methyl CpG hybrid capture for human specimens and ATACseq (assay for transposase-accessible chromatin with sequencing), respectively, and (3) genomic sequence and structure of all human participants. The assays are expected to provide insights into changes in biological processes as well as gene regulatory networks that occur in response to acute and chronic exercise. The GET assay component of MoTrPAC will involve comprehensive analyses of extensively curated rat and human MoTrPAC samples with an exercise intervention, contribute these data to public databases, help identify candidate molecular transducers, elucidate new mechanisms that might explain the human response to exercise, and cooperate with the Bioinformatics Center (BIC) to develop predictive models of the individual response to physical activity.

Proteomics

Proteins are important drivers of cellular structure, function, and signal mediation (Cox and Mann, 2011); thus, uncovering the pathways through which physical activity influences health requires analysis of the proteome and the critical signaling-associated posttranslational modifications of the proteome in various tissues. To date, a number of proteomic studies have shown important changes influenced by exercise (Burniston, 2008; Hoffman et al., 2015; Magherini et al., 2012; Sollanek et al., 2017). The majority of this work has focused on skeletal muscle, which is the tissue that actively performs the motions involved in exercise, and blood and plasma, which circulate signals systemically through the body and may be responsible for facilitating crosstalk between organ systems. Furthermore, this research is often performed in the context of diabetes because of the role of muscle and the interplay of exercise with insulin-resistance (Kleinert et al., 2018). Although these studies have largely been constrained to experimental models of exercise in animals or very small cohorts of human subjects, the results are tantalizing and have identified several proteins and signaling molecules that potentially play a key role in the response to exercise. The largescale and well-controlled preclinical and clinical protocols adopted for MoTrPAC will allow for expansion of this knowledge by providing a deeper interrogation of the proteomic response to acute and chronic exercise in numerous tissues from individuals across a range of fitness levels.

Importantly, proteomic analyses should be inclusive of not only protein expression but also the state of protein posttranslational modifications, such as phosphorylation or acetylation, because these chemical moieties can act as rapid integrators by dictating protein localization and enzymatic activity (Brandes et al., 2009; Choudhary et al., 2014; Emmerich et al., 2011; Hunter, 1995). Primarily, untargeted mass spectrometry methods and targeted aptamer-based detection techniques will be employed to probe changes in protein abundance and modifications induced by exercise. Given that distinct tissues present technological challenges to discovery-based proteomic analysis (e.g., dynamic range in skeletal muscle), state-of-the-art instrumentation and protocols, including tandem mass tag labeling and fractionation (Mertins et al., 2018), will be employed. Indeed, pilot discovery-based proteomics efforts with muscle

and other tissues have yielded robust datasets with levels of protein coverage exceeding previous studies, presenting a wealth of opportunities to elucidate proteomic response to exercise and integrate these findings with data obtained from GET and metabolomic studies of the same tissues.

Metabolomics

Complementing genomics, transcriptomics, epigenomics, and proteomic studies, MoTrPAC will also carry out a highly comprehensive mapping of exercise-associated alterations in the metabolome of both rats and humans. The metabolome is the total collection of biologically active small molecules in a given organism (Nicholson and Wilson, 2003). This includes endogenous molecules that are biosynthesized by metabolic networks in primary metabolism, molecules derived from diet or environmental exposures (the exposome; Wild, 2005), and molecules derived from the biosynthetic interactions with the microbiome. Metabolomics can either be ''targeted'' to a set of known compounds (e.g., certain acylcarnitines) or ''nontargeted,'' which attempts to detect and relatively quantify as many metabolites as possible (Dettmer et al., 2007). In the context of acute and chronic exercise, metabolomics can provide sensitive and dynamic phenotypic patterns that closely reflect cellular and molecular changes and will likely improve our understanding of the effects of exercise beyond the individual pathway level (Heaney et al., 2017).

A number of studies have documented profound metabolomic alterations associated with exercise (Fukai et al., 2016; Heaney et al., 2017; Lewis et al., 2010; Xiao et al., 2016), but these typically involve smaller cohorts (n < 100), are limited to only one (or a handful of) metabolomics assays, or focus primarily on alterations in energy production pathways. The vast chemical diversity of the metabolome, which includes lipids, sugars, amino acids, and myriad other molecule types, and its wide dynamic range (subnM to mM) implies that no single chemical assay can adequately profile all metabolites in one experiment (Smilde et al., 2005). To this end, MoTrPAC will employ a combination of nontargeted and targeted approaches for mapping the broader effects of exercise on both the metabolome and lipidome. These will range from triple-quadrupole-based liquid chromatography-mass spectrometry (LCMS) using stable isotope-labeled internal standards for absolute quantification to high resolution MS and tandem MS using reversed phase and hydrophilic interaction LC for mapping relative changes in both known and unknown molecular transducers. Targeted and nontargeted LCMS assays that focus on the nonpolar fraction of the metabolome (the lipidome) will also be leveraged to map exercise effects on lipid metabolism and oxidation (Nieman et al., 2013, 2014). It is expected that these studies will provide insights into energy metabolism and signaling molecules involved in the response to exercise.

