Published in final edited form as:

Shock. 2014 November ; 42(5): 407–414. doi:10.1097/SHK.0000000000000233.

ISOPROTERENOL INCREASES EFFICIENCY AND EFFECTIVENESS OF A LARGE FLUID BOLUS IN HEALTHY VOLUNTEERS

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Abstract

Background—The primary goal of fluid therapy is to maintain fluid homeostasis. Commonly used isotonic crystalloids are only marginally effective and contribute to fluid excess syndrome. In patients with decreased cardiovascular reserve, fluid therapy alone is not sufficient to maintain end-organ perfusion. Therefore, inotropes or vasoactive drugs are used to supplement fluid infusion. Recent animal data suggests that co-infusion of adrenergic agents modulate the distribution of fluid between the vascular and extravascular/interstitial compartments after a fluid bolus. We sought to determine if this effect would translate in humans by co-administering a beta (β)-adrenergic agonist with fluid.

Methods—Nine healthy volunteers (age 21–50 yrs) were randomly paired and received either a continuous isoproterenol infusion (ISO:0.05μg/kg/min) or 0.9% saline (control(CON)) 30min prior to a 25mL/kg 0.9% NaCl fluid bolus. Hemodynamics, ventricular volume and function and microcirculatory determinants (capillary filtration coefficient (CFC) and oncotic pressure) were measured. Vascular and extra-vascular volume (EVV) and fluid balance were determined.

Results—Compared to CON, ISO significantly increased heart rate (CON:64.2 ±4.1 bpm vs. ISO:97.4±5.7 bpm) and cardiac output (CON 4.4±0.7L/min vs. ISO:10.2±0.9) before fluid bolus. ISO significantly increased urinary output (ISO: 10.86±1.95 vs Control: 6.53±1.45 mL/kg) and reduced EVV (7.98±2.0 vs 14.15±1.1mL/kg). ISO prevented an increase in CFC (1.74±0.4 vs 3.21±0.4 mL/min/mmHg•10⁻³).

Conclusion—Isoproterenol, a non-selective β-adrenergic agonist, augments vascular volume expansion and eliminates EVV via enhanced diuresis, which may in part be due to enhanced endothelial barrier function.

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DISCLOSURES

All authors declare to have nothing to disclosure.

Keywords

Resuscitation; fluid therapy; β -adrenergic agonist; isoproterenol; Healthy volunteers

INTRODUCTION

The primary goal of fluid therapy is to restore and maintain organ perfusion. Perioperative intravenous fluid administration is generally considered to be safe, cost effective and life saving. However, crystalloid fluids, which are most commonly used, have varying effects on expanding vascular volume that depends on the patient's physiologic state and pathologic conditions (1,2). In general however, only a small portion of the infused crystalloid fluid remains in the vasculature. The remaining fluid volume is either lost in the urine or accumulates in the interstitial space as edema, which contributes to organ dysfunction and death (3,4).

The physiological mechanisms responsible for the rapid vascular escape of crystalloid fluids have been considered to be due to an increased capillary pressure and reduced colloid osmotic pressure. However, changes in capillary filtration coefficient (CFC), which is an index of hydraulic conductivity and microvascular surface area, can powerfully influence intravascular fluid retention (5,6).

Vasoactive and inotropic drugs are used to support cardiovascular system when fluid therapy alone cannot augment cardiovascular performance. Inotropic agents are often chosen to augment cardiac function (7), while beta-blockers decrease heart rate and blood pressure (8,9). Experimental evidence suggests that these agents alter the kinetics of fluid administration and distribution volume after a fluid bolus. Specifically, animal studies demonstrate that the increase in cardiac output from inotropic agents is not only due to enhanced myocardial contractility but also due to enhanced retention of fluid in the vascular compartment or preload (10,11). The present study determined the volume expansion properties and corresponding physiologic mechanisms of a beta (β) – adrenergic agonist in humans. We hypothesized that *β -adrenergic agonist, isoproterenol, would enhance intravascular volume expansion during and after fluid infusion in humans via modulation of CFC.*

MATERIALS AND METHODS

Study design

The Institutional Review Board and the Institute for Translational Sciences - Clinical Research Center (ITS-CRC) Scientific Review Committee of the University of Texas Medical Branch (UTMB) at Galveston reviewed and approved the protocol and experimental procedures of this study. Inclusion criteria included, men or non-pregnant women in good health, between 21 to 50 years of age. Subjects were excluded if they had a history of either atherosclerotic cardiovascular disease, cardiac conduction defects, neurologic, renal or lung diseases (e.g. asthma or chronic obstructive pulmonary disease). Additional exclusion criteria included subjects with known allergic reactions to sulfa drugs,

shellfish or iodine and if they were on medications that interacted with β -adrenergic receptor.

