Article Title

Cardiovascular responses during sepsis

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Running Head

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Abstract

Sepsis is the life-threatening organ dysfunction arising from a dysregulated host response to infection. Although the specific mechanisms leading to organ dysfunction are still debated, impaired tissue oxygenation appears to play a major role, and concomitant hemodynamic alterations are invariably present. The hemodynamic phenotype of affected individuals is highly variable for reasons that have been partially elucidated. Indeed, each patient's circulatory condition is shaped by the complex interplay between the medical history, the volemic status, the interval from disease onset, the pathogen, the site of infection and the attempted resuscitation. Moreover, the same hemodynamic pattern can be generated by different combinations of various pathophysiological processes, so the presence of a given hemodynamic pattern cannot be directly related to a unique cluster of alterations. Research based on endotoxin administration to healthy volunteers and animal models compensate, to an extent, for the scarcity of clinical studies on the evolution of sepsis hemodynamics. Their results, however, cannot be directly extrapolated to the clinical setting, due to fundamental differences between the septic patient, the healthy volunteer and the experimental model. Numerous microcirculatory derangements might exist in the septic host, even in the presence of a preserved macrocirculation. This dissociation between the macro- and the micro- circulation might account for the limited success of therapeutic interventions targeting typical hemodynamic parameters, such as arterial and cardiac filling pressures, and cardiac output. Finally, physiological studies point to an early contribution of cardiac dysfunction to the septic phenotype, however our defective diagnostic tools preclude its clinical recognition.

Didactic Synopsis

1. The hemodynamic modifications during sepsis and septic shock do not follow a common pattern in all patients. Moreover, in a given patient, the hemodynamic pattern changes as a function of time and interventions.
2. In humans, bacterial lipopolysaccharide in small doses triggers a systemic inflammatory reaction with a hyperdynamic pattern similar to that found in many septic patients.
3. Even if cardiac output is initially increased during infection, as an adaptation to the increased metabolic requirements, hypovolemia, either absolute or relative, and cardiac dysfunction may eventually lead to a fall of cardiac output.
4. Arterial pressure control may be lost despite an elevated cardiac output, due to:
    a. the inability of the vessels to vasoconstrict,
    b. and possibly malfunction of the central circuits responsible for pressure maintenance.
5. Microcirculatory alterations, elicited by bacterial products or by the dysregulated response of the host, may be present despite a preserved macrocirculation.
6. Blood flow distribution is markedly disturbed in sepsis and microcirculatory blood flow is often unable to match tissue metabolic demands, despite possibly increased blood flow towards the whole organ. This can be due to:
    a. disruption of the endothelium.
    b. imbalance between vasodilatory and vasoconstrictive substances.
    c. hyporeactivity of vascular smooth muscle cells to regulatory stimuli such as catecholamines.
    d. cellular rheological disturbances and microvascular thrombosis.
7. Disruption of the endothelial barrier may lead to tissue edema.

Didactic Legends

Figure 1. This figure illustrates the relation between central venous pressure (CVP) and cardiac index (CI) in 46 septic shock patients coming from the medical wards without vasopressor therapy studied by Winslow and coll. (581). Both very high and very low values of CVP and CI were recorded, mirroring the variety of hemodynamic patterns which can be found in sepsis. A previous study by McLean (310) suggested that in early septic shock the main determinant of the hemodynamic pattern is the volemic status of the patient at the onset of bacteremia. If the patient is hypovolemic, CVP and CI are low, if normovolemia is present, CVP and CI are both elevated. This kind of relation between CVP and CI was not confirmed by the study of Winslow and coll. Looking at the graph no proportionality between CVP and CI is seen. Although the volemic status is, beyond doubt, one of the determinants of the hemodynamic pattern in septic shock patients, it is not the only one, and many other factors, with a different weight in different patients, condition the cardiovascular performance.

Figure 2. Guyton's equilibrium diagram graphically shows that, at steady state, central venous pressure (CVP) and cardiac output (CO) depend on the characteristics of the heart and the pulmonary circulation, represented by cardiac function curves, and on the characteristics of the systemic circulation, represented by venous return curves. This figure illustrates various venous return curves. In all cases venous return curves are straight lines with a negative slope and a X intercept corresponding to mean systemic filling pressure (P_{msf}). However, the physical meaning of the slope is dependent on the model used to interpret these curves. If the peripheral circulation is modeled as an arterial compliance (C_a), arterial resistance (R_a), venous compliance (C_v) and venous resistance (R_v) in series as in panel A, the slope of the venous return curve is inversely proportional to the sum of R_v and of the product of R_a times the ratio between C_a and C_a+C_v. As $C_a<<C_v$, the relative weight of R_a is smaller than that of R_v. Systemic vascular resistance (SVR) is R_a+R_v. Because $R_a>>R_v$, it follows that the fall of SVR which may occur in sepsis does not necessarily imply a clockwise rotation of the venous return curve. In panel A a 50% isolated reduction of R_a causes the expected clockwise rotation of the venous return curve from VRC1 to VRC2. In contrast, the venous return curve rotates counterclockwise from VRC1 to VRC3 if an even greater fall of R_a is accompanied by an increase of R_v. In both cases (VRC2 and VRC3) SVR is reduced relative to VRC1. In panel B the systemic circulation is modeled as two compartments in parallel, each made by an arterial resistance (R_a), vascular compliance (C) and venous resistance in series (R_v).

Subscript number identifies the compartments. Compartment 2 has a greater compliance than compartment 1. If arterial resistances change in way so that the fractional perfusion of the compliant compartment (F_c) decreases, the slope of the venous return curve increases (from VRC1 to VRC2). If F_c increases, the slope of the venous return curve decreases (from VRC1 to VRC3). Thus, a change of blood flow distribution induced by bacterial products or by the response of the host is potentially able to markedly change the functional characteristics of the systemic circulation.

Figure 3. This figure illustrates, in terms of Guyton's equilibrium diagram, the elementary hemodynamic alterations induced by sepsis which may take place in the systemic circulation (panel A, B and C) and some of their possible combinations (panel D, E and F). The cardiac and venous return curve representing a healthy subject are indicated with thin lines (operating point C). Thick lines are used to indicate the modifications of the cardiac and venous return curves elicited by sepsis (operating point S). Cardiac output (CO) and central venous pressure (CVP) can be low, normal, or high depending on the combined effects of bacterial products on the cardiac and venous return curves or of the response of the host.

Figure 4. This figure illustrates the relation between the rate of norepinephrine infusion and mean systemic filling pressure (P_{msf}), resistance to venous return index ($R_{VR}I$), and systemic venous resistance index (SVRI), that is the resistance to venous return and systemic vascular resistance normalized by body surface area, in stable postoperative cardiac surgery patients (○) and resuscitated septic shock patients (●), obtained by pooling data from unrelated studies performed separately with the same technique (305, 306, 404). It is evident that for a given rate of norepinephrine infusion P_{msf}, $R_{VR}I$ and SVRI are less in septic shock patients than in post-surgery patients, indicating refractoriness of vessels to the action of catecholamines. The intercepts with the Y axis, obtained by extrapolation, correspond to the P_{msf}, $R_{VR}I$ and SVRI which would have been measured without vasopressors. When no norepinephrine is given, P_{msf} and SVRI are less in septic shock patients than in post-surgery patients. Considering the greater amount of fluids received by septic patients, it is likely that the difference in terms of P_{msf} shown by the upper panel between non-septic and septic patients underestimates the difference that would be present without resuscitation. In contrast to the obvious difference in terms of SVRI seen without norepinephrine between non-septic and septic patients, $R_{VR}I$ appears similar. Admittedly, the paucity of data available and the

arbitrariness of the extrapolation process prevent any firm conclusion. However, if these findings were confirmed by further research, they would suggest a different effect of sepsis on pre-capillary and post-capillary resistance vessels.

Figure 5. This figure illustrates, in terms of Guyton's equilibrium diagram, the effects of bacterial lipopolysaccharide (LPS) on the heart and systemic circulation. The cardiac and venous return curves existing before LPS administration are represented by continuous lines. Their intersection is point A, the operating point of the system. Broken lines show the effects of endotoxin. In healthy volunteers, without fluid loading, LPS causes an increase of cardiac output (CO) at constant (506) or decreased (322, 326) central venous pressure (CVP). The former possibility is shown by operating point E, which univocally implies a counterclockwise rotation of the cardiac function curve (from CFC_c to CFC_e). In contrast, several changes of the venous return curve compatible with an operating point at E are possible. The most likely possibility is a decrease of resistance to venous return (R_{VR}) without (VRC_e 1) or with (VRC_e 2) a concomitant decrease of mean systemic filling pressure (P_{msf}) (panel A). Panel B shows the unlikely possibility of an increase of P_{msf}.

Figure 6. This figure illustrates an indirect method to estimate glycocalyx thinning in the sublingual microcirculation in vivo. The method is based on the assessment of red blood cells deviation from the central flow toward the endothelial cells, a parameter thought to reflect the extent of red blood cells penetration in the glycocalyx. A series of sequential images of a selected portion of sublingual microcirculation are captured (A) by a sidestream darkfield camera, which visualizes red blood cells flowing in the vessels. 10 μm-spaced section (green) are drawn perpendicular to microvessels long axis (red) (B). Sections unsuitable for analysis (yellow), because of insufficient contrast, are discarded (C).

For each section, the distribution of red blood cells in time is analyzed, yielding median red blood cells column width (E), and perfused diameter (F). The perfused boundary region (PBR) is then calculated as half the difference between the two (the concept is depicted on D). This method takes advantage of the fact that the outer part of the glycocalyx tends to exclude flowing red blood cells (but not stationary ones), which therefore gather toward the center of the microvessel. As penetration of red blood cells in the glycocalyx is also a function of red blood cells velocity and deformability, it is unlikely that PBR is

univocally determined by glycocalyx thickness. From (282) with permission under the terms of the Creative Commons Attribution License.

Figure 7. The left part of this figure illustrates the relation between left ventricle volume and end-systolic pressure, that is the end-systolic pressure volume relation (ESPVR). This relation is approximately linear, and characterized by a volume-axis intercept, V_o, that is left ventricle end-systolic volume at zero transmural pressure, and by a slope, called end-systolic elastance (E_{es}), a robust preload-independent index of contractility. The end-diastolic pressure volume relation (EDPVR) is the curved line close to the volume axis. The actual end-systolic volume and pressure present when the left ventricle is coupled with the arterial vessels is graphically identified by the intersection between ESPVR and end-systolic pressure-stroke volume relation (ESPSVR), a straight line with a volume intercept corresponding to the end-diastolic volume, and a slope, called arterial elastance (E_a), corresponding approximately to the product of systemic vascular resistance and heart rate.

The right part of the figure shows the external work (EW) made by the left ventricle during each beat, corresponding to the area within P-V loop trajectory (A-B-C-D-A), and end-systolic elastic potential energy (PE), the area between ESPVR and EDPVR to left of EW. Total oxygen consumption of the ventricle is given by the oxygen consumption in unloaded conditions plus an amount proportional to EW+PE. From (477), with permission.

Figure 8. This figure illustrates the relation between left ventricle volume and pressure at the end of the diastole, when myocardium is relaxed (end-diastolic pressure volume relation, EDPVR). The physiological range is indicated by the dotted lines. In this range diastolic elastance of the ventricle, that is the ratio between pressure and volume changes, is very small for low diastolic volumes but increases progressively as end-diastolic volume is increased. Below the lower limit of the physiological range, transmural pressure is negative, indicating a tendency of the ventricle to expand. From (79) with permission.

Figure 9. This figure illustrates the effects of endotoxin administration in healthy volunteers on the relation between muscle sympathetic nervous activity (MSNA) and mean arterial pressure (MAP) (panel A), and on the relation between heart rate and MAP (panel B). Changes of MAP were induced by sodium nitroprusside, a vasodilator, and phenylephrine, a vasopressor. Measurements were repeated before (Δ)

and after placebo (∇), and before (□) and after (■) endotoxin. Placebo does not affect the relation between MAP and MSNA or the relation between MAP and HR. To the contrary, endotoxin rotate the MAP-MSNA relation downward, showing a decreased modulation of MSNA in front of MAP changes. Moreover, after endotoxin HR appears completely independent of MAP. From (465), with permission.

Figure 10. This figure illustrates the feedback circuit responsible for the baroreflex (only its orthosympathetic part is considered). A perturbation (P_d) of arterial pressure (AP) is sensed by carotid and aortic baroreceptors and this information is transmitted to the vasomotor center of the medulla oblongata. The vasomotor center modulates sympathetic nervous activity (SNA) in order to attenuate the perturbation. Physiologically, the reflex operates in closed loop conditions (panel A), meaning that the parameter sensed by baroreceptors is the same parameter which is modified by changes in SNA. If the pressure sensed by baroreceptors is artificially isolated from systemic arterial pressure (panel B), then it is possible to separately characterize the two parts of the circuit in terms of a) relation between pressure sensed by the baroreceptors (independent variable) and SNA (dependent variable) (mechanoneural arc or central arc), and b) relation between SNA (independent variable) and arterial pressure (dependent variable) (neuromechanical arc, or peripheral arc). The operating point of the system is the combination of arterial pressure and SNA which is compatible with both the central and peripheral arc (panel C). From (464), with permission.

Figure 11. This figure illustrates the effect of bacterial lipopolysaccharide administration (60 μg kg^{-1}) to anesthetized, vagotomized and mechanically ventilated Sprague-Dawley rats in terms of baroreflex characteristics assessed in open-loop conditions. Despite striking changes of the neural (panel B) and peripheral arcs (panel C), the total baroreflex arc changes modestly (panel A), because the effect of a greater sympathetic nervous activity (SNA) at any given carotid sinus pressure (CSP) (neural arc, panel B) are blunted by a lower arterial pressure (AP) at any given SNA (peripheral arc, panel C). From (523), with permission under the terms of the Creative Commons Attribution License.

Figure 12. This figure illustrates the effect of bacterial lipopolysaccharide administration (60 μg kg^{-1}) to anesthetized, vagotomized and mechanically ventilated Sprague-Dawley rats on the operating point of the baroreflex using a baroreflex diagram (SNA: sympathetic nervous activity; CSP: carotid sinus pressure; AP: arterial pressure). As time passes after lipopolysaccharide injection, the neural arc

progressively shifts rightwards, and the peripheral arc downwards, so the operating point moves from a (baseline) to b at 1 hour and to c at 2 hours, showing a progressive increase of SNA with little change of AP. From (523), with permission under the terms of the Creative Commons Attribution License.

Introduction

The use of word ‘sepsis’ dates back to ancient Greece, where it was used to describe “the decomposition of animal, or vegetable, or organic material” (160). It soon evolved to become a medical term, portraying the ‘worst case scenario’ of infections (or what became later known to be an infection). As such, the meaning of the term sepsis adapted to our knowledge about the pathophysiology of severe infection. In *Corpus Hippocraticum,* sepsis was regarded as a biological decay, occurring mainly in the colon and releasing dangerous principles that could potentially lead to “auto-intoxication” (160). For centuries, the focus was on the pathogen, taking the form of *miasmata* in Roman years, *animacules* in the early years of microscopy and finally Louis Pasteur’s *germs* (160). Since its inception, sepsis was considered interchangeable with infection, neglecting entirely the host response. The idea that our own immune machinery might be responsible for the complicated course of infections was conceptualized in the second half of the previous century (519), being the fruit of basic research on host response, the discovery of cytokines (160) and the dawn of intensive care medicine.

An American College of Chest Physicians / Society of Critical Care Medicine Conference held on August 1991 developed a definition to accommodate this new way of thinking (60). They defined sepsis as the systemic inflammatory response to infection, which might be accompanied by multiple organ system dysfunction (60). A set of clinical criteria, namely Systemic Inflammatory Response Syndrome (SIRS) criteria, was proposed for bedside recognition (60). However, in recent years it was felt that the meaning of sepsis neither depicted our current knowledge nor met our expectations (558). Specifically, basic research had shown that sluggish immune responses were associated with equally unfavorable outcomes compared to excessive ones (61). Additionally, it was quoted that sepsis – as it was defined – diverged from what clinicians consider as a “really bad infection”, by being too sensitive. An expert group redefined sepsis as a life-threatening multiorgan dysfunction arising from a dysregulated host response to infection and proposed a set of bedside criteria for its detection, known as the Sepsis-3 criteria (489).

According to recent research, on a global scale there were around 50 million incident cases and 11 million sepsis-related fatalities in 2017, a number corresponding to 1 in 5 deaths worldwide (449). Even though several lines of evidence suggest that sepsis mortality is decreasing (162, 323, 449), the burden

of the disease remains significant, especially in less developed parts of the world (449). Even in wealthy nations, though, the impact is considerable. In a recent US report, sepsis was the primary diagnosis of patients requesting emergency medical services, surpassing typical out-of-hospital crises, such as acute myocardial infarction and stroke (481), and is cited as the immediate cause in 1 every 3 hospital deaths or terminal exits to hospices (437).

Hemodynamic alterations in response to infection, in the form of septic shock, were first recognized in the 19th century in the works of both Laennec and Boise (9). However, it was not until the second half of the 20th century that the advent of endotoxin and cytokines, the development of reliable experimental animal models and the introduction of invasive hemodynamic monitoring in the clinical arena led to an abrupt expansion of our knowledge (211). It is our current understanding that the distinction between uncomplicated infection, infection with multi-organ dysfunction (i.e. sepsis by Sepsis-3) and septic shock is shaped by the extent of the hemodynamic alterations that are elicited in response to the pathogen. Indeed, even in the absence of clinically evident shock, multi-organ dysfunction is considered an issue of impaired tissue oxygenation, the latter being frequently the victim of a deranged microcirculation (12). Thus, current thinking implies there is no sepsis without macro or microcirculatory alterations. In clinical practice, however, cardiovascular failure is frequently defined by the level of arterial pressure or the dose of vasopressors administered but, even then, almost 2/3 of patients with sepsis are affected (556).

In this essay, the cardiovascular responses during sepsis are reviewed. For didactic purposes, the issue is primarily approached through the alterations that pertain to macrocirculatory parameters. Consequently, the vague borders between macro- and microcirculation are crossed, and the reader is gradually introduced to microcirculatory responses. Finally, the heart's response to the septic insult is analyzed with some emphasis being put on the echocardiographic evaluation of cardiac physiology during sepsis.

Variety of hemodynamic patterns in early septic shock

Earlier studies revealed a wide spectrum of hemodynamic patterns in septic shock patients early in the course of the disease before treatment (310, 554, 579), but attempts to categorize patients into well-defined groups characterized by definite clusters of hemodynamic alterations proved difficult. At one end

of the spectrum, circulation could be hyperdynamic, with high cardiac index (CI) coupled with low to normal systemic vascular resistance (SVR), but, at the other end, circulation could be hypodynamic, with a low CI coupled with normal to high SVR. In a series of 56 septic shock patients MacLean and coll. noted that central venous pressure (CVP) and volemia were elevated in hyperdynamic patients, but reduced in hypodynamic ones (310). These findings led the Authors to hypothesize that the hemodynamic response to the septic insult was primarily determined by the pre-existing volemic status: if the intravascular volume at the onset of bacteremia was normal or high the hyperdynamic response prevailed, if it was reduced, hypodynamic shock occurred. A similar wide range of CVP values in septic shock patients was reported by Wilson and coll., who also noted that CVP response to fluid infusion varied widely, apparently without relation to the pre-infusion CVP value (579). In the hyperdynamic septic shock patients studied by Villazon and coll. oxygen consumption ($\dot{V}_{O2}$) was decreased with a marked reduction of arterial-venous difference in oxygen content ($C(a\text{-}v)O_2$). In contrast, septic patients without shock presented a normal or elevated $\dot{V}_{O2}$ (554).

Some of the findings reported by these studies were confirmed by later works, while some others were not. The presence of a hyperdynamic hemodynamic pattern in some septic shock patients (2, 112, 577, 580, 200, 280, 310, 366, 397, 398, 404, 475) or a hypodynamic one in some others (423, 552) has been repeatedly confirmed. As suggested by Villazón and coll., $\dot{V}_{O2}$ is increased during sepsis and declines during the progression of the disease to septic shock (2, 260). To the contrary, the association found by McLean and coll. (310) between low CI and low CVP was not confirmed by subsequent studies (579, 581), suggesting that the low CVP measured by McLean and coll. in septic hypodynamic patients was probably related to the occurrence of severe volume depletion prior to the septic episode, but the hypodynamic pattern can occur even with a normal intravascular volume. Indeed, Wilson and coll. (579) found no clear relation between CI and CVP in septic patients, even after patients with liver disease or under vasopressors were excluded from the analysis. If any, CI tended to decrease with increasing CVP, though not significantly ($\Delta CI/\Delta CVP$=-0.18 L min^{-1} m^{-2} $mmHg^{-1}$, R=0.26, P=0.13). **Figure 1** shows the relation between CVP and CI assessed by Winslow and coll. (581) in a cohort of 46 septic shock patients: again no grouping is apparent, and CI decreases not significantly with increasing CVP ($\Delta CI/\Delta CVP$=-0.07 L min^{-1} m^{-2} $mmHg^{-1}$, R=0.17, P=0.25).

Equally diverse are the hemodynamic phenotypes in the pediatric population with septic shock. Reports describing the evolution of pediatric septic shock in its initial stages are lacking. Ceneviva and coll., using right heart catheterization, studied 50 consecutive patients with fluid-refractory septic shock and distinguished 3 hemodynamic profiles. The most frequent (58%) was characterized by low CI and high SVRI, to be followed by high CI-low SVRI and low CI-low SVR phenotypes, in equal proportions (92). These finding were in general accordance with the work of Pollack and coll. (415) and were later confirmed by other investigators, using non-invasive techniques (71, 124).

Several factors are potentially involved in the extreme variability of the reported cardiovascular response to sepsis in humans.

Sepsis frequently occurs in subjects with pre-existing diseases, who are often already hospitalized and under treatment, and the therapy the patients received before the occurrence of septic shock, particularly the amount of fluids administered, is usually not reported (396). Moreover, some time may be necessary for the recognition of the infection. As a result, a study population may be composed of patients with different underlying diseases, who received an unknown therapy and who come under observation after a variable time from the onset of the infection.

The multitude of microorganisms responsible for sepsis, and the different ways they come in contact with the host, are additional factors potentially able to condition the hemodynamic response of the cardiovascular system, because different pathogens may induce different patterns of immune and inflammatory mediators (343), which in turn may have different effects on hemodynamics.

However, in humans clear evidence that the hemodynamic response depends on the microorganism responsible of the infection is lacking, in line with the idea that the major determinant of the final hemodynamic state is the host response to bacterial products, rather than the specific characteristics of bacterial products themselves. Earlier works suggested that the hyperdynamic pattern arises more commonly in gram-negative than in gram-positive sepsis (51, 189), but this has not been confirmed in studies examining later stages of the disease in adults (3, 397, 577, 581) and in children (124). The possibility that aggressive fluid resuscitation and vasoactive drugs have obfuscated eventual differences in the hemodynamic patterns originally present, however, still exists.

In animals models some differences were noted between the cardiovascular effects of endotoxins from different bacteria or from different strains of the same bacterium, but overall the differences were not striking (110, 115, 300, 327, 365).

More importantly, bacterial products or mediators released in response to bacterial products are able to alter the functional properties of the different parts of the cardiovascular system at various degrees, and the final result depends on the magnitude of the functional changes of the different parts at any given time. Besides changing the rheological properties of the blood, sepsis can affect the vascular compartment by changing the permeability of the vascular walls, the tone of vascular smooth muscle and its responsiveness to intrinsic or extrinsic regulatory mechanisms. Absolute or effective hypovolemia can result from sepsis-induced fluid extravasation and relaxation of the capacitance vessels, respectively. Vasodilation can cause a decrease of arterial and venous resistances and, possibly, when it affects the vascular resistance of the different organs at various degrees, changes of the distribution of blood flow. Both the systolic and diastolic function of the heart can be abnormal, because of a decrease of contractility and an impaired diastolic relaxation. Finally, the control systems in charge of circulatory homeostasis maintenance appear to be altered.

