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Reactive hyperemia in the human liver

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Hinghofer-Szalkay HG, Goswami N, Rössler A, Grasser E, Schneditz D. Reactive hyperemia in the human liver. *Am J Physiol Gastrointest Liver Physiol* 295: 332–337, 2008. First published June 5, 2008; doi:10.1152/ajpgi.00042.2008.—We tested whether hepatic blood flow is altered following central hypovolemia caused by simulated orthostatic stress. After 30 min of supine rest, hemodynamic, plasma density, and indocyanine green (ICG) clearance responses were determined during and after release of a 15-min 40 mmHg lower body negative pressure (LBNP) stimulus. Plasma density shifts and the time course of plasma ICG concentration were used to assess intravascular volume and hepatic perfusion changes. Plasma volume decreased during LBNP (−10%) as did cardiac output (−15%), whereas heart rate (+14%) and peripheral resistance (+17%) increased, as expected. On the basis of ICG elimination, hepatic perfusion decreased from 1.67 ± 0.32 (pre-LBNP control) to 1.29 ± 0.26 l/min (−22%) during LBNP. Immediately after LBNP release, we found hepatic perfusion 25% above control levels (to 2.08 ± 0.48 l/min, $P = 0.0001$). Hepatic vascular conductance after LBNP was also significantly higher than during pre-LBNP control (21.4 ± 5.4 vs. 17.1 ± 3.1 ml·min^{-1}·mmHg^{-1}, $P < 0.0001$). This indicates autoregulatory vasodilatation in response to relative ischemia during a stimulus that has cardiovascular effects similar to normal orthostasis. We present evidence for physiological post-LBNP reactive hyperemia in the human liver. Further studies are needed to quantify the intensity of this response in relation to stimulus duration and magnitude, and clarify its mechanism.

hepatic; indocyanine green; orthostasis; splanchnic blood flow; autoregulation; lower body negative pressure

CENTRAL HYPOVOLEMIA, AS CAUSED by blood redistribution (e.g., orthostasis) or blood loss (e.g., trauma) can be simulated by application of negative pressure to the body from the iliac crest downward (lower body "negative" pressure, LBNP), as this leads to peripheral blood pooling while avoiding additional hydrostatic effects of upright posture (14). Driven by decreased load on cardiopulmonary and eventually arterial baroreceptors, neurohumoral readjustments occur. The splanchnic vascular bed is a major regulatory target because it represents a large regional vascular conductance and constitutes the primary blood reserve in cardiovascular "emergency" situations (11) Even low (≤20 mmHg) levels of LBNP suffice to induce sympathetic activation and reduce splanchnic perfusion (17), whereas higher stimulus levels (e.g., 50 mmHg) lower splanchnic vascular conductance as well, by as much as ≈30% (6, 33).

Reduced perfusion has local metabolic consequences. Vascular "escape" from sympathetic influence (9, 34) and the general concept of "reactive hyperemia" (20, 31) and autoregulation (38) are well established, but hepatic reactive hyperemia as such has not yet been reported.

Splanchnic ischemia is connected to hypotensive episodes especially under prolonged hypovolemic stress such as hemodialysis and ultrafiltration of excess body fluid (12, 36). We speculated whether a much shorter perturbation such as standard LBNP would also induce ischemia. We measured hepatic clearance of ICG as a surrogate for splanchnic perfusion before, during, and after LBNP and hypothesized that after LBNP-induced vasoconstriction, hepatic perfusion would not only return to but also actually exceed pre-LBNP control levels, owing to local effects of relative hypoperfusion induced metabolite accumulation that occurred during LBNP.

METHODS

The study was done in 14 healthy, male volunteers of moderate physical fitness, free from cardiovascular, renal, hepatic, and pulmonary diseases and not on any medication. The subjects abstained from use of tobacco, caffeine, alcohol, and heavy exercise for at least 48 h preceding each investigation and the subjects were their own controls. The Graz Medical University Research Ethics Committee approved the study protocol, and written, informed consent was obtained from each subject. Before the study, LBNP sham runs without blood sampling were carried out for familiarization to the study (24).

Protocols were conducted between 9 and 12 AM to minimize circadian influences on hemodynamic variables (29). The subjects were fasting and emptied the bladder before each study. An antecubital vein was cannulated, for blood sampling and administration of ICG.

