human experimentation: no accurate, quantitative data?

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this viewpoint addresses two issues in comparing data from humans and animals in studies of cardiovascular physiology: 1) accuracy of methods and 2) limits in extrapolating data from one species to another. you are invited to submit a brief commentary on this viewpoint, which will be reviewed by journal of applied physiology editors for possible publication in the journal. please limit your comment to 250 words and 5 peer-reviewed published references.

in a recent point:counterpoint debate in the journal of applied physiology, hainsworth and drinkhill (5) state: “unfortunately, as it is not possible to obtain accurate quantitative data from humans, we have to rely on results extrapolated from animal studies, mainly on dogs and cats.” the purpose of this viewpoint, which takes hainsworth’s unqualified comment at face value, is to rebut this notion. understanding of human physiology indeed rests in part on what we have learned from other species, but a great body of knowledge comes from studies performed on human subjects that entailed measurements as accurate and quantitative as those possible in work with animals.

extrapolation as a means of learning about one species from another has its limits (and dangers)—we would have little luck in understanding the problems for cardiovascular regulation in the giraffe from studies on dogs. no amount of extrapolation would have revealed particular features of human physiology that set this species apart. examples in cardiovascular physiology include the special hemodynamic problems that accompany changes in posture and exercise in humans. the uniqueness of human skin, an organ of temperature regulation, cannot be appreciated from studies of thermal balance in dogs or cats. nor can the overall problem of coping with the stress of prolonged exercise in humans be appreciated from studies with these laboratory animals.

the initial focus here is on some quantitative methods that have been employed in accomplishing our present understanding of human cardiovascular, metabolic, and thermal responses. these methods were key to resolving the dispute over species differences vs. methods.

methods of measurement

the advent of electromagnetic (em) and ultrasonic flowmetry led to dispute over different findings from animals and humans. one side saw problems as stemming from inaccuracies owing to “indirectness” and discontinuity of measurements from humans (13); the other side saw species differences as being primary (10).

determining accuracy for any measure of blood flow is difficult. k. l. franklin noted that “measurement of flow is difficult while that of pressure is easy, so our knowledge of flow is usually derivative” (cited in ref. 6).

in animals, we can place flow probes on major vessels. flowmeters permit continuous recording of instantaneous flow velocity, and in some cases flow, whereas the methods applied in humans were discontinuous and time averaged, putatively “indirect” (see below).

flowmetry—“direct” measures of flow?

ultrasonic and em flowmeters provided no more “direct” measurement of flow than the so-called indirect methods. the only direct measurements of flow were made when blood from a vessel was collected into a volumetric container over a timed interval. in 1894, g. n. stewart verified his dye dilution technique in this way. unfortunately, dynamic calibrations of flowmeters were initially unavailable owing to lack of high-capacity pulsatile flow pumps. later steady-state calibrations were made in vivo against the dye dilution method (2). mcdonald (6) noted substantial zero drift and also a deficient dynamic response in some flowmeters.

direct and indirect fick and dye dilution methods

as reviewed in refs. 10 and 14, simultaneous comparisons during the 1940s and 1950s of dye dilution and direct fick methods, which required catheterization, revealed no significant differences in cardiac output (co). improved rebreathing techniques (indirect fick) yield good agreement with dye dilution during exercise (8, 10, 14). all three measurements provided basically the same values for co in relation to oxygen consumption during rest and exercise, which eventually included maximal oxygen consumption, an objective measure of the functional capacity of the system to transport oxygen.

note that subject posture was a problem attending the original calibration of these three methods. catheterization for the direct fick measurements of co was most conveniently carried out with subjects supine, whereas rebreathing techniques were used with subjects seated on cycle ergometers. the dye dilution method was used in either supine or upright subjects. it took time to appreciate the large effects of posture on central hemodynamics. it is no surprise that disputes over methods were commonly divided according to whether the subjects were bipeds or quadrupeds (10).

precision of rebreathing methods was markedly improved by adding an inert reference gas to the rebreathing mixture and especially by application of mass spectrometry. precision of the dye dilution technique attended development of linear densitometers with adequate compensation for background dye
level and the discovery of a dye, indocyanine green, insensitive to changes in hemoglobin oxygenation. Recognition that spectral properties of blood change with posture, exercise, and temperature demanded multiple calibrations with precise dilutions of dye with appropriate blood samples. In 336 measurements of CO on six subjects, coefficient of variation was 4.7% (11).

**Central Blood Volume**

Nominally the volume of blood in the lungs and the cardiac chambers, central blood volume (CBV), is actually the volume of blood that is temporally equidistant from the sites of dye injection and blood sampling. If the catheters are placed, respectively, in the right atrium and aortic arch, then CBV calculated from CO and mean transit time (as tested in dogs) agrees with the direct anatomical measurement of this volume (see Ref. 11). In the 336 measurements in the six subjects mentioned above, coefficient of variation for CBV was 7% (11).

**Regional Blood Flow**

In humans, and to some extent in primates, redistribution of blood flow is an important adjustment to upright posture, exercise, and heat stress (9). In dogs it is not, except when cardiovascular function is diminished (13). Again, some attributed differences to species, others to poor measurement accuracy.