Exosomes

Exercise is a potent stimulus that has broad ranging systemic effects that are indubitable (Egan and Zierath, 2013). One prevailing hypothesis is that circulating extracellular vesicles termed exosomes play an important role in carrying training-induced protein, mRNA, and microRNA (miRNA) cargo between organs as a means of integrating responses to exercise (Safdar and Tarnopolsky, 2018)(Figure 4). Many techniques have been developed for isolating exosomes from plasma to analyze their molecular cargo (Barrachina et al., 2019). Importantly, these techniques have been used to demonstrate that acute exercise increases the abundance of a wide variety of exosome-associated proteins related to metabolic and immune regulation (Whitham et al., 2018). Exosome isolation and analysis of MoTrPAC specimens will further investigate these effects by describing how exosome content is modulated in response to endurance and resistance exercise. The identification of protein and RNA signatures associated with exercise will shed light on exosome-mediated interorgan crosstalk and provide a framework for studies to characterize the systemic response to physical activity.
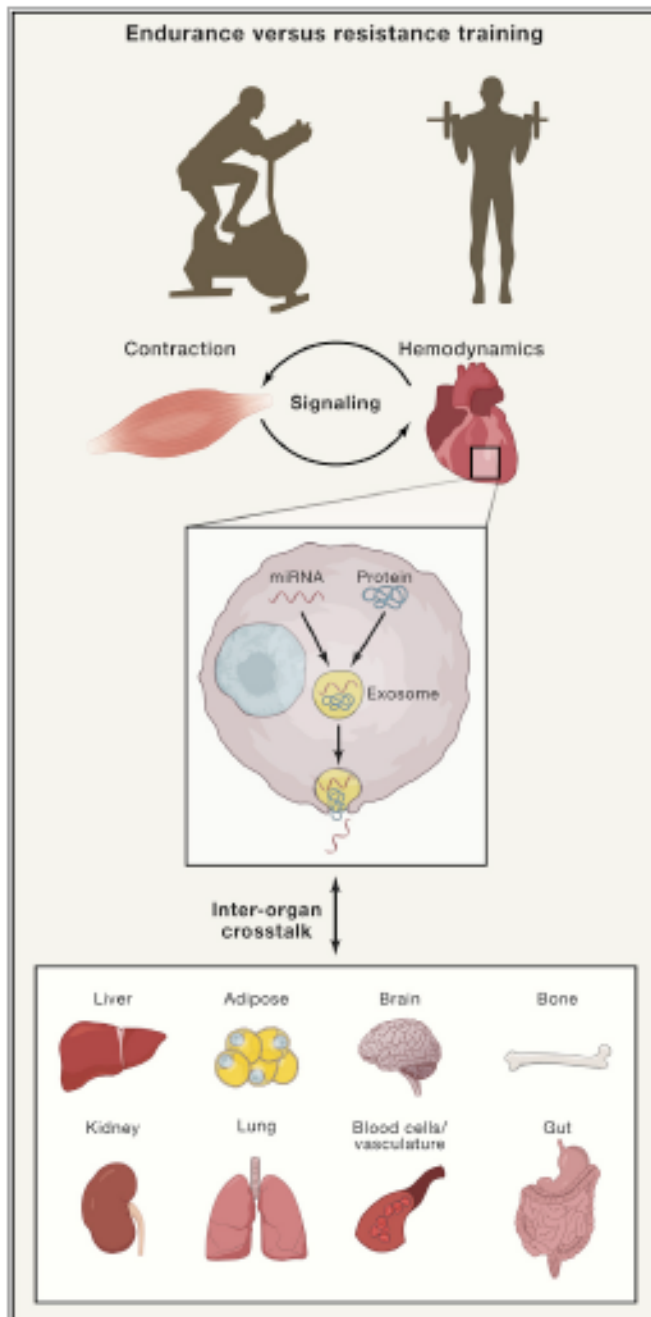
Figure 4. Role of Exosomes in Integrating the Exercise Response across Organ Systems

Exosomes are small extracellular vesicles (EVs) that are packaged with functional molecular cargo and released from most cell types. Exosomes released in response to exercise can be transported in the blood to various other tissues (adipose, brain, liver, etc.) where their contents can be released and have biological impact. Protein and miRNA cargo of circulating EVs from MoTrPAC samples will be analyzed to provide additional insight into this emerging biological phenomenon. Figure design by Jill K. Gregory, Mount Sinai.

Bioinformatics Center

The immediate goals of MoTrPAC will be vested in the 'omic platforms used and the data being generated, the quality of this data, both meta and experimental, and how it will be utilized to map the molecular transducers involving the responses to acute and chronic exercise. Data from each assay will be collected at the BIC and analyzed using consistent bioinformatic and analytic pipelines, whenever possible. This will improve reproducibility, interpretability, and ease in data harmonization across sites. Assay-specific quantitative data will undergo quality control assessment and be normalized to reduce undesirable sample-to-sample variation, minimize batch effects, and deal with analyte heteroskedasticity typically observed in molecular abundance datasets. Relative levels of analytes will be determined and the changes in molecules and pathways in response to exercise deduced.