Study preparation and experimental procedures

This was a prospective, randomized, paired, study performed in nine healthy volunteers at ITS-CRC, UTMB. Volunteers were studied on two separate visits. Volunteers were instructed not to eat or drink after midnight, the day before the study. On the day of the study, the volunteers reported to the ITS-CRC. Vital signs (HR, non-invasive blood pressure measurement and blood saturation) and weight was obtained after the subject emptied their bladder. The subjects were positioned supine in a hospital bed. A three lead ECG, pulse oximeter (Nellcor N600, Covidien PLC, Dublin, Ireland) and blood pressure cuff were placed and connected to a clinical monitor (Viridia 24CT, Hewlett Packard Inc., Palo Alto, CA, USA). Two venous catheters (18 G, B. Braun AG, Melsungen, Germany) were placed in each arm (either the median cubital vein or a forearm vein) for drug and fluid administration, respectively. An arterial catheter (20 G, Abbocath, Hospira Inc., Lake Forrest, IL, USA) was placed in the radial artery under local anesthesia (1% Lidocaine, 2mL, Hospira Inc., Lake Forrest, IL, USA) and connected to a pressure monitoring kit (Transpac, Abbott Laboratories Inc., Abbott Park, IL, USA) with a 0.9% NaCl (Baxter Healthcare Corp., Deerfield, IL, USA) pressure bag.

Each subject underwent two randomly assigned infusion protocols that were separated by at least 7 days. The specific time points and interventions were (Figure 1):

T minus 60 (T-60), after monitors and catheters were placed. Measurements were obtained prior to drug infusion.

T minus 30 (T-30), a continuous infusion of isoproterenol (ISO: 0.05 μ g/kg/min; Hospira Inc., Lake Forrest, IL, USA) or placebo control (CON: 0.9% NaCl at 10 mL/h; Baxter Healthcare Corp., Deerfield, IL, USA) was begun, which continued until study end T120. Between T-30 and T0 measurements for plasma volume were obtained.

T zero (T0), start of the 25 mL/kg 0.9% saline fluid bolus (Baxter Healthcare Corp., Deerfield, IL, USA)

T twenty (T20), end of fluid bolus

T sixty (T60), 60 min after start of fluid bolus

T one hundred twenty (T120), end of study and cessation of drug infusion or placebo

Details of the specific measurements and timing are described in detail below.

Hemodynamic and echocardiography measurements

Measurements for heart rate (HR), mean arterial blood pressure (MAP) were recorded at T-60, T-30, T0, T5, T10, T15, T20, T30, T60, T90 and T120. Echocardiographic (ventricular volume and function) parameters were measured by transthoracic echocardiography using a 3.5 MHz transducer and ultrasound machine (Vivid 7 PRO BT04, General Electric Medical Systems Inc., Milwaukee, WI, USA). Left ventricle (LV) area and length were interrogated in parasternal long axis position. The Modified Simpson's rule was

applied for calculation of end-diastolic (EDV), end-systolic volume (ESV). Stroke volume (SV) was determined from the calculation of EDV – ESV and the ejection fraction (EF%) was determined by SV/EDV. Cardiac output (CO) was calculated from SV • HR. Systemic vascular resistance (SVR) was calculated from MAP/CO • 80. Echocardiographic parameters were measured at T-60 min, T-30 min, T0, T20, T60 and T120.

Volumetric and fluid measurements in mL/kg

The distribution of the 25 mL/kg 0.9% saline fluid bolus was calculated in three compartments (plasma, urine and interstitial volume (extravascular volume)) using mass balance after T0. Cumulative urinary output (UO) was measured via an ultrasound bladder scanner (BVI 3000, Verathon Inc., Bothell, WA, USA). Measurements were obtained at T0, T20, T40, T60, T90 and T120. Initial PV was determined with the spectrophotometric detection of indocyanine green (ICG, Akron Inc., Lake Forrest, IL, USA) using optical densitometry bound to plasma proteins (12). Specifically, 5mg ICG was injected intravenously at T-15. Blood samples were taken every minute for six minutes. The amount of ICG bound to plasma proteins (PV_{ICG}) was measured at 840nm using spectrophotometry (DU 800, Beckman Coulter Inc., Brea, CA, USA). The change in PV (ΔPV) is directly proportional to the initial plasma volume + changes in hematocrit ($\Delta PV \sim PV_i +$

