# Translational physiology in practice

*'Knowing is not enough; we must apply. Willing is not enough; we must do'*
Johann Wolfgang von Goethe

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Translational research has become the preferred model worldwide for organizations supporting biomedical science. As a field, we should look for opportunities to integrate translational approaches into physiological research to most effectively gain insight into mechanisms of interest, study relations between genotype and phenotype, establish the efficacy of interventions, and maximize the impact of our work on public health.

'Fortunately, many functions can now be modelled and studied using translation-friendly techniques in preclinical models and human subjects'

Translational research has received a lot of attention in some corners of the biomedical research community over the past decade. Traditionally, translational research has been viewed as a process in which experimental observations made at the basic science level using preclinical models (cell culture and animal models) are eventually tested in humans. However, contemporary views of translational research emphasize a dynamic 'continuum' of observations from basic science to the clinical research setting ('T1 translation'), then to medical practice/clinical guidelines ('T2 translation') and, finally, to the community, where the findings serve to inform public health policy ('T3 translation') (Fig. 1). This process should operate *bi-directionally*, i.e. observations made initially in populations or in medical practice can be 'reverse translated' to gain insight into mechanisms of action via controlled studies performed in a clinical research centre or basic research laboratory (Fig. 1). Because research at these various levels requires different skill sets, personnel and infrastructure, it often involves multi-disciplinary collaborations. The importance of translational research is reflected by the recent establishment of the new translational offices and programmes within biomedical research organizations in the EU and worldwide, including the National Center for Advancing Translational Science in the US. (www.ncats.nih.gov).

There is a growing demand for translational research in physiology. In the following sections, we discuss this important need and how our laboratory approaches translational physiological research.

## Translational physiology

Physiology, the study of function of living organisms, can be effectively investigated using translational strategies. Indeed, since a pivotal initial commentary by John Hall in 2002 (Hall, 2002), several editorials have emphasized the need for translational approaches in physiology. What wasn't clear in these commentaries, however, was exactly *how* to apply translational research practices in physiological research. To help address this question, we recently published a full-length perspective on the topic (Seals, 2013). In that article, we advanced the concept of translational physiology as a framework to study function from molecular events all the way up to informing public health policy, with the ultimate goal of attaining optimal physiological function in populations of both healthy adults and patients with clinical disorders (Fig. 2). Key concepts, experimental approaches, opportunities, roadblocks and

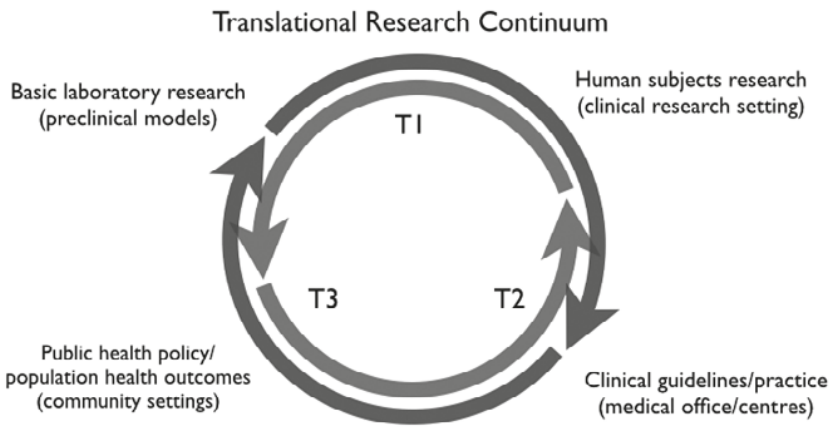


*Figure 1.* The dynamic and bi-directional translational research continuum. Observations from basic science (T1) can be translated to the clinical research setting and eventually applied in clinical practice (T2) and public health policy (T3). Alternatively, T3 or T2 observations may be used to drive discovery or investigations of underlying mechanisms at the T1 level. Reproduced from Seals (2013).