Representation of sepsis-induced elementary hemodynamic alterations on Guyton's equilibrium diagram

Sepsis-induced hemodynamic alterations can be fruitfully visualized in terms of Guyton's equilibrium diagram (192). In this representation, the functional characteristics of the right heart, pulmonary circulation and left heart are depicted by cardiac function curves, which show the relation between the preload of the right ventricle, indexed by CVP (independent variable), and the output of the left ventricle, CO (dependent variable). On the same diagram venous return curves, also called vascular function curves (287), show the functional characteristics of the systemic circulation, displaying the relation between CO (independent variable) and CVP (dependent variable). The CVP-axis intercept of a venous return curve corresponds to the mean systemic filling pressure (P_{msf}), that is the ratio between stressed volume and the compliance of the systemic circulation. In symbols:

$$P_{msf}=\frac{V_{B,sys}-V_{us,sys}}{C_{sys}} \quad (1)$$

where $V_{B,sys}$, $V_{us,sys}$ and C_{sys} are the intravascular volume, the unstressed volume and the compliance of the systemic circulation, respectively. The analog parameter for the pulmonary circulation is mean pulmonary filling pressure (P_{mpf}) defined as the stressed volume of the pulmonary circulation ($V_{B,pul}$-$V_{us,pul}$) divided by its compliance (C_{pul}). In symbols

$$P_{mpf}=\frac{V_{B,pul}-V_{us,pul}}{C_{pul}} \quad (2)$$

Mean circulatory filling pressure (P_{mcf}), *"the pressure that would be measured at all points in the entire circulatory system if the heart were stopped suddenly and the blood were redistributed instantaneously in such a manner that all pressures were equal"* (192), is given by

$$P_{mcf}=\frac{C_{sys}P_{msf}+C_{pul}P_{mpf}}{C_{sys}+C_{pul}}=\frac{V_B-V_{us}}{C_{tot}} \quad (3)$$

where V_B, V_{us} and C_{tot} are blood volume, unstressed volume and compliance of the whole circulation. In dogs, P_{mpf} exceeds P_{mcf} by ~3 mmHg (199), while P_{msf} is just slightly less than P_{mcf}, because of the greater compliance of the systemic relative to the pulmonary vascular bed (192, 445, 446). Blood distribution between the systemic and pulmonary circulation is determined by the elastic properties of the circuits and by the contractility of the left and right hearts. A decrease of right heart contractility relative to that of the left heart causes translocation of blood from the pulmonary to the systemic circulation, decreasing P_{mpf} relative to P_{msf}. A decrease of left heart contractility relative to that of the right heart has opposite effects (304).

The slope of a venous return curve is minus the reciprocal of the resistance to venous return (R_{VR}) and its physical meaning is heavily dependent on the model which is used to interpret it (178). If the venous return curve is interpreted in the context of a model made of in-series resistances and compliances (193, 536), R_{VR} is the cumulative resistance of the systemic circulation weighted by systemic compliances according to their distribution (193). In symbols:

$$R_{VR}=\frac{R_1C_1+(R_1+R_2)C_2+...+(R_1+R_2+...R_n)C_n}{C_1+C_2+...+C_n} \quad (4)$$

where R and C are the resistance and the compliance of each part of the systemic circulation numbered from the venae cavae to the aorta. In a simpler model in which the systemic circulation is

represented by an arterial compliance (C_a), arterial resistance (R_a), venous compliance (C_v) and venous resistance (R_v) in series (192), R_{VR} is given by

$$R_{VR}=R_v+R_a\frac{C_a}{C_a+C_v} \quad (5)$$

For a discussion of different in series model, see (178). Examples of venous return curves are shown in **Figure 2** (panel A). Variation of the slope of the venous return curves are proportional to the changes of SVR only if both R_a and R_v change proportionally. If it is not so, the changes of SVR tend to mirror those of R_a, and the changes of R_{VR} those of R_v.

In models made by in series elements, as those considered until now, no redistribution of blood flow can occur. To take into account this possibility, the cardiovascular system should be represented with elements in parallel. Caldini and coll. (84) used a two-compartments model to analyze the effects of epinephrine administration on the systemic circulation of dogs studied with a constant-flow, right-heart-bypass preparation. Each compartment was in parallel with the other and characterized by three elements in series: an arterial resistance (R_a), a vascular compliance (C) and a venous resistance (R_v). The distribution of the blood flow is dependent on the resistance offered by one compartment (R_a+R_v) relative to the other. As R_a is much greater than R_v, the relative magnitude of precapillary resistances in the two compartments is a primary determinant of blood flow distribution. Similarly to Guyton's model, in Caldini's model the relation between CVP and CO (the venous return curve) is linear, and its intercept with the CVP axis is P_{msf}. The reciprocal of the slope of the venous return curve is minus the sum of the products of the fractions (F) of blood flow in the two compartments times venous resistance, weighted by compliances, that is

$$R_{VR}=\frac{F_1C_1R_{v1}+F_2C_2R_{v2}}{C_1+C_2} \quad (6)$$

In panel B of **Figure 2** different venous return curves have been calculated using reported data for a dog (84). The slope of venous return curves increases if the fraction of blood flow to the non-compliant compartment increases. It is evident that the slope of venous return curves may heavily change without a concomitant variation of SVR.

Whatever the model used to interpret the characteristics of venous return curves, the intersection between the vascular and cardiac function curves represents the only equilibrium point possible for the whole cardiovascular system.

The elementary hemodynamic alterations induced by sepsis are represented in panels A, B and C of **Figure 3**.

Sepsis-induced fluid extravasation or relaxation of capacitance vessels causes P_{msf} to decrease, shifting the venous return curve to the left. In the absence of change in the cardiac function curve, the new equilibrium point will move from C (control condition) to S (sepsis), with a decrease of both CVP and CO (**Figure 3**, panel A).

Vasodilation of resistance vessels does not affect P_{msf}, but decreases R_{VR}, causing a clockwise rotation of the venous return curve. In these settings both CVP and CO will increase relative to the control condition (**Figure 3**, panel B). A similar change of the venous return curve can take place if redistribution of blood flow from a compliant to a non-compliant compartment occurs.

Sepsis-induced myocardial dysfunction causes the cardiac function curve to rotate clockwise, decreasing CO but increasing CVP (**Figure 3**, panel C).

Note that hypotension may be present in all these situations, due to the fall of SVR or CO in a variable degree.

If these alterations are combined, the numbers of hemodynamic patterns further increase (**Figure 3**, panels D, E and F), and the final hemodynamic pattern which will emerge in a given patient depends on the predominant alterations at a given time. The simultaneous presence of effective hypovolemia and myocardial dysfunction may further decrease CO but normalize CVP (**Figure 3**, panel D). A concomitant myocardial dysfunction and vasodilation increases CVP in the presence of low CO (**Figure 3**, panel E). When myocardial dysfunction is modest and vasodilation involves both resistance and capacitance vessels, both CO and CVP may be even normal (**Figure 3**, panel F).

Cardiovascular changes in LPS-treated healthy volunteers and during the progression from infection-triggered systemic inflammatory response to septic shock

The observation of the natural history of the disease is made difficult, or even impossible, by the time it takes to recognize the presence of sepsis, and by its dynamic and rapidly progressive nature, which warrants a prompt intervention. That's why data regarding hemodynamics in resuscitated patients with septic shock are abundant, but much less information is available regarding the transition from infection-triggered systemic inflammatory response to sepsis and septic shock. Moreover, until recently, assessment of hemodynamic measurements required placement of instruments which are associated with measurable morbidity and mortality (94), and are not indicated for routine use. Finally, research in this field is impeded by the difficulty of reproducing the features of the human disease in experimental animal models (149, 440). This markedly limits the possibility to use the information gathered in animal experiments to understand sepsis in humans, and warrants extreme caution when therapies, developed in animal models, are translated into clinical interventions, as suggested by the impressive number of failed clinical trials (440).

With so many confounding factors, to define the natural history of the cardiovascular septic response is an extremely challenging task, and it should not be surprising that contradicting opinions exist in the literature. At one end there is the belief that septic shock starts hyperdynamic (179, 555), on the other that the hyperdynamic pattern is a consequence of volume expansion (207, 423).To this regard, some useful hints can be obtained from the studies investigating the effects of bacterial products in healthy humans, in particular endotoxin.

Hemodynamics after endotoxin administration in healthy humans

In humans and in animals the host reaction to bacterial products has been experimentally studied by administering low doses of purified typhoid vaccine (67) or bacterial endotoxin (352, 506). This topic has been extensively reviewed elsewhere (26, 169, 297, 582). Lipopolysaccharide (LPS) is the main biologically active component of endotoxin, a part of the outer cell membrane of gram-negative bacteria. Its administration to healthy human volunteers has been used as a model of systemic inflammatory reaction. Injection of a dose as low as 2 ng kg^{-1} in healthy volunteers is able to elevate body temperature, heart rate (HR) and white blood cell count (85) enough to meet three of the four SIRS criteria (60, 286), that is 1) temperature >38°C or <36°C, 2) HR >90 beats per minute, 3) respiratory rate >20 breaths per

minute or $PaCO_2$ <32 mmHg, and 4) white blood cell count > 12,000 mm^{-3}, <4,000 mm^{-3}, or >10% immature (band) forms. Additionally, LPS triggers the release of proinflammatory and anti-inflammatory cytokines, like TNFα, IL-6, IL-8, IL-10, and of counter-regulatory hormones, as cortisol and epinephrine (85). Many, but not all, of these findings are commonly seen in septic shock patients (1, 562) and in different animal models of sepsis (141, 196, 246, 255, 434, 527).

The administration of LPS to previously healthy humans or animals to simulate septic and septic shock conditions has been criticized (149, 167, 440). Indeed, it is unlikely that a purified toxin, produced by a single class of microorganisms (gram-negative bacteria), and administered as a bolus or by constant infusion, can fully simulate the complex effects of the intermittent release of exogenous substances within the host, as it happens in sepsis. Even if LPS injection triggers changes of plasma cytokines and hormones similar to those obtained in more realistic septic models, for example cecal ligation and puncture (CLP), the kinetic and magnitude of cytokine production and release are different (434). To this regard, consensus guidelines recommended against the use of LPS challenge as a model of sepsis (291, 384).

However, the hemodynamic pattern observed in humans after LPS administration is evocative of that of many septic patients. Moreover, a condition closely resembling septic shock can be induced in humans by sufficient amounts of LPS. The effects of the self-administration of 1 mg *Salmonella minnesota* endotoxin in a human subject were described in a case-report (111). This dose is 3750 times higher than the usual dose of 4 ng kg^{-1} administered to healthy human subjects. At admission to the emergency department, 2 hours after endotoxin injection, the subject had profound hypotension (42/20 mmHg), which persisted after aggressive volume expansion (~4L) and dopamine infusion. Invasive hemodynamic measurements performed 9 hours after the admission, during treatment with fluids, dopamine and norepinephrine, showed low systemic vascular resistance index (SVRI) (10 mmHg L^{-1} min m^2), elevated CI (5.0 L min^{-1} m^{-2}) together with reduced pulmonary wedge pressure (PWP) (3 mmHg). Clearly, treatment preceded the hemodynamic measurements, so it is impossible to say if CI was elevated before fluid expansion. However, the decrease of SVRI described is of such magnitude that cannot be explained by the effect of fluid infusion alone (264). Before discharge, the patient developed disseminated intravascular coagulation, abnormalities suggestive of mild organ dysfunction and non-cardiogenic

pulmonary edema. Twelve hours after the injection, serum endotoxin concentration was very low (38 pg ml^{-1}) and decreased thereafter, despite profound hypotension, indicating that presence of endotoxin in the blood is not a necessary condition for septic shock to persist. Indeed, endotoxin is found in the blood in ~40% of septic shock patients. In ~60% of patients, endotoxin is not detected even with repeated sampling (114).

Humans are extremely sensitive to the effects of endotoxin relative to other species, and the dose administered to healthy volunteers (2-6 ng kg^{-1}) is several orders of magnitude smaller than the doses routinely used in animal experimental studies (149). Clearly, the dose administered to experimental animals is selected as to induce fully developed shock with multiorgan dysfunction, while that given to humans causes just transient hemodynamic impairment without profound hypotension. However, if the dose given to mice and humans is titrated in order to produce a comparable effect in terms of plasma IL-6, mice still requires a much larger dose (250 times greater) (102).

Moser and coll. (352) administered 0.45 μg of LPS in 10 healthy males without previous fluid loading (the weight of the subjects was not reported, if a weight of 70 kg is assumed, the dose should have been around 6 ng kg^{-1}). LPS injection was followed by a ~30 minutes prodromal phase in which body temperature remained invariant. After that, temperature rose sharply during the following 2 hours (chill phase), reaching a peak at about 3 hours (flush phase) to decline later (defeverscence phase). The increase of temperature was rather variable in each individual. Arterial pressure did not change during the prodromal and chill phase but decreased by 14% during the flush phase. Hypotension appeared despite a marked increase of CO, indicating a proportionally greater fall of SVR. The increase of CO took place because of accelerated HR, as stroke volume (SV) remained unchanged or declined. The fall of MAP and SVR was not paralleled by a concomitant decrease of mean pulmonary artery pressure (PAP) and pulmonary vascular resistance (PVR), which increased and remained unchanged, respectively. Note that in this study CVP and PWP were not systematically measured (PWP was measured in 6 subjects only) and assumed to be zero to calculate SVR and PVR. As the difference between PAP and PWP can be modest, this procedure might have overestimated PVR.

A similar dose of LPS (4 ng kg^{-1}), given to 9 healthy volunteers, had comparable effects, causing progressive hypotension despite increasing CI (506). At 3 hours from LPS administration, CVP was

unchanged. At the same time point, PAP and PWP were not different from control values, suggesting, together with the increase of CI, a decrease of PVR. Surprisingly, at each time point pulmonary vascular resistance index (PVRI) was not statistically different between control subjects (who received saline) and subjects who received LPS. The limited number of experimental subjects and the extreme variability of PVRI (360) may account for this paradoxical finding. In another study using similar protocol and techniques, LPS actually decreased both CVP (-49%) and PWP (-36%) at 3 hours leaving PAP unaffected. PVRI tended to increase but not significantly (322).

These hemodynamic effects of endotoxin appears to be dose dependent, as hypotension may not ensue if lower doses are administered in healthy volunteers (152, 335). Note that the described hemodynamic changes take place without significant changes of pH or arterial saturation. $C(a\text{-}v)O_2$ remains constant (352) or tends to decrease (322). $\dot{V}_{O2}$ increases after LPS administration (322, 352), but is not correlated to body temperature, indicating that the increased metabolic demands are not univocally determined by the febrile reaction (352). Indeed, prevention of the temperature rise with ibuprofen does not abolish the rise of $\dot{V}_{O2}$ (322).

The studies above clearly show that 4-6 ng kg^{-1} of LPS in healthy human subjects are able to induce a hyperdynamic state characterized by a tendency for hypotension, a markedly decreased SVR and elevated CO at constant (352, 506) or decreased preload (322, 326). As it will be discussed, contractility is increased (326) or unchanged at 3 hours (506), and, at 5 hours, unaltered (326) or depressed in the presence of volume loading (265, 322, 506).

Hemodynamic changes during the transition from infection-triggered systemic inflammatory response to septic shock

The few prospective studies unraveling the natural history of the disease suggested that an early hemodynamic alteration in a consistent number of septic patients is a decrease of SVR. Robinson and coll. (441) studied 18 patients without hepatic or cardiac diseases undergoing transurethral resection or cystoscopy, procedures at risk for septic complications. After the procedure 5 patients developed a positive limulus assay and one experienced infection by gram-positive bacteria. SVR decreased in all 6 patients. In 3 patients CI increased, in 2 remained constant and in 1 decreased, so that, overall, mean CI

was unchanged. In only two patients did MAP fell below 75 mmHg. In a similar study performed by Gunnar and coll. before and after genitourinary instrumentation (189), the patients who developed gram-negative bacteremia displayed a greater decrease of SVR and increase of CI than those who developed gram-positive bloodstream infection. Similar results were reported by Blain and coll. (51). However, in the study by Gunnar and coll. (189) on patients with established shock, opposite results were reported: those with gram-positive infections had a greater CO and lower SVR than those with gram-negative infections, prompting the Authors to hypothesize a time-dependent effect of endotoxin, i.e. early vasodilation with late cardiac depression.

More recently, CI and SVRI have been measured with non-invasive pulse contour analysis before any treatment in 127 patients presenting at the emergency department with systemic infection (371). Patients were, on average, normotensive (MAP 80±20 mmHg). Most of them (57%) had a high CI (4.0±0.6 L min^{-1} m^{-2}) and reduced SVRI (20.7±4.4 mmHg L^{-1} min m^2), but in a considerable percentage (39%) CI was low (2.5±0.5 L min^{-1} m^{-2}) and SVRI elevated (32.5±6.5 mmHg L^{-1} min m^2). Chronic heart failure was more prevalent in the second than in the first group (32 versus 11%). Few patients (4%) presented with markedly reduced CI (1.4±0.8 L min^{-1} m^{-2}) and extremely increased SVRI (70.6±18.5 mmHg L^{-1} min m^2).

These studies clearly indicate that different hemodynamic patterns coexist during the early part of the course of the infection, when arterial pressure is still kept well inside the physiological range.

Many patients present with elevated CO and low SVR, but not all. By itself, a decrease of SVR and an increase of CO is not specific of sepsis but can be a physiological adaptation to an increase of $\dot{V}_{O2}$. In a landmark study of Kreymann and coll. resting metabolic rate was increased (+50±14%) during infection and relatively declined in the presence of sepsis (+24±12%) or septic shock (+2±24%). Similarly to what happens in healthy subjects after LPS injection (352), temperature elevation was not correlated to the increase of resting metabolic rate (260).

Even if, in the absence of a pre-existing cardiac disease, CO is normal or elevated at the beginning of the septic process, decreases of the effective intravascular volume and worsening of cardiac function can cause CO to fall. In a series of 18 patients in septic shock with hyperlactatemia (5.9 mmol L^{-1}) who received just 0.7 L of fluids in the preceding 8 hours, Rackow and coll. (423) found normal SVR (14.2 mmHg L^{-1} min) and low-normal CI (2.49 L min^{-1} m^{-2}). Nine of these patients had a pre-existing cardiac

disease, but CI was also reduced (2.24 L min^{-1} m^{-2}) if only patients without pre-existing cardiac disease were considered. The reduction of CI was due to low stroke volume index (SVI) (24.9 ml m^{-2}) in the presence of increased HR (101 beats min^{-1}) and was associated with normal CVP (5.7 mmHg) and PWP (6.5 mmHg), suggesting a combined vascular and cardiac dysfunction. A fall of CI (from 4.38±1.57 to 2.83 L min^{-1} m^{-2}) was observed by Kreymann and coll. in a small sample of patients resuscitated with colloids up to a CVP of 12 mmHg or a PWP of 15 mmHg during the progression from sepsis to septic shock (260). The decrease of CI was paralleled by a decline of $\dot{V}_{O2}$.

More recently Guarracino and coll. studied a series of 55 septic patients before volume expansion who received just a small amount of fluids (up to 250 ml) during instrumentation (186). All patients were hypotensive (MAP<65 mmHg) and had a serum lactate concentration greater than 2 mmol L^{-1} (the group average was 5.1 mmol L^{-1}). CI was low (2.1±0.1 L min^{-1} m^2), right atrial pressure (RAP) was at the upper reference limit (7.6±1.4 mmHg) and SVRI, calculated using the mean values of MAP, RAP and CI, was 23.6 mmHg L^{-1} min m^2, at the lower limit of reference range.

In both the study of Rackow and coll., and of Guarracino and coll. (186, 423) the preload of the right ventricle was normal or at the upper reference limit, indicating that cardiac dysfunction is in part responsible of the low CI early in the course of the disease.

In summary, it is likely that early in the course of sepsis hemodynamic pattern can be hyper- or hypodynamic. The initial hyperdynamic pattern can be regarded as an adaptative response to the increased metabolic requirements and progress towards a hypodynamic one as effective hypovolemia, and cardiac dysfunction develop. However, if cardiac dysfunction elicited by bacterial products or mediators produced during the host response is prominent, the hypodynamic pattern prevails. After volume loading, the hyperdynamic pattern is commonly reported (2, 112, 475, 577, 580, 200, 280, 310, 366, 381, 397, 398, 404).

Effects of sepsis on intravascular volume and on capacitance vessels

Absolute hypovolemia

Absolute hypovolemia may be present in septic shock patients because of poor fluid intake or a precedent fluid loss, secondary to the underlying disease or previous surgical procedure (22, 310). Inappropriate polyuria may further reduce intravascular volume (103). Additionally, alterations of endothelial permeability may lead to extravasation of plasma fluid to the interstitial space.

A significant relation between total blood volume and CI has been reported by Weil (572), showing that a low CI is associated with a low intravascular volume and higher mortality. Similar observations were already reported by McLean and coll. (310). It is possible that this inverse relation between intravascular volume (or cardiac output) and mortality reflected incomplete resuscitation and cardiovascular support, because more recently a very high mortality has been reported in septic shock patients with high CI and markedly reduced SVRI (552).

In healthy volunteers, small doses of LPS (2 ng kg^{-1}) do not change systemic microvascular permeability, measured as transcapillary escape rate of I^{125}-albumin, venous occlusion strain-gauge plethysmography or bioelectrical impedance (542), but this does not rule out that larger doses, or prolonged exposure time may elicit a change of microvascular permeability, as suggested by the 15 L cumulative fluid balance measured in 72 hours from the autointoxication with 1 mg of endotoxin in a laboratory worker as previously described (111).

It is well known that acute respiratory distress syndrome (ARDS), a frequent complication of septic shock, is characterized by an increase of pulmonary capillary permeability (11). However, less information is available regarding permeability of systemic capillaries in septic shock humans, despite the fact that the occurrence of generalized edema is not rare. This issue has been addressed by measuring colloid osmotic pressure in lymph or edema fluid from subcutaneous tissue (Π_E) and in serum (Π_S) in 35 critically ill patients with severe generalized edema, 16 with sepsis and 19 without (135). Π_S was similar in septic and non-septic patients (14.6±2.1 versus 15.8±3.4 mmHg, respectively), while Π_E and the ratio Π_E/Π_S were higher in the former (2.4±0.7 mmHg and 0.165±0.0.052) than in the latter group (1.3±0.7 mmHg and 0.084±0.048). It is possible that capillary hydrostatic pressure was greater in septic than in non-septic patients due to fluid resuscitation. This possibility however cannot explain these results because an

increase of the driving pressure leading to extravasation would dilute interstitial fluid, reducing Π_E and Π_E/Π_S.

An increased microvascular water permeability has been found in hyperdynamic septic shock patients without overt generalized edema using a venous congestion plethysmograph (101). Briefly, with this technique an inflatable cuff is placed around the leg and the volume changes of the calf are estimated by changes of its cross-section. When the cuff is inflated quickly with a certain pressure (P_{cuff}), there is a fast volume increase of the calf (ΔV_a) reflecting the distension of vessels of the local circulation followed by a progressive slow volume increase secondary to increased filtration (J_v). Filtration capacity (K_f) is given by the ratio between ΔJ_v and ΔP_{cuff} for P_{cuff} greater than isovolumetric venous pressure (P_{vi}), the cuff pressure at which filtration is balanced by reabsorption (163). Septic shock and control critically ill patients were studied after resuscitation and stabilization. K_f was markedly greater in septic (6.6±0.4 10^{-3} ml min^{-1} 100 ml of $tissue^{-1}$ $mmHg^{-1}$, K_fU) relative to non-septic patients (3.5±0.3 K_fU). By itself, a change of K_f may reflect a change of permeability, of the surface area available for exchange or both. Thus, an enhanced recruitment of previously unperfused capillaries is an alternative explanation of the increased K_f measured in septic patients. As local capillary recruitment was not assessed in this study, a definitive conclusion is not possible. Relative to healthy volunteers or acutely ill patients without sepsis, septic patients with preserved CI have a lower proportion of sublingual perfused small vessels (118), as it happens for skeletal muscles and small bowel mucosa vessels in CLP rat models (146, 269). If the patients studied by Christ and coll. (101) had a similar decrease of perfused vessels despite the much larger CI, the measured K_f would reflect a marked increase of small vessels permeability.

Alterations of vascular permeability in sepsis have been investigated using animal models. A progressive loss of intravascular volume has been documented in dogs treated with endotoxin (409). In sheep, infusion of *Pseudomonas* strains causes the protein content of the lymph from the lungs to increase (73, 74). Similar effects can be obtained in the same animal model administering LPS (72). In a porcine model, LPS administration caused hemoconcentration (207).

It is difficult to define the impact of the sepsis-induced increased permeability in terms of absolute hypovolemia during early sepsis or septic shock, as studies directly assessing intravascular volume after careful selection of participants are lacking. Intravascular volume was measured in earlier studies, but

patients with pre-existing hypovolemia for reasons unrelated to the septic process were not excluded (310).

It is likely that absolute hypovolemia secondary to fluid extravasation is not prominent in the majority of septic shock patients early in the course of the disease, as the expected hemoconcentration is rarely evidenced. Indeed, hematocrit is usually normal at presentation (564). In a recent study (233), Jansma and coll. measured hemoglobin concentration in 296 patients with sepsis or septic shock and in 320 patients without sepsis admitted to the ED. Patients with conditions potentially able to affect hemoglobin concentration, like previous blood losses, surgical interventions, anemia, hematological malignancies, were excluded from the study. Hemoglobin concentration was, on average, normal (~ 14 g dL^{-1}) and similar in septic and control patients. Also, the fraction of patients with hemoconcentration was similar in both groups (2-3%). However, the interpretation of these results is not straightforward, as in sepsis the absolute amount of red blood cells (RBC) can decrease, due to either increased clearance secondary to cell membrane alterations or decreased production consequent to a concomitant renal dysfunction or bone marrow suppression (5, 21, 36, 407). It is thus possible that absolute hypovolemia is actually present, but the simultaneous decrease of red cell volume and plasma volume keeps hemoglobin concentration and hematocrit invariant. The administration of large amounts of fluids during resuscitation contributes to the fall of hemoglobin concentration usually seen later in the disease (233).

