Teaching fluid shifts during orthostasis using a classic paper by Foux et al.

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Hypovolemia

Hypovolemia refers to the condition of a significant reduction in circulating blood volume. A sudden drop in blood volume reduces venous return and stroke volume, which creates a drop in blood pressure. At this point, the baroreflex responds by the combination of an increase in heart rate, heart muscle contractility, systemic resistance, and a reduction in venous blood volume. The precise combination of control responses varies among individuals and, if insufficient to support blood pressure, can result in hypotension. Hypovolemia can lead to dizziness and fainting due to reduced blood flow to the brain. Clearly, hypovolemia represents a central challenge to the cardiovascular control system. Hypovolemia can be induced by traumatic hemorrhage, by excessive reduction of blood volume during dialysis treatment, and by an effective hypovolemic state due to the pooling of blood in the legs during orthostasis, which reduces the dynamic circulating blood volume. LBNP induces a controlled version of such blood pooling in the lower extremities and thus can be used to study hypovolemia and its consequences in an experimental setting.

LBNP: Physiological Testing, Clinical, and Spaceflight Applications

In 1965, Stevens and Lamb (20) introduced the LBNP method, a method that has proven to be a useful tool in medical research, especially in regard to (1) inducing orthostatic stress (20), (2) studying cardiovascular responses to hemorrhage (2), (3) preventing cardiovascular deconditioning (6), and (4) studying blood pressure regulation. In addition, LBNP has been used to assess pharmacological and physiological interventions on cardiovascular reflexes in patients with congestive heart failure and to simulate situations such as renal and splanchnic adaptations to stress and reduced cardiopulmonary function.

Orthostatic intolerance after manned spaceflight is common. LBNP has become an integral part of spaceflight research. For example, LBNP can be used to evaluate orthostatic tolerance. In addition, LBNP partially counteracts the cephalad shift of blood and body fluids that occurs in microgravity and consequently improves orthostatic tolerance after bedrest and head-down tilt as well as during spaceflight. In fact, LBNP has been used as a countermeasure against deconditioning during spaceflight (10) and also to simulate gravity during exercises in subgravity conditions (11).

Fluid Shifts During Simulated Orthostasis (LBNP)

Several papers have discussed fluid shift during orthostasis (1, 14, 19); one paper that stands out is that by Foux et al. (5), which was published in the Journal of Applied Physiology in
1976. Study by these researchers is part of the American Physiological Society (APS) Legacy project (http://www.the-aps.org/publications/legacy/).

In their classic paper, Foux et al. (5) assessed fluid shifts with LBNP. They reported that there is a shift of blood from the circulatory system and translocation of fluids from the circulation to the tissues, which could total up to 1–2 kg. They also observed that the fluid shift between tissue and vascular spaces was “proportional to the pressure gradient (induced by LBNP) and exponential with time.” Using LBNP set to −30 mmHg for 30 min, they observed two phases of fluid shifts: a rapid change due to the application of LBNP and representing the shift of fluid between vascular regions and a slower shift due to vascular-interstitial fluid exchange (Figs. 1 and 2).

More recent findings (14) have shown that blood volume changes during and after LBNP follow a more complex time course. Rapid blood volume changes are caused by an altered balance of Starling forces, which depend on hydrostatic as well as externally applied forces, as with LBNP. The exchanged fluid contains some protein, resembling lymphatic fluid in its protein concentration (15–17). Conventional hematocrit-to-hemoglobin ratio measurements may fail to reveal these transient volume changes. Therefore, we used a method that gives both high precision and measuring frequency, i.e., the mechanical oscillator technique, which allows for continuous high-precision recording of hematocrit changes in humans (18). We found that the mass density of whole blood and plasma decreases with early LBNP, indicating a transient ≈3% blood volume gain, before density rises as expected (Fig. 3) (14). Immediately after LBNP, on the other hand, density increases further, indicating an additional ≈1.5% hemoconcentration, before the expected return toward baseline control (14). This might suggest reflex-driven transient filtration effects: the sudden unloading of central pressure receptors with LBNP may cause fluid gain (inward filtration) preceding hemoconcentration during LBNP. After LBNP has ceased, receptor loading may cause additional fluid loss before inward filtration (hemodilution) predominates.