$Hematocrit_{after/initial}$) (12). Arterial blood for hemoglobin and hematocrit were taken prior to, during and after the fluid bolus to calculate vascular volume expansion. Specifically, samples were obtained at start of fluid bolus and every two minutes during the fluid bolus, every five minutes until T60 and then every 30 min until T120. Plasma Volume (PV) along with hemoglobin/hematocrit at specific time points was used to calculate PV expansion that occurred after the fluid bolus. Change in extravascular volume (EVV) over time (ΔEVV) was calculated from UO, plasma volume and total fluid administered: $EVV = \text{infused volume} - (\Delta PV + UO + \text{fluid infused})$ (11,13).

Microcirculatory measurements

The capillary filtration coefficient (CFC) was determined *in vivo* using venous congestion plethysmography (VCP), as described by Gamble and Christ (14,15). In brief, step-wise increases in venous pressure were performed by applying a pressurized cuff on the thigh and a mercury-silastic strain gauge transducer on the calf (Hokanson EC6 strain gauge plethysmograph, D.E. Hokanson Inc., Bellevue, WA, USA) (Figure 2a). The change in limb volume/girth, measured by the strain gauge transducer, represents the net fluid filtration or J_v (in which each % = 1 mL/100 mL tissue) if the exceeded filtration pressure of P_v (via the pressurized cuff in mmHg) is held for several minutes. Thus, CFC was determined from the % girth over time (net fluid filtration (J_v)) divided by the changes in venous pressure (ΔP_v) as: $CFC = J_v / \Delta P_v$. Three separate inflation pressures (~ 30, 45 and 60 mmHg), each sustained for three min, were performed before drug infusion, after drug infusion but before fluid bolus, during and immediately at end of fluid bolus, one hour after fluid bolus and study end (T-60, T-12, T8, T20, T60 and T120). The collection of these measurements provided insight into derangements in the Starling Equation, as described below.

The amount of pressure applied to the cuff, limb girth % and time (min) were digitally sampled and stored using Powerlab (ADInstruments Inc., Colorado Springs, CO, USA). The

CFC was determined from an offline analysis using the shallow slope from the change of % limb girth and time (Figure 2b). VCP measurements at each time point took approximately nine to twelve minutes to complete. The CFC from each of the three inflation pressures was averaged to determine the CFC for each time point.

Blood samples, taken at the same time points as CFC measurements, for total protein and albumin were measured using a protein analyzer (Vitros Fusion 5.1, Ortho Clinical Diagnostics Inc., Raritan, NJ, USA). The plasma colloid osmotic pressure (COP_{pl}) was estimated using a derived formula from these constituents as previously described in detail (16).

Statistical analysis

Students t-test and two-way ANOVA were used for data analysis. Bonferroni post hoc comparison was used when applicable. Statistical analysis was performed with *Prism 4 for Mac* (GraphPad Software Inc.). Data are expressed as mean $\pm$ S.E.M. Significance was set as $p < 0.05$.

RESULTS

Baseline Characteristics

There were no significant differences between ISO or Control groups in any of the investigated variables at start of the experiment (T-60 to T-30).

Hemodynamics (Table 1) and cardiac function (Figures 3a+b)

HR and CO was significantly higher at all time points after baseline (BL) for the ISO group. HR reached its highest peak ($\approx 175\%$) immediately following the fluid bolus. Similarly, CO increased after ISO and fluid bolus with a peak increase exceeding 250% of BL. MAP showed no significant differences at any time point between the two groups or to BL. Compared to BL and control, the SVR decreased after ISO and further declined following the fluid bolus. The SVR decreased in the control group immediately after the fluid bolus but thereafter, it returned towards BL.

End-diastolic volume (EDV), in both groups, increased after fluid bolus (Figure 3b). However, only the ISO group significantly augmented EDV, when compared to BL (-60 min: 130 ± 7 vs 20 min: 171 ± 12 mL). The fluid bolus was associated with a small increase in End-systolic volume (ESV) (Figure 3a). ISO blunted this effect on ESV and by study end, significantly reduced ESV compared to control (120 min ISO: 36 ± 5 mL vs 120 min control 60 ± 4 mL). Interestingly, the increase in stroke volume (SV) [$\text{SV} = \text{EDV} - \text{ESV}$], for ISO, was due to larger increase in EDV than decrease ESV.