In resuscitated septic patients, intravascular volume has been found similar to that of critically ill non-septic controls, but in the former patients interstitial water is increased relative to the latter, suggesting an alteration of vascular permeability (460).

In conclusion, in septic patients, vascular permeability is increased, likely late in the clinical course of the disease. The exact role of this phenomenon in shaping hemodynamics is still to be determined.

Relative hypovolemia

During sepsis excessive vasodilation of capacitance vessels may lead to relative (or effective) hypovolemia. Effective volemia can be fruitfully quantified in terms of P_{msf} or P_{mcf} (445, 446). However, quantitative data in humans are scanty. This is a problem because species-specific responses to bacterial

products are frequently reported, and extrapolation of results obtained with animal research to humans is at best difficult.

In humans assessment of P_{msf} by the stop-flow technique is feasible only in particular situations (236, 468). Three alternatives methods are available (576). The first method takes advantage of the effect of intrathoracic pressure on CO in mechanically ventilated patients during several post-inspiratory pauses. At different lung volumes, pairs of CVP and CO values are recorded, and the regression line is extrapolated at zero flow yielding the so called P_{msf}-hold (305). Alternatively, on the assumption that the same value of intravascular pressure after equilibration would be measured by stopping the flow in the whole body or in a representative part of it, P_{msf} can be assessed as the intravascular pressure present after ~30 s from the rapid inflation of a cuff placed around the arm at a pressure level above systolic pressure (P_{msf}-arm). Finally, P_{msf} has been estimated in terms of mean systemic filling pressure analogue (P_{msa}). It should be underlined that this parameter is an empirical estimate of P_{msf}, which is calculated from three parameters, MAP, CVP and CO, using coefficients set on the assumption of a fixed ratio between arterial and venous compliance and resistance (400). All these assumptions can be violated in sepsis, so it is unlikely that values of P_{msa} actually reflect absolute P_{msf} in this condition.

A study comparing the three methods in postoperative cardiac surgery patients found no differences between P_{msf}-hold and P_{msf}-arm (19.7±3.9 versus 18.4±3.7 mmHg) but a substantially lower P_{msa} (14.7±2.7 mmHg) (307). Note that the values of P_{msf}-hold and P_{msf}-arm are greater than those measured with the stop-flow technique both in animals (178, 193, 194) and humans during cardioverter/ defibrillator implantation (236, 468), and moribunds (435). The reason for this discrepancy is unclear: in part, it may be related to the different case-mix and the different treatments patients received before measurements were made (307), but a recent study directly comparing P_{msf}-hold and P_{msf} measured with a stop-flow technique in pigs, confirmed that P_{msf}-hold overestimates P_{msf}, at least in some conditions (44). A study in dogs showed that P_{msa} is able to track the changes of intravascular volume, but changes of P_{msa} were systematically less than those of P_{msf} assessed independently using cardiopulmonary interactions (283).

P_{msf} has never been measured in healthy volunteers before and after LPS administration and its eventual changes cannot be deduced from available data.

During early sepsis, before resuscitation, P_{msf} has been assessed in terms of P_{msa} only (186). The average value found (13 mmHg) is somewhat lower than that measured in non-septic patients (91, 191). However, doubts about the ability of P_{msa} to reflect P_{msf} and the lack of a control group prevent a definitive conclusion. After volume expansion performed according to Surviving Sepsis Guidelines (SSG) (30 mL kg^{-1} saline solution) (438) P_{msa} increased (up to 15 mmHg), as expected. These 2 mmHg probably underestimate the true increase of P_{msf}, as a previous study showed that the changes of P_{msa} are ~50% of the concomitantly measured changes of P_{msf}-hold (307).

P_{msf}-hold has been assessed in septic shock patients with normal CI (3.28±0.76 L min^{-1} m^{-2}) and CVP (8±4 mmHg) only after volume loading and treatment with two levels of norepinephrine infusion (0.19 and 0.30 µg kg^{-1} min^{-1}) (404). P_{msf}-hold increased with increasing dose of norepinephrine (from 26±10 to 33±12 mmHg) as expected. These values are higher than those measured in postoperative cardiac surgery patients with the same technique (305). In order to get rid of the confounding effect of different treatments in **Figure 4** P_{msf}-hold, resistance to venous return index ($R_{VR}I$) and SVRI measured in postoperative cardiac surgery patients (305, 306) and in septic shock patients (404) have been represented as a function of the rate of norepinephrine infusion. Assuming a linear relation between norepinephrine infusion rate and P_{msf}-hold, the intersection between the regression line and the Y-axis gives the value of P_{msf}-hold that would have been measured without norepinephrine infusion (**Figure 4**, upper panel). This value is somewhat lower in septic shock patients (13.9 mmHg) than in post cardiac surgery patients (15.8 mmHg), despite the presumably greater amount of fluids received by septic shock patients. These values are likely to be qualitatively but not quantitatively valid, because P_{msf}-hold is known to overestimate P_{msf} (44). P_{msf} has also been measured in septic ICU patients immediately after death, and found not different from that measured in the other categories of ICU patients (435), however it is likely that eventual differences in P_{msf} were obfuscated by the terminal condition of the patients and the prolonged treatments they received before the exitus.

Overall, these studies suggest that P_{msf} is reduced during early septic shock, but the extent of the reduction cannot be easily defined, as this parameter has never directly measured before treatment, expect in the form of P_{msa}.

The third determinant of P_{msf}, together with total intravascular volume and unstressed volume is vascular compliance [see equation (1)]. Vascular compliance has been characterized in human patients with sepsis both at the level of the skeletal muscle circulation (23, 24) and of the whole circulation (499). At both levels, vascular compliance has been found reduced. This is surprising, because the major effect of active venoconstriction is believed to be a reduction of the unstressed volume, and not stiffening of the vessels (178). Additionally, stiffening of the vascular bed should result in an increase of P_{msf}, and some evidence suggests that P_{msf} is decreased in many septic patients.

When assessed at the level of the forearm using a plethysmographic technique, before volume expansion and vasopressor treatment, Astiz and coll. (24) found that a marked decrease of vascular compliance in septic shock patients relative to septic patients without shock and non-septic subjects. Moreover, the volume of the forearm increased less in septic shock patients when the pressure in the occlusion cuff was risen to 30 mmHg. This kind of response is not specific of sepsis, as an increase of vascular elastance has been measured with similar techniques in other pathological conditions, as heart failure or severe anemia (483). The Authors interpreted these findings as evidence for increased venous tone in early sepsis, related to orthosympathetic and hormonal stimulation together with alterations of vascular responsiveness (362). However, equating vascular elastance with venous tone is not warranted, as the change of unstressed volume secondary to venous smooth muscle activation is preponderant over the changes of elastance (446). One may speculate that the results obtained by Astiz and coll. (23, 24) were the result of an increase of unstressed volume, shifting the pressure-volume relation of forearm capacitance vessels to the left and moving the operating point closer to the flat part of the curve with increased elastance, so that a smaller additional volume could be accommodated by rising cuff pressure.

Total effective vascular compliance (C_{tot}), assessed as the ratio between the changes of total blood volume (ΔV) and the changes of CVP (ΔCVP) before and after a 6 min infusion of 450 ml of iso-osmotic and iso-oncotic gelatin (499), has been found decreased in septic patients relative to non-septic patients (68 ± 17 ml $mmHg^{-1}$ versus 99 ± 18 ml $mmHg^{-1}$), independently of concomitant treatment with vasoactive drugs. However, in these settings $\Delta V/\Delta CVP$ equals C_{tot} only if CO remains constant (178), and CO increased after volume infusion in both non septic and septic patients. In this case, $\Delta V/\Delta CVP$ overestimates C_{tot} by an amount proportional to the slope of the cardiac function curve ($\Delta CO/\Delta CVP$) and

to $1/R_{VR}$. While ΔCO/ΔCVP was similar in the two groups, R_{VR} was not measured. If the latter parameter was substantially less in septic than non-septic patients, this would have resulted in a greater ΔCVP in septic relative to non-septic patients, potentially accounting for part of the measured difference in C_{tot}.

In animal studies, the response in terms of P_{msf} is not uniform, and probably depends on the species investigated and on experimental conditions. In dogs, endotoxin (1 mg kg^{-1}) rapidly (~30 min) shifts the venous return curve leftwards, indicating a decrease of P_{msf}. As blood volume does not change immediately and as the slopes of the relations between P_{msf} and blood volume are relatively constant, the decrease of P_{msf} should be due to an increase of unstressed volume, rather than to a change of blood volume or total compliance (409).

In pigs, P_{msf} measurements during endotoxemia provided different results relative to those found in dogs. Hiesmayr and coll. (207) administered endotoxin at a dose of 5 μg kg^{-1} per hour for one hour, and 2 μg kg^{-1} per hour for the following 6 hours. After 5 hours from the beginning of endotoxin infusion P_{msf} was increased from 8.1±1.9 to 9.9±3.2 mmHg, despite a concomitant rise of hemoglobin concentration probably reflecting a decrease of intravascular volume. On the assumption that total intravascular volume was reduced by ~20%, as suggested by the rise of hemoglobin concentration, a substantial active decrease of unstressed volume or a decrease of total vascular compliance or a combination of both should have taken place in order for P_{msf} to rise. Active venoconstriction is suggested by the concomitant marked increase of SVR, which maintained normotension despite a fall in CO. Note that this hemodynamic pattern is markedly different from that seen in healthy humans treated with LPS (322, 352, 506) or in many hyperdynamic septic patients (101, 147, 394). In pigs, acute pulmonary hypertension may limit the output of the right ventricle and cause, together with a preload decrease presumably secondary to venous pooling, CO to fall, especially if the rate of LPS administration is elevated (313). One may wonder if these differences are due to the proportionally greater dose of LPS given to the experimental animals relative to humans, but this is not the case, because in the same porcine model lower doses of LPS fail to elicit an hyperdynamic response (207). If, in pigs, the rate of endotoxin infusion is increased (10 μg kg^{-1} for two hours), the animals become unable to compensate completely for the decrease of CO by increasing SVR, which does not change relative to pre-endotoxin level, and hypotension develops. At the same time, the inability to modulate the tone of vascular smooth muscle

becomes manifest also at the level of capacitance vessels, and P_{msf}, instead of increasing as when a lower dose of endotoxin is administered, remains unchanged or decreases slightly (from 10.8±1.6 to 9.7±1.7 mmHg) (313).

Blood pooling or venous pooling is a poorly defined term which has been used to generically indicate pooling of blood in the peripheral parts of the circulation. It can be secondary to an increase of unstressed volume or to an increase of venous resistances, especially in the splanchnic bed. Indeed, the latter possibility takes place acutely in dogs when very high doses of endotoxin are administered. In this species 3-5 mg of endotoxin cause a fall of CI in front of unchanged or decreased inferior vena cava pressure together with a rise of portal vein pressure, indicating blood trapping in the hepatic circulation (52, 212, 571). These findings have, however, not been reproduced in other animal sepsis models, and probably are not prominent in septic patients. In a study by Kuida and coll. no significant increase in splanchnic blood after endotoxin infusion was found in cats, monkeys and rabbits (263). Similarly, Ujhelyi and coll. found no splanchnic blood pooling in septic swine using Tc^{99m}-labelled erythrocytes and radionuclide imaging (537). The reasons for these discrepancies are unclear and in part species-specific: besides the dose of endotoxin administered, the modality of administration seems important, as in dogs administration of endotoxin as a bolus or slow infusion triggers markedly different hemodynamic responses (109, 110).

In conclusion, while some evidence suggests a decrease of P_{msf} in septic patients which is partially counteracted by fluid-loading, little is known about the changes of this parameter during the course of the disease. Translation of the results obtained in animal models to humans is risky at best, due to the variety of responses assessed in different animal species and in different experimental conditions.

Effects of sepsis on resistance vessels

In terms of Guyton's equilibrium diagram, the increase of CO at constant or decreased CVP observed in LPS-treated healthy volunteers (326, 352, 506) implies a change of both the functional properties of the systemic circulation and of the heart. In **Figure 5** the equilibrium point before the LPS injection is point A corresponding to a CO of 5.5 L min^{-1} and a CVP of 5 mmHg. After the injection, CO increases up to 10 L min^{-1} and CVP does not change, so the equilibrium point shifts from point A to point E. Note that an

increase of CO is not usually present in endotoxemic animal models (90, 153, 188, 207, 237, 313, 523, 584), unless fluids are administered (70, 100, 313), severely limiting the utility of animal models to understand LPS-induced hemodynamic changes in humans.

The new condition is possible only if the cardiac function curve rotates counterclockwise (from CFC_c to CFC_e). Several factors are potentially responsible for this phenomenon. Arterial vasodilation usually occurs after LPS administration in healthy humans (322, 326, 352, 506), and the decrease of the afterload to the left ventricle may contribute to elevating CO at a given CVP (222). Contractility itself can be modified by bacterial products or substances produced by the host in response to the infection, or by reflex activation. The changes of contractility are discussed in the section "Effects of sepsis on heart function". HR increases both after LPS administration (152, 465, 506) and in septic conditions (2, 24, 200, 231, 234, 398, 423, 554, 577). A concomitant cardiac dysfunction (506) is not excluded by a counterclockwise rotation. If LPS depresses the contractility of the myocardium, its influence should have been more than compensated by the concomitant decrease of afterload and the increase of nervous and hormonal stimulation.

Several different venous return curves are compatible with a new equilibrium point at point E. At least in theory, there can be an isolated decrease of the R_{VR} with constant P_{msf} (VRC_e1), a decrease of R_{VR} and P_{msf} (VRC_e2) or no change (or increase) of R_{VR} with increased P_{msf} (VRC_e3). In physiological conditions, modulators of the vascular tone, as the orthosympathetic system, affect both the arterial and venous side of the circulation (178), even if the extent of the response is quantitatively different (241, 334). For this reason, the decrease of SVR, which has been measured after LPS (352, 506), is suggestive of a decrease of R_{VR}, but not probative. If the systemic circulation is represented by an arterial compliance (C_a), arterial resistance (R_a), venous compliance (C_v) and venous resistance (R_v) in series, SVR is just R_a+R_v, but R_{VR} is given by equation (5). Unless the changes of R_a cause redistribution of CO away from the compliant compartment (312, 340), a fall of R_a is expected to have a much smaller effect on R_{VR} than a fall of R_v, and consequently on CO (314) (**Figure 2**)**.** Unfortunately, R_{VR} and P_{msf} have not been measured in healthy humans after LPS administration, and, even if a decrease of R_{VR} is likely, it is not possible to say which possibility takes place.

Data relative to R_{VR} in septic patients are scanty. In untreated patients with sepsis or septic shock venous return curves have been built estimating P_{msf} in terms of P_{msa} but the lack of a control group prevents any firm consideration regarding the specific effects elicited by sepsis on the venous return curve (186).

Persichini and coll. measured R_{VR} with the inspiratory holds method in 16 resuscitated septic shock patients at two different rates of infusion of norepinephrine (404). A decrease of the rate of infusion from 0.30 to 0.19 μg kg^{-1} min^{-1} led to a similar decrease of $R_{VR}I$ and SVRI (from 6.5 to 5.2 mmHg L^{-1} min m^2, -17%, from 25.3 to 21.0 mmHg L^{-1} min m^2, -20%, respectively). By itself, a decrease of R_{VR} tends to increase CI, but CI fell from 3.47 ± 0.86 to 3.28 ± 0.76 L min^{-1} m^{-2} due to a proportionally greater decrease of P_{msf}. In the absence of a control group, these results can be tentatively compared with those obtained by Maas and coll. using the same technique in 16 stable postoperative cardiac surgery patients (306). A decrease of the rate of infusion of norepinephrine from 0.08 to 0.04 μg kg^{-1} min^{-1} caused $R_{VR}I$ and SVRI to decrease, the former from 9.7 to 6.6 mmHg L^{-1} min m^2, -32%, and the latter from 48.0 to 35.7 mmHg L^{-1} min m^2, -26%. By looking at **Figure 4,** it is evident that the rate of rise of P_{msf}, $R_{VR}I$ and SVRI with increasing norepinephrine is markedly less for septic shock patients than for post cardiac surgery patients. Extrapolation of the regression lines indicates that, without vasopressors, in fluid-resuscitated septic shock patients SVRI is lower than in post-surgery patients but, surprisingly, $R_{VR}I$ is similar. Admittedly, the paucity of data available and the arbitrariness of the extrapolation process prevent any firm conclusion. However, if these findings were confirmed by further research, they would suggest a different effect of sepsis on precapillary and postcapillary resistance vessels.

The interpretation of the hemodynamic events triggered by LPS injection in healthy subjects or by sepsis in patients is further complicated if parallel elements are used to describe the cardiovascular system. Indeed, a marked increase of the slope of the venous return curve can take place if the fraction of blood flow to the compartment with the greater compliance decreases relative to the fraction of blood flow to the compartment with the lower compliance, even if SVR does not change appreciably (84). Such an occurrence is potentially able to contribute to the increase of blood flow, which is seen in healthy subjects after LPS administration or in hyperdynamic septic patients, but only limited and partially conflicting information is available (182).

The increase of plasma concentrations of epinephrine and norepinephrine which has been measured in healthy subjects after LPS (335, 465) or in septic patients (16, 40, 185, 285) is potentially able to change blood flow distribution. If the increase of circulating catecholamines caused vasoconstriction in the splanchnic circulation but vasodilation in the skeletal muscles in humans as it happened in the dogs of Caldini and coll. (84), the consequent redistribution of blood flow towards the non-compliant compartment would increase the steepness of the venous return curve.

However, vascular response to catecholamines is impaired in the presence of endotoxin (50, 411) or in sepsis (**Figure 4**), and this, together with the known interspecies differences (169), prevents any prediction concerning the effects of epinephrine on blood flow distribution in humans.

Vascular tone is also modulated by local orthosympathetic activity. Indeed, muscle sympathetic nerve activity (SNA) decreases after 4 ng kg^{-1} LPS administration in humans (465): if splanchnic SNA was not decreased proportionally, this would result in a blood flow redistribution similar to that described by Caldini and coll. (84). Contrary to this idea, a study conducted on healthy volunteers receiving ~4 ng kg^{-1} of *E. coli* endotoxin showed that splanchnic blood flow increases by ~ 90% after LPS administration peaking at 3 hours after the injection, while indexes of leg blood flow were unchanged (152). It should be noted that in this study no hypotension was observed, and cardiac output was not measured, so the comparison between these results and those previously reported (352, 506) is not straightforward.

In patients with a normodynamic septic shock (CI 3.7±0.5 L min^{-1} m^{-2}) the percentage of forearm blood flow to CO was similar to that found in non-septic critically ill patients with similar CO (CI 3.6±0.2 L min^{-1} m^{-2}) (23). A similar percentage was measured in septic shock patients with a hyperdynamic circulation (CI 5.5±0.6 L min^{-1} m^{-2}) (22). Another study found no difference in terms of percentage of CO perfusing the splanchnic circulation between critically ill and hyperdynamic septic patients (112). In severe sepsis patients with hypotension (MAP 65±12 mmHg) and hyperdynamic circulation (CI 5.0±1.9 L min^{-1} m^{-2}), perfusion to skeletal muscles, as indexed by tibialis anterior, was decreased relative to non-septic patients with a smaller CI (4.1±1.2 L min^{-1} m^{-2}), arguing against a redistribution of blood flow to the non-compliant compartment (366).

In summary, what happens to the distribution of blood flow in LPS-treated or septic humans is still unclear. If redistribution to the splanchnic compartment actually takes place, as suggested for LPS-

treated healthy subjects by (152) and for hyperdynamic patients by (366), the slope of venous return curve should decrease and CO fall. As CO is actually increased, either the effects of blood flow redistribution to the splanchnic compartment are more than compensated by the fall of arterial and particularly of venous resistances, or by an increase of P_{msf}. Alternatively, redistribution can take place not between different organs but at the level of different parts of the microcirculation. The increase of plasma lactate after LPS injection in healthy subjects (335) or in septic patient with an elevated central oxygen saturation (168) is suggestive of this possibility, but whether this mechanism has an important role in shaping hemodynamics is still to be determined.

Regarding R_{VR} and P_{msf}, the information gathered in animal studies cannot be directly translated to humans, due to the different effects of endotoxin in different species and the variety of experimental settings.

In a pig model characterized by normotension and decreased CO, endotoxin infusion (5 $\mu g \cdot kg^{-1}$ h^{-1} for the first hour and 2 $\mu g \cdot kg^{-1}$ h^{-1} for 6 hours) markedly raised R_{VR} (from 5.7±1.2 to 13.3±5.7 mmHg L^{-1} min) together with P_{msf} (from 8.1±1.9 to 9.9±3.2 mmHg) and SVR (from 63±12 to 128±43 mmHg L^{-1} min) (207), suggesting a reflex compensation to maintain normotension in front of a fall of CO. A greater dose of endotoxin (10 μg kg^{-1} for two hours) without concomitant fluid-loading in the same animal species produced hypotension without changes of R_{VR} and SVR, as if reflex activation was no longer able to compensate for the marked reduction of CO (313).

Similar changes of SVR and R_{VR} can take place if arterial and venous resistances changes by the same proportion (assuming that compliances are invariant). Interestingly, in the study of Hiesmayr and coll. (207) the changes of SVR paralleled those of R_{VR} after endotoxin administration (+108 and +135%, respectively) and fluid infusion (-29 and -28%, respectively). Similarly, both SVR and R_{VR} did not change significantly with greater doses of endotoxin in the same animal species (313). When LPS (1 mg kg^{-1}) was administered to dogs which had received an unspecified amount of fluids, SVR and R_{VR} decreased on average by 51 and 25%, respectively (409). If parallel changes of SVR and R_{VR} take place also in humans, the fall of SVR previously described implies a concomitant decrease in R_{VR}. However, when endotoxin (10 μg kg^{-1} h^{-1}) was administered to pigs together with enough fluids to keep right atrial pressure (RAP) at 5 mmHg, SVR fell (from 29.8±6.4 to 12.9±1.9 mmHg ml^{-1} min) and R_{VR} did not change

significantly (from 1.7±0.5 to 1.4±0.2 mmHg ml^{-1} min) (313). In the same preparation N^{w}-nitro-L-methyl ester (L-NAME) increases SVR more than R_{VR} both in the presence (+294 and +129%, respectively) and in the absence (+196 and +107%, respectively) of fluid-loading. In another study from the same group, L-NAME elicited similar effects (117). Overall, these experimental studies warn against the assumption that changes of SVR are always paralleled by similar changes of R_{VR}.

The Microcirculation in Sepsis

Introduction

Historically, sepsis research and management have focused on monitoring and reversing macrocirculatory alterations. Nevertheless, the correction of parameters such as MAP and CI often fails to resolve or even prevent organ dysfunction. Indeed, a recent study highlighted that many septic shock patients, after fluid resuscitation, present an impairment in oxygen use, rather than a macrocirculatory defect of oxygen transport (168). Additionally, fluid loading and vasopressors do not seem able to restore microcirculatory parameters late in the disease, irrespective of changes of MAP and CI (41, 281, 319, 383). In some animal models vasopressors might even exacerbate local blood flow regulation disturbances (259, 279). For these reasons, in the last decades, focus has shifted towards the microcirculation, which appears markedly altered both in humans and in animal models. Regarding the functional aspects of the microcirculation, see (288, 419).

Distribution of microcirculatory perfusion in sepsis

Our ability to visualize the human microcirculation in vivo was severely limited until the development of hand-held vital microscopes (HVMs) in the 1990s. Orthogonal polarization spectral imaging (OPS) first, and then sidestream dark-field (SDF) imaging and incident dark field illumination (IDF) have gathered a lot of interest amongst researchers (223, 224). De Backer and coll. took advantage of OPS to evaluate sublingual microcirculation in terms of perfused large (diameter >20 μm) or small vessels (diameter <20 μm) in subjects without sepsis and in septic patients. With this technique vessels can be visualized only if they contain RBC (183). These Authors found a significant decrease of the density of small vessels in

septic patients, suggesting that some of the vessels were collapsed. Moreover, the proportion of perfused small vessels in these subjects was reduced, due to the increase number of nonperfused or intermittently perfused vessels. Finally, the heterogeneity of the distribution of blood flow in the different areas visualized was almost two times greater in septic patients relative to healthy volunteers, potentially contributing to oxygen extraction impairment (565). Survivors demonstrated a higher portion of perfused vessels compared to non-survivors (118). Other studies using the same technique, confirmed these findings (55, 456, 531).

A critical point of this approach to the study of the microcirculation is whether the changes of sublingual microcirculation in sepsis reflect those of more important sites, as splanchnic microcirculation. Some animal studies provided an acceptable correlation between alterations in the sublingual and gut microcirculation (157, 548), but not always (131). In humans the alterations of sublingual microcirculation during sepsis do not reflect those of the splanchnic circulation during early sepsis or in response to fluid challenges (55, 134). In clinical practice, HVM techniques, which are relatively time-consuming and require considerable expertise in image interpretation, have not gained wide acceptance, despite the prognostic value of sublingual microcirculation monitoring (456, 524) and the possibility to use changes of sublingual microcirculation as a surrogate markers of fluid resuscitation responsiveness (531).

