Developing a “dry lab” activity using lower body negative pressure to teach physiology

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Abstract

In this paper we assessed how lower body negative pressure (LBNP) can be used to teach students the physiological effects of central hypovolemia in the absence of the LBNP and/or a medical monitor using a “dry lab” activity using LBNP data that have been previously collected. This activity was performed using published LBNP papers, with which students could explore LBNP as an important tool to study physiological responses to central hypovolemia as well as consider issues in performing an LBNP experiment and interpreting experimental results. The activity was performed at the All India Institute of Medical Sciences, New Delhi, with 31 graduate students and 4 teachers of physiology. Both students and teachers were provided with a set of questionnaires that inquired about aspects related to the structure of the activity and how this activity integrated research and knowledge, as well as aspects related to motivation of the students and teachers to perform the activity. Our results from student and teacher surveys suggest that a “dry lab” activity using LBNP to teach physiology can be an important tool to expose students to the basics of systems physiology as well as to provide useful insights into how research is performed. Providing insight into research includes formulating a research question and then designing (including taking into account confounding variables), implementing, conducting, and interpreting research studies. Finally, developing such an activity using LBNP can also serve as a basis for developing research capacities and interests of students even early in their medical studies.

critical thinking; “dry lab”; group work; LBNP; student-focused teaching

INTRODUCTION

Lower body negative pressure (LBNP) is a noninvasive procedure that is typically applied in supine subjects. Application of negative atmospheric pressure on the lower body (via seal application at iliac crest) leads to a suction effect and enhanced vascular compliance leading to blood pooling in the lower limbs, reductions in venous return, and central hypovolemia (Fig. 1; Ref. 1).

Compensatory increases in sympathetic activity in response to the central hypovolemia ensure that arterial pressure and cerebral perfusion is maintained. LBNP effects are reversible, as the release of LBNP is always followed by a rapid return of blood from the legs to the thorax, increases in arterial pressure, and, consequently, decreases in heart rate. Therefore, LBNP can be an important tool to teach students aspects related to blood pressure regulation, cerebral blood autoregulation, autonomic system responses, as well as the role of stress hormones and volume regulating hormones (see Refs. 1, 2).

Tilt table testing is widely used in clinical laboratories and/or in teaching to assess effects of central hypovolemia. Head up tilt table-induced changes in systemic and cerebral vascular resistance have been shown to be similar to those that occur during LBNP (3). In laboratories where LBNP and medical supervisions are available, LBNP can be used as a teaching tool for simulating central hypovolemia in physiology and medical school curricula. Such a learning activity where students can be directly involved in designing and performing LBNP protocols can be used to explore the complex nature of cardiovascular control, modulation of blood flows in different vascular beds such as splanchnic region (4, 5) and “simulated orthostasis” (6, 7). It also can illustrate that LBNP-induced central hypovolemia leads to hemodynamic and neurohormonal responses (2, 8), which vary in different individuals and from one environment to another in the same individual. Indeed, LBNP alone or in combination with head up tilt (HUT) has been used to assess systemic responses as subjects reach the end of their cardiovascular stability and develop a presyncopal state (9). Coagulatory activities have also been studied during LBNP (6, 10, 11) and at presyncope (12). Studying the impact of LBNP activity in the laboratory setting can also reinforce the need to identify possible confounding variables that can potentially affect cardiovascular and hormonal responses associated with acute
reductions in central blood volume (see Ref. 13) and related to participant inclusion and exclusion criteria in LBNP studies (14). In addition, such a learning activity can illustrate concepts related to the design of LBNP protocols and the identification of appropriate laboratory conditions related to specific LBNP protocols (1, 7).

Teaching Goals When Using LBNP to Teach Systems Physiology

The following goals can be addressed when learning about LBNP:

1) Highlight the different uses and applications of this noninvasive physiological tool. The students should be able to follow the several uses of this innovative tool over the years. These range from the usage of LBNP to create a dry operating field to its usage in spaceflight and as an important tool to understand systems physiology.