This means that instead of a simple approximation, corresponding to first-order kinetics (5), more complex modeling might be in order, mirroring the biphasic filtration and blood volume responses described above (14–18).

![Graph of the fluid shift during lower body negative pressure (LBNP) set to −30 mmHg for 30 min. LBNP was removed at 30 min. Two phases were observed: a fast rise due to the application of LBNP and representing the direct shift of fluid between vascular regions (ΔG1) and a slower process involving the fluid shift due to vascular-interstitial fluid exchange (ΔG2) (for 1 subject averaged over 5 trials). Δp, pressure difference. [From Ref. 5.]](image1)

![Graph of each component (ΔG1 and ΔG2) of the fluid shift during various levels of LBNP averaged over all four subjects. [From Ref. 5.]](image2)
Other Aspects of LBNP Integrated Into Our Teaching Seminar Plan

Discussion of the classic Foux et al. paper together with such current research (14–18) can be used to teach undergraduate and graduate students basic concepts of fluid regulation. In addition, they can be given an appreciation of the historical progression of the experimental process. In this context, it is also interesting to note that at the time of the Foux et al. research, LBNP application was being viewed more as a clinical treatment tool rather than as a diagnostic or experimental tool.

The physiological responses to LBNP are complex and often involve multiple organs. Thus, no one approach can cover the entire spectrum of possible control interactions, and the seminar supplements the work and findings of Foux et al. by teaching students important aspects of LBNP that have emerged since that original work (8, 14–18). As illustrated by the discussion above, by incorporating these additional aspects of LBNP methodology and comparing these methods and subsequent experimental results with the original work of Foux et al., the students come to better understand complex physiological principles, key mechanisms of cardiovascular regulation, and the process of experimentation that reveals these principles and mechanisms. The additional concepts that are incorporated into this teaching seminar structure, along with the relevant references, have been elaborated elsewhere (8). In brief, the teaching points can be described as follows.

LBNP-induced responses depend on the pressure applied and the exposure duration.

The extent of lower body blood pooling and the central venous pressure decline are directly related to LBNP intensity, with an approximate slope of 1-mmHg central venous pressure/10-mmHg LBNP. Approximately 500–1,000 ml of fluid move from central to peripheral regions within 5 min under the application of 20- to 40-mmHg LBNP.

LBNP has varying effects on blood flows in different body regions.

Leg fluid volume more than doubles when LBNP is elevated from 20 to 40 mmHg, with the change due exclusively to venous filling. LBNP of 50 mmHg reduces splanchnic blood flow by ~32%, with a further decrease as the negative pressure increases. When LBNP is released, reactive hyperemia occurs in the hepatic circulation (13).

LBNP magnitude affects different receptors. Effects on cardiac and arterial baroreceptors depend on LBNP intensity: splanchnic perfusion is mainly influenced by arterial baroreceptors, whereas muscular, cutaneous, and renal vasoconstric-
tions are driven by both arterial and cardiopulmonary receptor stimulation (for a review, see Ref. 8). LBNP < 20 mmHg changes peripheral vasoconstriction and increases heart rate mainly by affecting cardiopulmonary baroreceptors. Higher LBNP influences both the arterial and cardiopulmonary receptors, thereby reducing forearm and splanchnic blood flow and pulse pressure. Nevertheless, whether LBNP < 20 mmHg allows for the exclusive study of cardiopulmonary receptors is controversial.

**Pressure duration/increments in ramps.** Short-term LBNP, applied rapidly, can be a tool for studying sympathetic control reflexes. Most cardiovascular changes occur within 3–5 min of LBNP application. LBNP duration of ≥20 min evokes additional neurohormonal responses. One hour of LBNP reduces central, forearm, and splanchnic blood flow without changes in mean aortic or pulse pressure due to the activation of the renin-aldosterone system, even with very low levels (10 mmHg). See Ref. 8 for a detailed discussion.

Foux et al. (5) demonstrated that varying levels of LBNP will affect response courses and dose-response patterns (see Fig. 2); therefore, the varying pressures and protocols applied in different studies make it difficult to compare the associated hemodynamic and hormonal changes. When designing LBNP protocols, it is important to ascertain which protocol will enhance a particular reflex/response pertinent to the study focus. Some of these protocols are shown in Table 1.