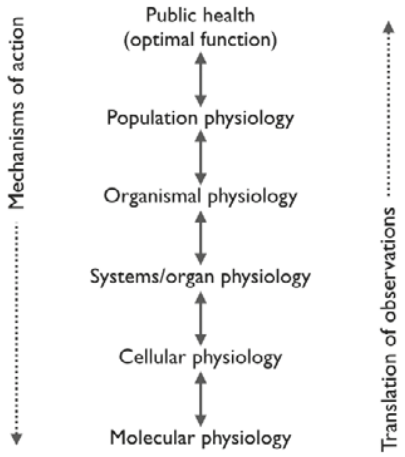


*Figure 2.* A framework for translational physiology. Physiological function can be studied from molecular events to public health with the goal of optimizing function in healthy adults and patients with clinical disorders. Reproduced from Seals (2013).

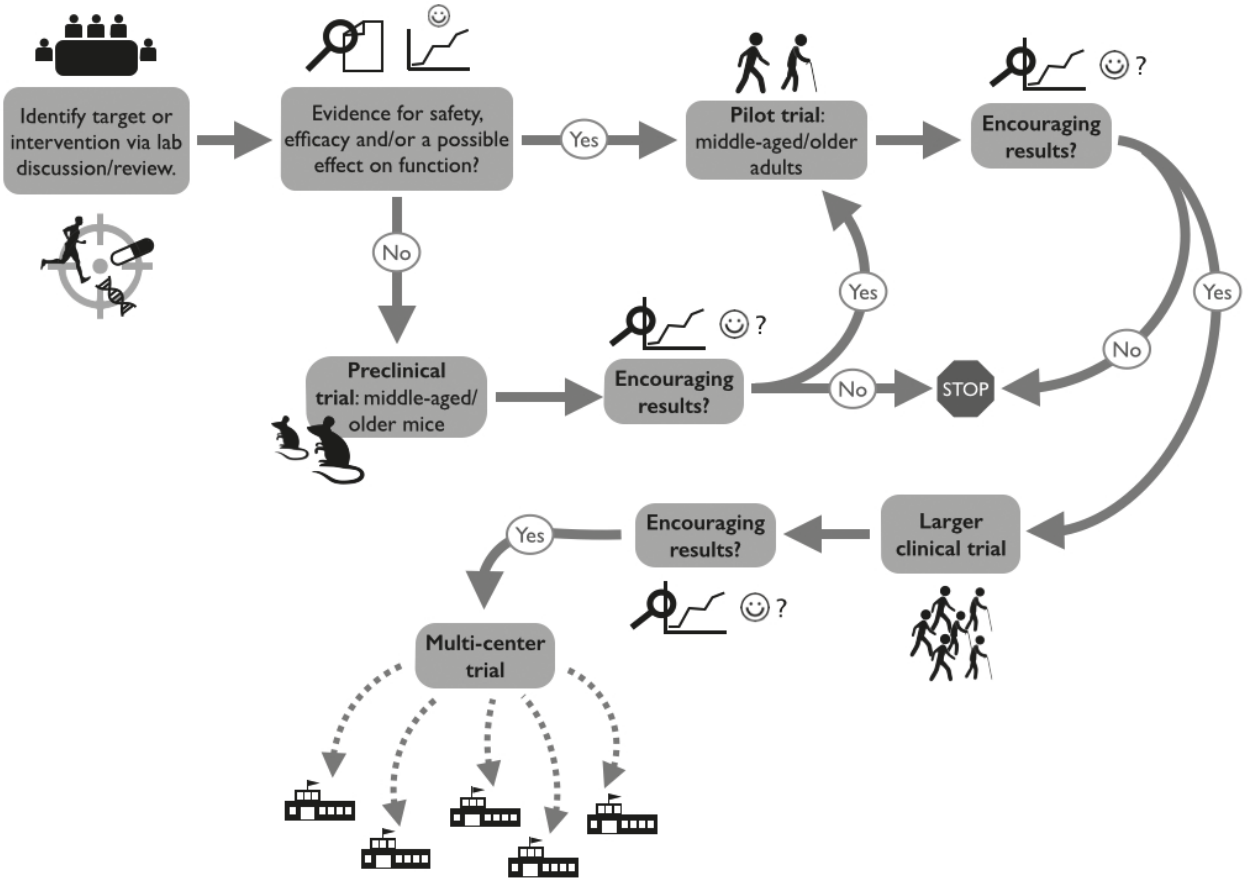


*Figure 3.* One possible approach to translational physiology. We identify potential targets or interventions that may improve physiological function during ageing, and evaluate them for translational potential. If they appear promising, we may proceed directly to pilot clinical trials; otherwise, we might first test for efficacy in preclinical studies in mice. In either case, experimental results are used to inform our decision about how and if to proceed to higher levels of translation, with the ultimate goal of identifying therapies and interventions with the potential for broad application.

‘Translational physiology offers new opportunities for studying function from molecular events to populations of humans, with direct relevance to clinical practice and public health’

strategies for success all were discussed in some detail.

As noted in our paper (Seals, 2013), several compelling examples of successful translational investigations in physiology already exist, one of which is the study of dietary sodium intake and arterial blood pressure. Following the discovery of a relation between dietary sodium and blood pressure in human populations in the early 1960s, a series of ‘upward’ translational investigations were performed culminating in studies of dietary sodium restriction for reducing blood pressure in various groups. These findings led to the establishment of new clinical guidelines for daily sodium intake, as well as to changes in public health policy in some countries to reduce sodium in processed foods. At the same time, the epidemiological observations stimulated *reverse* translational studies of mechanisms underlying the effects of sodium intake on blood pressure in both clinical and preclinical models. Thus, over the past 50+ years, the full translational scope of investigation from T1 to T3 has been conducted on this clinically relevant issue in physiology.

## Unprecedented opportunities

Today, there are unprecedented opportunities to conduct translational physiological research on a large scale. The ability to manipulate genes and signalling pathways in cells and experimental animal models has revolutionized basic research in biology, including physiology. Similarly, recent developments in high throughput molecular analysis (‘omics’) and systems biology, combined with the wide availability of samples from human subjects, is creating new horizons for conducting investigations in population physiology. With these novel technologies, we are in a position to bridge key gaps in translational