Investigators across the consortium will conduct a series of integrative analyses with the end goal of creating a map of molecular transducers of physical activity and understanding the dynamic biological changes that occur in response to acute and chronic exercise (Figure 3). Unbiased statistical analysis will be performed to understand the variability in the data, thereby revealing significant differential responses possibly interacting with other clinical variables (e.g., sex, age, and VO_2max). Systems biology methods and network algorithms will be used to correlate the different 'omics and provide interpretable modules, focusing on regulatory processes. This will include basic interpretation of differential abundance patterns via network and pathway enrichment analyses. Additional analysis will be performed to understand the correlation structure between 'omics, detecting latent subject classes with different temporal responses to exercise, and network propagation techniques to highlight tissue-specific response (Amar and Shamir, 2014; Amar et al., 2015; Cowen et al., 2017; Gallant et al., 2013; Hofree et al., 2013; Jo et al., 2016; Schulz et al., 2012). In addition to identifying differential molecular responses and their associations with physiological changes (e.g., molecules and pathways that are associated with VO_2max), MoTrPAC will attempt to build predictive models of the effects of exercise on change in these parameters. Plans are in place to study the effects of age, sex, race, and exercise type on these changes. To enhance interpretability, the results from all analyses above will be summarized using multiple visualization techniques.

Data and Resources Dissemination

As an NIH Common Fund Program, MoTrPAC aims to provide a foundation for further research to be used by the broad biomedical research community. The goal is to share all MoTrPAC data that does not compromise personal health information (PHI) for general research use. All of the produced raw and processed data, analysis pipelines, and results will be made rapidly available to the scientific community through the MoTrPAC Data Hub (https://motrpacdata.org) and, whenever suitable, in the appropriate public repositories (e.g., Metabolomics Workbench for metabolomics data and Database of Genotypes and Phenotypes for sequencing data). We plan to follow the FAIR (findable, accessible, interoperable, and reusable) data principles (Wilkinson et al., 2016) to ensure that the data are widely available to and usable by the scientific community. In addition, we will be collaborating with the Common Fund Data Ecosystem (https://commonfund.nih.gov/dataecosystem)teamontools,techniques, and external user training to maximize the utility of the MoTrPAC data to the scientific community. The MoTrPAC Data Hub itself is a cloud-native application which uses a service-oriented architecture. It is being hosted on the Google Cloud Platform. In addition to providing direct access to data, there will be tools for customizable web-based data visualizations provided on the site.

Scientific Opportunities/Ancillary Studies

Although many activities are occurring within MoTrPAC, there will be opportunities to expand the breadth and depth of MoTrPAC. These opportunities include (1) additional interrogation of clinical metadata derived from animal and human studies and through the 'omics applications and the interpretation of the data beyond what has been proposed and approved through the main MoTrPAC study, (2) addition or expansion of data collection to the current parent protocol, and (3) accession of the biorepository for remaining biospecimen samples obtained from the animal and human studies to perform complementary analyses on these tissues. The MoTrPAC Ancillary Study policy and proposal template are available at https://motrpac.org/ancillarystudyguidelines.cfm.

Study Challenges

There are many challenges associated with a large multicenter project generating a wide variety of data types. Standardization procedures, operation manuals, and quality control steps are in place to reduce the variation in exercise performance and evaluation and sample collections of the animal and human samples. Participant and clinic burden are a concern given the large scope and complexity of MoTrPAC; the consortium has made strategic choices regarding the protocol design and biospecimens sampling to help mitigate these challenges. Similarly, standardization and quality control steps are in place for the data generated using multiple 'omics platforms even for similar data types (e.g., metabolomics and proteomics). An initial implementation phase (described above) was also put in place for the human studies to further evaluate the adult protocol during the early stages of recruitment, data collection, and analysis to identify any unforeseen issues.

Integrating heterogeneous data types across MoTrPAC will require sophisticated tracking, data normalization, and analytic approaches. MoTrPAC will generate large amounts of data, and many measurements may not meet statistical significance at the individual molecule level but may do so at the pathway level. State-of-the-art analytic tools are in place to help manage the depth and breadth of data analysis, and these tools will continue to evolve as MoTrPAC progresses. Finally, the data are complex; ensuring that they are accessible and understandable to the broad scientific community in a timely fashion is essential for this project to be successful. Protection of participant clinical data (PHI, genomics data) will be given the highest priority to protect each individual's identity. Incorporation of useful visualization tools as well as active engagement with the broader scientific community will be equally important to fully capitalize on the MoTrPAC project.

Summary

When complete, MoTrPAC will deliver a map of the biological molecules and pathways underlying the systemic effects of acute and chronic exercise. The data, which will ultimately be made freely available to the scientific community, will provide unprecedented opportunities to begin to understand the pathways by which physical activity influences health. In the future, it is expected that the knowledge gained will allow researchers and health professionals to develop personalized exercise recommendations and provide insights into molecular targets that could be manipulated to mimic some of the effects of exercise in persons unable to do so.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j. cell.2020.06.004.

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