Urinary Output (UO) and Volume expansion (Figures 4a–c)

ISO had pronounced differences on the distribution of the 25 mL/kg 0.9% NaCl fluid bolus compared to control. ISO was associated with a greater diuresis compared to control. Cumulative UO was significantly higher with ISO compared to control after 60 min (ISO: 7.6 ± 1.6 vs Control: 3.6 ± 1.1 mL/kg) and 90 min (ISO: 9.5 ± 1.8 vs 5.7 ± 1.3 mL/kg). The fluid

bolus resulted in both a peak, which was transient and occurred immediately at the end of the bolus and a sustained plasma volume expansion, which was greater with ISO versus control. ISO resulted in significantly higher values at 90 and 120 min, which at study end was 6.3 ± 0.4 vs 4.3 ± 0.9 mL/kg, for ISO and control, respectively. The fluid bolus resulted in extra-vascular expansion (EVV), which was significantly reduced by ISO compared to control at 60 min (ISO: 11.0 ± 1.4 vs Control: 16.3 ± 0.7 mL/kg), 90 min (ISO: 8.6 ± 1.5 vs Control: 15.6 ± 1.2 mL/kg) and 120 min (ISO: 8.0 ± 2.0 vs Control: 14.2 ± 1.1 mL/kg). The effect of ISO or control on vascular volume efficiency (VVE – calculated as PV divided by $fluid_{in}$ over time) showed that ISO significantly enhanced fluid retention in the vascular compartment control (Fig 5a). Additionally, ISO significantly altered the distribution ratio – calculated as PV divided by EVV (Fig 5b). For control group, following the fluid bolus, more fluid was retained in the EVV. On the other hand, ISO preferentially distributed fluid in the vascular compartment. Further, after T60, an inversion in fluid distribution was observed. This effect was due to plasma volume expansion and/or extravascular volume contraction.

Microcirculatory determinants (Figures 6a+b)

The fluid bolus in control group resulted in a large significant increase in the CFC during and immediately after the fluid bolus, which partially returned to basal levels towards study end. ISO significantly attenuated the response of the bolus on the CFC and was significantly lower than control at study end (ISO: 1.7 ± 0.4 vs Control: 3.2 ± 0.4 mL/min/mmHg $\cdot 10^{-3}$). COP_{pl} significantly fell in both groups after the fluid bolus. The nadir occurred at 20 min. There was partial restoration in COP_{pl} to basal levels by study end. There was a significant difference between ISO and control at end of fluid bolus (ISO 18.3 ± 0.9 vs Control: 16.3 ± 0.3 mmHg).

DISCUSSION

Intravenous fluid, most commonly isotonic crystalloid administration is used to treat perioperative fluid losses. However, volume substitution using isotonic crystalloid is not very efficient and only a small portion of the infused volume remains in the circulation. Consequently, excessive volumes are needed to restore vascular volume losses resulting in interstitial accumulation, which contribute to significant morbidity e.g. peripheral edema, pulmonary edema, GI dysfunction and decreased wound healing (3,4). Large volume crystalloid resuscitation has been reported to disrupt the glycocalyx, which further exacerbates vascular volume loss (17,18).

Our primary finding was that isoproterenol (ISO) increased the efficiency of the crystalloid substitution. Specifically, ISO enhanced vascular volume expansion after a fluid bolus. Additionally, ISO increased urinary output, thereby reducing extravascular volume. ISO also increased end-diastolic volume (EDV). Although the precise mechanism for the effects of ISO on preferentially expanding vascular volume expansion is not known, our findings suggest that ISO enhances endothelial barrier function since the capillary filtration coefficient (CFC) was reduced following ISO and fluid loading.