research by combining insight from several domains of investigation, including mechanisms of physiological function and dysfunction, variability in subjects’ baseline function and responses to stress/intervention, and the relations between genotype and phenotype (Seals, 2013). Utilizing new developments in molecular analysis of biological samples and non-invasive physiological monitoring, along with established observational approaches, will allow us to extend investigation of physiology from cells, tissues and individual organisms to populations of humans. In so doing, we can expand the traditional boundaries of clinical epidemiology (population health and disease) to ‘physiological epidemiology’ (population function) (Seals, 2013). Extending our scope of study to populations also will provide opportunities to better determine how the environmental factors to which we are chronically exposed (aging, education, income, social networks, culture, pollution, etc.) influence human physiology, functional status, disease risk and mortality.

## Translational physiology in practice: assessing interventions to improve function

One important application for translational research, including translational physiology, is the need to develop new strategies to prevent and treat clinical diseases and delay adverse ageing processes. Our laboratory, for example, is interested in optimizing and preserving physiological function during adult ageing with the ultimate goal of extending healthy lifespan (‘healthspan’). The need for this work is reflected by the strong interest in the biomedical industry to advance drugs for treating age-associated diseases. This is an extensive process requiring efforts throughout the translational continuum, from identifying promising signalling pathways, developing screening tests for compounds that may activate (or inhibit) those pathways, screening numerous compounds, and eventually testing for safety and efficacy in preclinical models and then in humans (Collis, 2013). Conventional drug development increasingly has become a prohibitively lengthy and expensive process, involving resources far beyond the scope of academic physiology laboratories. As such, we have adopted our own translational physiological research approach for establishing effective strategies to preserve function with ageing. Most of our work over the past decade has involved assessing the effectiveness of various interventions for improving vascular function in late middle-aged and older (MA/O) adults with baseline dysfunction. We test the efficacy of both healthy lifestyle behaviours and novel nutraceutical compounds with potentially healthy lifestyle-‘mimicking’ effects for enhancing physiological function with ageing. To accomplish this, we employ a variety of translational approaches, beginning with the original idea and advancing to clinical trial testing.