Experiments were carried out in a semidark, quiet room maintained at 24°C and humidity at 55%. A padded pair of tightly connected chains was used to stabilize and maintain an exact sealing position at the exact level of the iliac crest within the LBNP box (14). The box was equipped with a footrest that was individually adjusted before LBNP was commenced. A pillow supported the head to avoid stimulation of the otolith organs, which has been reported to increase muscle sympathetic nerve activity and calf vascular resistance (21).

Baseline data were collected for 30 min in the supine position, with the seal in place, before LBNP to allow for reequilibration of gravity-related fluid shifts (16). Pressure within the box was lowered electronically by a pump within 10 s and monitored by an electronic gauge (24). LBNP (−40 mmHg) lasted for 15 min because any longer period affects LBNP tolerance (15). During LBNP the subjects were instructed to avoid movements of the lower limbs and to breathe normally. The post-LBNP observation period lasted another 15 min. The time course of the experimental protocol is shown in Fig. 1.

Blood volume and hepatic perfusion. ICG (25 mg) was injected at two times, 20 min before and 7 min into LBNP, with sufficient time between injections for ICG to be completely cleared from the blood stream. Whereas the ICG disappearance following the first injection

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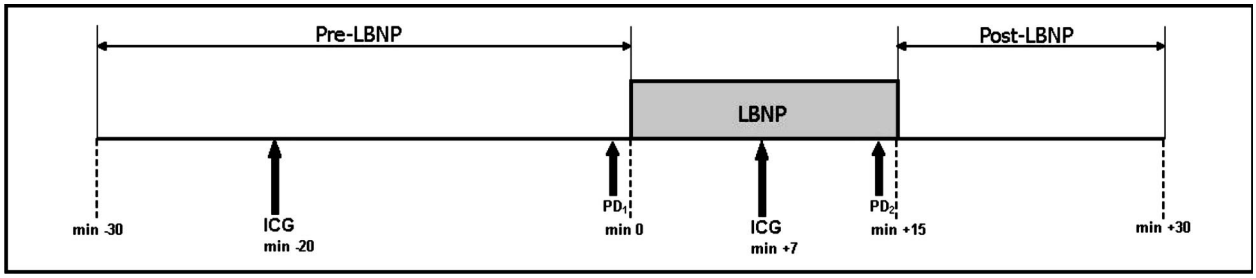

Fig. 1. Experimental protocol. ICG, indocyanine green injection. Blood was collected for plasma density (PD) measurements.

served to establish pre-LBNP baseline conditions, the second injection was timed to observe the change from LBNP to post-LBNP conditions. ICG concentration was measured noninvasively by the DDG-2001K Dye Densitometer (Nihon Kohden, Tokyo, Japan) via a nose probe, and the initial hemoglobin concentration was measured by standard techniques. Blood volume and hepatic blood flow were calculated from the amount of ICG injected and the measured ICG disappearance rate. The rate constant k_1 for ICG disappearance following the first injection was determined from fitting a straight-line model to the time series of logarithmically transformed ICG concentrations. To examine the changes in rate constants following the release of LBNP, the second ICG data series was split into two periods, a first period of 7 min preceding and a second, subsequent period of 8 min immediately following the release of LBNP. The ICG data collected during these two periods were separately fit to determine the rate constants k_2 and k_3 and compared with the rate constant k_1 determined at baseline. In each study, the slopes of the elimination curves were separately calculated. The corresponding slopes k_1, k_2, and k_3 were calculated by least-squares fits and compared.

ICG clearance K_{icg} was used as a surrogate for hepatic (splanchnic) blood flow and calculated as

$$K_{icg,t} = k_t V_{b,t} \quad (1)$$

where V_b refers to blood volume and the index t indicates conditions at baseline ($t = 1$), during LBNP ($t = 2$), and after LBNP ($t = 3$).

Absolute blood volume at baseline ($t = 1$) was determined from ICG mass M_{icg} injected and from ICG concentration c_0 at the time of injection according to

$$V_{b,1} = \frac{M_{icg}}{C_0} \quad (2)$$

Absolute blood volumes at times $t = 2$ and $t = 3$ were determined from $V_{b,1}$ and relative plasma volume changes by the following relationship

$$V_{b,t} = V_{b,1}[1 + \Delta V_{p,t}\%(1 - H_1)] \quad (3)$$

where H_1 refers to the hematocrit at baseline and where $V_{p,t}\%$ refers to the relative plasma volume change computed from mass densitometry. Relative changes in plasma volume ($V_{p,t}\%$) were calculated from changes in plasma density (ρ)

$$V_{p,t}\% = \frac{\rho_1 - \rho_t}{\rho_t - 1{,}008} \quad (4)$$

where indices refer to conditions at baseline ($t = 1$), during LBNP ($t = 2$), or after LBNP ($t = 3$), and where 1,008 refers to the density of the filtered fluid (19). Plasma mass density was measured with a DMA 602 MW (Paar KG, Graz, Austria) as described elsewhere (18). Hematocrit was measured in duplicate by a standard technique.