In line with human studies investigating sublingual microcirculation, animal studies have consistently shown significant microcirculatory alterations in sepsis. Lam and coll. applied intravital microscopy to assess the microcirculation of an extensor digitorum longus muscle preparation in CLP rats, and found 36% reduction of perfused capillary density, a 265% increase in stopped-flow capillaries and an increased spatial distribution heterogeneity of perfusion (269). This kind of alterations has also been assessed in the ileal mucosa of anesthetized pigs after *P. aeruginosa* infusion (229) and in the cerebral microcirculation of sheep after intra-abdominal injection of autologous feces (514). Interestingly, in the latter study, changes in functional capillary density appeared before the decline of MAP, supporting the notion that microcirculatory dysfunction occurs before the onset of macrocirculatory derangements.

The differential behavior of the different parts of the microcirculation in septic conditions has been assessed in animal studies only.

In skeletal muscle microcirculation, larger arterioles constrict and smaller arterioles dilate during sepsis in hyperdynamic (105, 106, 298) and hypodynamic rat models (53, 105, 298). In the latter condition, smaller arterioles' diameter may also not change (53). In these preparations, venules' diameter is invariant (105, 106, 298) or transiently decreases in hypodynamic animals (298). The different behavior of larger and smaller arterioles may be related to the different factors controlling the diameter of these vessels, that is neurohumoral signals for larger and local signals for smaller arterioles. Interpretation of these findings is problematic because in these hyperdynamic models MAP does not fall as in septic patients, but remains stable (298) or increases (105, 106). In hypodynamic models, MAP falls transiently (298) or for a more extended period (53, 105) but usually with an increase of SVR (105), indicating that control of vascular tone is in part maintained. Moreover, local metabolic activity is usually not assessed, and it is therefore difficult to figure out which part of the response is adaptative. Indeed, in a presumably hypodynamic model, arteriolar behavior during endotoxemia was indistinguishable from that observed after a hemorrhage producing a similar hypotension (53). Endotoxin does not seem able to change directly smooth muscle tone in larger murine arterioles, as local exposure to endotoxin does not elicit a change in diameter. To the contrary vasoconstriction appears when endotoxin is administered systemically (172). Substances produced by vascular segments upstream likely contribute to the modulation of arteriolar tone in sepsis. In the same study, larger arterioles' vasodilation was triggered when arterioles were perfused with endotoxin with an aortic segment placed upstream. Interestingly, endothelium-derived nitric oxide (NO) or prostaglandins were not the vasodilators responsible for the loss of basal tone, because the vasodilatatory response persisted when the upstream vessel (an aortic segment) was stripped of the endothelium or treated with a cyclooxygenase inhibitor (172). A similar dependence of larger arterioles' tone on upstream released factors in endotoxemic conditions has been demonstrated in an ex-vivo human cremaster muscle preparation (87).

The response of the microcirculation to sepsis in animal models appears markedly heterogeneous and partially dissociated from changes of regional perfusion. In awake rats, Whitworth and coll. described "progressive arteriolar constriction" in the microcirculation of ileum during hyperdynamic sepsis induced by live *E. coli* infusion, which led to a gradual reduction of microvascular blood flow (575). Blood flow to the ileal mucosa, measured with microspheres, decreased also in a presumably hypodynamic model

(anesthetized rats injected with 10 mg kg^{-1} of LPS). In these experiments no significant microcirculatory perfusion changes in other intestinal compartments were detected (254). In anesthetized mechanically ventilated pigs, in which sepsis was induced by fecal peritonitis, the relation between microcirculatory flow (measured with laser doppler flowmeters) and the regional flow (measured with ultrasound flowmeters) has been assessed during the hypodynamic phase and after fluid resuscitation (hyperdynamic phase). CO and superior mesenteric artery blood flow similarly fell and increased during the hypodynamic and hyperdynamic phase. During the hypodynamic phase, relative to regional perfusion, microcirculatory blood flow was decreased in the pancreas, increased in the jejunum, and unchanged in stomach, liver, colon and kidney. In contrast, after fluid resuscitation microcirculatory flow increased less than regional blood flow at the level of pancreas, liver, colon and kidney (208).

The pulmonary microcirculation during sepsis has received a lot of attention due to its involvement in acute lung injury and ARDS. Blood flow distribution is regulated at this level by hypoxic vasoconstriction, the impairment of which may contribute to the deterioration of gas exchange. Indirect evidence for hypoxic vasoconstriction impairment in human sepsis is given by the similar distribution of perfusion in septic ARDS patients and in healthy controls (474). Similarly, in anesthetized, intubated sheep, ventilated with a constant tidal volume of 10 ml/kg, 5 cmH_2O PEEP and FiO_2 of 1.0, infusion of *E. coli* LPS does not change perfusion distribution, and hypoxemia is larger than expected from the loss of aerated tissue, suggesting that hypoxic vasoconstriction is not effective in reducing ventilation perfusion mismatch (148). Apparently in humans blunting of hypoxic vasoconstriction is not characteristic of ARDS patients, occurring also in patients with pulmonary edema due to cardiac or renal failure (474). The specific effect of bacterial products on hypoxic vasoconstriction has been dissected in a mouse model. In normal mice, occlusion of the left mainstem bronchus causes diversion of blood flow to the right lung secondary to an increase of left lung vascular resistance, a phenomenon which is markedly blunted after 10 mg kg^{-1} *E. coli* endotoxin administration. This behavior cannot be attributed to the presence of edema, which was absent in LPS-treated mice (539). The molecular mechanisms responsible for hypoxic vasoconstriction have been recently reviewed (132, 181).

The microcirculation of the kidney is also impaired in sepsis, but significant controversy exists regarding the exact cause of sepsis-induced acute kidney injury despite normal or even increased organ

blood flow, especially during hyperdynamic sepsis (275). A study by Lankadeva and coll. in sheep revealed some intriguing information on the subject. Fiber optic probes in the renal medulla and cortex of the animals allowed monitoring of local tissue perfusion and tissue oxygen tension. After *E. coli* infusion, medullary blood flow and oxygenation decreased by ~50%, despite a 67% increase in total renal blood flow. On the other hand, blood flow and tissue partial oxygen tension increased in the cortex. Even more surprisingly, medullary blood flow and oxygen tension were further reduced with norepinephrine administration (279).

Overall, in septic patients and animal models, sepsis causes a reduction of perfused microvessels together with heterogeneous spatial distribution of perfusion, involving different organs to various degrees. These microcirculatory abnormalities are only partially related to changes of macrocirculatory parameters.

The described microcirculatory alterations originate at multiple levels. Together with malfunctioning of intrinsic and extrinsic regulatory mechanisms, activation of the clotting cascade, and alterations in red blood cell and white blood cell deformability lead to capillary “clogging”, creating capillary areas with zero or intermittent flow, further hampering local perfusion-demand matching. The concomitant dysfunction of endothelial glycocalyx may lead through an increase of permeability to interstitial edema, further worsening gases and nutrient exchange between the tissues and the blood.

Disturbances of intrinsic mechanisms regulating the microcirculation in sepsis

Under normal conditions, microcirculatory blood flow is tightly regulated to achieve adequate perfusion of tissue cells through adjustments of arteriolar smooth vascular muscle tone. Smooth muscle tone is controlled by a number of mechanisms, based on mechanical (stress and strain), metabolic and neurohumoral signals (427). During sepsis these mechanisms may fail, producing a condition which is indicated with the poorly defined term vasoplegia, and which often manifests clinically as hypotension due to abnormally low SVR despite the maintenance of normal or even increased CO (271). Vasoplegia includes phenomena such as reduced vascular smooth muscle tone, alterations in vasomotion and, finally, reduced response to nervous, hormonal and pharmacological stimulation, believed to be triggered by damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns

(PAMPs) (271). The loss of microcirculatory regulation is thought to eventually lead to the clinical presentation of refractory septic shock.

Alterations of the intrinsic mechanisms regulating microvascular blood flow during sepsis in humans have been assessed in terms of reactive hyperemia, that is the transient rise in local blood flow which follows tissue ischemia. Briefly, perfusion to a part of the body (upper or lower limb) is interrupted using a cuff inflated at a suprasystolic pressure for 3-5 minutes, and the increase of blood flow upon deflation of the cuff is compared with the preinflation value. Perfusion can be assessed directly by plethysmography (21, 24) and laser Doppler flowmetry (366), or indirectly by near-infrared spectroscopy (NIRS) (127). Applying an air plethysmograph to the forearm Astiz and coll. found a reduced hyperemic response in septic shock patients with reduced pre-occlusion perfusion before fluid resuscitation (24) and in hyperdynamic septic shock patients (21). In the latter study however, peak blood flow after the occlusion was comparable to that of healthy volunteers, and the reduced increment was largely due to a greater pre-occlusion blood flow in septic patients. Using laser Doppler flowmetry the hyperemic response was found reduced at the level of tibialis anterior muscle (366) and of the skin (586). Similar results were obtained by NIRS at the thenar skeletal muscle (127).

As reactive hyperemia is mediated by multiple mechanisms (485), it is not possible to identify on the base of these experiments which regulatory mechanism is impaired and to what extent. The finding of Neviere and coll. (366) of a similar rate of increase of blood flow immediately after the release of the occlusion in septic and non-septic subjects suggests that myogenic regulation, believed to be responsible of the initial phase of the hyperemic response, is somewhat preserved.

Animal studies confirmed to some extent the alterations of microcirculatory regulation observed in septic patients. The ability of the microcirculation to adapt to local tissue oxygen level in sepsis was investigated using a hind limb extensor digitorum longus muscle preparation in a fluid-resuscitated CLP mouse model. Red blood cells and oxygen supply rate were heterogeneously distributed, with some capillaries exhibiting very high RBC and oxygen supply rate, while others were flow-stopped. The response time within hypoxic capillaries was three-fold greater in CLP mice relative to sham animals. This regulation disturbance was associated with a decrease of RBC O_2-dependent ATP efflux, a local adaptive mechanism in areas of hypoxia that allows for upstream vasodilation, presumably through NO-mediated

smooth muscle relaxation (38). In a larger animal model (pigs), hemorrhage caused gut capillary transit time heterogeneity to decrease in control animals, but to increase in the presence of endotoxemia, impairing oxygen extraction (217).

Several factors can play a role in the genesis of these alterations. In the last decades research has focused on the endothelium, as dysfunction of these cells is potentially able to explain many of the microcirculatory findings in sepsis through multiple mechanisms.

Cell-to-cell electrical signaling in sepsis

Cell-to-cell electrical communication allows an electrical signal to spread bidirectionally in the endothelial monolayer, so that a stimulus on a particular part of the microcirculation can elicit its effects at some distance (conducted response) and trigger a coordinated response. Indeed, when a vasodilator that does not trigger a conductive response is used on a daughter arteriole, the effect on parent arterioles and therefore local blood flow is minimal. Conversely, when vasodilation is induced together with a conducted response, blood flow increases substantially because of vasodilation of the parent arteriole together with the non-stimulated daughter arteriole (267). In vitro and in vivo animal experiments have shown that endotoxin is able to reduce intercellular electrical coupling through a tyrosine kinase-dependent mechanism, so to decrease the conducted response triggered by direct electrical stimulation (534).

Conduction of electrical signals at the level of smooth muscle and endothelial cells contributes to the genesis of "*vasomotion",* periodic oscillations in microvascular tone that are autonomously generated from within the vascular wall (417). The functional purpose of these oscillations remains almost as elusive as their mechanism. Indeed, some lines of evidence suggest that vasomotion exerts a protective role during periods of ischemia (227, 448). As such, one would expect vasomotion to be increased in septic states. In the jejunum of endotoxemic pigs the frequency of oxygen partial pressure oscillations increased relative to control animals in front of a decrease of mucosal tissue oxygen tension (387). In humans published data are too scanty to attempt any generalization: Young and Cameron found an increased amplitude of skin blood flow oscillations at 0.1-0.15 Hz frequencies using laser Doppler flowmetry in septic patients (586), but Neviere and coll. with the same technique did not observe any vasomotion in the skeletal muscle of septic patients, despite being present in control subjects (366).

Nitric oxide in sepsis

A number of vasoactive substances are produced by endothelial cells, including vasodilators and vasoconstrictors (413). For the purposes of this review, special attention will be given to NO, since NO is believed to play a pivotal role in the pathophysiology of sepsis-induced microcirculatory dysfunction, being potentially able to affect a plethora of microvascular processes, among which microvascular tone, vascular permeability, erythrocyte deformability, leukocyte adhesion, platelet aggregation and formation of microthrombi, and unresponsiveness to vasoactive drugs (4, 36, 37, 249, 262, 393, 557). For more information regarding the physiology of NO, see (56, 126, 150, 350).

NO production has been assessed by total plasma concentration of nitrogen oxides, $[NOx]_p$, including nitrates (NO_3^-) and nitrites (NO_2^-) (353). Doses of endotoxin between 2 and 4 ng kg^{-1} administered to healthy volunteers do not elicit a change of $[NOx]_p$ (6, 203, 493), but tend to increase urinary excretion of NOx (203). In septic shock patients $[NOx]_p$ are usually elevated relative to healthy controls, unless sepsis develops post-trauma (18, 40, 486, 490, 505, 518, 583, 123, 128, 143, 166, 176, 203, 228, 373). The amount of NOx measured in patients is however usually smaller than that measured in rodent models (165, 521). An increase of $[NOx]_p$ in septic patients does not necessarily imply an increased production of NO, as may also be due to a decrease of urinary clearance (563). Some studies suggested that elevation of $[NOx]_p$ is the result of increased NO synthesis (18, 123, 143, 583), but others were unable to measure an increase of the production rate (302, 553).

Exhaled NO increases after 2 or 4 ng kg^{-1} of endotoxin in healthy volunteers (493) and in endotoxemic animal models (333, 501). Probably it is produced locally, because is not accompanied by an elevation of $[NOx]_p$ (333, 493) and increases when pulmonary blood flow is reduced (501). In septic shock patients without respiratory tract infection exhaled NO is not different relative to control subjects without systemic inflammation, despite increased $[NOx]_p$ in the former group. To the contrary, exhaled NO is higher in patients in whom sepsis originated from a pulmonary infection (518). In ARDS patients, despite the existence of an inflammatory process, exhaled NO is decreased (69). The discrepancy between these results and those collected in healthy volunteers may be related to the multiple determinants of NO recovered in exhaled air, especially in mechanically ventilated subjects, including hypoxia, hypercapnia,

volume and composition of the fluid lining lower airways, prostaglandins, and direct damage to small airway epithelial cells producing NO (108).

In humans little is known about the tissues responsible for the production of NO appearing as NOx in the blood, and about the modality with which NO is produced. Cells of the immune system are likely responsible for part of the increase, because NO synthase activity in PMN isolated from septic shock patients is elevated, especially in those patients with three or more dysfunctional organs (177), and NO production from blood mononuclear cells is increased (428). However, a report provided evidence of reduced expression of mRNA for inducible (iNOS) and endothelial NO synthase (eNOS) in peripheral blood mononuclear cells and mesenteric arterial smooth muscle (429). As the amount of synthases actually produced was not quantified, and NO production not measured, it is not possible to say if these cells were producing the extra amount of NO expected or not, as production of NO depends also on the availability of substrate, for example arginine, which can be increased due to increased membrane transport (428). Two studies suggested overproduction of NO by mesenteric arteries from septic patients (502, 533), in line with a vascular origin of NO. No human data however are available to identify with certainty if the NO so produced comes from endothelial or smooth muscle cells or both. In an in vitro model, bovine aortic endothelial cells, after only one minute incubation with endotoxin, produced a substance able to decrease platelet aggregation, presumably NO, indicating that endothelial cells are a potential source of NO during endotoxemia (459). However, no change of NO production was found in another study upon acute exposure to endotoxin in the same cells (359). Conversely, in the same study, incubation with endotoxin for 1 hour caused NO production to decrease in a dose dependent manner. In another study, 4 hours after the injection of 10 mg kg^{-1} and 2 mg kg^{-1} endotoxin in rats and rabbits, respectively, contraction of isolated aortic rings induced in vitro by phenylephrine and angiotensin II was reduced, independently of the presence of endothelium, and the normal contractile response was restored by treatment with N^{G}-nitro-L-arginine, a NOS inhibitor (540). Wang and coll., using a rat CLP model, showed a markedly depressed vasodilatatory response to acetylcholine in large arterial vessels and, indirectly, in the microcirculation of intestine at 5 and 20 hours from sepsis induction, despite an unchanged response to nitroglycerin (567). Apart from indicating that increased basal NO production may be one of the factors involved in blunting of vasoconstrictive response, these results raise the question of

whether a significant part of the excess of NO is not produced by endothelial cells, rather by the smooth muscle. Also an in vitro study provided a similar suggestion (311).

On the base of animal models, it has been hypothesized that iNOS is overexpressed during sepsis (431). Indeed, bacterial products and sepsis-induced mediators as IL-1, IL-6, TNF, IFN-γ, in various combinations, are known to induce iNOS expression in different tissues, including smooth muscle cells and hepatocytes (350). The resulting uncontrolled, calcium-independent NO synthesis would lead to vasodilation and unresponsiveness to vasoconstrictors (120, 225, 522). Moreover, differential expression of iNOS would cause inappropriate vasodilation of particular vascular beds which would become overperfused relative to others, with the consequent formation of functional shunts (349, 436).

Not all animal models are consistent with this hypothesis. In an endotoxemic rat model the changes of iNOS activity and $[NOx]_p$ appear partially dissociated from the changes of vascular reactivity and total peripheral conductance (165, 512). Moreover, in carefully controlled laboratory experiments on small and large mammals, endotoxin causes exhaled NO to rise after an interval (30-100 min) (218, 333, 493, 501), which is too short for iNOS transcription and translation, a process which takes several hours (388). Indeed in a porcine model, after a 2 hours infusion of 10 μg kg^{-1} h^{-1} endotoxin, iNOS was undetectable in lung, liver, kidney, diaphragm, ventricles, aorta and vena cava (333).

Evidence of increased iNOS expression in humans during sepsis is scarce. In isolated hepatocytes cytokinic stimulation can induce calcium and calmodulin independent production of NOx (372), and iNOS mRNA has been detected in a number of human tissues (202, 242, 303, 330, 392, 432, 457), but this does not necessarily imply that iNOS is produced in big amounts in sepsis. iNOS or its mRNA do not increase in the plasma after administration of 1-2 ng kg^{-1} of *E.coli* endotoxin to healthy volunteers (411). iNOS mRNA increases in urinary cells from healthy subjects after LPS injection and in septic patients (203). However, while this report provides some evidence of a possible association between renal iNOS induction and proximal tubular injury during sepsis, it sheds no light on whether iNOS is also induced in other tissues, or whether that induction has hemodynamic and microcirculatory consequences. In septic patients expression of iNOS appears compartmentalized at the site of inflammation (15).

It is not therefore surprising that attempts to manipulate NO production in human sepsis have been made both aiming to reduce (296, 569) and to increase NO bioavailability (49, 54, 251, 301, 530). For a

recent review of these studies see (270). Up to date, these attempts were unsuccessful to restore a normal microcirculation or to decrease mortality.

A phase II trial with non-selective nitric oxidase inhibitor, N^G-methyl-L-arginine, showed earlier resolution of shock and a trend toward improved outcome (29, 569), but a multicenter, randomized, placebo-controlled, double-blind phase III study testing the same molecule, in similar but not identical conditions, was prematurely discontinued because of increased mortality in the treatment arm (296). N^G-methyl-L-arginine, besides reducing $[NOx]_p$, increased SVR while reducing the requirements for vasoconstrictive drugs. Note that the latter result does not imply that responsiveness to catecholamines was restored, because N^G-methyl-L-arginine acts as a vasoconstrictor also in healthy volunteers (541). The patients treated with the drug exhibited an excess of mortality related to decreased cardiac output, cardiac failure, and cardiogenic shock.

A generalization of these results is a daring task, as the relation between the different models with the in vivo condition is often difficult to establish. There is abundant evidence that the intrinsic regulation of the microcirculation is abnormal in sepsis, but the exact combination of the mechanisms responsible is yet to be defined with precision in human sepsis and in experimental models.

Disturbances of extrinsic mechanisms regulating the microcirculation in sepsis

In septic patients, nervous and hormonal stimulation is elevated despite normal or reduced SVR, as indicated by high levels of circulating catecholamines, aldosterone and renin (16, 40, 185, 285). Moreover, the rate of increase of SVR with increasing catecholamine infusion is generally smaller in septic than in non-septic patients (**Figure 4**). Indeed catecholamine-resistant, refractory hypotension is an early cause of death in septic patients (138).

Vascular hyporeactivity to various agents is not a specific feature of sepsis, as it arises also in other pathological conditions, as during vascular decompensation associated with hemorrhagic shock (525). Indeed, vasodilatatory shock may appear as a late event whenever shock is severe and long-lasting (274).

The response of arterial and venous vessels to several vasoactive substances has been studied in human endotoxemic models. After 4 hours from the injection of a small dose (1 or 2 ng kg^{-1}) of *E. coli*

endotoxin in healthy volunteers, systolic blood pressure (SBP) increased less than in control subjects during phenylephrine infusion (4 μg kg^{-1} h^{-1}). At the level of the forearm, LPS blunted norepinephrine-induced vasoconstriction and acetylcholine-induced vasodilation, while the effects of glyceryl trinitrate and N^G-methyl-L-arginine were unchanged (411). An antioxidant, vitamin C, restored in this model a normal response to acetylcholine (412). In a different human model, in which a systemic inflammatory response was generated by *Salmonella typhi* vaccine, the vasodilatatory response of forearm blood flow to endothelium-dependent vasodilators, acetylcholine and bradykinin, was similarly blunted. Again, the effects of endothelium-independent vasodilators (verapamil and nitroglycerin) were unchanged (210). In another study, investigating dorsal hand veins, local application of endotoxin at a concentration of 20 ng mL^{-1} had no effect on resting vein size, which probably was maximal in those experimental conditions, but markedly attenuated norepinephrine-induced vasoconstriction up to 3 hours from endotoxin exposure. Endotoxin also abolished vasoconstriction induced by deep breath, an effect mediated by an increase of orthosympathetic tone (42). Neither locally applied N^G-methyl-L-arginine or pretreatment with a cyclooxygenase inhibitor restored norepinephrine-induced vasoconstriction. Conversely, pre-treatment with hydrocortisone prevented endotoxin-induced hyporesponsiveness (50).

These experiments clearly show that endotoxin can induce vascular hyporeactivity in arteries and in veins, blunting the effects of nervous and hormonal stimulation, but do not allow definite conclusion regarding the mechanisms mediating this effect. Restoration of the vasoconstrictive effects of norepinephrine on a dorsal hand vein by hydrocortisone, but not by cyclooxygenase or NO synthase (NOS) inhibitors suggests that, at the level of the veins, prostaglandins or NO hyperproduction were not directly responsible for the refractoriness to the effect of norepinephrine. Indeed, a human in vitro study showed that LPS induces NO overproduction, blunting the vasoconstrictor response to phenylephrine, in internal mammary arteries, but not in saphenous veins, in which hyporesponsiveness to vasoconstrictors is induced by a NO-independent mechanism (520). Interestingly, an experimental study using a porcine endotoxemic model suggested that N^G-methyl-L-arginine partially restores arterial but not venous responsiveness to norepinephrine (117). These human studies also indicate that in endotoxemic conditions vascular smooth muscle responds normally to vasodilators like NO and calcium-agonists, but not to vasoconstrictive agents as catecholamines.

In resuscitated septic shock patients, phenylephrine increases MAP less than in healthy subjects, a phenomenon partially reversed by administration of 50 mg hydrocortisone (40). In this study there was no correlation between the parameters characterizing the phenylephrine-MAP relation and plasma renin, aldosterone and norepinephrine/epinephrine levels, suggesting that vascular hyporeactivity was unrelated to nervous or hormonal hyperstimulation. In another study, septic shock patients with decreased adrenal reserve showed hyporesponsiveness to norepinephrine (13).

Vascular hyporesponsiveness to catecholamines in septic conditions has been described in different animal models, including mice (239, 240), rats (28, 33, 213), rabbits (540), cats (401), sheep (48), and pigs (68, 117). Similarly, blunting of angiotensin-induced vasoconstriction is a common finding in different animal models (27, 77, 86, 278, 467, 516). A reduced response to vasopressin in experimental sepsis models is not a universal finding. In some studies the vasoconstrictor response to vasopressin was preserved (7, 33, 43), but in others not (140, 214, 467). Importantly, responsiveness of vessels of septic animals to vasopressin and its time-course appear dependent on the regional circulation which is investigated (516), In septic patients plasma levels of vasopressin are usually reduced (273), and vasopressin has been proposed as an additional therapeutic agent in these subjects (451).