**Splanchnic blood flow.** Methods to measure flow and volume of this, the largest of regional circulations, were credited to Bradley and coworkers (see Ref. 14). Measurement was by dye clearance and extraction (Fick principle), but extraction (sampled via a catheter in an hepatic vein) was usually determined from only one of the four hepatic veins. However, simultaneous sampling from different hepatic veins in humans during rest and exercise revealed only small differences in dye concentration and thus in computed splanchnic blood flow (SBF; Ref. 7). Furthermore, in simultaneous comparisons of flows measured by dye clearance with 1) flows measured by EM flowmeters; 2) those obtained directly by timed measurements; and also those derived by different indicators with widely differing extraction coefficients all had good agreement (8, 14). With exercise, hepatic dye extraction rises ~8%, thus changes in clearance rate alone provide valid estimates of reductions in SBF (7).

Finally, the dynamic properties of the splanchnic system enable one to follow reliably rapid changes in SBF using arterial-venous sampling intervals of 2 min or longer (7).

**Renal blood flow.** Discussion of renal blood flow (RBF) is essentially a recapitulation of that of SBF, except a kidney is supplied by just one artery and one vein from which PAH clearance and extraction can be measured. Normally, PAH extraction is ~95% and remains the same as RBF falls in proportion to the severity of exercise (just as SBF), thus minimizing the need of continuing to measure extraction except in patients with heart failure and renal disease, which decrease extraction.

As with SBF, direct measurements (timed collections of blood) and simultaneous measurements by EM flowmeter and PAH clearance and extraction gave remarkably similar values during non-steady-state conditions caused by graded hemorrhage (see Fig. 2 in Ref. 8). Measurements of renal venous outflow in humans by thermal dilution yielded values that agreed exactly with measurements of RBF by clearance (plus extraction) methods during exercise by Grimby and by Castensfors (9; see Figs. 6–18).

**Muscle blood flow and cutaneous blood flow.** These organs afford no convenient measuring site like a single collecting vein, so the Fick principle cannot be applied. Clearance rates of injected isotopes and the technique, for skin, of transcaneous Doppler flowmetry have revealed relative changes in flow, but absolute flow unaccompanied by contributions from other tissues has not been readily obtainable except by microsphere techniques in animals.

Saltin’s group (1) developed a thermal dilution technique for measuring femoral venous outflow from human quadriceps during mild to peak exercise restricted to that muscle group. Any contamination from skin and inactive thigh muscle was minimal (during measurements the lower leg circulation was always occluded). The great value of these experiments was that they revealed for the first time the enormous potential for blood flow in human muscle when flow was not limited by the pumping capacity of our small hearts. Peak values for 1 kg of human quadriceps matched peak values from microsphere-based measurements in dog, horse, and pigs during whole body exercise (Chapt. 7 in Ref. 9).

The human cutaneous vasculature has the second biggest potential flow of the peripheral vasculature, in terms of the rise in CO that accompanies cutaneous vasodilation along with the consequential reduction of SBF, RBF, and muscle blood flow (MBF; Ref. 8). With venous occlusion plethysmography (VOP), total flow in one forearm [MBF + cutaneous blood flow (SkBF)] could be compared with flow in the other forearm, treated with epinephrine to reduce SkBF to zero. The findings supported using VOP as a quantitative means of determining SkBF increases insofar as the assumption that underlying MBF remained constant (as also indicated by isotope clearance from muscle; see Ref. 8) could be relied on (8). This approach enabled a comparison of total CO increase with VOP-based peak values per unit surface area of skin. The agreement was possibly fortuitous given the likelihood of significant regional variations in SkBF responses. Nonetheless, estimates that SkBF reaches peak values in humans far above values obtained via microspheres in other species (8) are supported. Also, estimated total cutaneous vascular volume during maximum vasodilation is close to that of the splanchnic region.

**SPECIES DIFFERENCES AND PROBLEMS CAUSED BY EXTRAPOLATIONS TO HUMANS**

Two major disputes concerning Starling’s Law of the Heart and active redistribution of blood flow pitted thinking of human physiologists and cardiologists against that of many (not all) animal physiologists. But the findings of both groups were valid: extrapolation of findings from dogs to humans caused major misunderstandings.

**Starling’s Law: Posture and Exercise**

The long battle concerning the applicability of Starling’s law to intact animals and humans was recently reviewed (10). A commonly held view was that, with exercise, CO is raised by
increases in heart rate (HR) and SV, but Rushmer and colleagues (11a) observed no increments in left ventricular volume and SV with voluntary exercise in dogs. They and others (10) concluded that Starling’s Law is applicable only to exposed or isolated hearts, not intact animals; CO is raised in response to exercise solely by raising HR and contractile force (no muscle pump). However, Guyton et al. (3) argued this was not possible; their attempts to raise CO by pacing were counteracted by falling central venous pressure (CVP) and SV owing to the flow-dependent redistribution of blood volume from central to peripheral vessels. Later, this was confirmed in intact dogs during rest and exercise by Sheriff et al. (12), and by Bevegard et al. (see Ref. 12) in humans.