**Plasma volume.** As mentioned above, the time course of LBNP-induced filtration and blood volume changes is complex (biphasic) both after the beginning and ending of LBNP (14). Therefore, calculations of plasma volume changes after LBNP should be done at the appropriate times.

Plasma volume changes with supine LBNP are not influenced by the hydrostatic leveling of filtration forces, which would occur with any change from a supine position to an upright position except at the point of “hydrostatic indifference” (12). Therefore, LBNP-derived plasma volume changes are exclusively caused by transcapillary fluid shifts due to microvascular changes brought about by the effect of external suction applied on the lower parts of the body, depending on the exact sealing position (7).

### Teaching Seminar Characteristics

The seminar is directed at undergraduate and graduate students with a background in physiology or biology. The instructor will begin by summarizing the Foux et al. paper, including the methodology and results, and providing some additional inputs regarding LBNP testing, emphasizing the teaching points given above and the changes in physiological knowledge and research approaches that have occurred since the paper appeared. Students then will receive the Foux et al. paper and work in groups to understand the importance of the paper, critically appraise the findings and find solutions to some questions (see **Sample Questions for Discovery Learning**).

In this way, the students learn how carefully planned experiments, such as those of Foux et al., can advance science. In addition, the teaching seminar also encourages critical thinking. As the development of critical thinking is hard to prove, we assess it via analytical reasoning and fair-mindedness testing (as detailed in http://www.criticalthinking.org/resources/assessment/index.cfm).

### Student Learning Outcomes

After completion of the interactive seminar, students will be able to:

1. Explain what types of fluid shifts occur during orthostasis
2. Explain the differences between HUT and LBNP
3. Explain which homeostatic compensatory responses occur during central hypovolemia to maintain blood pressure, for example, how the heart rate, cardiac output, and regional blood flows change due to the fluid shifts
4. Clearly understand why the technical aspects of LBNP protocol and testing are important
5. Plan a LBNP study and limit the confounding variables
6. Interpret the data generated, taking into account the experimental limitations
7. Encourage critical thinking regarding basic physiological principles of cardiovascular regulation

### Sample Questions for Discovery Learning

**Question 1.** How much fluid shifts toward the lower body during standing?

**ANSWER.** This depends on the duration of standing. When a healthy person stands, 10–15% (~650–700 ml in a person weighing 70–80 kg) of blood is rapidly pooled in the legs. LBNP of 40 mmHg causes similar fluid shifts as during standing (as detailed in Ref. 8).

**Question 2.** What are the advantages of LBNP over HUT?

**ANSWER.** LBNP does not involve a change of body position. Therefore, vestibular effects of postural change are avoided.

### Table 1. Studying the effects of LBNP using specific protocols

<table>
<thead>
<tr>
<th>To Study</th>
<th>Recommended LBNP Protocol</th>
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<tbody>
<tr>
<td>Unloading of cardiopulmonary baroreceptors; fluid displacement to peripheral vascular compartments</td>
<td>Mild level, constant LBNP pressure, short duration</td>
</tr>
<tr>
<td>Forearm or splanchnic blood flow; leg interstitial fluid pressure changes</td>
<td>Mild level, constant pressure, long duration</td>
</tr>
<tr>
<td>Sympathetic activity; “quick” hormone responses</td>
<td>Mild level, pressure increase (ramp), short duration</td>
</tr>
<tr>
<td>Volume-regulating hormone responses</td>
<td>Mild level, pressure increase (ramp), long duration</td>
</tr>
<tr>
<td>Regional blood flows; sex differences in orthostatic tolerance</td>
<td>Moderate level, constant pressure, short duration</td>
</tr>
<tr>
<td>Vagal activity during LBNP-induced hypotension</td>
<td>Moderate level, constant pressure, long duration</td>
</tr>
<tr>
<td>Near-maximal vasoconstrictor responses</td>
<td>Moderate level, pressure increase (ramp), short duration</td>
</tr>
<tr>
<td>Splanchnic and renal blood flows; the renin-aldosterone system; orthostatic tolerance testing</td>
<td>Moderate level, pressure increase (ramp), long duration</td>
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Specific protocols are required to assess specific aspects. For example, to study renal vasoconstriction might require a different protocol (level of pressure, constant or increasing pressure, how long the pressure must be applied) compared with studying forearm vasoconstriction. LBNP, lower body negative pressure. [Modified from Ref. 8.]
**Question 3.** Why should the blood pressure cuff be held at the heart level during HUT? [When answering this question, please consider the hydrostatic indifference point (12).]