## One laboratory’s approach

Our approach begins by identifying lifestyle behaviours (most often, exercise, energy intake or diet composition) or pharmacological compounds that might improve function during ageing (Fig. 3). If there is sufficient evidence for safety and possible efficacy (i.e. improvement in function) in the scientific literature, the intervention can be directly tested in MA/O adult humans. If not, preclinical studies are performed in MA/O mice to obtain such evidence. If the results are encouraging, we then develop protocols for testing the treatment in human subjects.

In our work, ideas for investigation are based on identifying possible therapeutic targets and interventions that may influence (activate/inhibit) those targets. There are

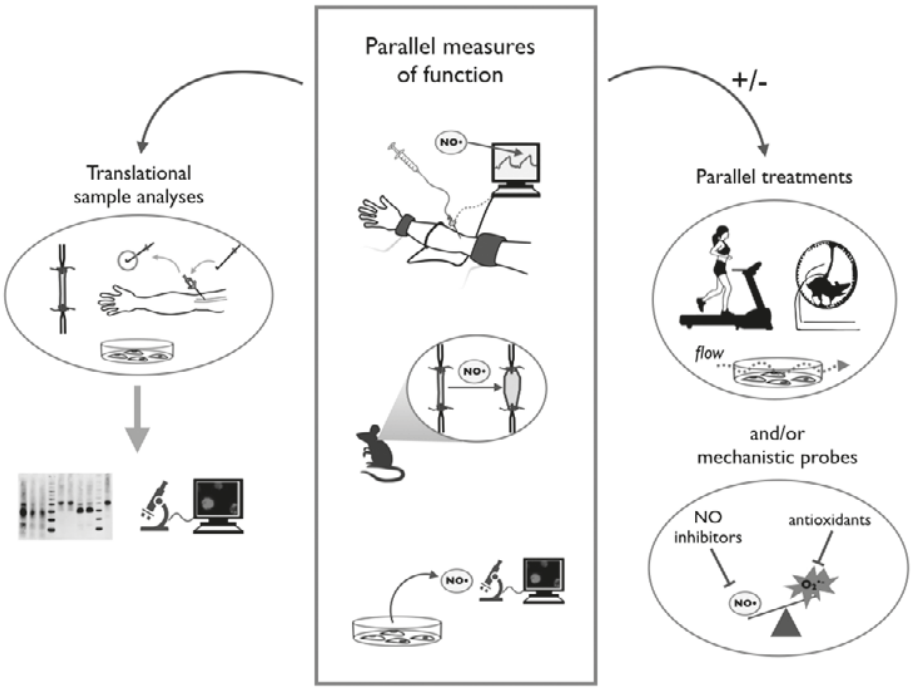


*Figure 4.* Translational research techniques: an example. Middle panel, top to bottom: vascular function mediated by nitric oxide (NO) can be measured in human subjects (acetylcholine[ACh]-induced increases in forearm blood flow), in arteries isolated from mice (Ach-induced carotid artery dilation), and in vascular cell culture (Ach-induced NO production). Left: In all three models, cells or biological samples can be isolated and analysed using similar techniques (biochemical protein analyses, microscopy, etc.). Finally (right), treatments or interventions (e.g. aerobic exercise, which can even be simulated even in vascular cell culture) can be studied in all models, and mechanisms underlying observed differences in function can be dissected using pharmacological agents (e.g. NO inhibitors or antioxidants that scavenge superoxide [$O_2^-$]).