Vascular volume expansion and fluid distribution

Perioperative fluid losses, which can be aggravated by increased venous pressure e.g., heart failure and microvascular permeability e.g., inflammation and infection, lead to reduced circulating volume. Repletion of vascular volume is commonly achieved by infusing isotonic crystalloid. Anesthesia and critical care textbooks suggest that isotonic crystalloid distributes passively and proportionally to the relative size of the interstitial and vascular compartments. This is likely the genesis for the 3:1 rule, which suggest that for every one mL of blood, 3 mL of crystalloid is needed (19). The Starling Equation has been used to describe the rapid redistribution of infused crystalloid:

$$J_v = CFC [(P_c - P_{if}) - \sigma(\Pi_p - \Pi_{if})]$$

Where J_v represents the fluid filtration, CFC is capillary filtration coefficient, P_c is the capillary pressure, P_{if} is interstitial hydrostatic pressure, σ is the reflection coefficient for protein, Π_p is plasma colloid osmotic pressure and Π_{if} interstitial colloid osmotic pressure.

This equation, in part, explains the transient nature of a crystalloid fluid bolus, since rapid crystalloid administration increases P_c and reduces Π_p . However, the Starling equation cannot sufficiently account for all the effects that occur during fluid resuscitation. For example, Chappell and colleagues (18) proposed changes to the classic equation in a “revised Starling principle”, to incorporate the relevance of the glycocalyx by establishing the pressures Π_p and Π_{if} to Π_{esl} (oncotic pressure within the endothelial surface layer) and Π_b (oncotic pressure beneath the endothelial surface layer). The glycocalyx is the inner lining of the vascular endothelium and presents therefore the second important barrier line beside the endothelial cells (20,21). Chappell *et al* further summarize that the endothelial glycocalyx can bind free plasma and they form together the endothelial surface layer and that an oncotic gradient seems to be present directly across the endothelial surface layer, that defines finally vascular integrity, so that the presence of this layer should be the basic requirement for a physiologic barrier function (21). Additionally Svensen *et al*, (22) showed that in septic and burn-injured sheep that the intravascular retention of infused fluid is paradoxically maintained despite increased microvascular protein permeability and a reduced plasma-to-tissue oncotic gradient (22). Thus, isotonic crystalloid components due to their small size can leak regardless of the state of the endothelial barrier. It is interestingly in this context, that crystalloids and colloids show the same volume effect when infused in septic patients. Whether ISO could stabilize the glycocalyx is not known. Others have demonstrated that pharmacologic agents alter the intravascular retention after a fluid bolus (10,11,13). Stephens *et al*. (11) showed that dobutamine enhanced vascular volume expansion after a fluid bolus in sheep. Similarly, Vane *et al* showed that a co-infusion of isoproterenol greatly enhanced the peak and sustained vascular volume expansion after a fluid bolus (10). Interestingly, these authors reported that isoproterenol resulted in an anti-diuresis. Other adrenergic agents, such as phenylephrine (an α_1 -agonist) and esmolol (a β_1 antagonist), resulted in significant vascular volume loss (plasma volume contraction) when co-infused during and after a fluid challenge (10,13). These studies suggest that control blood-to-tissue transport of fluid can be modulated by the state of the patient, the rate and timing of the infusion and pharmacologic adrenergic agents.

Our results are consistent with previous sheep studies in regard that the co-infusion of the β -receptor agonist isoproterenol with a fluid bolus in healthy humans reduces the escape of fluid to the extravascular compartment. In contrast to Vane et al (10) we observed that ISO resulted in a prominent diuresis, thereby decreasing EVV (less interstitial fluid accumulation). Our results suggest that ISO preferentially enhances the efficiency of the fluid bolus. Surprisingly, vascular volume expansion occurs with a contraction of EVV. Thus, ISO alters the distribution volume of a fluid bolus.

Microcirculatory determinants

Venous congestion plethysmography was used to determine the capillary filtration coefficient (CFC). This technique is well established in humans. Our basal CFC measurements were consistent to previous reports of CFC in healthy humans (14,15). In the non-treated control group, during fluid loading, CFC increased nearly two-fold. The increase in CFC may explain the rapid expansion of extravascular fluid we observed in the control group for our study. Isoproterenol reduced CFC and attenuated its response to fluid loading, which likely explains the greater vascular volume expansion and efficiency of ISO on the fluid bolus (Fig 5a). The lower CFC is consistent with enhanced endothelial barrier function. The β -adrenergic receptor has been shown to play a role in the microvascular fluid regulation (11,23,24). Mechanisms for this effect have been demonstrated in animals. Adamson *et al*, demonstrated that hydraulic conductivity of intact capillaries is reduced by the β -adrenergic agonist ISO, resulting in decreased transvascular fluid flux and vascular fluid retention (5,6,11). Activation of cyclic adenosine monophosphate (cAMP), via β -adrenergic receptor, results in enhanced endothelial barrier function in both health and disease (25,26). Others have reported that cAMP activation by ISO can also aid in clearance of pulmonary edema (27). ISO also resulted in a slightly greater, albeit significant, plasma protein concentration [COPpl] at end fluid bolus suggesting that ISO conferred some decrease in macromolecular permeability. Together, these physiologic determinants favor fluid retention during the fluid bolus.