2) Identify variations in LBNP experimental protocols and the motivations for such variations. LBNP-induced responses depend on the type of LBNP application (ramp vs. step up protocol) and pressure applied as well as the duration of LBNP application. Therefore, varying the LBNP protocol can be used to study different physiological systems. For example, lower levels of LBNP when applied for brief periods do not lead to changes in cerebral blood flow whereas high levels of LBNP applied even briefly (<5 min in some cases) can lead to cerebral hypoperfusion, confusion, and impending presyncope (7–9, 15). Presyncope is rapidly followed by syncope, where the participant can lose consciousness. In summary, each LBNP protocol can be used to study different systems and to assess the role of different aspects (e.g., heart rate, hormones, autonomic system) in blood pressure responses and cerebral autoregulation (16, 17).

3) Recognize the individual variability in physiological compensatory response to central hypovolemia. This is an important aspect for learning physiology. While the body is excellent at maintaining homeostasis, each person responds to central hypovolemia in a specific way. For example, some individuals respond to the initial central hypovolemia with increases in heart rate while others react more with increases in peripheral resistance responses. Interestingly, despite each person having different strategies for responding to hypovolemia, the strategies appear fairly constant. Indeed, our results and those of others have shown that the LBNP tolerance and the hormonal responses are reproducible even when the LBNP tolerance is tested after 1 yr (18).

4) Understand how cardiovascular and neurohormonal responses vary during and following different levels and durations of LBNP exposure. LBNP application can be used to teach the students that the initial responses to central hypovolemia, aimed at maintaining the blood pressure, are autonomic in nature whereas LBNP application over longer periods leads to volume regulating hormones. The latter responses play important role in the maintenance of blood pressure during persistent central hypovolemia.

5) Learn how to design, implement, and interpret results from an LBNP experiment. This is an important goal, as the students begin to understand the role of confounding variables in understanding physiology. By learning about the role of confounding variables such as age, sex, height, physical fitness, etc. in blood pressure regulation (1, 19) and coagulation (6) during LBNP-induced central hypovolemia, the student is able to understand systems physiology.

Ultimately, such learning activities will also provide a basis for students to perform their own future independent research in both the basic sciences and medical sciences (15) and across disciplines (20). For instance, Batzel and

Figure 1. Overview of the mechanical effects and physiological responses of lower body negative pressure (LBNP). FVC, forced vital capacity; FEV1, forced expiratory volume in 1s; FRC, functional residual capacity; LVED, left ventricular end-diastolic volume; IVC, inferior vena cava; MAP, mean arterial pressure. [Reproduced from Ref. 1.]
colleagues (20) have used LBNP research and data obtained from LBNP studies to bridge different perspectives of the physiological and mathematical disciplines in students and graduates with different educational backgrounds.

Teaching Systemic Integrative Physiology via a “Dry Lab” Activity Using LBNP Data

LBNP is, however, not available in every laboratory. It also requires the careful assessment of the subject’s physiological parameters during LBNP via a medical monitor as syncope and arrhythmias, both of which may be life threatening, may occur (albeit rarely). Therefore, there is a need to develop a “dry laboratory” activity in which LBNP could be used as a teaching tool even when direct access to an LBNP and/or medical monitor is unavailable. Such a “dry laboratory” activity will be effective in addressing the educational goals outlined above.

The “dry laboratory” activity presented here was performed using a published LBNP paper (1), with which students could explore LBNP as an important tool to study physiological responses to central hypovolemia as well as consider issues in performing an LBNP experiment and interpreting experimental results.

The activity proposed in this paper involves important learning methodologies for students, as it incorporates significant elements of critical thinking, discovery learning, group work, and student interactions (21, 22). An important part of this activity is that students and facilitators should interact freely and gain insights into each other’s thinking processes, prior knowledge about the topic and physiology in general, as well as identify the need to emphasize group work and learning by doing (23). It has been reported that students often lose attention during lectures, and one of the contributing factors toward this is the teaching style of the teacher and how learning material is presented (24). To address these issues, the activity proposed in this paper aims at providing the teachers with an innovative approach (“dry laboratory” activity) to teach systems physiology. Use of innovative approaches in teaching have been recommended previously by Biggs and Tang (25). While LBNP was used as the focus of the “dry laboratory” activity presented here, the format and structure of the “dry laboratory” activity could be extended to other realms in physiology (see for instance, Ref. 26). This activity also builds on other activities that have been performed by physiology instructors to improve the understanding of systems physiology (27, 28).