several sources for ideas. We draw heavily from the basic science literature. Indeed, although we are considered a translational research laboratory, most of the papers discussed in our weekly laboratory meeting/journal club involve basic research, both original research articles and reviews. We attempt to identify, early on, novel molecular/cellular signalling pathways that modulate our physiological function of interest, as well as compounds that could be used to induce the desired effect on those pathways and improve function. In many cases, the signalling pathway of interest may not have been shown previously to influence function *per se* (e.g. improve vascular endothelial function), but rather to alter a key determinant of that function (e.g. increase nitric oxide [NO] bioavailability). This approach requires constant surveillance and assessment of the translational potential of a dynamic, continually expanding scientific literature based on genetic and molecular biological observations, i.e. a literature that often supersedes our technical expertise and intellectual comfort zone. Nevertheless, we have found basic research to be a rich source of ideas for innovative translational studies. To facilitate this process, we seek students and postdoctoral fellows from a range of backgrounds: biochemistry to clinical research to the pharmaceutical industry. Once in the laboratory, trainees become informed on topics of interest from a basic science perspective up to community health, and develop the research and intellectual skills necessary to conduct their work using translational research principles.

In the end, potential therapeutic targets and interventions can be identified from observations made at the preclinical, clinical or even *epidemiological* (population) levels. Referring to the earlier example, the epidemiological finding of a relation between dietary sodium intake and blood pressure led to trials testing the efficacy of dietary sodium restriction for lowering blood pressure in patients with essential hypertension (Mozaffarian *et al.,* 2011). Similarly, clinical trials and mechanistic investigations on the potential benefits of omega-3 fatty acid supplementation were triggered by epidemiological observations in native Eskimo populations (Mozaffarian *et al.,* 2011). Ideas also can be generated from findings not directly related to the function of interest. In our work in vascular ageing, we may explore the efficacy of an intervention that has been shown to improve insulin resistance in patients with type 2 diabetes mellitus, or extend lifespan in *C. elegans* or other basic models of ageing. The essential question in these situations is whether or not the putative treatment likely influences a key biological process modulating the target function.

When considering a possible intervention stemming from work in preclinical models, it is important to consider the feasibility for translation to humans. Is there a synthetic or natural version of the compound that can be delivered to human subjects at a dose and for a duration that would be both safe and effective? For physiologists interested in improving function in humans, determining the regulatory/approval status of the agent also is important to consider. In our laboratory, if there is no obvious compound that is either already approved for use in humans or that could be approved by an institutional review board in a timely manner, generally we do not expend the time, effort and resources to conduct preclinical studies on that agent. The ultimate goal must be to test efficacy in trials on humans because of the high historic rate of 'false positive' findings when studies in preclinical models are tested in humans. Many basic science laboratories establish preclinical evidence for a therapeutic target and, in some cases, a possible treatment compound, and then move on to the next potential target. However, full translational assessment of a treatment to improve and/or preserve physiological function requires establishing efficacy in human populations.

Fortunately, many functions can now be modelled and studied using translation-friendly techniques in preclinical models and human subjects. In our work, similar measurements of vascular function (including endothelium-dependent dilation and large elastic artery stiffness) can be made in mice, physiological studies of small groups of healthy adults, and larger clinical trials (Donato *et al.,* 2011; LaRocca *et al.* 2012; Seals, 2013). The same is true for muscle function, glucose–insulin signalling, metabolism and many other areas in physiology. Integrative motor function assessment batteries used in physiological, clinical and epidemiological investigations in humans can be modelled in rodents (Justice *et al.,* 2014), and continuous *in vivo* monitoring of numerous physiological variables can be conducted using telemetry in animal models and contemporary sensor technology in human populations.