Hemodynamics

The 25 mL/kg crystalloid fluid bolus resulted in modest, non-statistically significant, increases in heart rate and cardiac output and decreases in systemic vascular resistance. Mean arterial blood pressure was unchanged. The infusion of isoproterenol with and without a fluid bolus resulted in large increases in heart rate, stroke volume and consequently cardiac output. MAP was not altered due to reduced systemic vascular resistance. The observed hemodynamic effects of isoproterenol were in concurrence with previous reports of other β -adrenergic and inotropic agents (11,28) and anticipated, since isoproterenol is a non-specific β -adrenergic agonist. On the other hand, isoproterenol resulted in a surprising effect on systolic function. Specifically, we observed that the increase of ejection fraction and stroke volume was due to a greater increase in EDV than reduction in ESV immediately following the fluid bolus. The enhanced EDV was consistent with isoproterenol effect on vascular volume expansion. Interestingly, even though isoproterenol induced tachycardia, which should lessen diastolic time and cardiac filling, preload accommodation was preserved. Isoproterenol has been shown to increase cardiac compliance (29), which could also account for the increased efficiency we observed following the fluid bolus (Fig. 5a).

Isoproterenol dose and timing

The rationale for the ISO pretreatment window beginning 30 min prior to fluid bolus was based on previous work by our group (10, 11, 13) and others (5). Specifically, the continuous infusion dose and time was selected based on our previous animal work (10). In the present study, a more modest infusion dose of ISO was chosen: 1) for safety reasons and 2) as a means to induce microcirculatory effects while trying to minimize the macrocirculatory or hemodynamic effects. ISO has a short plasma half-life (< 5 min) and effective duration of thirty min or less. We began our infusion at 0.05 µg/kg/min and titrated downward as to not exceed a greater than 50% increase in basal heart rate. Our final dose was 0.03 ±0.1 µg/kg/min. One limitation of the current study is using a specified dose and timing of the continuous infusion of ISO. We are currently, investigating if even lower doses of ISO can yield similar effects on vascular expansion. While it is not specifically known the amount of time that is needed systemically activate endothelial adhesion and reduce permeability, Adamson et al, demonstrated, in isolated perfused vessels exposed to isoproterenol, a fairly rapid reduction in conductivity (5–10 min) with peak reductions occurring 20–30 min after onset of exposure (5). It is likely that some ‘priming’ is needed by agents that activate intracellular cAMP, such as beta-adrenergic agonists and phosphodiesterase inhibitors, in order to activate adhesion structures and tighten endothelial barrier function prior to the fluid bolus. We chose a 30 min pre-treatment window to ensure that there would be sufficient amount of drug exposure in the volunteers at time point T0, prior to the fluid bolus. While it is not known if both ISO and the fluid bolus were infused at the same time and together, our data suggests that the peak effect of vascular volume expansion would be shifted, delayed or potentially prolonged, since activating endothelial adhesion and reducing water permeability by ISO would occur after the fluid bolus has begun. Further studies are needed to address the volume expansion properties of a concomitant infusion of isoproterenol and fluid.

Limitations

The primary limitation in this study was that these observations were made in normovolemic healthy volunteers. We acknowledge that the effects of a fluid bolus e.g., greater transcapillary refill could be very different in a hypovolemic circulatory state. The effectiveness of co-infusing isoproterenol with fluid to preferentially expand plasma over interstitial volume in shock, in which the sympathetic nervous system is already activated, is not known. This initial study was performed in healthy volunteers in order to investigate the basic effects of isoproterenol and fluid bolus without hypovolemia. We are currently exploring the interaction of isoproterenol and other agents in volunteers undergoing hypovolemia. Clinically, adrenergic agents are often co-infused with fluids to assist with cardiovascular function e.g., sepsis or patient’s with heart failure. Inotropic agents, for example, are usually administered to augment cardiac function in patients in heart failure. Heart failure is a chronic disease with compensatory mechanisms that often facilitates fluid retention. Interestingly, a significant subset of heart failure patients are admitted to the hospital with decompensated heart failure due to hypovolemia (30). However, in most cases, fluid therapy is the first line treatment and adrenergic agents are added once fluid alone cannot achieve hemodynamic goals. Rarely are adrenergic agents used initially to treat hypovolemia. Future studies comparing: 1) vascular volume expansion of a fluid bolus