**METHODS**

The “dry laboratory” activity with LBNP was designed and implemented as a collaboration activity between the Medical University of Graz, Austria and the All India Institute of Medical Sciences (AIIMS), New Delhi, where the activity was performed. Approval from the Ethics committee of AIIMS, New Delhi was obtained for the study.

**Details about the Activity Setting**

This activity was carried out in problem-based learning setting, in which small groups (including graduate students and teachers) were aided by a coordinator. The groups consisted of four to six persons. The group members were not known to each other before this activity. The activity involved participation of both 1): graduate students (n = 31); and 2): facilitators (medical teachers, including those at different level of academic standing: assistant professors: n = 2 and professors: n = 2).

**Performing the “Dry Lab” Activity with LBNP Data**

The exercise involved three steps:

- **Assigned prework.**
  A recent review presented in *Physiological Reviews* by the lead author of this paper (1) was provided to the participants a week before the activity took place. The recipients were told to read the paper carefully, including aspects related to effects of LBNP, important factors that could affect the results, and possibly the applications of LBNP.

- **Procedure during the “dry laboratory” activity** (including discussions by the coordinator of the activity).
  The participants were willing to participate in the discussion from the beginning of the activity as they were aware of the format of the activity (see above). No icebreakers were, therefore, required during the activity itself.

The activity included several steps:

1. A 5 min- short presentation of the planned “dry lab” activity by the coordinator of the course. The PowerPoint presentation provided an overview of LBNP, including historical aspects and its broad application from space-flight to its usage as a research tool.

2. The students and teachers were then placed in groups. Teachers were told to listen to the discussions in their groups, challenge the students thinking and guide them with open-ended questions (see below);

3. Clear topics and areas for discussions were incorporated into 5- to 10-min blocks. These were presented in each group as open-ended questions (taking into account the teaching goals of this exercise, as outlined above) and were classified as follows:
   - **Question #1**: What are the effects of LBNP? (10 min allowed)
   - **Question #2**: What are the possible applications of LBNP? (10 min allowed)
   - **Question #3**: How would you go about planning an LBNP experiment? That is, what are the aspects, including confounding variables, that you need to consider when you are planning an LBNP experiment? (20 min allowed)

Following the individual group discussions, the coordinator of the activity then reviewed the discussions. The “dry laboratory” activity was completed when important points from discussions related to questions #1–3 above were summarized by showing the figures and tables from the published paper (1).
At the end of “dry laboratory” activity.
Having engaged the students and teachers in exploring and implementing LBNP research via discussions related to questions #1-3, the coordinator concluded the “dry laboratory” activity by showing the students real data reflecting LBNP-induced hemodynamic responses in subjects not developing presyncope (Fig. 2) and then showing the students data on hemodynamic responses from subjects depicting cardiovascular collapse, with development of presyncopal signs and symptoms (Fig. 3). These data provided visual illustration of what LBNP research can reveal regarding human physiology.

Wrap-up/conclusions.
A final summary of the role of the “dry laboratory” activity using LBNP in the learning process was made. The students were asked to identify which aspects they found difficult to answer/understand and which aspects surprised them most. In addition, any other questions raised by the students or teachers were then addressed.

Both students and teachers were then provided with a questionnaire (detailed in RESULTS) that inquired about aspects related to the structure of the activity, and how this activity integrated research and knowledge, as well as aspects related to motivation of the students and teachers to perform the activity. The questionnaires were given to the participants immediately after the activity. The survey evaluated the LBNP activity and also requested the participants to provide recommendations for further improvement of the activity. Responses were rated on a scale of 1–5, where 1 = “strongly agree” and 5 = “strongly disagree.” Outlines of the aspects that were asked in the questionnaires administered to the students and teachers are provided in Tables 1 and 2, respectively.

RESULTS
The total “dry laboratory” activity lasted 2 h. The activity was completed by 31 graduate students and 4 teachers of physiology. All the participants confirmed that they had completed their pre-work.