Multiple mechanisms, reviewed in (274, 482), are implicated in vascular hyporeactivity. Besides inactivation of catecholamines by superoxide (308), at the level of the membrane α_1-adrenergic receptors have consistently been shown to be downregulated during the late phases of sepsis in human hepatocytes from septic patients (221) and in murine LPS and CLP models (240, 331, 470). This downregulation is mediated via sepsis-associated pathways including relative adrenal insufficiency (320, 538) and promoter activity suppression at the transcriptional level by proinflammatory cytokines (470). Abnormal hyperpolarization of vascular smooth muscle cells seems to be another contributing factor. A number of different potassium channels, calcium-activated, voltage-dependent, inward rectifier and ATP-sensitive, appear to play a role (266). Activation of these channels due to reduced intracellular ATP, local acidosis, circulating sepsis-associated molecules (atrial natriuretic peptide, calcitonin gene–related peptide, and adenosine), and electrical stimuli via gap-junction through the endothelium, leads to increased potassium efflux, hyperpolarization and inhibition of voltage-dependent Ca^{2+} channels opening, with a net reduction of extracellular calcium influx (180, 422). Alterations in several intracellular pathways

that modulate smooth muscle contraction are also responsible for reduced responses to vasoconstrictive agents. Rho-associated protein kinase (ROK)-dependent inhibition of myosin light-chain phosphatase (MLCP) activity is dampened in CLP mice, leading to reduced vasoconstrictive responses to K^+, thromboxane A_2 receptor activation and protein kinase C (PKC) activation (430). Finally, NO may play a role, inhibiting smooth muscle contraction via activation of MCLP, and of potassium channels on the plasma membrane (17, 58, 357, 422). Moreover, depletion of NO synthase cofactors (L-arginine and tetrahydrobiopterin) may cause NO synthase to produce reactive oxygen species as superoxide and hydrogen peroxide, forming, together with NO, reactive nitrogen species, as peroxynitrite (385). Hydrogen peroxide induces smooth muscle relaxation by activation of soluble guanylate cyclase (338) and by hyperpolarizing cell membrane (31, 201).

Despite the number of experimental works focused on vascular reactivity in sepsis, the interplay of the different mechanisms in humans remains poorly understood, as indicated by the limited results of the clinical trials attempting to manipulate these mechanisms. Indeed, glucocorticoids, antinflammatory drugs, antioxidants, inhibitors of potassium channels and vasopressin were unable to improve survival substantially (14, 47, 158, 348, 361, 452, 513, 529, 547, 568).

Microvascular walls in sepsis

Bacterial products and molecules produced during the host response may alter several functional characteristics of microvascular walls (30). In physiological conditions, endothelium is lined by the glycocalyx, a meshwork of membrane-bound proteoglycans and glycoproteins, whose thickness (from 0.5 to more than 1 μm) increases with increasing diameter of the vessel (332, 544). Its position at the interface between endothelial cells and blood enables it to condition a number of biological functions relevant during sepsis, including shear-stress mechanotransduction and shear-dependent vasodilation, capillary barrier function, coagulation and regulation of the interactions between immune cells and the endothelium. Moreover, in the microcirculation its thickness is in the same order of magnitude of the diameter of the vessels, and therefore it can condition local hemodynamics (95, 144, 418–420, 433, 458).

Although alterations of the glycocalyx is not a phenomenon specific of sepsis, having been detected in a number of different pathological conditions (113, 282, 321, 354, 560), its degradation in the

experimental settings leads to microcirculatory alterations very similar to those detected in the sublingual microcirculation of septic patients (82).

As direct visualization of the glycocalyx in vivo is technically demanding, glycocalyx shedding can be assessed indirectly by measuring plasma levels of syndecan-1 and heparan sulfate, two constituents of the glycocalyx whose plasma levels have been found inversely proportional to glycocalyx thickness in an hemorrhaged rat model (526). In septic patients, during the first 24 hours of observation, syndecan-1 levels were markedly increased relative to patients without sepsis after major abdominal surgery or to healthy volunteers. Relative to healthy controls, also heparan sulfate was increased during sepsis, but less than in post-surgery patients. A relation between systemic inflammation and glycocalyx shedding was suggested by the positive correlation between plasma IL-6 and syndecan-1 (500).

Recently, additional evidence for glycocalyx shedding and thinning in septic patients has been gathered via SDF assessment of RBC deviation from the central flow toward the endothelial cells in the sublingual microvessels, a parameter called perfusion boundary region (PBR), thought to reflect the extent of RBC penetration in the glycocalyx (**Figure 6**) (447). This method takes advantage of the fact that the outer part of the glycocalyx tends to exclude flowing RBC, which therefore gather toward the center of the microvessel. As penetration of RBC in the glycocalyx is also a function of RBC velocity (559) and deformability (364, 476), it is unlikely that PBR is univocally determined by glycocalyx thickness.

Plasma components contribute to the formation of the luminal part of the glycocalyx, and a change of plasma composition can lead to degradation of this layer (418). Albumin appears important in the preservation of the glycocalyx (336), but it is not the only plasma molecule involved (220, 472, 591). However, in hypoalbuminemic septic patients, albumin supplementation does not reduce microvascular permeability, possibly because the albumin concentration required to maximally reduce permeability is quite low (316), and albumin administration to keep a serum albumin level of 30 g L^{-1} do not improve survival up to 90 days (83). A matter of concern is the possible damage to the glycocalyx after aggressive resuscitation with crystalloids (317, 318). Indeed volume expansion, besides changing plasma composition, can trigger the release of atrial and brain natriuretic peptide, mediators able to induce glycocalyx shedding (75, 76).

An increase in permeability is known to occur in the rat myocardial, liver, intestinal, lung and kidney microvasculature after exposure to LPS or in CLP models (170, 190, 219, 277, 585, 593).

Not only glycocalyx degradation, but also several alterations of endothelial cells have been associated with increased permeability and interstitial edema in in vitro and in vivo models of sepsis. Alterations of endothelial adherens junctions, mainly consisting of VE-cadherin, is induced by proinflammatory cytokines and LPS, impairing VE-cadherin-mediated adhesion (469, 480). Incubation of human endothelial cells with TNF-α or LPS results in endothelial disruption, with A disintegrin and metalloproteinase domain-containing protein 10 (ADAM10)-mediated production of endothelial cadherin fragments (sVE-cadherin) (151). An increase of serum levels of VE-cadherins in septic patients has been repeatedly measured (151, 587, 594).

Another mechanism potentially able to impair the barrier function of the endothelial layer is apoptosis, Evidence of endothelial apoptosis in the systemic circulation in in vivo models is scarce, because apoptotic endothelial cells rapidly detach from the vessel wall and are quickly cleared by the circulation (215). In in vitro models of sepsis, bacterial products may or may not trigger endothelial apoptosis, apparently depending on the type of bacterial product, the dose and the cell line used (215). Endothelial apoptosis may be also triggered by cytokines produced in response to bacterial products (405, 566). Stimulation of pulmonary microvascular endothelial cells in vitro with TNF-α, interferon γ and IL-1β induces apoptosis with a caspase-dependent mechanism, together with impaired barrier function. Co-culture with polymorphonuclear cells worsens the impairment of barrier function but apparently does not increase endothelial cells apoptosis (566). Recently, endothelial cell death with features consistent with cell apoptosis has been directly observed in the pulmonary microcirculation of a CLP murine model using intravital microscopy (170, 171). The extent of cell death correlated with impairment of endothelial barrier function assessed by albumin leakage (170). Furthermore, shedding of microparticles, i.e. membrane fragments from apoptotic cells (endothelial cells, platelets, and leukocytes) exert a proinflammatory and prothrombotic effect (159, 375, 376).

In conclusion multiple mechanisms, among which glycocalyx shedding, disruption of adherens junctions and apoptosis may undermine endothelial barrier function, leading to tissue edema, and potentially generating local hypoxia by an increase of oxygen diffusion distance (116, 230).

Rheology, microthrombosis and immunothrombosis in sepsis

Alterations of the microvessels walls are not the only responsible for the abnormal state of microcirculation in sepsis. Microcirculatory flow may be worsened by abnormal changes of the rheological characteristics of the blood: decreased RBC and white blood cell deformability (34), increased RBC aggregation (46) and coagulation disturbances may contribute to the impairment of the microcirculation (247, 292, 402).

As erythrocyte diameter in the absence of external forces (~8 μm) exceeds that of smaller capillaries, considerable deformability is required for the erythrocyte to flow through the capillaries and deliver oxygen to the tissues. During sepsis, the biconcave shape of the erythrocytes may be partially lost, with the appearance of echinocytes, disintegrating erythrocytes and erythrocyte aggregates (378). The shape of RBC becomes more spherical, and the decrease of surface-to-volume ratio leads to reduction of deformability. These changes take place together with a decrease of sialic acid molecules on the membrane (406), increasing the tendency to form aggregates during low shear conditions (232). In the clinical setting, the aforementioned morphological changes are suggested by an increased erythrocyte distribution width, a strong predictor of mortality in septic patients (252, 261, 453). Besides cell geometry, erythrocyte deformability is determined by membrane properties, and cytoplasmic viscosity (342, 408), which may be altered during sepsis by a number of factors, including 2,3 diphosphoglycerate (511), NO (37, 62), intracellular calcium (356, 380) and reactive oxygen species (309, 378, 416). In animal models, decreased deformability of RBC leads to macro and microcirculatory dysfunction (276).

Polymorphonuclear leukocytes also become stiffer and less deformable in septic patients. Activation of human polymorphonuclear leukocytes by exposure to inflammatory cytokines (e.g. TNF-a) confers an increased cell rigidity (130), possibly impairing their flow in the microcirculation. In animal models this process leads to polymorphonuclear leukocytes sequestration in organs like the lung and the liver (129, 344). Excessive activation of neutrophils leads to the release of granules containing enzymes and mediators able to activate endothelial cells and platelets, besides directly injuring the endothelial layer (484).

Widespread activation of platelets and of the coagulation cascade within the microcirculation is thought to lead to microthrombosis during sepsis. In healthy volunteers, small amounts of LPS cause platelet

activation. When 2-4 ng kg^{-1} LPS are administered to healthy humans, thrombocytes count falls by ~10-15% at 1-2 hours, independently of the administration of paracetamol, aspirin, heparin or a thrombin inhibitor (289, 473, 503, 504). The rapid decline in platelet count can be due to removal of activated platelets from the circulation by adhesion to leukocytes or endothelial cells, as suggested by increased formation of platelet-leukocyte aggregates (503).

In a large cohort of septic patients, about 50% experienced thrombocytopenia, defined as a platelet count of <150,000 μL^{-1}. One of four thrombocytopenic patients presented disseminated intravascular coagulation (546). Activated platelets rapidly lose surface P-selectin, a glycoprotein expressed on the surface of endothelial cells and platelets (590), which can be recovered in the blood (337). Indeed, a strong positive correlation has been found between plasma P-selectin and disseminated intravascular coagulation scores in septic patients (351). The tendency for microthrombosis is further enhanced by endothelium-mediated activation of the extrinsic coagulation pathway, thrombin formation and complement activation (45, 209). Neutrophil extracellular traps (NETs) are web-like chromatin structures which are released from neutrophils and act by trapping microbes, blood cells and activating platelets (164, 439). Apart from forming an intravascular bactericidal net, this process also triggers intravascular thrombosis, through interactions with platelets and enzymes in an immune and thrombotic process, described as immunothrombosis (137).

Effects of sepsis on heart function

Introduction

Bacterial products or endogenous substances produced by the host as a response, besides changing the coupling conditions between the heart and the vessels, can markedly affect the systolic and the diastolic function of the heart.

In the past, it was common opinion that, in the absence of a pre-existing heart disease, cardiac dysfunction becomes prominent only in the later stages of the disease (206, 299, 570). However, a number of studies has provided evidence that, at least in a certain number of patients, sepsis-induced

cardiac dysfunction has a strong impact on patients' hemodynamics early at admission (186, 187, 234, 235, 552).

Assessment of the systolic and diastolic properties of the heart

Systolic and diastolic properties of the heart have been fully reviewed elsewhere (104, 494, 561). Here we will discuss in brief only some aspects, which will be useful to characterize the effect of sepsis on heart function.

Systolic properties of the heart

The systolic function of a ventricle can be characterized in the pressure-volume (PV) plane by the end-systolic pressure volume relation (ESPVR) (79, 454), which shows the univocal relation of ventricular end-systolic pressure (P_{es}) and ventricular end-systolic volume (ESV) (**Figure 7**). Experimentally, it has been found that this relation is roughly linear and common for ejecting and isovolumic contractions. Thus, the ESPVR is characterized by only two parameters, E_{es}, that is the slope of ESPVR, and the dead volume (V_0), that is the volume of the ventricle at end-systole when transmural pressure is zero. The latter parameter is not directly measured and is obtained by extrapolation of the regression line describing the experimentally measured data points.

It should be kept in mind that the simplicity of this model can be deceiving, as the ESPVR is not completely afterload-independent (25, 155, 328, 495), its shape can deviate from a straight line (81, 243, 339), and it may be affected by the ventricle size and shape (39, 198). Initially, it was believed that changes of the systolic function (or contractility, if all the other determinants of systolic function are invariant) are mirrored in the PV plane by changes of E_{es} only, without large shifts of V_0 (455), but later it became evident that a change of systolic function can manifest itself as a shift of the ESPVR without a change in slope (79, 244, 492, 508).

The sensitivity of E_{es} to changes of contractility is modest relative to other indices (245) and in healthy humans the inter-subject variability of E_{es} and especially V_0 is considerable. In the 29 healthy subjects by Starling (496) E_{es} was 3.51±1.26 mmHg ml^{-1} (range: 1.65, 5.61 mmHg ml^{-1}) and V_0 1±23 ml (range: -23, 81 ml). Somewhat smaller values of E_{es} (2.3±1.0 mmHg ml^{-1}) were measured in another study (97). At least in dogs, E_{es} and even more V_0 show a significant intra-subject variability (154).

Assessment of E_{es} requires the measurement of the ESPVR, which can be determined by invasively recording the P-V loops corresponding to several heart beats while repeatedly changing the loading conditions, by transiently occluding (97) or compressing (59) the vena cava to reduce preload, or by pharmacological manipulations of afterload (19, 329, 496). In the last decades methods allowing an approximate estimation of the ESPVR on a single-beat base have been published (96, 488, 515), potentially obviating the need of manipulation of the loading conditions. However, single-beat methods are empirical approximations, as they either assume constant volume-axis intercept, or a V_0 equal to zero (290), or draw the additional information required for the calculation of E_{es} from a simulated P-V relation, which is based on load-dependent parameters (96, 487, 488, 515). In a carefully controlled laboratory study (253), single-beat derived E_{es} showed small biases relative to the multi-beat assessment of the same parameter (from -0.3 to 0.5 mmHg ml^{-1}), but the limits of agreement of different single-beat methods (±2 SD) spanned from ±2.6 to ±3.8 mmHg ml^{-1}, showing an unacceptable lack of precision.

In clinical studies, P_{es} is not usually measured directly using a catheter in the left ventricle, but estimated through measuring arterial pressure more peripherally, invasively or non-invasively. P_{es} is then approximated to 90% of systolic arterial pressure (SAP), to MAP or to the pressure at the dicrotic notch (96, 184, 248, 345, 346). Even if the correlations between all these variables are usually high and biases modest (347), it is likely that these successive approximations introduce noise in P_{es} assessment.

The need to manipulate preload in order to assess the characteristics of the ESPVR led to the substitution of E_{es} and V_0 with surrogate parameters, as the ratio between P_{es} and end-systolic volume of the left ventricle (ESV_{LV}) or end-systolic volume index of the left ventricle ($ESVI_{LV}$) (P_{es}/ESV_{LV} or $P_{es}/ESVI_{LV}$) and the ratio between SAP and ESV_{LV} or $ESVI_{LV}$ (SAP/ESV_{LV} or $SAP/ESVI_{LV}$), practically, single points estimates of E_{es} (89, 368, 425, 491). A common assumption when using these surrogates is that V_0 is negligible. If V_0 equals zero, P_{es}/ESV_{LV} coincides with E_{es}. If it is not so, however, errors, together with some degree of load-dependence, are introduced (96, 257).

Due to the difficulty of measuring load-independent indexes of systolic function, a big amount of research in sepsis and septic shock has been performed using indices of systolic function which are heavily dependent on loading conditions, for example ejection fraction (EF) (245). It is thus useful to

briefly review the dependencies of this index, using the conceptual framework of the ESPVR. For an exhaustive discussion of this topic see (442).

On the pressure-volume plane, afterload can be quantified by effective arterial elastance (E_a), the ratio between P_{es} and SV (248, 509, 510). Graphically, E_a is the slope of the line connecting the end-diastolic volume (EDV)-zero pressure point with the P_{es}-ESV point (**Figure 7**), and corresponds, approximately, to the product of SVR and HR (510). When measured invasively in healthy humans by Starling and coll. (496), E_a averaged 2.32±0.61 mmHg ml^{-1}.

On the other hand, EF is defined as

$$EF = \frac{SV}{EDV} = \frac{SV}{ESV+SV} = \frac{\frac{P_{es}}{E_a}}{\frac{P_{es}}{E_{es}}+V_0+\frac{P_{es}}{E_a}} = \frac{E_{es}}{E_a+\frac{V_0(E_{es}E_a)}{P_{es}}+E_{es}} \quad (7)$$

If V_0 is set to zero, equation (7) becomes

$$EF = \frac{E_{es}}{E_a+E_{es}} = \frac{1}{\frac{E_a}{E_{es}}+1} \quad (8)$$

or

$$\frac{E_a}{E_{es}} = \frac{1}{EF} - 1 \quad (9)$$

It is evident that EF is markedly dependent on arterial load (E_a), besides contractility (E_{es}) and that if V_0 is assumed to be negligible, the information provided by EF is equivalent to that provided by E_a/E_{es}, a parameter used to assess ventricular-arterial coupling.

Other load-independent indices of systolic function, apart from E_{es} and V_0, are the slope and intercept of the relationship between EDV and stroke work (SW) (173) (preload-recruitable SW), and of the relationship between the maximum change of intraventricular pressure with time (dP/dt_{max}) and EDV (293, 294). These indices, however, have not been used in human sepsis studies.

Apart from left ventricle fractional shortening (FS) and EF derived from left ventricular (LV)-trace, a number of echocardiographic parameters have been developed for non-invasive assessment of LV systolic function.

Tissue Doppler imaging (TDI)-derived peak systolic mitral annular velocity (Sm) reflects LV longitudinal shortening during systole. In an animal model, Sm displayed a stronger correlation with dP/dt_{max} than did EF_{LV} (478). According to theoretical and experimental analysis, Sm is a coupling parameter positively

affected by preload and contractility, and negatively affected by afterload (535). The degree of its afterload dependence in clinical settings is somewhat controversial, with some studies supporting Sm dependence on afterload (63, 205, 377) and some afterload-independence (10, 57, 390).

Myocardial performance index (MPI) of LV uses measurements from mitral inflow and left ventricular outflow Doppler tracings, i.e. isovolumic contraction time (IVCT), ejection time (ET), isovolumic relaxation time (IVRT) and is calculated as:

$$\mathrm{MPI} = \frac{\mathrm{IVCT} + \mathrm{IVRT}}{\mathrm{ET}} \tag{10}$$

Systolic dysfunction prolongs IVCT and shortens ET. At least in pigs, MPI is also dependent on loading conditions (98, 197) and dissociated from E_{es} (98).

Mitral annular plane systolic excursion (MAPSE), an M-mode index standing for the linear distance of mitral systolic movement towards the LV apex, shows a significant decrease in patients with septic shock and might be a more sensitive marker of LV systolic failure than EF (592).

Speckle tracking is a recently developed echocardiographic tool, which evaluates LV deformation over time, tracking user-selected regions of the myocardium (“speckles”). During systole, the LV shortens along the longitudinal and circumferential dimensions, while its wall thickens in the radial dimension. Strain is a measure of myocardial deformation of a segment in relation to its original dimension and it is expressed as a percentage. Global longitudinal strain (GLS) and global circumferential strain (GCS) respectively express LV longitudinal shortening and LV circumferential shortening. In septic pediatric patients, strain imaging detected subtle changes in LV systolic function prior to overt decline of EF (35). Boissier et al. showed that a reduced GLS is inversely correlated with afterload indices in septic shock patients (57).

Two-dimensional (2D) strain analysis cannot illustrate rotational motion of the ventricles because it perceives ventricular structure in only two dimensions. However, myocardial fibers display a complex spatial orientation and contract simultaneously in different axes. Three-dimensional (3D) is a novel echocardiographic modality assessing LV surface deformation in three dimensions. Recently, parameters obtained with this technology have been compared with invasively assessed indexes of contractility as E_{es} and dP/dt with promising results (284).

Diastolic properties of the heart

The diastolic properties of the cardiac chambers can be described in both static and dynamic terms. The static properties of the ventricles have been characterized by the end-diastolic pressure volume relation (EDPVR) (79, 104), which represents the relation between the pressure and the volume at the end of the diastole, in the relaxed state. Below a certain residual volume (the volume of the chamber at zero transmural pressure) the chamber recoils outward, but for most of the physiologic volume range the ventricles recoil inward (**Figure 8**). Above residual volume, pressure increases with volume roughly exponentially, causing ventricular diastolic stiffness (dP/dV) or compliance (dV/dP) to be markedly volume-dependent (8). In humans the ratio between the diastolic filling pressure and ventricular volume has been used as a rough estimate of the passive elastic properties of the ventricle (506). However, because of the non-linear shape of EDPVR, any single-point estimate of diastolic compliance cannot correspond to the elastance of the ventricle at a given ventricular end-diastolic volume. More recently, single-beat methods has been developed to characterize diastolic ventricular properties (424, 517).

In healthy subjects compliance of the right ventricle is maximal at low-normal filling volumes, and decreases very fast at higher volumes as the filling limit is approached (424). At comparable volumes the left ventricle is stiffer than the right (517), and the shape of its EDPVR is markedly curvilinear in the whole volume range (403).

The EDPVR has never been assessed in septic shock patients. In rats made septic by intraperitoneal injection of fecal slurry, EDPVR shifted to the left and rotated counterclockwise, indicating an increase of the stiffness of the left ventricle and a decrease of its residual volume (145). Diastolic stiffening of the left ventricle has been noted also in anesthetized rabbits 36 hours after treatment with LPS (600 μg kg^{-1}) (32).

During the isovolumic phase of the diastole and part of the ejection phase the ventricle relaxes, and this dynamic behavior contributes to diastolic performance. The time-constant of ventricular relaxation during the isovolumic phase of diastole can be measured invasively (573). When an intraventricular catheter is not available, this parameter can be estimated by echocardiography (466).

TDI-derived early diastolic mitral annulus velocity (e') and the ratio of the peak trans-mitral inflow velocity in early diastole (E) to e' (E/e') are the most commonly used markers of LV diastolic function (363, 379). Irrespective of its clinical usefulness (295), it should be underlined that E/e' is just a surrogate

for more physiologically robust indices of diastolic function, and its alterations cannot be directly related to alterations of the EDPVR or relaxation properties of the myocardium (532).

Cardiac effects of endotoxin in healthy volunteers

In an attempt to eliminate the many confounding factors present in patients with clinical sepsis and to avoid the interspecies differences which characterize animal models of sepsis, cardiac function has been investigated in healthy humans. The results of these studies were not completely consistent.

Two studies, one by Mathru and coll. (326) using transthoracic echocardiography, and one by Suffredini and coll. (506) using right heart catheterization and radionucleotide cineangiography, assessed cardiac function in healthy volunteers after 3 hours from the administration of 4 ng kg^{-1} of LPS without fluid loading. LPS triggered the expected hyperdynamic state, with a decrease in MAP (14-18% of baseline) and an increase in CI (53-66%). Left preload decreased in one case (326) and remained unchanged in the other (506). EF of the left ventricle (EF_{LV}) was increased in both studies (6-9%). As EF_{LV} is loosely dependent on preload but markedly dependent on afterload (245), it is impossible to say, on this base only, if systolic function was enhanced, unchanged or depressed. According to Mathru and coll., velocity of circumferential fiber shortening (VCFc), endocardial wall systolic velocities measured by TDI and $SAP/ESVI_{LV}$ were markedly increased, suggesting enhancement of systolic function. To the contrary, the small decrease of stroke work index of the left ventricle (SWI_{LV}) measured by Suffredini and coll. at the same time point is suggestive of deterioration of systolic function, because SWI_{LV} is relatively independent of afterload (which was decreased) and dependent on preload, which, estimated by PWP, was unchanged. Overall, both studies did not detect any clue of diastolic dysfunction at 3 hours. In both studies, no diastolic enlargement of the left ventricle was seen (326, 506). Additionally, IVRT and time constant for left ventricular relaxation were unchanged.

At 5 hours, in the absence of fluid loading, $SAP/ESVI_{LV}$ and EF_{LV} returned to control values while VCFc remained somewhat elevated. Also, endocardial wall systolic velocities remained elevated. IVRT and time constant for left ventricular relaxation were not different from control values (326).