before and after adrenergic agonists [inotropic agents like isoproterenol] or 2) fluid balance in patients receiving earlier treatment versus later treatment with adrenergic agents need to be performed in order to determine if these agents can improve the efficiency of fluid resuscitation. Thus, interactions with inotropes and fluid therapy merit further investigation.

Hemodynamic parameters are frequently used to guide resuscitation efforts (31). Due to the co-infusion of ISO in this study these common guiding parameters could not be used, because the administration of isoproterenol, in general, improves cardiac function and optimizes myocardial perfusion directly by dilatation of coronary arteries and indirectly by increasing cardiac oxygen consumption (32). ISO is additionally beneficial through reducing afterload by decreasing systemic vascular resistance (33).

We focused on the CFC and COP_{pl} as determinants of transvascular fluid flux. These methods were chosen due to their reproducibility, ease of measurement and validity in clinical subjects (14,15). CFC, using venous congestion plethysmography (VCP), requires the subject to remain still for several min at each time point, which was possible in our healthy volunteers, but could be problematic in patients that are shivering or non-cooperative. VCP is an indirect assessment of the CFC. Direct CFC measurements require small vessel [venule] cannulation, which would be invasive and non-practical in awake volunteers. Additionally, we used albumin and total protein to estimate the COP_{pl} , as previously reported (16). The COP_{pl} is directly related to circulating macromolecules. Other endogenous proteins are essentially non-contributory to oncotic pressure. Since we did not administer exogenous fluid solutions with larger molecules, such as starch or dextran, we assumed that the serum protein and albumin concentration reflected COP_{pl} at each time point. We acknowledge that other microcirculatory determinants such as P_c , P_{if} and COP_{if} were not measured. This was due to technical limitations and/or likelihood that these forces would not yield additional contributory information as to the transvascular fluid flux we observed with isoproterenol. Specifically, direct P_c requires invasive cannulation and isolation. Indirect measurements such as venous pressure and cardiac compliance (diastolic function) are now being pursued but were not measured in these volunteer studies. P_{if} and COP_{if} can be measured in vivo. P_{if} has been found to strong determinant of trans vascular fluid flux in some disease states that disrupt interstitial matrix e.g., burn injury (34), but most often only minimally fluctuates, even during fluid loading (34). Measurement of COP_{if} requires intermittent sampling over a long period of time. The time points of our measurements are more rapid than the turnover of interstitial protein and thus detecting differences over time or between groups would not be feasible (35).

Finally, we observed that the infusion of isoproterenol with and without fluid was associated with tachycardia, which could be detrimental for many perioperative ill patients e.g., coronary artery disease. We are currently investigating whether a lower effective/non-fixed dose of isoproterenol can similarly augment vascular volume expansion without tachycardia. Alternatively, it remains to be determined if other pharmacologic agents that activate cyclic AMP activity e.g. milrinone or pentoxifylline also have the ability to enhance volume expansion without adverse hemodynamic consequences. An important observation was that ISO showed an increase of the EDV and likely improves cardiac filling, however, diastolic indices of cardiac filling were not measured.

Safety—During our study, there were not any adverse events due to ISO such as cardio-pulmonary complications e.g., arrhythmias, pulmonary edema or other problems due to interventions. Our protocol, which included the use of arterial line – arterial pressure monitoring, constant ECG monitoring, moderate isoproterenol dose and fluid bolus, was approved by UTMB's IRB.

Conclusion

Fluid therapy is a dynamic process that involves a rapid and reversible modulation of the CFC. We have shown that a co-infusion of ISO with rapid fluid administration leads via an increased plasma volume expansion, diuresis and resultant decrease in overall EVV, likely representing interstitial fluid. The precise mechanistic pathway that regulates this process is unknown, but is likely related to barrier function and microcirculatory homeostasis e.g., glycocalyx. Further studies are warranted to explore precise mechanisms and if similar results can be safely obtained in patients.