In the “dry lab” activity, there were some aspects that the students found more difficult to answer. These included the following:
1) Aspects related to the usage of LBNP in spaceflight.
2) Unclarity regarding mechanical effects of LBNP versus physiological effects.
3) Aspects related to LBNP sealing position and why this was important.
4) Aspects related to influence of gender on the physiological responses. While most of the students speculated that differences across the sexes exist, most of them were surprised that there are sex-based steroids effects on the hemodynamic responses seen during hypovolemia. That is, there are differences in cardiovascular responses in males and females during LBNP-induced hypovolemia.
5) Another aspect that the students found surprising was related to the influence of different phases of the

Figure 2. Lower body negative pressure (LBNP) application without development of presyncope. LBNP10, 20, 30, and 40: LBNP at –10 mmHg, –20 mmHg, –30 mmHg, and –40 mmHg.
menstrual cycle on the cardiovascular responses. That is, how each phase of the menstrual cycle could potentially affect the hemodynamic responses.

Some of the questions asked by the students included the following:

1) How is LBNP superior to artificial gravity?
2) Is it possible to combine LBNP with other perturbations such as exercise?
3) Is there a need to control the temperature and humidity in the LBNP chamber?
4) Could different types of dressing worn by the participants (e.g., shorts vs. trousers; with- and- without socks) have an effect on the observed responses?

Tables 3 and 4 show the assessment scores of the activity as performed by students and teachers. Table 3 outlines the responses of the students and researchers (males: 12; females: 19) with respect to the “dry laboratory” activity performed. Table 4 outlines the responses of each teacher/facilitator that were involved in the activity.

## DISCUSSION

During the “dry” laboratory activity, both the students and the teachers were seen to be enthusiastically engaged in discussions. It was also very encouraging to see the students interacting freely with their peers and teachers, especially during the open-ended questions. Some of these are now discussed.

### Discussions Related to the Effects of LBNP (Question #1)

- The emphasis here was to make the participants realize that there were mechanical effects of the LBNP application per se as well as the physiological responses that arise due to LBNP application.
- An important focus was to have participants realize that LBNP affected not only the cardiovascular system but also other systems. For example, students discussed aspects relating LBNP and cerebral blood flow. Since cerebral blood flow depends on carbon dioxide levels in the blood, there is a need to ensure that no deep breaths or rapid inhalations occur during LBNP application.
- Another aspect highlighted in this question was that the effects of LBNP vary in response to both the amount, and the duration, of the applied LBNP level.

This discussion was important as it outlined the need to fine-tune the LBNP protocol (LBNP amount, LBNP duration of application, etc.) according to the research question being proposed. For instance, if the aim is to assess presyncope in the test subjects, then there is a need to apply the LBNP...
beyond -40 mmHg and to increase this in steps until presyncope signs and symptoms occur (7–9, 15).

Applications of LBNP (Question #2)

This discussion ranged from historical aspects of LBNP, including its usage in clinical medicine, as a tool to study effects of central hypovolemia and as a countermeasure to prevent space flight induced deconditioning (1, 7).

Planning an LBNP Experiment (Question #3)

This discussion ranged from protocol design to considerations of the potential confounding variables that need to be incorporated in the study. Discussed were aspects such as the effects of fasting states and sex hormones on physiological responses, the need for careful inclusion and exclusion criteria, and location of LBNP sealing position (29) among others. The need to perform sample size calculations, obtain ethical permissions, and define the statistical approaches for data analysis before the commencement of the study were emphasized.

From exploring the above three questions the participants could better understand and interpret the impact of LBNP as well as gain insight into key issues related to experimental design. At the end of the discussions, the participants were better prepared to appreciate the information provided in the real data sets reflecting presyncope.

As physiological knowledge is of fundamental importance for understanding pathophysiology, pharmacology, as well as most aspects of internal medicine, we believe laying down such an approach toward learning of physiological concepts in systems biology will go a long way in raising the knowledge and research potential of participants of such activity.