After 3 hours from LPS administration, Suffredini and coll. infused 2 L normal saline during the next 2 hours. CVP, PAP, and PWP increased similarly in LPS-treated and in control subjects and similarly

declined towards baseline values when the infusion was stopped. To the contrary, in LPS-treated subjects but not in control subjects, MAP, after a transient increase concomitant with volume expansion, continued to decline, and at 6 hours it had decreased by 20%. This occurred because of a reduction in CI together with persistently low SVR. In the last part of the experimental period, EF_{LV} and SWI_{LV} were depressed relative to controls, as was the ratio between peak systolic pressure and $ESVI_{LV}$. Moreover, end-diastolic volume index of the left ventricle ($EDVI_{LV}$) was increased in LPS-treated relative to control subjects. The latter finding should be taken carefully, as the difference in $EDVI_{LV}$ between control and LPS-treated subjects at baseline (on average 13 ml m^{-2}) was similar to the increase of the same parameter due to LPS and fluid infusion (on average 16 ml m^{-2}). In other words, the change of $EDVI_{LV}$ due to LPS was of the same order of magnitude as the natural variability of the parameter. Actually, a more recent study did not confirm the increase of $EDVI_{LV}$ at the same time point after the same saline infusion protocol (322).

The effects of LPS on healthy subjects were also investigated during aggressive volume loading (265). In these experiments 4 ng kg^{-1} of LPS were administered immediately before the infusion of 1 L hr^{-1} for 3 hours and the additional infusion of 0.5 L hr^{-1} for 2 hrs. Relative to placebo, LPS at 3 hours caused a marked increase of CI together with a proportional fall of SVR, so that MAP was unchanged. The CI elevation was accounted for mostly by the increase of HR. During the next 2 hours, CI declined but remained elevated relative to baseline. SVRI did not rise proportionally, so MAP fell. Relative to baseline and placebo, $EDVI_{LV}$ was decreased at 3 and 5 hours. Conversely, $ESVI_{LV}$ fell at 3 hours and returned to baseline at 5 hours. Indexes of contractility like EF, VCFc, $SAP/ESVI_{LV}$ and $P_{es}/ESVI_{LV}$ were increased at 3 and depressed at 5 hours.

The problem in assessing the effects of LPS on heart function in these experimental models is twofold. The effects of LPS on the heart are obfuscated by the concomitant increase of orthosympathetic and hormonal stimulation which can by itself change both the systolic and the diastolic properties of the cardiac muscle. Thus, an eventual depressant action of LPS on myocardium can be evidenced only if LPS-induced depression of contractility is greater than the enhancement of the same parameter operated by the increase of orthosympathetic and hormonal stimulation. Second, assessment of systolic and diastolic properties of the heart is by no means straightforward, because most of the available indices are

more or less dependent on the coupling with the circulation (245). A comprehensive discussion on this topic can be found in (104). LPS, by changing the characteristics of the systemic circulation, alter the coupling between the pump and the vessels, and thus apparently alter cardiac function.

These problems are highlighted by a recent study in mice (237). Contractility was evaluated by conventional estimators, namely EF_{LV}, SW_{LV} and dP/dt_{max} and by more load-independent indices, that is E_{es}, dP/dt_{max} normalized by end-diastolic volume of the left ventricle (EDV_{LV}), and maximal P_{es} in percentage of the control value at the same ESV_{LV}. Measurements were done 2, 6 and 20 hours post *E. coli* LPS administration, at doses of 1, 5, 10 or 20 mg kg^{-1}. In this model, LPS decreased MAP, increased CO and decreased SV. At 2 hours after the injection, preload, indexed by end-diastolic pressure of the left ventricle (EDP_{LV}) and EDV_{LV}, was decreased, and afterload, indexed by total effective E_a increased. At the same time point EF_{LV}, SW_{LV} and dP/dt_{max} were depressed, indicating a decrease of contractility. To the contrary E_{es} and dP/dt_{max} normalized by EDV_{LV} were increased relative to baseline, suggesting an increase of contractility, while maximal P_{es} at iso-ESV_{LV} was unchanged! Actually, LPS while increasing E_{es} also increased V_0, so that with increasing doses of LPS the ESPVR was steeper but shifted to the right. When the effects of LPS on contractility were assessed using P_{es} at iso-ESV_{LV}, no change was found relative to baseline. Thus the conclusion is that, in this mouse model, LPS does not depress contractility; rather it may increase it, as suggested by the increased dP/dt_{max} normalized by EDV_{LV}, possibly due to the effects of enhanced orthosympathetic stimulation (410), or the direct effect of increased HR (156).

Overall, there is no evidence that in the absence of fluid loading 4 ng kg^{-1} of LPS in healthy humans depresses systolic or diastolic functions 3 hours after the injection (326, 506). Rather, at this time point EF and $SAP/ESVI_{LV}$ are increased, but in front of a decreased afterload. At 5 hours, the only study which assessed systolic and diastolic functions without concomitant fluid loading found them unchanged, as indexed by $SBP/ESVI_{LV}$, isovolumic relaxation time and relaxation time-constant (326).

Note that, on assumption of a positive V_0, $SAP/ESVI_{LV}$ should increase with increasing afterload (88). The fact that afterload was decreased and $SAP/ESVI_{LV}$ increased, suggests an increase of contractility (326). However, the assumption of a positive V_0 should not be taken for granted because in humans,

negative V_0 have been measured (496, 515), presumably due to the non-linearity of the ESPVR, and LPS has demonstrated the ability to shift V_0, at least in an animal model (237).

In the presence of fluid loading, some clues of systolic dysfunction emerges at 5-8 hours from LPS administration (265, 506). To the contrary, strong evidence of LPS induced diastolic dysfunction in healthy volunteers is lacking. Interestingly, in dogs TNFα, which is produced in humans after LPS administration (322, 506), elicits a biphasic effect on contractility, which is not altered by complete β-adrenergic blockade: an early increase followed by a late decrease (358). At present, it is unknown if TNF has a similar effect in humans.

Cardiac dysfunction in sepsis and septic shock

The interest in the effects of sepsis on the heart was boosted in the 80s by a series of papers showing profound but reversible alterations of both systolic and diastolic functions in septic patients.

Parker and coll., combining thermodilution with radionuclide cineangiography, studied for 10 consecutive days 20 septic shock patients, treated, in addition to antibiotics and methylprednisolone, with intravenous fluids in order to maintain a PWP of 12-15 mmHg, dopamine in case of persisting hypotension and norepinephrine if the required dopamine dose exceeded 20 μg kg^{-1} min^{-1} (397). Thirteen patients survived septic shock and 7 died due to refractory hypotension. On average, CI was elevated and SVRI was low, more in non-survivors than in survivors. SV was comparable in the two groups. Initially, EF_{LV} was markedly depressed in survivors (0.32±0.04) and higher in non-survivors (0.55±0.05). EF_{LV} increased back to normal values in survivors (0.55±0.05) at the end of the study (5-10 days later), while it did not change until the exitus in non-survivors. In survivors, $EDVI_{LV}$ and $ESVI_{LV}$ were markedly elevated at day 1 and normalized before discharge. In contrast the same parameters were normal in non-survivors, despite similar end-diastolic filling pressures. These abnormalities were not noted in a control group of 32 critical patients without sepsis. On the base of these results, it was proposed that the dilation of the left ventricle represents a kind of compensatory response (preload-adaptation) to maintain a normal stroke volume in the face of a decrease in contractility (138). Not all these findings were confirmed by following studies.

As suggested by Parker and coll. (397), systolic dysfunction is a frequent occurrence in septic shock, and reductions in EF_{LV} or SWI_{LV} (136, 234, 235, 280, 374, 394, 395, 423, 552) or, more recently, E_{es} (186) were consistently reported. Both EF_{LV} or SWI_{LV} are load-dependent estimators of contractility (104, 245, 442). With increasing afterload, EF_{LV} markedly decreases, being almost invariant in a wide range of $EDVI_{LV}$, while SWI_{LV} is relatively unaffected by afterload, increasing almost linearly with $EDVI_{LV}$. In septic shock patients MAP is abnormally low and, because of this, an increase of EF_{LV} would be expected. If EF_{LV} falls, it indicates an abnormal contractility. Similarly, a fall of SWI_{LV} cannot be attributed to a change of the loading conditions if preload remains high because of fluid loading. An increase of HR can cause EF_{LV} to decrease, but the reduction of EF_{LV} in septic shock patients is usually greater than expected even considering the concomitant tachycardia (139).

The interplay between heart contractility and arterial load in determining EF_{LV} may also explain part of the extreme variability of the prevalence of systolic dysfunction, assessed with this parameter, in septic shock patients (from 18 to 65%) (397, 552). The prevalence is apparently greater when patients are examined later after the admission, probably reflecting the administration of drugs causing vasoconstriction and increasing the afterload of the left ventricle (549, 550). Given the great variability of both preload and afterload during septic condition, a load-independent estimator of contractility would be very useful for the purpose of characterizing systolic dysfunction. E_{es} is a good candidate, but for its interpretation V_0 should also be assessed (78, 79, 442).

Alterations of EF_{LV} were found to be reversible in survivors not only when EF_{LV} was measured with cineangiography (394, 395, 397, 399), but also using echocardiography (235, 551). The association between low EF_{LV} and survival found by Parker and coll. (395, 397) was not confirmed by subsequent studies (161, 216, 234). The discrepancy between the different studies may be, at least in part, related to the variety of hemodynamic patterns present in septic shock patients. The non-survivors of Parker and coll. (397) had lower SVRI, despite a greater rate of infusion of norepinephrine than survivors (34.0±13.0 versus 1.6±1.0 μg min^{-1}). On one hand, the more profound refractory vasoplegia of these patients may have conditioned the prognosis, and on the other the decrease of afterload may have masked an existing systolic dysfunction by causing EF_{LV} to increase (234, 442).

Regarding the impressive increase of $EDVI_{LV}$ measured by Parker and coll. (up to ~ 135 ml m^{-2} in the whole cohort, more than 150 ml m^{-2} in survivors) (397), similar values were measured by the same group with the same technique in another study (394). This time, however, $EDVI_{LV}$ (and also $EDVI_{RV}$) was abnormally elevated in both survivors and non-survivors. As expected EDVIs returned back to normal by the time patients recovered (6-14 days), but, at variance with what previously reported (397), there was a concomitant decrease of CVP and PWP, suggesting that at least part of the increase of the ventricular end-diastolic dimensions observed at admission could be due to an increased filling pressure, and not to a sepsis-induced alteration of the passive mechanical properties of the chambers. A non-significant trend towards higher $EDVI_{LV}$ in septic shock patients was found in another study exploring the response to fluid infusion in patients with sepsis and septic shock using the same radionuclide cineangiography technique (374). Before the infusion $EDVI_{LV}$ was 90±6, and 109±7 ml m^{-2} in patients with sepsis without shock, and patients in septic shock, respectively. The latter value is substantially smaller than those reported (~135 ml m^{-2}) in previous studies (394, 397).

The hypothesis of preload-adaptation in sepsis was revaluated using transesophageal echocardiography by Vieillard-Baron and coll. (552). This group studied 40 septic shock patients, whose post-resuscitation hemodynamic profile was hypokinetic (CI<2 L min^{-1} m^{-2}, n=7), normokinetic (2<CI<4 L min^{-1} m^{-2}, n=27) or hyperkinetic (CI>4 L min^{-1} m^{-2}, n=6), under hemodynamic support by a vasoactive agent and positive-pressure mechanical ventilation. On average, $EDVI_{LV}$ was 61±17 ml m^{-2}, similar to that measured in the same laboratory in 50 healthy volunteers (71±15 ml m^{-2}). On the other hand, EF_{LV} was 49±15%, reduced relative to control values (69±7%). These results confirmed previous works using similar techniques, showing a normal left ventricular end-diastolic volume in septic shock patients (234, 414). It is difficult to reconcile these results with those of Parker and coll. (394, 397). In part, these different results may have been a consequence of the different methodologies used to measure $EDVI_{LV}$. Thermodilution can overestimate CO (370), particularly at low flows (543). Using radionuclide cineangiography, $EDVI_{LV}$ is obtained as the ratio between SVI, assessed by the pulmonary catheter as CI HR^{-1}, and EF. Thus, an overestimation of SVI would lead to an overestimation of $EDVI_{LV}$. However, the patients of Parker and coll. with elevated $EDVI_{LV}$ had high, not low CI, averaging ~4 L min^{-1} m^{-2} (397). On

the other hand, echocardiography, especially if transthoracic, can underestimate ventricular volumes (528).

Other studies, using transthoracic (234, 235, 272) or transesophageal (65, 550) echocardiography found increases in left ventricle dimensions in patients with a decreased LV_{EF} relative to those with a preserved LV_{EF}, the differences were however much smaller than those initially reported (397).

In the last decades echocardiography has become a frequently used tool for the evaluation of systolic function in septic conditions. Almost 2/3 of patients with sepsis or septic shock were found to have echocardiographic evidence of myocardial dysfunction, affecting either LV or RV and either the systolic or the diastolic cardiac properties (421). Attempts have been made to associate several echocardiographic indices with patient outcome. Regarding EF_{LV}, two recent meta-analyses showed absence of sensitivity and specificity in predicting mortality in patients with sepsis and septic shock (462, 479). Among TDI-derived indices, the average systolic velocity measured at the mitral annulus (S_a) has been shown to independently predict mortality in medical ICU patients (574). MPI worsening during the first 24 hours after diagnosis of severe sepsis or septic shock was associated with 90-day mortality in 47 medical critically ill individuals, even after correcting for severity of illness, fluid and vasopressor use (369). Studies investigating the role of GLS as predictor of mortality in septic subjects have yielded conflicting results, either positive (93, 367, 389), or negative (121, 226, 382, 589). A 2018 meta-analysis by SanFilippo and coll. showed that GLS predicted mortality in septic patients (while at the same time EF_{LV} did not) (463).

Among indices pertaining to the right chambers, a recent meta-analysis, including five studies using different techniques, found no significant difference in EF_{RV} and RV end-diastolic dimensions between survivors and non-survivors (similar results were obtained for the LV) (216). $ESVI_{RV}$, however, predicted in-hospital mortality in patients with sepsis or septic shock (272). TDI-derived RV peak systolic velocity (RV-Sm) was found to be significantly decreased in non-survivors with sepsis (161). More sophisticated modalities, such as speckle tracking, have been implemented for evaluation of RV function in septic patients. Orde et al. showed that severe reduction of RV free wall strain was an independent predictor of 6-month mortality in patients with sepsis or septic shock (382). Nevertheless, the contribution of RV in

septic cardiomyopathy remains an open field for research, particularly with novel echocardiographic modalities.

Echocardiographic studies provided evidence that diastolic function is also frequently impaired in sepsis (64, 65, 231, 355, 414). E/e' and e' have been assessed as markers of LV diastolic dysfunction in patients with sepsis. A 2015 meta-analysis by SanFilippo and coll. reviewed seven observational studies and found that diastolic dysfunction, detected with e', was significantly associated with mortality in septic patients (462), while a later meta-analysis by the same author, including 18 original studies, attributed the same property to E/e' (461).

In conclusion systolic LV and RV systolic dysfunction is frequently present in septic patients. Diastolic dysfunction has never been assessed in terms of EDPVR in patients, but echocardiographic studies strongly suggest its impairment.

The pathogenesis of cardiac dysfunction in sepsis is reviewed elsewhere (324, 450, 588).

Effects of sepsis on the coupling between the heart and the vessels

In the last decade, the coupling between the left ventricle and the arterial compartment (ventricular-arterial coupling, VAC) during sepsis has been characterized in terms of the ratio between E_a and E_{es} (E_a/E_{es}).

On theoretical grounds, the relation between E_a and E_{es} determines the efficiency, that is the ratio between the external work produced by the ventricle and its metabolic cost (80, 507) (**Figure 7**). For a given set of conditions, efficiency is expected to be maximal when E_a/E_{es}=0.5 (80), a value close to that measured in healthy subjects (19, 20, 496). A laboratory study on isolated dog hearts showed that maximal efficiency remains high in a broad range of E_a/E_{es} (0.3-1.3), corresponding to EF ranging from 40 to 80%, indicating that in physiological conditions the ventricle operates in conditions of maximal metabolic efficiency, and that only marked deviations of E_a or E_{es} from normality may cause a substantial decline of E_a/E_{es} (122). For an overview of the limitations of this approach in evaluating ventricular-arterial coupling see (99).

In septic shock patients before (186) and after fluid resuscitation (187) E_a/E_{es} is abnormally high, mainly due to a depressed E_{es}. It is doubtful that this elevated E_a/E_{es} can be responsible, on average, for

a marked efficiency reduction and therefore a significant rise in the heart energy expenditure, because the fall of efficiency at these E_a/E_{es} values is modest (80, 122). However, the scatter of individual values was high, with E_a/E_{es} reaching values of 2-2.5 (186). Interestingly, in (186) E_{es} increased by ~16% after volume-loading performed according to SSG (438), possibly due to reversal of hypotension, increased coronary perfusion and improved heart performance. While this occurrence is possible in septic patients before treatment, coronary perfusion does not seem to be a limiting factor in myocardial performance after resuscitation (107, 125).

It should be kept in mind that E_a is a composite parameter, depending both on the characteristics of the arterial tree and on heart rate. Only further studies will be able to tell if E_{es}/E_a provides additional information useful to improve the clinical management of septic patients.

Effects of sepsis on cardiovascular regulation

Cardiovascular variability is abnormal in sepsis and septic shock, suggesting that in these conditions autonomic modulation of cardiovascular parameters is impaired. Heart rate, systolic and diastolic pressure variability have been investigated by Annane and coll. in 20 septic patients without or with septic shock (16). The results were compared with those collected in normal subjects during passive 70° upright tilting, a maneuver known to trigger autonomic and humoral activation. Indeed, upright tilting increased norepinephrine, epinephrine, renin and aldosterone plasma concentrations to levels similar to those found in patients with septic shock. In contrast to the apparent similarity of orthosympathetic activation, suggested by the comparable norepinephrine plasma concentrations, the areas under the curve of HR and of diastolic blood pressure power spectrum, and their components at low frequencies were markedly decreased in septic and septic shock patients relative to controls. Moreover, the square root of the ratio between the low-frequency component of HR and systolic blood pressure, an index of baroreflex sensitivity (386), was depressed in septic shock patients relative to tilted healthy subjects. Baro- and chemoreflex sensitivities were found depressed in another study (471).

A decreased variability of HR is not specific of sepsis, as it has been reported in a number of pathological conditions, like chronic obstructive pulmonary disease (498) and chronic heart failure (195),

or after major surgery (256, 268). Also, the association between depressed heart rate variability (HRV) and mortality which has been found in sepsis (119) is not characteristic of this disease (497).

Similar alterations of HRV appear in normal volunteers after LPS administration (465), even before the rise of body temperature (174), suggesting that this experimental model can be valuable in understanding how autonomic regulation changes in septic conditions.

The effects of 4 ng kg^{-1} of *E. coli* LPS on muscle SNA, HR, and arterial pressure were studied on 14 volunteers by Sayk and coll. (465). Baroreflex was assessed with incremental doses of sodium nitroprusside and phenylephrine.

After 90 min, endotoxin decreased muscle SNA and increased HR at constant MAP. Interestingly, the change of muscle SNA was inversely correlated with serum TNF-α concentration, and directly correlated with serum IL-6 concentration. As MAP was unchanged, this reduction of muscle SNA cannot be attributed to the baroreflex, unless of a set-point change. In humans, cardiopulmonary receptors plays a major role in the control of the vascular resistance of the limbs, presumably by modulating muscle SNA (238, 596). However, cardiopulmonary baroreceptors cannot explain the decrease of muscle SNA measured by Sayk and coll., because after endotoxin administration to healthy humans CVP remains constant (506) or decreases (322, 326), and a reduced stimulation of cardiopulmonary receptors is expected, if any, to increase, not to decrease, muscle SNA (315). It appears, therefore, that in humans endotoxin is able to modulate autonomic output independently of the information coming from the peripheral sensors.

The ability of LPS or of bacterial products to modulate sympathetic output has been repeatedly confirmed in animal models. However, the measured changes of SNA are markedly dependent on the species investigated and on the experimental conditions. In cats receiving a dose of 1 mg kg^{-1} of *E. coli* endotoxin, preganglionic SNA of the splanchnic nerve fell, potentially contributing to the concomitant hypotension (258). Other studies in rats and sheep showed that visceral SNA increases in septic conditions or after treatment with LPS, whether indexed by splanchnic (523, 545), mesenteric (545), cardiac or renal (426) sympathetic activity. Notably, the increase of visceral sympathetic activity was present whether CO was elevated and SVR reduced (426), or CO was normal or reduced and SVR normal or increased (523). Experiments in rats showed that the markedly increased SNA measured after

a high dose of LPS (20 mg kg^{-1}) is only partially dependent on the concomitant hypotension, pointing to a central medullary effect of LPS or mediators triggered by LPS (391, 545). Indeed sympathoadrenal activation after LPS (5 mg kg^{-1}) occurs in rats even after sinoaortic baroreceptor denervation (595).

Even if the fall of muscle SNA at constant MAP measured by Sayk and coll. indicates that endotoxin affects centrally regulated sympathetic outflow, how overall sympathetic outflow changes remains unclear. A decrease of muscle SNA does not immediately imply that overall sympathetic activity is reduced. Indeed, at the same time point, circulating norepinephrine concentration was increased about six-fold relative to baseline, in front of a two-fold increase of circulating epinephrine, similarly to what happens to critically ill patients with systemic infection (16, 185, 285), suggesting an increase, rather than a decrease, of overall sympathetic activity. Plasma norepinephrine concentration, by itself, is not a good indicator of SNA, as it may change due to both a change of spillover or of clearance, or it may remain constant if spillover and clearance change proportionally (142, 175, 578). However, it has been shown that in critically ill patients with systemic infection both norepinephrine spillover and clearance are elevated (285). If this happened also after LPS injection in healthy volunteers, the measured elevation of plasma norepinephrine concentration would be strongly suggestive of an increased overall sympathetic activation. In this case, the reduction of muscle SNA implies that SNA is enhanced in another body region. Unfortunately, in our knowledge, human data to this regard are not available. It is not impossible that bacterial products or substances produced by the body in response to bacterial products cause different alterations of the autonomic control of different parts of the body. In sheep intravenous infusion of live *E. coli* induces a progressive increase of cardiac SNA, but an initial fall followed by a progressive rise of SNA at the level of the kidney (426).

When in healthy volunteers the baroreflex was tested with sodium nitroprusside infusion, MAP decreased more and muscle SNA increased less post-LPS than post-placebo (**Figure 9**, panel A), suggesting that baroreflex sensitivity had been reduced by endotoxin (465). Generalization of these findings to patients in septic conditions is, however, difficult and much depends on whether one assumes that muscle SNA reflects overall sympathetic SNA or not. In the former case, these findings suggest that LPS-induced disruption of the baroreflex control over vasculature is a potential contributor to the hypotension which can develop in septic conditions. In the latter case, little can be said about the role of

baroreflex in sepsis, as the central suppression of muscle SNA should be counterbalanced by enhancement of SNA somewhere else, and hypotension primarily develops due to refractoriness of the vessels to the action of catecholamines. An example of a differential modulation of the baroreflex has been provided by experiments in chronically instrumented sheep, in which infusion of *E. coli* alters the range of the baroreflex-mediated changes of cardiac SNA, but not that of renal SNA (426).

In the human subjects studied by Sayk and coll. endotoxin decreased muscle SNA by 50%, without any change of MAP, questioning the role of muscle SNA in the maintenance of MAP in these experimental conditions. However, such apparent insensitivity of MAP to a fall of muscle SNA may have been due to the concomitant increase of circulating catecholamines, potentially able to cause vasoconstriction, as the MAP response to phenylephrine in these subjects was preserved. Muscle vasculature may be only secondarily involved in the baroreflex control of arterial pressure in humans (315). During common carotid occlusion, limb vasculature resistance does not increase despite a rise of MAP (443). To the contrary, visceral vascular resistance appears to be very responsive to arterial baroreceptor stimulation (238), suggesting that also in humans SNA is modulated differently in different parts of the vasculature.

Notably, the changes of HR in human volunteers after endotoxin were completely unrelated to MAP (**Figure 9**, panel B) (465). In chronically instrumented sheep, infusion of *E. coli* increases cardiac SNA in the lower and mid part of the arterial pressure range, but at high pressures cardiac SNA is completely suppressed, as in the control conditions. Nonetheless, at these pressures HR is two-times greater after *E. coli* administration indicating that, apart from cardiac SNA, other factors, possibly a change of the vagal tone or a local effect of the pathogens on the heart, are responsible of the increase of HR measured in this experimental model (426). Indeed in vitro and in vivo studies have shown that endotoxin interacts with hyperpolarization-activated cyclic nucleotide-gated cation (HCN) channels, increasing their sensitivity to the effect of catecholamines (133, 597). Whether this mechanism is active also in humans remains to be elucidated.

Recently, the baroreflex response after LPS has been characterized in depth in a new analytic framework proposed by Sato and coll. (464) using a technique based on the decomposition of the feedback loop into a mechanoneural arc (neural arc) and a neuromechanical arc (peripheral arc). The

former is characterized by the relation between carotid sinus pressure (CSP) (input) and SNA (output), while the latter by the relation between SNA (input) and MAP (output) (**Figure 10**). In vagotomized, mechanically ventilated Sprague-Dawley rats under urethane and α-chloralose anesthesia (523) LPS (60 μg kg^{-1}) caused a time-dependent upward shift of the neural arc with a concomitant increase in the amplitude of the operative range. The peripheral arc underwent time-dependent modifications with a marked decrease in slope, which at 2 hours was less than half the control value (**Figures 11 and 12**). The operating point thus shifted towards higher SNA without a change of MAP.