So, why does SV not rise with exercise in dogs, whereas a 40% increase accompanies exercise onset in humans, remaining nearly independent of subsequent increases in work intensity? It took time for the pieces to come together. Before that, one group argued that the problem was the methods used in humans; others argued that it was species differences. The latters’ points were as follows.

Point 1. Dogs have ~70% of total blood volume at or above heart level and heart volume (and SV) are close to maximum owing to pericardial constraints (10).

Point 2. Upright humans have ~70% of their total blood volume below heart level, and 70% of this is in compliant veins; CBV, CVP, and SV are low until leg muscle contraction immediately restores these variables to supine resting values, i.e., increasing SV ~ 40% (3, 12).

Point 3. In both species, the near constancy of SV with increasing exercise intensity is abolished by cutting the pericardium. Both CO and SV are higher and exceed previous maximal values, thus unveiling the full effect of the Starling relationship (9, 10).

**Redistribution of RBF**

Many species vasoconstrict visceral organs if tipped upright, certainly humans do (9). The debate about regional vasoconstriction centered on exercise during which dogs show little or no vasoconstriction, whereas dogs do whether upright or supine (for reviews see, Refs. 10 and 13). The arguments pro and con were the same as those above.

Brief histories of how regional blood flow is affected by exercise are found in Ref. 10 and 14. In 1936, Grande and Rehberg (cited in Ref. 10) first quantified reductions in glomerular filtration rates induced by exercise. This was the forerunner of numerous measurements thereafter of RBF by PAH clearance during exercise, heat stress, etc. All showed progressive and marked reductions in RBF relative to exercise intensity, best related to HR and sympathetic nervous activity. The story from humans of both RBF and SBF can best be told by Figs. 6–3 and 6–18 in Ref. 9. These figures show data from hundreds of measurements that show that SBF and RBF decrease as HR rises during exercise: 1) in neutral and hot environments; 2) before and after physical conditioning; 3) with different muscle groups. The slopes, intercepts, and r values are all basically the same, even including data from four different laboratories [all intercepts for resting data are shifted leftward (Figs 6–3 from Ref. 9)].

Others have confirmed some of these changes in humans by transcutaneous ultrasonic methods. Also, norepinephrine spill-over and plasma rennin activity, indexes of sympathetic nervous activity, all rise in close inverse proportion to blood flow (Chapt 6, Ref. 9). Tidgren and colleague’s (Chapt 6, Ref. 9) measurements of renal venous renin, norepinephrine, and neuropeptide Y also show the close correspondence to flow reduction. All of these measurements were closely parallel to level of sympathetic activity and HR.

A series of studies by Rushmer and his colleagues (for review, see Ref. 13) during the 1960s that applied EM and ultrasonic flow probes to a renal and a mesenteric artery in dogs and, later, primates found no decrements in SBF or RBF even during severe exercise.

The misunderstanding caused by extrapolating these results from dogs to humans was substantial. Vatner (13) resolved the argument by showing that surgical impairment of oxygen delivery in the dog (which normally has a threefold greater cardiac pumping capacity per kilogram than a human) produced a humanlike redistribution of CO during exercise.

**Human Temperature Regulation**

It suffices to say here that temperature regulation in humans during heat stress at rest or during exercise is so fundamentally different from that of other species that extrapolation from animals has no meaning (8). Humans’ copious sweating capacity coupled with a uniquely potent active vasodilator mechanism leads to a dramatic shift in blood volume from a hot core to a cooler periphery (Fig. 1). This shift of flow and volume provides remarkable thermoregulation while at the same time putting great demands on the cardiovascular system, which must meet competing demands for oxygen transport (8, 11).

**SUMMARY**

Many investigators have carefully examined the methods, especially invasive ones, used on humans and have devoted much time calibrating and testing them, sometimes on their-

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**Fig. 1. Schematic illustration of redistribution of blood volume in a heat-stressed upright human. In contrast, dogs and most species have small cutaneous vasodilation and volume redistribution. In dogs, hydrostatic effects are small. Vasodilation occurs primarily in the tongue. Reproduced from Ref. 8.**
selves. To paraphrase Hainsworth (4), the accuracy or precision of any method depends on the skill of the operator and the cooperation of the subject (in itself a major species difference). It is true that Hainsworth’s (and Rothe’s) skillful means of quantifying active as opposed to passive changes in venous volume in animals are too invasive to be applied to humans; nevertheless they contribute to our basic understanding of venous function in all species.

The methods described in this viewpoint were central to the debate on whether basic differences in responses of humans vs. dogs were due to species differences or to deficient methods used on humans. These disputed methods are still basic to cardiovascular physiology having worked well over decades on humans and animals. They are quantitative and share precision with newer methods, although their temporal resolution and discontinuity can be disadvantages in dynamic conditions. Of course, modern flowmeters lack these two disadvantages and have contributed greatly to our understanding of flow dynamics, but they have not altered the basic relationships among CO, regional blood flow, oxygen uptake, HR, and sympathetic activity so firmly established by the traditional methods used on humans (9, 10).

REFERENCES