**Answer.** To ensure that blood pressure measurements are done at the height of the same arterial hydrostatic indifference level while supine and upright, the blood pressure cuff should be held at the heart level (perhaps by a system of Velcro straps).

**Question 4.** How is the regional blood flow affected by LBNP?

**Answer.** Differential blood pooling occurs in different body compartments depending on the level of LBNP suction and the duration of application (see Table 1). For example, as shown in Table 1, different protocols might be required to study renal blood flow compared with forearm blood flows.

**Question 5.** What are the physiological mechanisms to be considered when studying fluid shifts induced by LBNP?

**Answer.** The LBNP suction level applied, duration of application, whether pressure is constant or a ramp pattern, level of sealing application, etc.

**Question 6.** Why is the sealing position of the LBNP important? Sealing positions have varied from the chest to the umbilicus to the pelvis. Could this influence the responses? [When answering this question, it is important to remember that the splanchnic vascular bed represents the largest regional vascular conductance and constitutes an important blood reserve (3) and is sensitive to baroreceptor stimulation.]

**Answer.** A previous study (7) determined that seal location would affect thoracic electric impedance, hepatic blood flow, and central cardiovascular responses to LBNP. It was hypothesized that LBNP with an upper abdomen sealing location (lowest palpable rib in the midclavicular line, i.e., including the splanchnic area in the region exposed to LBNP) compresses the abdomen, provides suction on the splanchnic area, and creates a larger cardiovascular stress than does an iliac crest sealing position.

This study found that some differences were present even before the application of LBNP. Central blood volume was apparently reduced at rest when the seal was placed in the upper abdominal position, affecting heart rate and splanchnic blood flow during baseline and causing a larger increase in heart rate during LBNP.

**Future Directions**

In collaboration with mathematical researchers, we also plan to provide the students some aspects of mathematical modeling and the important predictive values modeling provides. This is detailed in Ref. 1.

The cardiovascular system involves many subsystems, which interact in complex ways. The interactions of control mechanisms responding to blood volume perturbations or stresses such as induced by LBNP result in many potential combinations of control responses, and mathematical models can be used to quantify these interactions both for educational purposes and clinical application. Modeling examples and aspects of mathematical modeling applicable to the study of cardiovascular regulation related especially to blood volume control and simulation of LBNP and HUT effects, which we intend to include in our teaching seminars, are based on our previous work. For example, LBNP-induced physiological data from double sealing (7) were modeled (19), as were the data from presyncopal runs (Refs. 9 and 4, respectively).

**Conclusions and Recommendations**

Specific research objectives can require quite different LBNP methodologies, and, for research results to be optimized, the precise details of LBNP application have to be considered. Therefore, using the elegant classic paper of Foux et al., the seminar seeks to incorporate LBNP methodologies developed subsequent to that paper plus additional physiological responses that have been subsequently observed. Comprehensive protocols and carefully controlled studies will reduce confounding variables and allow for an optimal extraction and elucidation of the physiological responses that are being investigated. In addition, comparison of the classic results with later scientific developments will help students appreciate how scientific research proceeds and how scientific ideas evolve. This experience should contribute to developing open mindedness and flexibility when carrying out research.

Students will learn about the technical aspects of how to do the experiments (learning objective 4) and use the knowledge from technical aspects of the protocols to identify if the experiments done by Foux et al. and ourselves (7, 9, 14) have taken these into account (learning objective 6). All these processes will empower students to not just accept the results as they are presented but rather think “how” and “why” certain aspects were done and indeed to be able to independently decide whether criteria for good clinical and laboratory practice were followed. In this way, critical thinking is encouraged (learning objective 7) in the aspects of understating physiological principles and whether experiments were conducted appropriately.

Finally, we also teach the students to consistently challenge what has been reported and question the findings of investigators rather than accepting everything that has been reported in our seminars. We try to reveal for the students the mistaken paths taken, the process of hypothesis formulation, what to do with perplexing or negative results, and the ability to persuade those who disagree with published findings, including those from our laboratory.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**REFERENCES**