Hemodynamics. Beat-to-beat arterial blood pressures and hemodynamic variables such as stroke volume and heart rate were derived from the arterial pulse wave measured at the level of the digital artery by use of the Finometer (Finapres Medical Systems, Arnhem, The Netherlands) and presented as means for each phases final 5 min. Cardiac output was calculated as the product of stroke volume and heart rate. The Finapres technology is becoming increasingly used in physiological and clinical research (35).

Statistical analysis. Variables were tested for normality by the D'Agostino and Pearson omnibus normality test and expressed as mean value ± SD. Repeated-measures ANOVA with Bonferroni post hoc or the Friedman test with Dunn post hoc testing were used to test for changes in all tested variables and parameters with orthostatic loading. For all tests, significance was set at $P \leq 0.05$. All analyses were performed using GraphPad Prism 5 software (GraphPad Software San Diego, CA).

RESULTS

Fourteen subjects (25 ± 4 yr, 76 ± 8 kg, 1.75 ± 0.05 m, and 1.72 ± 0.15 m^2 body surface area) completed the study. Linear relationships with a negative slope k were observed when the logarithms of ICG concentrations were plotted vs. time (Fig. 2*A*) so that a standard exponential decay model could be used to describe the process of ICG elimination measured at baseline.

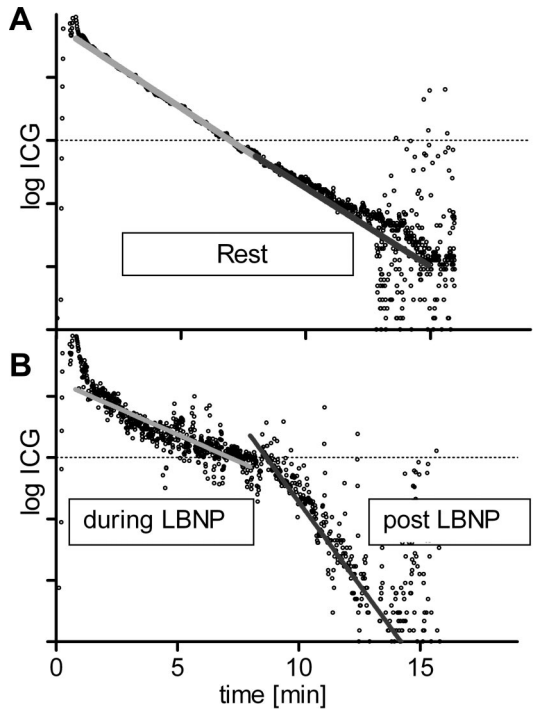

Fig. 2. Example for $\log_{ICG}$ vs. time slope determination by a standard exponential decay model. *A*: pre-lower body negative pressure (LBNP) baseline. *B*: a distinct slope change occurred when ICG clearance was examined during vs. after LBNP.

The exponent of this model refers to the rate constant k of the elimination process. However, when ICG clearance was examined shortly before and after releasing LBNP there was a distinct change in slope, which became steeper (more negative) from this point onward (Fig. 2*B*).

We found a mean slope of -0.28 ± 0.05 before (k_1 control); -0.24 ± 0.03 during (k_2); and -0.37 ± 0.09 min^{-1} after LBNP (k_3) with correlation coefficients of 0.93 ± 0.07, 0.88 ± 0.06, and 0.77 ± 0.08, respectively. The slope k_3 obtained after LBNP was significantly steeper than k_2 and k_1 measured during baseline and LBNP, respectively ($P < 0.01$). From the k values we computed 1.67 ± 0.32, 1.29 ± 0.26, and 2.08 ± 0.48 l/min of hepatic perfusion; LBNP-induced blood volume loss compared with control has been taken into account. Perfusion values were significantly different from pre-LBNP control (Table 1). Hepatic vascular conductance as computed from hepatic blood flow over arterial mean pressure was 17.1 ± 3.1 before, 13.8 ± 2.7 during, and 21.4 ± 5.4 ml·min^{-1}·mmHg^{-1} after LBNP ($P < 0.0001$).