Acknowledgments

This study was supported by a beginning in Aid Grant from the American Heart Association (AHA #06650744). This study was conducted with the support of the Institute for Translational Sciences -Clinical Research Center (ITS-CRC) at the University of Texas Medical Branch (UTMB) at Galveston, TX. Supported in part by a Clinical Translational Science Award (# UL1TR000071) from the National Center for Research Resources, National Institutes of Health.

The authors also thank volunteers and ITS-CRC staff of the research team for their contribution.

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Figure 1. Schematic of the study protocol timeline

Baseline data was recorded at -60 min. At -30 min, a continuous infusion of isoproterenol [ISO] or placebo control [CON] was begun. At 0–20 min, a fluid bolus (30 mL/kg 0.9% NaCl) was infused. The effects of drug infusion on the fluid bolus were followed for 120 min. Arrows indicate major measurements (CFC, COP, hemodynamics and volumetrics).

Figure 2. Capillary filtration coefficient measurement

Schematic of patient setting with mercury silastic strain gauge (a) and sample of measured results and calculation (b).

Figure 3. Echocardiographic measurements and calculations

EDV (a), ESV (b). Black solid line: control. Gray dashed line: Isoproterenol. * $p < 0.05$ vs.

Control; † $p < 0.05$ vs -60.

Figure 4. Fluid measurements and calculations

Urinary Output (a), Plasma Volume (b) and Extravascular Volume (c). Black solid line: control. Gray dashed line: Isoproterenol. * $p < 0.05$ vs. Control.

Figure 5. Vascular expansion efficacy – VVE calculated as change in plasma volume/fluid in (A) and *Fluid bolus distribution ratio*, calculated as change in plasma volume/EVV (B) during the study period. Control: black solid line. Isoproterenol: gray dashed line. * $p < 0.05$ vs. Control

Figure 6. Microcirculatory measurements

CFC (a) and COP_{pl} (b). Black solid line: control. Gray dashed line: Isoproterenol. * $p < 0.05$ vs. Control; † $p < 0.05$ vs -60.

Hemodynamic measurements

Table 1

Volunteers received continuous infusion of isoproterenol [ISO] or placebo [CON] 30 min before a 30 mL/kg 0.9% NaCl bolus infused over 20 min, then observed for 120 min.

Parameter	Group	-60 min (BL)	0 min	20 min	60 min	120 min
HR (bpm)	ISO	65 ± 4	97 ± 6 ^{*,†}	113 ± 3 ^{*,†}	101 ± 4 ^{*,†}	102 ± 5 ^{*,†}
	CON	64 ± 5	64 ± 4	74 ± 5	68 ± 4	67 ± 3
MAP (mmHg)	ISO	84 ± 3	87 ± 2	81 ± 3	82 ± 1	84 ± 3
	CON	83 ± 3	81 ± 3	82 ± 2	83 ± 3	85 ± 1
CO (L/min)	ISO	5.3 ± 0.6	10.2 ± 0.8 ^{*,†}	13.5 ± 0.9 ^{*,†}	10.2 ± 1.4 ^{*,†}	8.7 ± 0.9 ^{*,†}
	CON	4.5 ± 0.6	4.4 ± 0.7	6.1 ± 0.5	4.9 ± 0.5	4.6 ± 0.6
SVR (dyn [*] sec/cm ⁵)	ISO	1430 ± 180	703 ± 81 ^{*,†}	506 ± 45 [†]	696 ± 111 [†]	869 ± 124 ^{*,†}
	CON	1626 ± 237	1635 ± 221	1111 ± 135	1302 ± 191	1648 ± 207

HR: Heart rate, MAP: Mean arterial pressure, CO: Cardiac output, SVR: Stroke volume ratio during the study period.

^{*} p<0.05 vs. Control;

[†] p<0.05 vs -60.

Table 2**Volunteer demographic values**

Body Surface Area [BSA: $(\text{kg} \cdot \text{cm}/3600)^{0.5}$].

	ISO	Control	Significance
	Mean $\pm$ SEM	Mean $\pm$ SEM	
Age (yrs)	34.3 $\pm$ 2.7	33.7 $\pm$ 2.5	NS
Sex (m:f)	3 : 6	3 : 6	NS
Weight (kg)	83.5 $\pm$ 4.9	83.2 $\pm$ 5.1	NS
Height (cm)	169.6 $\pm$ 2.1	169.8 $\pm$ 2.1	NS
BSA (m²)	1.97 $\pm$ 0.1	1.97 $\pm$ 0.1	NS