## Translational insight into mechanisms of action

Several options are available to investigate mechanisms of action using translational approaches. As an example (Fig. 4), in an initial study of a potential therapy for vascular ageing using our mouse model, function in arteries from treated and untreated animals can be assessed *ex vivo* in the presence and absence of NO production (using inhibitors of NO synthases), superoxide bioavailability/oxidative stress (using antioxidant compounds or inhibitors of oxidant enzymes), or the treatment compound itself (Lesniewski *et al.,* 2011; Fleenor *et al.,* 2012). Biochemical characterization can be performed on vascular tissue from the same animals, with or without further *ex vivo* treatments. Similarly, in human subjects, local arterial infusion of NO synthase inhibitors, antioxidant compounds (vitamin C) and other agents can be used to 'pharmaco-dissect' the mechanisms influencing vascular function, and endothelial cells from arteries or veins can be obtained for further treatment and biochemical analysis (Shenouda *et al.,* 2011; Jablonski *et al.,* 2013; Kaplon *et al.,* 2013). Moreover, plasma, whole blood, and circulating blood cells can be assessed for clues regarding the molecular events involved using either conventional or newer high-throughput analyses. Finally, complementary cell culture experiments involving genetic and molecular manipulation of pathways of interest can be performed to provide more direct cause and effect evidence for the role of particular processes in the functional changes observed (LaRocca *et al.,* 2012; Shenouda *et al.,* 2011).

## Integrative model

Using a combination of the translational approaches described above and highlighted in Fig. 3 and 4, we have developed an *integrative model* for studying the effects of various factors on physiological function (e.g. ageing), the effects of treatments to improve function, and the underlying mechanisms involved. When data in humans are not available to support an initial hypothesis, we perform preclinical studies in mice *using assessments of function that are directly translatable to humans*. Whereas a pilot study to obtain preliminary data in human subjects might take multiple years given present regulatory approval procedures, this can be done within a matter of months and at a fraction of the expense in mice. These preclinical studies also can help to guide safety, dosing and study design components for a first trial in human subjects, and may provide insight into mechanisms of action, for which protocols and measurements can be integrated into the planning for the clinical trial.

Based on these preclinical results, an initial 'pilot' trial can be conducted in healthy adults with the physiological dysfunction of interest (vascular dysfunction, insulin resistance, impaired exercise capacity or motor function, etc.) to assess both safety and efficacy of the proposed intervention (Fig. 3). This initial study can provide preliminary results from which to determine the number of subjects needed to achieve statistical power in subsequent studies, and may offer early insight into mechanisms of action. A larger trial can then be conducted and, if the results are confirmed, an experimental basis can be created for a multi-centre clinical trial studying high-risk adults or patients with diagnosed clinical disease. Using preliminary data from each of these steps, grant applications can be developed in parallel with this investigative model.

Several alternative approaches exist. For example, the process may begin with cell culture experiments involving screening of compounds aimed at a particular target pathway, leading to testing in animal models, and so forth, as is common in the development of pharmaceuticals (Collis, 2013). In a different context, if preclinical studies are not possible and the intervention appears safe, initial evidence supporting an effect in humans might be obtained using acute administration or short-term treatment combined with a crossover design (subjects serving as their own controls) rather than using a longer treatment period. Of course, an absence of treatment effect with acute/short-term approaches could be due to insufficient treatment duration, requiring conducting the longer intervention trial you sought to avoid in the first place! Finally, in some cases, preliminary evidence supporting an intervention trial can be obtained from cross-sectional comparisons of function in groups of humans who chronically differ in the factor of interest (physical activity, dietary sodium intake, nutraceutical or pharmaceutical use, etc.).

## Conclusions and challenges

Translational physiology offers new opportunities for studying function from molecular events to populations of humans, with direct relevance to clinical practice and public health. Translational approaches allow important questions to be answered more completely, and offer a potential funding advantage in the present environment in which peer reviewers are being asked to weigh the biomedical significance of the proposed work in order to differentiate among many meritorious applications. Although greater implementation of translational strategies in physiology will require overcoming numerous challenges (Seals, 2013), such efforts hold considerable promise for increasing the societal impact of our science and our competitiveness for extramural grant support.

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