Table 1. Questionnaire administered to the students for their assessments of the “dry lab” activity

| Using LBNP for TEACHING |
|-------------------------|
| Student Evaluation Form |

I am a/an: o Physiologist o Mathematician o Others
I am a: o Physiologist o Mathematician o Others

Please place an x next to your impressions of the items listed below:

| Item | Strongly Agree | Agree | Neutral | Disagree | Strongly Disagree |
|------|----------------|-------|---------|----------|-------------------|
| 1. The course met my expectations. | o | o | o | o | o |
| 2. I will be able to apply the knowledge learned | o | o | o | o | o |
| 3. Objectives were identified & followed | o | o | o | o | o |
| 4. Content was organized and easy to follow | o | o | o | o | o |
| 5. Materials distributed were useful | o | o | o | o | o |
| 6. Speakers/teachers were knowledgeable | o | o | o | o | o |
| 7. The teachers were motivated | o | o | o | o | o |
| 8. Quality of education was good | o | o | o | o | o |
| 9. Speakers met the course objectives | o | o | o | o | o |
| 10. Critical thinking and student-teacher interactions were encouraged | o | o | o | o | o |
| 11. Adequate time was provided for question and answers | o | o | o | o | o |
| 12. How do you rate the course overall? | o Excellent | o Good | o Average | o Poor | o Very Poor |

LNBP, lower body negative pressure.

Table 2. Questionnaire administered to the teachers for their assessments of the “dry lab” activity

| Using LBNP for TEACHING |
|-------------------------|
| Teacher Evaluation Form |

I am a/an: o Physiologist o Mathematician o Others
I am a: o Physiologist o Mathematician o Others

Please place an x next to your impressions of the items listed below:

| Item | Strongly Agree | Agree | Neutral | Disagree | Strongly Disagree |
|------|----------------|-------|---------|----------|-------------------|
| 1. The event structure met my expectations | o | o | o | o | o |
| 2. Student pool was diverse | o | o | o | o | o |
| 3. Mix of research and knowledge well balanced | o | o | o | o | o |
| 4. The content prepared was about right for the educational level | o | o | o | o | o |
| 5. The resources available were useful | o | o | o | o | o |
| 6. The students were knowledgeable | o | o | o | o | o |
| 7. The students were motivated | o | o | o | o | o |
| 8. I found the event useful for myself | o | o | o | o | o |
| 9. Students asked many interesting questions | o | o | o | o | o |
| 10. Adequate time was provided for questions and answers | o | o | o | o | o |
| 11. How do you rate the overall impact? | o Excellent | o Good | o Average | o Poor | o Very Poor |

LNBP, lower body negative pressure.
Indeed, given the complex interactive nature of biological systems, the presentation of systems biology demands in-depth, interactive, and exploratory thinking.

Additionally, some authors have suggested that preunderstanding is a prerequisite for meaningful learning (30). It is important to clarify that preunderstanding is not only reflecting the previous knowledge about a subject from a learner’s point of view but also includes the assumptions/attitudes as well as the learning processes and styles of the student as well as his/her motivation (30, 31). From laboratory experiments, it has been shown that the brain organizes the information it receives in such a way that it can be used for recall or for future use (32). Overall, new knowledge must be added on or be connected to prior knowledge (31, 32); it is only then that the new knowledge becomes integrated in the learner as meaningful. Therefore, students are required to have basic knowledge of physiology (especially the regulation of cardiovascular variables) before performing this "dry laboratory" activity. While meaningful learning here was performed using a "dry laboratory" activity, other approaches toward such learning can include concept maps (33), case seminars (34), using interactive lectures in problem-based learning (30) and learning about cognitive processes associated with problem solving (35).

Finally, the activity proposed here also emphasizes an additional type of learning: application-directed learning. In this type of learning, the student thinks of ways in which knowledge can be used in practice (22). As an example, students will work in groups and examine the literature provided and critically examine it together and then come up jointly with answers to aspects that could be used by the group when they are conducting further research in physiology (e.g., confounding variables).

Lastly, in the unprecedented scenario of global epidemic restricting classical classroom teaching and practical exercise, alternatives that can be performed through online classrooms are extremely important for uninterrupted education (36). In this sense also, the methodology of the "dry laboratory" activity for LBNP may be replicated in virtual classroom setting. This model shows immense potential for online learning with experts and students from different background and locations coming together for effective learning via virtual platform.

**Limitations**

The sample size of the students and teachers was limited. Furthermore, this study was performed in only one country (India). We propose that future studies should include undergraduate medical students as well as be performed with graduate students and teachers in different settings (e.g., in European institutions). This will serve not only to increase the sample size but also provide important insights into the perceptions of such activity in students at undergraduate and graduate level and in different countries and across different ethnic groups.