This elegant study clearly shows that a relatively small dose of LPS is able to a) markedly decrease the responsiveness of the systemic circulation to sympathetic stimulation, so that for a given SNA a lower arterial pressure ensues, and b) reset the baroreflex neural arc, so that for a given MAP SNA is higher (**Figure 12**).

Regarding point a), several mechanisms are potentially responsible for the time-dependent decrease of the slope of the peripheral arc. At 60 min after LPS the slope of the relation between SNA and SVR was significantly depressed, suggesting a decrease of the responsiveness of the vasculature to sympathetic stimulation (**Figure 11**). Moreover, the relationship between SNA and CO was unchanged at 60 min but shifted downwards at 120 min, presumably due to a decrease of contractility combined with a reduction of the stressed volume of systemic circulation (**Figure 11**).

The exact reason why LPS resets the baroreflex neural arc upwards is unknown, but upward resetting is in line with previously collected evidence. Indeed, bacterial products or endogenous substances produced in response to bacterial products can elicit changes at the level of the central nervous system inducing sympathoexcitation (204, 250, 325, 545).

Even if the work of Tohyama and coll. has important limitations (the animals were vagotomized), it is significant because it dissects the different factors involved in the alterations of the baroreflex during systemic inflammation, showing that the overall effect of LPS is the result of quantitative changes both at central and peripheral level. In rats, baroreflex sensitivity measured from the relation between MAP and HR during phenylephrine infusion has been found increased after a low dose of LPS (10 μg kg^{-1}) (444) and decreased after a higher one (325). The dose-dependency of LPS effect on baroreflex may be due to different quantitative changes of the neural and peripheral arcs. Time and dose-dependent modifications

of the neural and peripheral arcs may be also involved in the complex pattern of response to LPS in rats. When *E. coli* LPS is administered at a dose of 1.6 μg kg^{-1} in rats with precollicular brainstem transection, initially MAP is unchanged, CO increases and SVR decreases. After ~1 hour, there is a tendency for CO to return to pre-LPS value, SVR increases above baseline, so MAP tends to increase. Note that in this model HR is constant and the increase of CO is due to an increase of SV (105). When the dose is increased to 10 mg kg^{-1}, LPS causes CO to fall despite an increase in HR, SVR to increase, and MAP to decrease (341). Even larger doses (30-40 mg kg^{-1}) cause a similar cardiovascular response (66, 105), together with a fall in CVP (66).

The work of Tohyama and coll. warns against simple interpretations of the experimental findings, clearly showing that endotoxin alters both the control system and the effectors responsible for arterial pressure maintenance. The importance of this phenomenon in shaping septic patients' hemodynamics is doubtful, because in humans the effects of vascular hyporesponsiveness seems preponderant. However, if modulation of sympathetic output to the various organs is different, as in some animal models, the consequences on the macro- and microcirculation may be remarkable. Further research is therefore warranted in order to fully characterize how the autonomic control changes in septic conditions and the way in which these changes affect the course of the disease.

Conclusion

Despite decades of study, key aspects of sepsis are yet to be elucidated.

The hemodynamics during sepsis is shaped by multiple physiopathological processes going on at the same time. Their combinations may be different in different patients and change during the natural course of the disease or as a result of the therapy the patients receive. The differences frequently reported between human and animal sepsis may be the product of the different relative weight of each pathological process in the different species and in the different models. This is not to say that in this field animal research is useless. Animal research is still essential to dissect the response to the septic insult in its multiple components and in their interactions. However, the pathophysiological processes identified should be regarded as mere possibilities, and their effective role in human sepsis should be independently assessed before attempts of translation are made. Moreover, animal research is essential

in the development of minimally invasive techniques which can be used in septic patients to track the role of each pathophysiological process for the identification of relevant therapeutic targets. Most of the alterations of the pathophysiological processes seem to be both beneficial and detrimental for the host, and their manipulation is at best risky if made disregarding their effective impact in a given clinical condition.

From the perspective of the human cardiovascular system several features of sepsis still remain obscure.

1) Although the existence of several hemodynamic patterns is well-established, work remains to be done in order to understand why some patients follow a certain path and others a different one. *Does this depend on the pathogen, the reaction of the host, or both?* Probably the nature of the pathogen plays a relatively minor role, since different pathogens trigger the same cytokine cascade (365). *But if it is so, what genetic patterns condition the response of the host?* It is tempting to imagine that a low cardiac output appears during sepsis because of a preexisting heart disease. A pre-existing cardiac disease may be present in some cases, and, if it is not already known, it may be difficult to identify when the patient is in septic conditions. However, this explanation appears valid only for a limited number of patients (423). Other vascular factors are involved, but it is still difficult to identify the different mechanisms which are operating in any given patient and their relative weight in any given patient at any given time.
2) The vascular changes occurring in septic humans still await for a complete characterization. In particular, the understanding of the behavior of the venous side of the systemic circulation is not complete, due to the known difficulties in measuring the relevant parameters in this vascular compartment. In particular, the venous side of the splanchnic circulation, especially at the level of the liver, may play a critical role in shaping the hemodynamic profile.
    a. *What are the changes of the venous resistance in the course of the disease?*
    b. *Does venous resistance change differently in different organs?*
    c. *What happens exactly to the unstressed volume and the compliance of the capacitance veins in the organism as a whole and in the different organs?*

3) The microcirculation appears overtly important in the genesis of multi-organ dysfunction, and a number of alterations of the microcirculation in septic conditions have been highlighted. However, much work remains to be done in the relative importance of these disturbances in humans, so that their functional significance can be appreciated.
    a. *What are the hemodynamic effects of the alterations of the distribution of blood flow in the microcirculation in sepsis?*
    b. *How are these alterations distributed in the different organs?*
    c. What is the relative importance of the mechanisms which affect the microcirculation in septic patients in the different organs?
    d. To what extent does maldistribution of blood flow in the microcirculation reduce oxygen availability to parts of the tissues?
4) The hypotension which characterizes septic shock obviously points to a defect of the mechanisms responsible for the maintenance of arterial pressure. But the specific role, in humans, of the malfunction of the neural circuitry responsible for the baroreflex or of the hyporesponsiveness of peripheral vessels to nervous or hormonal stimulation is not well characterized. Probably hyporesponsiveness plays a major role, but a quantitative assessment is still to be done. Moreover*, what is the relative importance of these alterations at the level of the different organs?*

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Figure Legends

Figure 1. Relation between central venous pressure (CVP) and cardiac index (CI) in 46 medical septic patients drawn according to the data reported by Winslow and coll. (581).

Figure 2. Venous return curves built using a model with elements in series (panel A) or in parallel (panel B). The arrangement of the different elements is indicated by the inserts.

For the in-series model, R_a is arterial resistance, R_v venous resistance, SVR systemic vascular resistance and R_{VR} resistance to venous return, calculated according to equation (5). In panel A the control condition corresponds to VRC1 (R_a, R_v, SVR and R_{VR} are 16.7, 0.8, 17.5 and 1.1 mmHg L^{-1} min, respectively). Halving of R_a causes SVR and R_{VR} to decrease to 9.2 and 1.0 mmHg L^{-1} min, respectively and rotates clockwise the venous return curve from VRC1 to VRC2. If R_a decreases four times relative to its control value but R_v doubles, SVR becomes 5.8 and R_{VR} 1.7 mmHg L^{-1} min, respectively, and the venous return curve rotates counterclockwise to VRC3.

For the in parallel model, R_a is arterial resistance, R_v venous resistance and C compliance. The subscripts 1 and 2 refer to the non-compliant and to the compliant compartment, respectively. F_c is the percentage of cardiac output (CO) perfusing the compliant compartment. In panel B VRC1 was built using published data relative to a dog (84). Calculated SVR is 67 mmHg L^{-1} min. If R_a of the non-compliant compartment is halved and that of the compliant compartment is doubled, so that F_c decreases from 54 to 25%, SVR decreases (57 mmHg L^{-1} min) and the slope of the venous return curve increases (VRC2). If R_a of the non-compliant compartment is doubled and that of the compliant compartment is halved, so that F_c increases from 54 to 80%, SVR decreases to the same amount as before (57 mmHg L^{-1} min) but the slope of the venous return curve decreases (VRC3).

Figure 3. Equilibrium diagram showing the effects of sepsis-induced absolute or effective hypovolemia (panel A), vasodilation of resistive vessels (panel B), myocardial dysfunction (panel C), absolute or effective hypovolemia and myocardial dysfunction (panel D), vasodilation of resistive vessels and myocardial dysfunction (panel E) and absolute or effective hypovolemia and vasodilation of resistive

vessels (panel F). Thin lines correspond to the control conditions, thick lines indicate sepsis-induced alterations of the cardiac and venous return curves. Reflex compensation is not shown.

Figure 4. Relation between the rate or norepinephrine infusion and mean systemic filling pressure (P_{msf}), resistance to venous return index ($R_{VR}I$) and systemic vascular resistance index (SVRI) in stable postoperative cardiac surgery patients (○) and septic shock patients (●). The values corresponding to zero norepinephrine were obtained by extrapolation. Data are from three unrelated studies performed separately on cardiac surgery patients (305, 306) and septic shock patients (404).

Figure 5. Guyton's equilibrium diagram representing the functional properties of the heart and of the systemic circulation of a healthy human subject before and after the injection of LPS. Before the injection, the intersection of the cardiac function curve (CFC_c) and the venous return curve (VRC_c) is at point A. After the injection, the equilibrium point moves from point A to point E. An increase of cardiac output (CO) without changes of central venous pressure (CVP) implies unequivocally a counterclockwise rotation of the cardiac function curve (to CFC_e) but is compatible with different modifications of the vascular function curve. Or resistance to venous return (R_{VR}) decreases proportionally more than mean systemic filling pressure (P_{msf}) (panel A), or P_{msf} increases proportionally more than R_{VR} (panel B). The measured decrease of systemic vascular resistance (SVR) after bacterial lipopolysaccharide (LPS) administration suggests (but does not prove) that the possibility shown by panel A is more likely.

Figure 6. Glycocheck algorithm on endothelial perfused boundary region (PBR) determination and microvascular perfusion properties. A) Red blood cells (RBC) are detected through reflection of light emitting diodes by hemoglobin. Images captured by the sidestream darkfield camera are sent to the computer for quality checks and assessment. The black contrast is the perfused lumen of the vessels. B) In each recording, the software automatically places the vascular segments (green), every 10 mm along the vascular segments (black contrast). C) After the acquisition, for the analysis, the software undergoes several quality checks in the first frame of each recording, to select vascular segments with sufficient quality for further analysis. Invalid vascular segments (yellow) are distinguished from the valid vascular segments (green). During the whole recording session of 40 frames, the percentage of time in which a particular valid vascular segment has RBCs present is used to calculate RBC filling percentage. D) Depiction of the concept of glycocalyx thickness by lateral RBC movement is shown here. E) For each

vascular segment, the intensity profile is calculated to derive median RBC column width. F) Then, the distribution of RBC column width is used to calculate the perfused diameter, median RBC column width, and subsequently the PBR. From (282) with permission under the terms of the Creative Commons Attribution License.

Figure 7. Pressure volume (P-V) relations (left) and P-V-area (right). Points at top left corners of loops are end-systolic P-V points. Line through points is end-systolic pressure-volume relation (ESPVR), and its slope and its volume-axis intercept are E_{es} and V_0 respectively. Effective arterial elastance (E_a) is slope of end-systolic pressure-stroke volume relation (ESPSVR). PVA is the area in P-V diagram that is circumscribed by ESPVR, end-diastolic P-V relation curve, and systolic segments of P-V trajectory (A-B-C-D-A, right). External work (EW) is the area within P-V loop trajectory (A-B-C-D-A), and end-systolic elastic potential energy (PE) is the area between ESPVR and end-diastolic P-V relation curve to left of EW. From (477), with permission.

Figure 8. The end-diastolic pressure volume relationship (EDPVR) is nonlinear, having a shallow slope at low left ventricular (LV) volume range and a steeper slope at higher LV volume range. At subphysiological (sub) volumes the EDPVR turns toward negative LV pressures. From (79) with permission.

Figure 9. Correlation between mean arterial blood pressure and muscle sympathetic nervous activity (MSNA) (*A*) or heart rate (*B*) of the endotoxin (□, preinjection; ■, postinjection) and placebo groups (▵, preinjection; ▿, postinjection); (§§$P < 0.01$). Please note that physiologically the stimulus-response curve of the baroreflex is rather sigmoid, not linear. However, linear regression helps to visualize the apparent differences of vascular baroreflex-sensitivity (MSNA) and uncoupling of heart rate. The slope, *y*-intercept and regression coefficient (R^2) of the linear best-fit lines are: ***A*** □: $y = -2.0173 \times +208.54$; $R^2 = 0.9932$; ▪: $y = -0.7895 \times +80.573$; $R^2 = 0.8427$; ▵: $y = -1.8098 \times +184.73$; $R^2 = 0.9478$; ▿: $y = -2.1008 \times +218.47$; $R^2 = 0.9928$. ***B*** □: $y = -0.9325 \times +144.71$; $R^2 = 0.9756$; ▪: $y = -0.0817 \times +91.208$; $R^2 = 0.0654$; ▵: $y = -1.1491 \times +166.6$; $R^2 = 0.9629$; ▿: $y = -1.4322 \times + 193.89$; $R^2 = 0.9628$. From (465), with permission.

Figure 10. Sympathetic arterial baroreflex system in closed-loop (A) and open-loop (B) conditions. P_d indicates external disturbance to arterial pressure (AP). In open-loop conditions, relationship between baroreceptor pressure (BRP) and sympathetic nerve activity (SNA) and that between SNA and systemic

arterial pressure (SAP) can be quantitatively measured. When the two curves characterizing the two relationships are plotted on an equilibrium diagram, intersection of the two curves is supposed to be operating point of AP and SNA under closed-loop conditions of feedback system (C). From (464), with permission.

Figure 11. Open-loop characteristics of the baroreflex under bacterial lipopolysaccharide (LPS). Open-loop static characteristics of the total baroreflex arc (A), neural arc (B), peripheral arc (C), CSP to HR relationship (D), CSP to SVR relationship (E), CSP to CO relationship (F), SNA to SVR relationship (G), and SNA to CO relationship (H) obtained at baseline (dotted line with white circles, ○), and 60 min (thin solid line with diamonds, ◊) and 120 min after LPS (thick solid line with black circles, ●). Data are expressed as means ± SEM (n = 10). CSP, carotid sinus pressure (mmHg); AP, arterial pressure (mmHg); SNA, sympathetic nerve activity (a.u.); HR, heart rate (bpm); SVR, systemic vascular resistance (mmHg min ml^{-1}); CO, cardiac output (ml min^{-1}). From (523), with permission under the terms of the Creative Commons Attribution License.

Figure 12. Baroreflex equilibrium diagram under bacterial lipopolysaccharide (LPS). Averaged baroreflex equilibrium diagram at baseline (dotted line with white circle, operating point a), and at 60 min (thin solid line with diamond, operating point b) and 120 min after LPS injection (thick solid line with black circle, operating point c). SNA, sympathetic nerve activity (a.u.); CSP, carotid sinus pressure (mmHg); AP, arterial pressure (mmHg). From (523), with permission under the terms of the Creative Commons Attribution License.

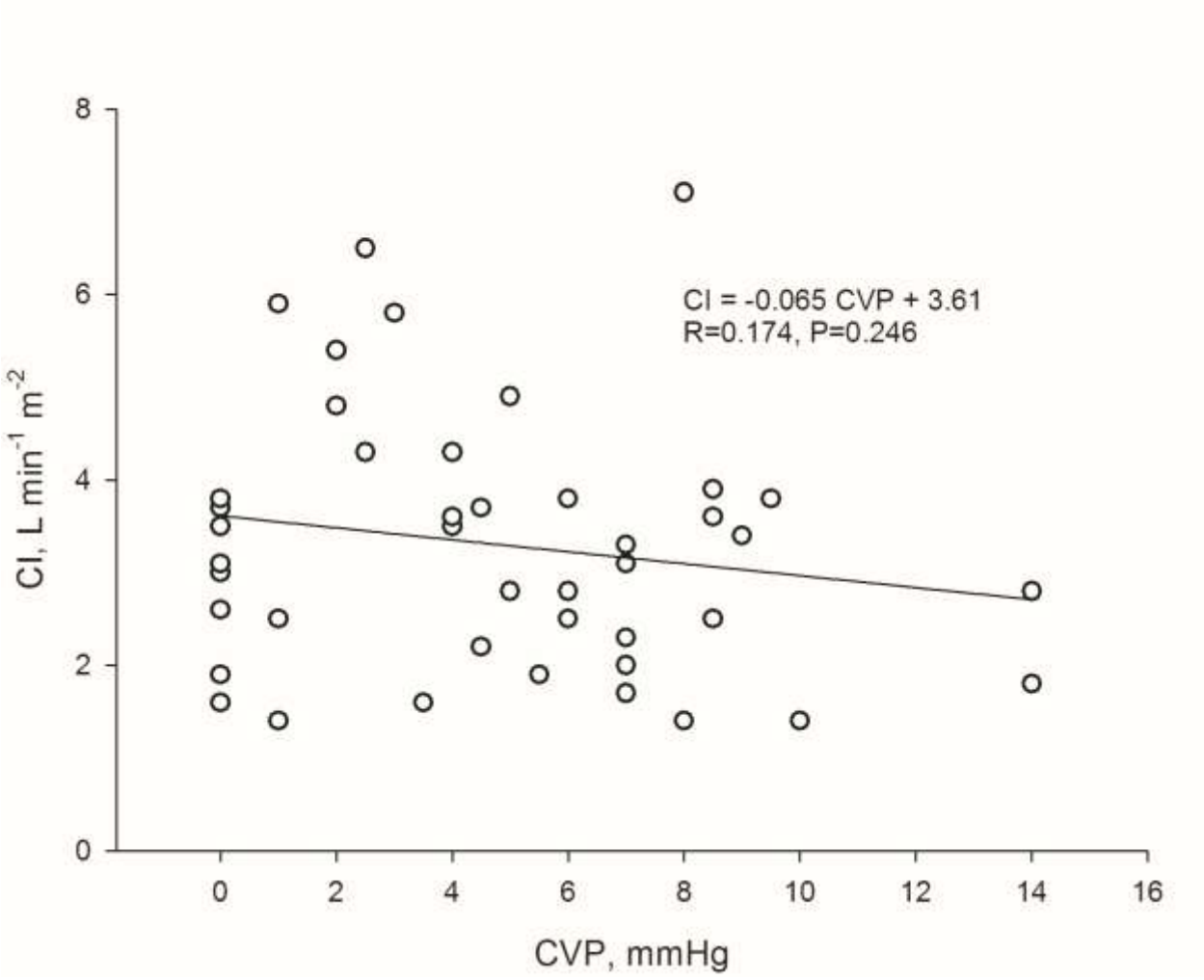

CI = -0.065 CVP + 3.61
R=0.174, P=0.246
CI, L min-1 m-2
CVP, mmHg
0
2
4
6
8
10
12
14
16

FIGURE 1

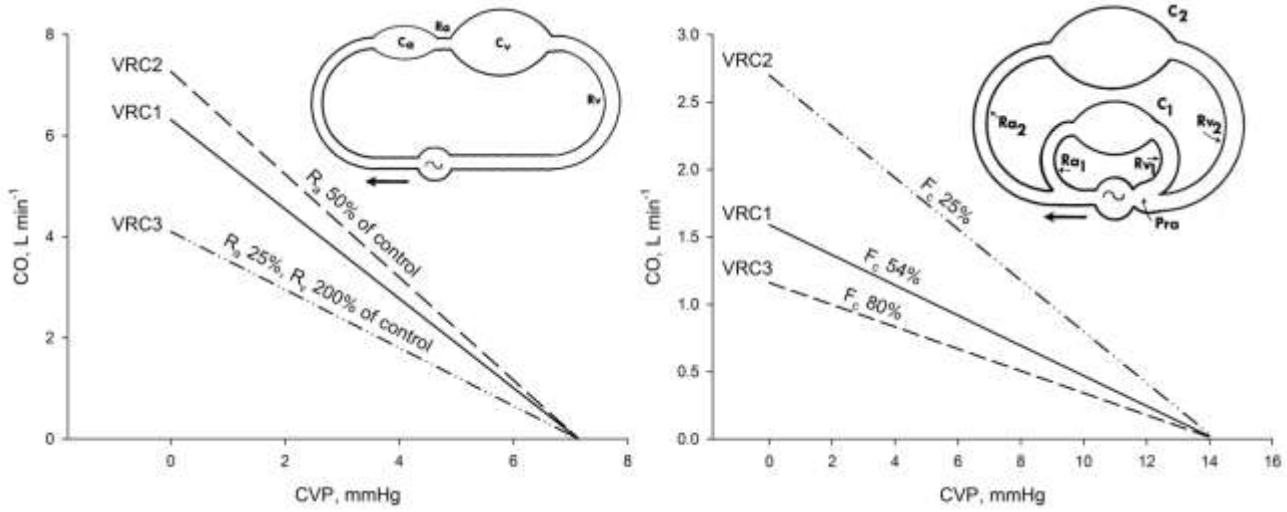

VRC2
VRC1
VRC3
R_s 50% of control
R_s 25%, R_c 200% of control
CO, L min^{-1}
CVP, mmHg
C_a
R_a
C_v
R_v
C_2
C_1
Ra_2
Rv_2
Ra_1
Rv_1
Pra
F_c 25%
F_c 54%
F_c 80%

FIGURE 2

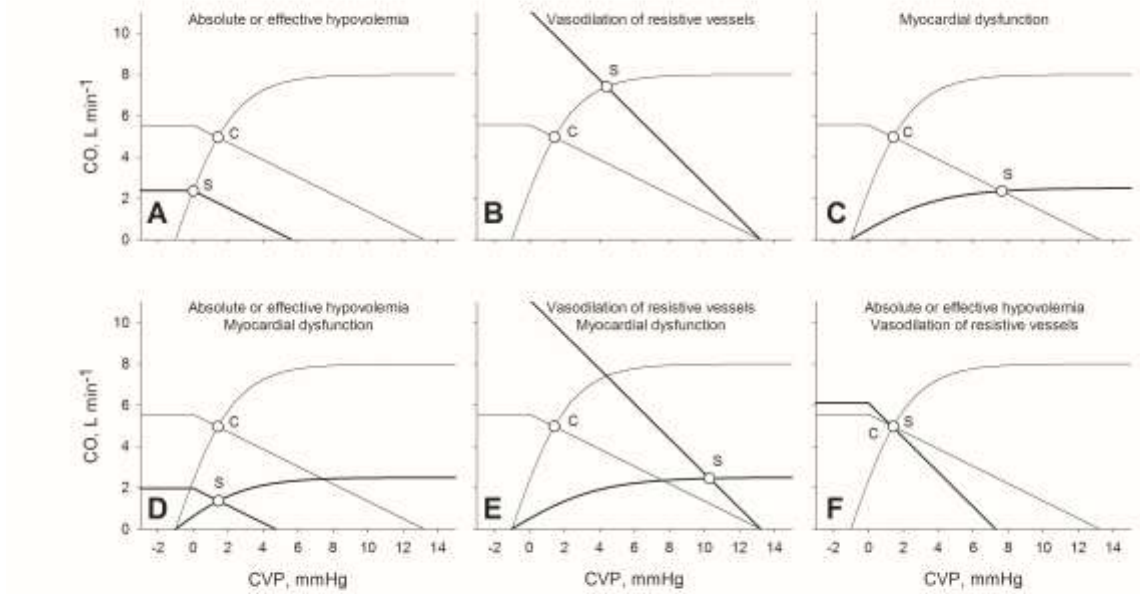

Absolute or effective hypovolemia
Vasodilation of resistive vessels
Myocardial dysfunction
Absolute or effective hypovolemia
Myocardial dysfunction
Vasodilation of resistive vessels
Myocardial dysfunction
Absolute or effective hypovolemia
Vasodilation of resistive vessels
CO, L min-1
CVP, mmHg
A
B
C
D
E
F
C
S