Another aspect to be considered is the high scores of the evaluations from the students and teachers from India. It is common knowledge that the students from developing countries are more generous in their award of marks in questionnaires. They are also less critical of teaching methodologies and as they hold teachers often in the highest regard and are, therefore, reluctant to provide low scores in their evaluations of teaching and teaching-related activities.

**Conclusions of the “Dry Laboratory” Activity, Recommendations, and Future Directions**

The students and teachers were asked to evaluate the "dry laboratory" activity. Based on the results provided in the tables, it can be seen that the activity provided a very useful and novel learning experience for both the students and teachers. The results demonstrate that, even without the availability of a LBNP, teachers can effectively guide the students to reliably understand physiological principles and systems physiology while supporting the deeper learning of the students.

**Box 1** summarizes the details about the lesson, how to conduct it, as well as the exact steps involved. This can help other educators to use this dry laboratory LBNP activity as a teaching tool.

**ACKNOWLEDGMENTS**

We thank the participants for enthusiasm and willingness to take part in this "dry lab" activity.

**Table 4. Results of the teachers’ evaluations of the “dry lab” activity**

| Q | Categories                                    | Means | SD  |
|---|-----------------------------------------------|-------|-----|
| 1 | The event structure met my expectations      | 1.5   | 0.6 |
| 2 | Student pool was diverse                     | 1.8   | 0.6 |
| 3 | Mix of research and knowledge well balanced  | 1.3   | 0.6 |
| 4 | The content prepared was about right for the educational level | 1.3 | 0.6 |
| 5 | The resources available were useful          | 1.5   | 0.6 |
| 6 | The students were knowledgeable              | 1.8   | 0.6 |
| 7 | The students were motivated                  | 1.7   | 0.6 |
| 8 | I found the event useful for myself          | 1.3   | 0.6 |
| 9 | Students asked many interesting questions    | 1.5   | 0.6 |
| 10| Adequate time was provided for questions and answers | 1.5 | 0.6 |
| 11| How do you rate the overall impact           | 1.5   | 0.6 |

Responses were rated on a scale of 1–5, where 1 = “strongly agree” and 5 = “strongly disagree.”
Box 1. Dry laboratory LBNP activity

Activity type: Problem-based learning setting, in which small groups are aided by a facilitator/teacher/expert.
Participants: 4–6 per group; the group members need not know each other before this activity is carried out.

Carrying out the activity:
A) Preactivity assignment: A recent review presented in Physiological Reviews by Goswami et al. (1) is provided to the participants a week before the activity.
B) Procedure during the “dry laboratory” activity (including presentation of coordinator):
1) A 5 min- short presentation of the planned “dry lab” activity (coordinator): overview of LBNP and applications;
2) The students work in the predefined groups of 4-6 per group;
3) Clear topics for discussions incorporated into 5- to 10-min blocks;
4) Facilitator listens to each group discussions and challenges the students thinking and guides them with open-ended questions;
Question #1: What are the effects of LBNP? (10 min allowed);
Question #2: What are the possible applications of LBNP? (10 min allowed); Question #3: How would you plan an LBNP experiment? (20 min);
5) Following the group discussions, facilitator asks each group to present their answers to the above questions;
6) Activity completed when important points from discussions related to questions #1–3 above are summarized by facilitator.
C) At the end of “dry laboratory” activity: Facilitator shows real data reflecting LBNP-induced hemodynamic responses in subjects not developing presyncope and then data on hemodynamic responses depicting cardiovascular collapse, with development of presyncopal signs and symptoms.
D) Wrap-up/conclusions: Students asked to identify which aspects they find difficult to answer/understand; questions from the students are then addressed.
E) Administering the questionnaire: Students provided a questionnaire to assess the activity and to provide recommendations for further improvements.

Future studies should compare the learning outcomes of students who use the “dry laboratory” LBNP activity teaching method with students who are taught cardiovascular physiology using an actual LBNP.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

N.G., A.S., and K.K.D. conceived and designed research; N.G., A.S., and K.K.D. performed experiments; N.G., A.S., and K.K.D. analyzed data; N.G., prepared figures; N.G. drafted manuscript; N.G., A.S., and K.K.D. interpreted results of experiments; N.G. prepared figures; N.G. drafted manuscript; N.G., A.S., and K.K.D. approved final version of manuscript.

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