FIGURE 3

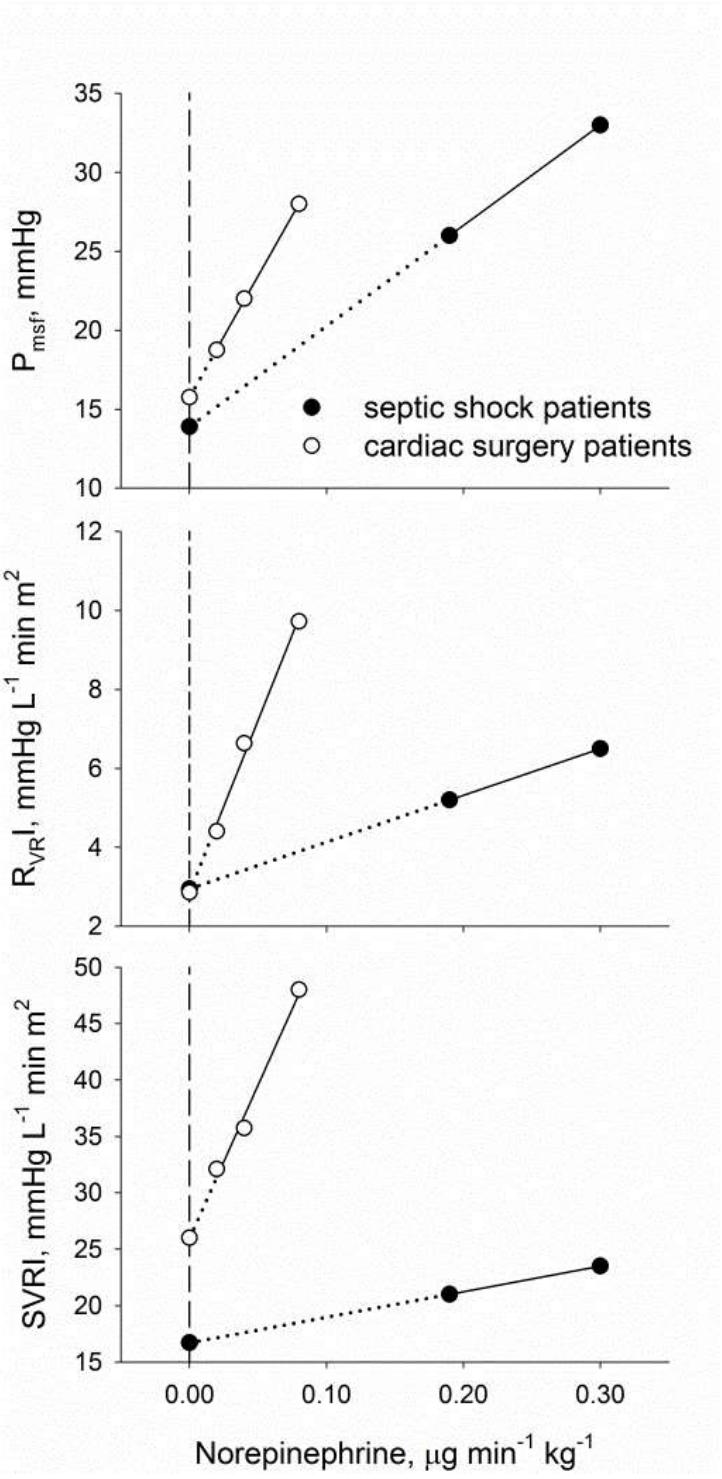
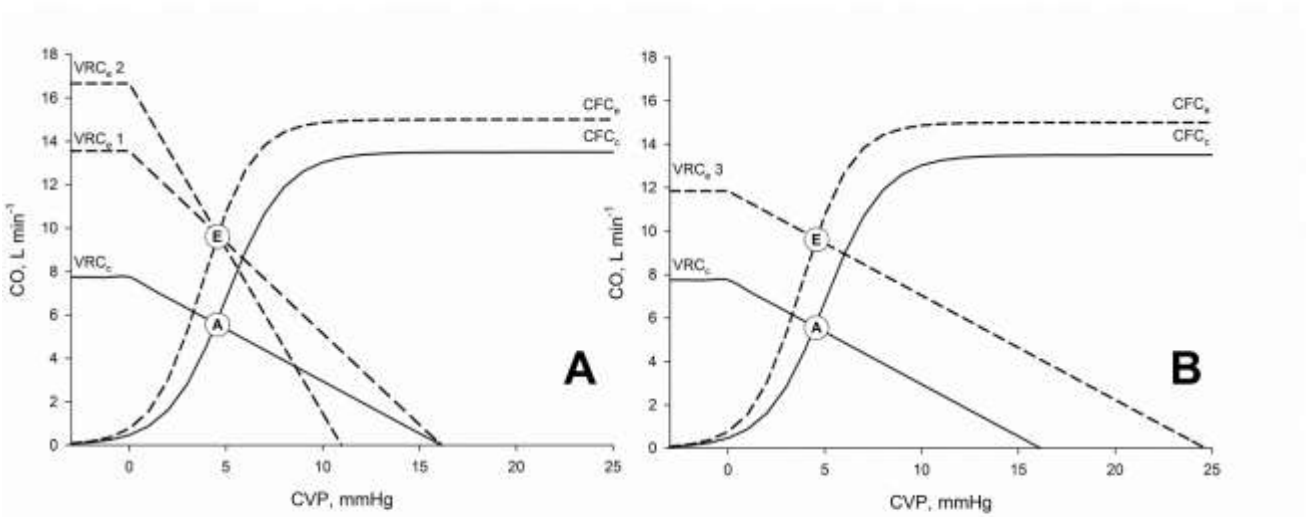

35
30
25
20
15
10
P_{msf}, mmHg
septic shock patients
cardiac surgery patients
12
10
8
6
4
2
$R_{VR}I$, mmHg L^{-1} min m^2
50
45
40
35
30
25
20
15
SVRI, mmHg L^{-1} min m^2
0.00
0.10
0.20
0.30
Norepinephrine, μg min^{-1} kg^{-1}

FIGURE 4



CFC$_c$: control cardiac function curve
VRC$_c$: control venous return curve
VRC$_e$ 1: vascular function curve after endotoxin (R_{VR} is reduced and P_{msf} is unchanged)
VRC$_e$ 2: vascular function curve after endotoxin (both R_{VR} and P_{msf} are reduced)
VRC$_e$ 3: vascular function curve after endotoxin (P_{msf} is increased and R_{VR} is unchanged)

FIGURE 5

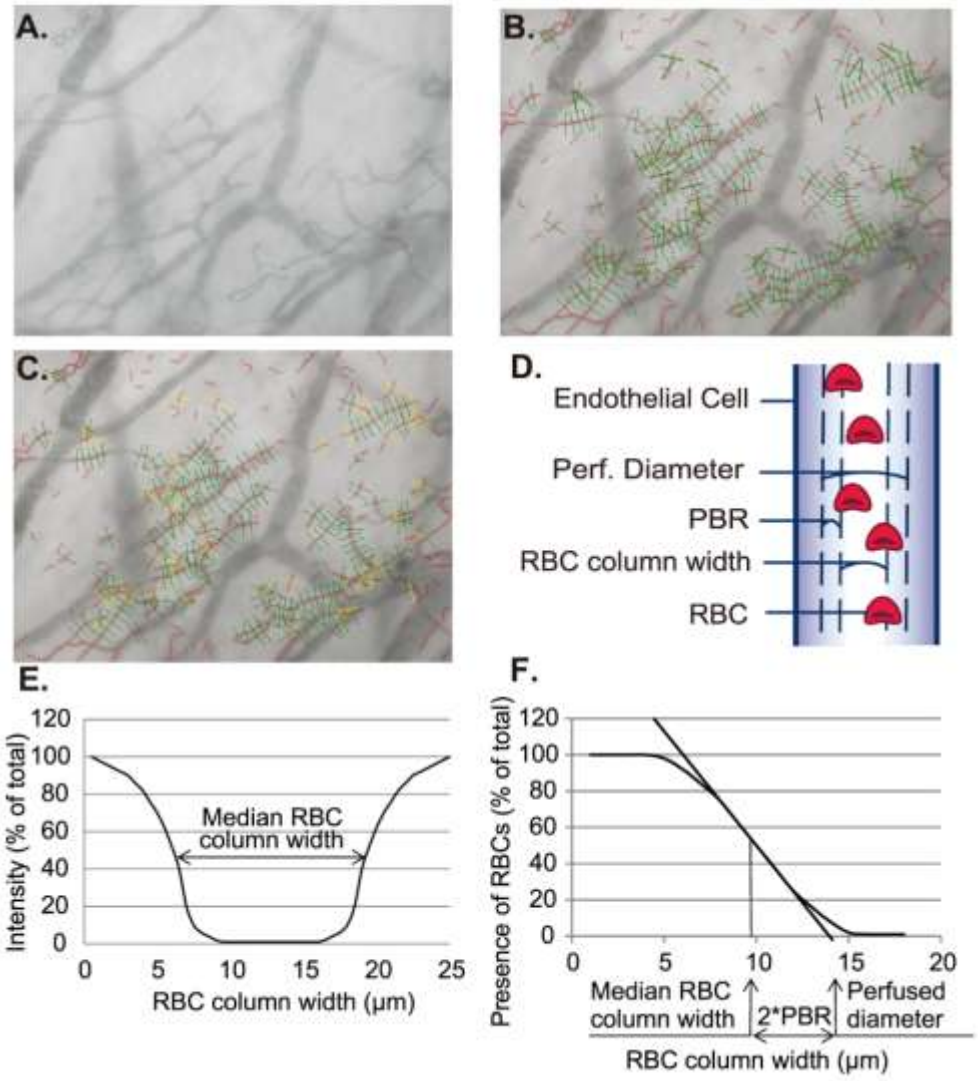

A.
B.
C.
D.
Endothelial Cell
Perf. Diameter
PBR
RBC column width
RBC
E.
Intensity (% of total)
120
100
80
60
40
20
0
Median RBC column width
0
5
10
15
20
25
RBC column width (µm)
F.
Presence of RBCs (% of total)
120
100
80
60
40
20
0
0
5
10
15
20
Median RBC column width
2*PBR
Perfused diameter
RBC column width (µm)

FIGURE 6

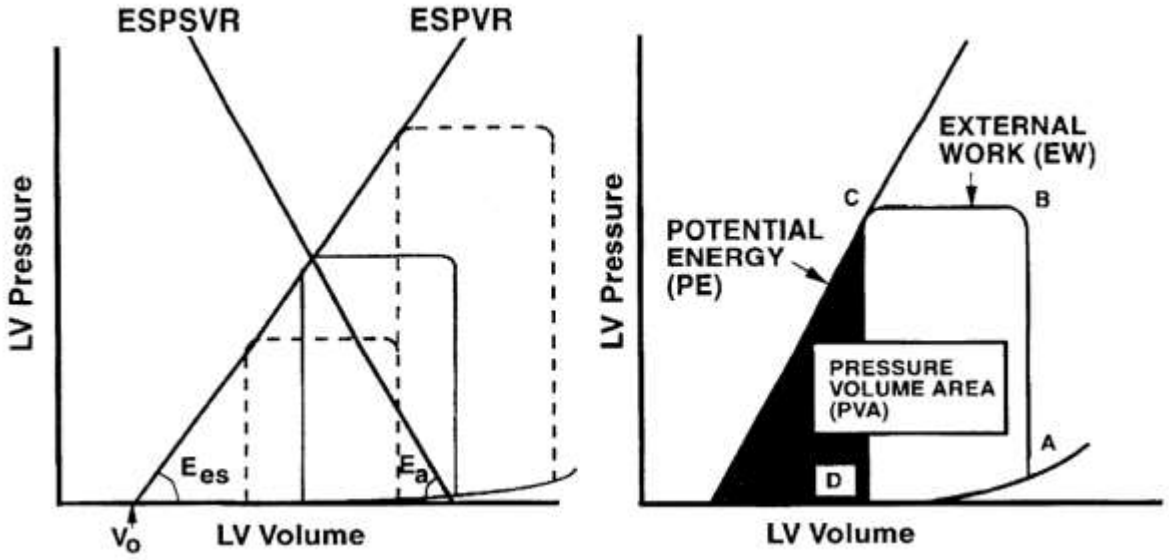

ESPSVR
ESPVR
LV Pressure
E_{es}
E_a
V_o
LV Volume
LV Pressure
EXTERNAL
WORK (EW)
C
B
POTENTIAL
ENERGY
(PE)
PRESSURE
VOLUME AREA
(PVA)
A
D
LV Volume

FIGURE 7

Figure 7

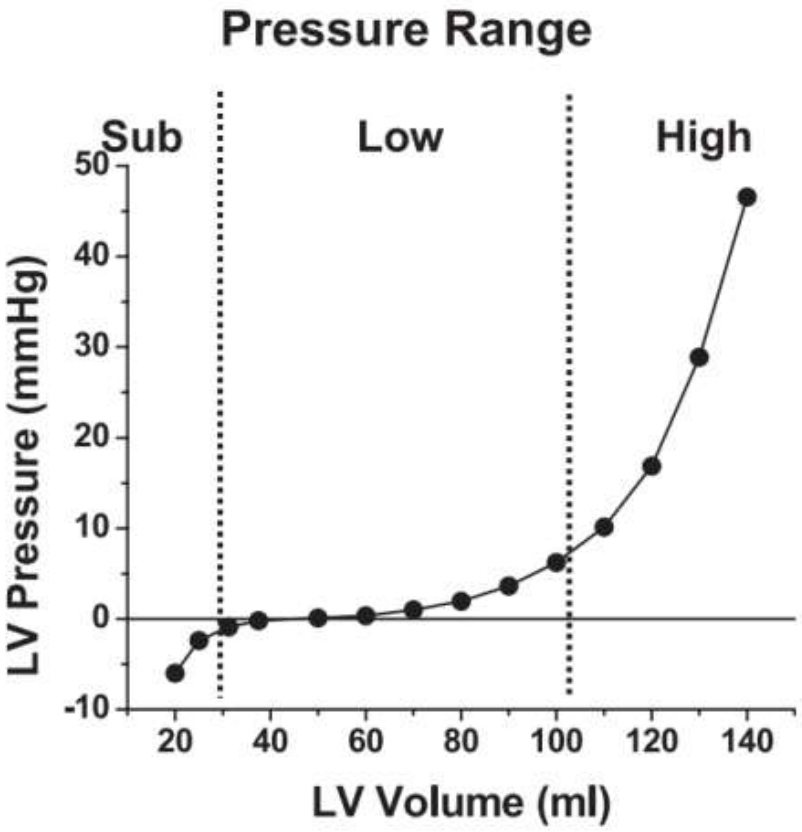


FIGURE 8

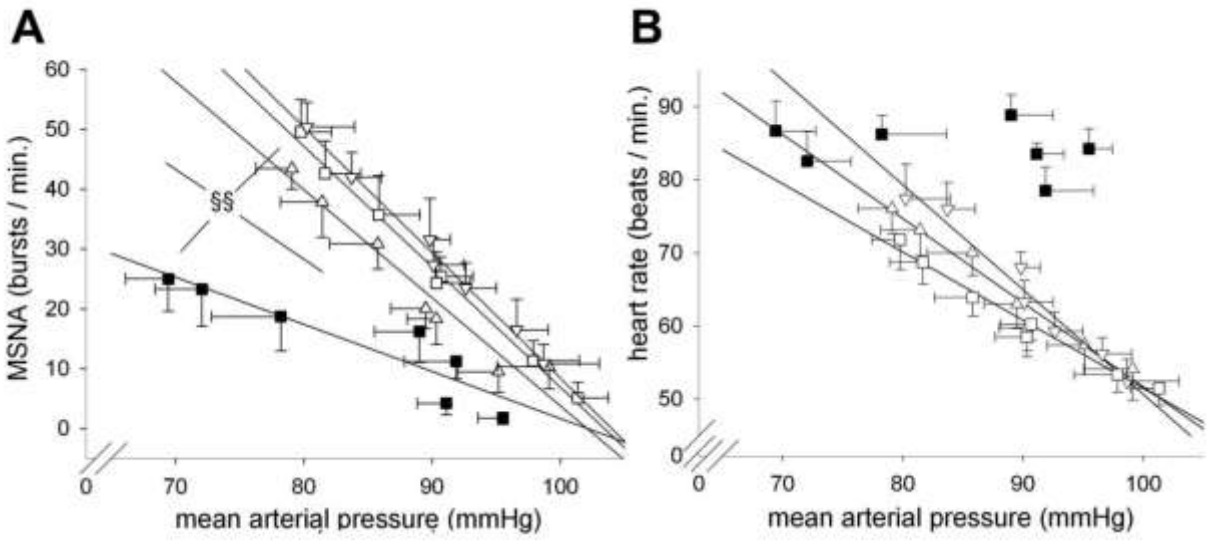

A
MSNA (bursts / min.)
60
50
40
30
20
10
0
§§
0
70
80
90
100
mean arterial pressure (mmHg)
B
heart rate (beats / min.)
90
80
70
60
50
0
0
70
80
90
100
mean arterial pressure (mmHg)

FIGURE 9

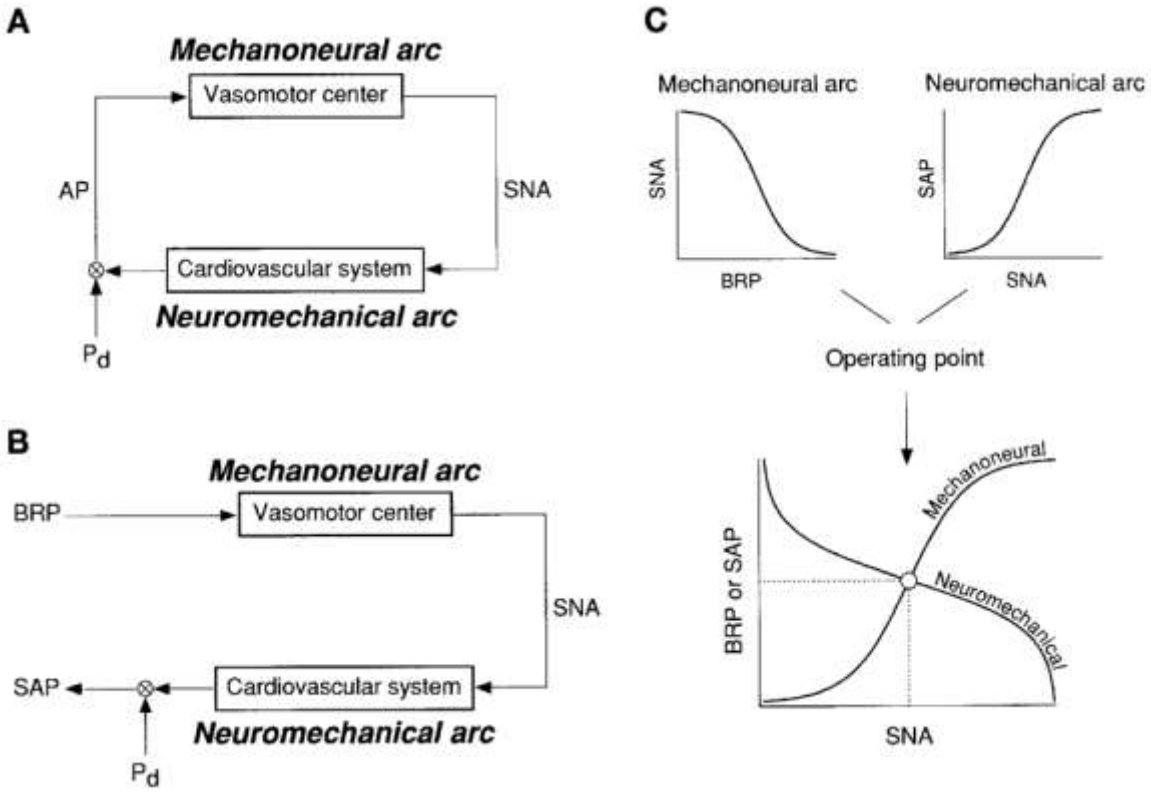

A
Mechanoneural arc
Vasomotor center
AP
SNA
Cardiovascular system
Neuromechanical arc
Pd
B
Mechanoneural arc
BRP
Vasomotor center
SNA
SAP
Cardiovascular system
Neuromechanical arc
Pd
C
Mechanoneural arc
Neuromechanical arc
SNA
BRP
SAP
SNA
Operating point
Mechanoneural
Neuromechanical
BRP or SAP
SNA

FIGURE 10

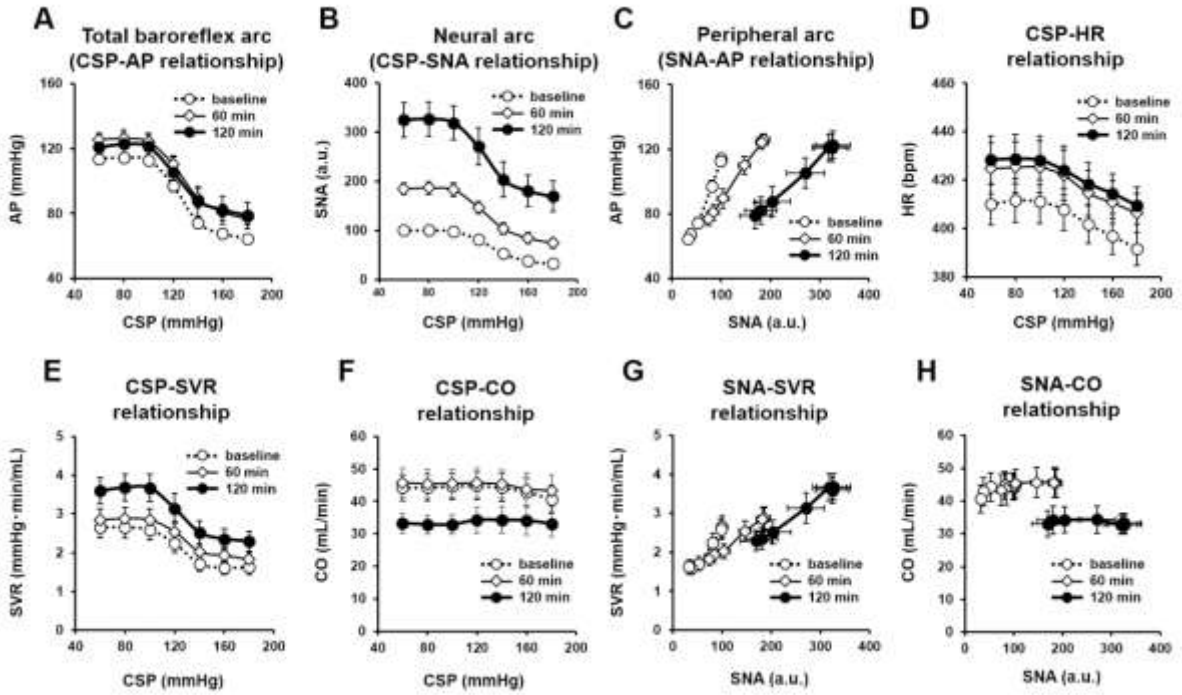

A
Total baroreflex arc
(CSP-AP relationship)
AP (mmHg)
CSP (mmHg)
B
Neural arc
(CSP-SNA relationship)
SNA (a.u.)
CSP (mmHg)
C
Peripheral arc
(SNA-AP relationship)
AP (mmHg)
SNA (a.u.)
D
CSP-HR
relationship
HR (bpm)
CSP (mmHg)
E
CSP-SVR
relationship
SVR (mmHg·min/mL)
CSP (mmHg)
F
CSP-CO
relationship
CO (mL/min)
CSP (mmHg)
G
SNA-SVR
relationship
SVR (mmHg·min/mL)
SNA (a.u.)
H
SNA-CO
relationship
CO (mL/min)
SNA (a.u.)
baseline
60 min
120 min

FIGURE 11

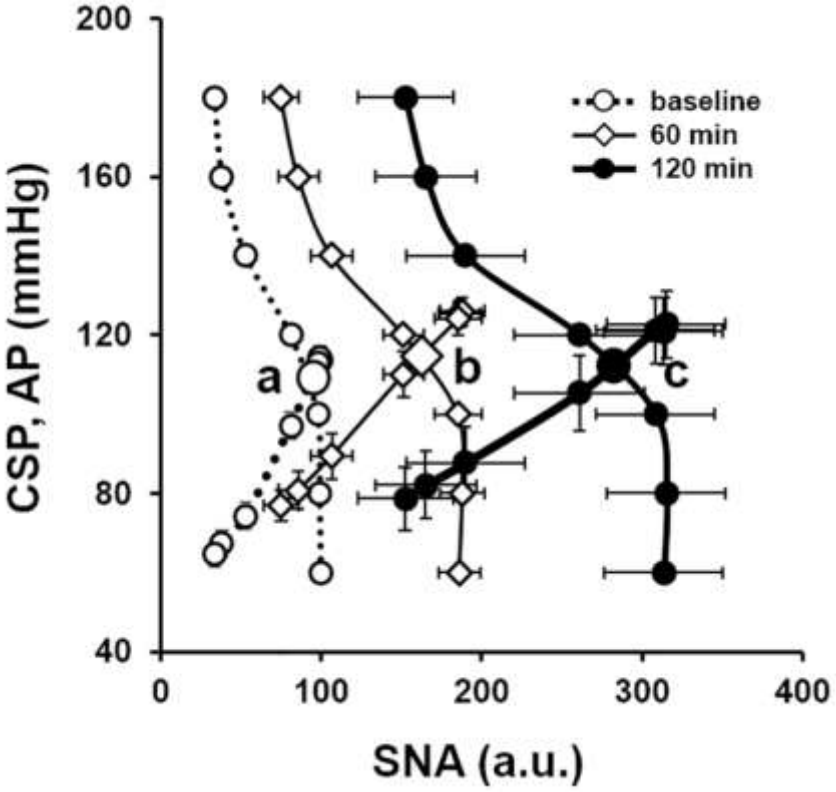

200
160
120
80
40
CSP, AP (mmHg)
0
100
200
300
400
SNA (a.u.)
baseline
60 min
120 min
a
b
c

FIGURE 12