LBNP-induced hemodynamic effects, as well as changes in plasma density, are indicated in Table 1. Heart rate increased by 14% and total peripheral vascular resistance by 17%; cardiac output decreased by 15%, stroke volume by 25%, systolic pressure by 8%, pulse pressure by 22%, and mean arterial pressure by 3%. The 1.23 g/l increase in plasma density indicated a 10% plasma volume reduction. At the end of the post-LBNP recovery period, cardiac output was 9% below pre-LBNP control values.

DISCUSSION

In this study we report evidence of LBNP-induced reactive hyperemia in the hepatic circulation in healthy humans. Hepatic hyperemia was observed during the recovery period after a 15-min 40 mmHg LBNP maneuver in young healthy men in a relaxed supine position. We used the ICG disappearance method to assess hepatic perfusion (8, 40) during and following LBNP (17, 39). It has been shown that the hepatic extraction of ICG dye remains constant throughout LBNP (33), supporting our approach.

Table 1. *Hemodynamic, ICG, and plasma density data*

	Pre-LBNP	LBNP	Post-LBNP
HR, bpm	66.7±8.4	76.0±9.2‡	63.8±7.4
CO, l/min	6.5±1.7	5.5±1.4‡	5.9±1.4†
SV, ml	97.1±21.8	72.4±18.1‡	94.2±20.3
TPR, mmHg·s^{-1}·ml^{-1}	0.95±0.27	1.11±0.29*	1.05±0.25
SBP, mmHg	130.8±5.3	120.2±10.2‡	129.5±11.3
DBP, mmHg	78.7±7.0	79.7±7.8	79.6±7.8
MAP, mmHg	97.4±5.2	94.2±8.0	97.9±8.6
PP, mmHg	52.1±7.3	40.6±8.0‡	49.9±7.8
ρ, g/l	1019.21±0.47	1020.44±0.48†	1019.40±1.12
k, min^{-1}	−0.28±0.05	−0.24±0.03	−0.37±0.09‡
HBF, l/min	1.67±0.32	1.29±0.26†	2.08±0.48‡
HBF/CO, %	27.3±7.3	25.1±7.0	36.3±9.1‡
C, ml·min^{-1}·mmHg^{-1}	17.1±3.2	13.8±2.7†	21.4±5.4‡

Values are means ± SD ($n = 14$). ICG, indocyanine green; LBNP, lower body negative pressure; HR, heart rate; CO, cardiac output; SV, stroke volume; TPR, total peripheral resistance; SBP, systolic; DBP, diastolic; MAP, mean arterial pressure; PP, pulse pressure; ρ, plasma mass density (37.0°C); k, ICG disappearance rate; HBF, hepatic blood flow; HBF/CO, percentage of hepatic blood flow from the cardiac output; C, hepatic vascular conductance. Significant differences to pre-LBNP control: *$P <$ 0.05, †$P < 0.01$, ‡$P < 0.001$.

Determination of ICG clearance. We assessed rate constants for ICG disappearance by fitting a linear model to the time series of logarithmically transformed dye concentrations. For the pre-LBNP control period, this was done after a first intravenous injection. A second intravenous ICG application was timed during the early LBNP period (Fig. 1) to determine, in an optimized fashion, ICG disappearance both during and after LBNP after the same bolus injection. The resulting time courses (log $_{[ICG]}$ vs. time) showed a distinct increase in slope with ceasing LBNP (Fig. 2). Immediately following LBNP, we regularly observed a short, transient peak in dye concentration before the second (steeper) part of ICG disappearance started (Fig. 2). However, this effect is so short-lived that it did not influence the post-LBNP curve fitting.

Hepatic blood flow calculation from ICG disappearance is on the basis of a single-pool model with constant elimination rate, essentially characterized by hepatic blood flow. The solution of this model provides an exponential decay in blood ICG concentrations which, after logarithmic transformation results in a classic plot as shown in Fig. 2*A*. Indeed, the data closely followed this model in the baseline experiment with very high correlation coefficients, before the application of LBNP, suggesting that hepatic blood flow was constant during this part of the study.

When the injection was repeated at the end of LBNP the pattern was different. Elimination rate was not constant and significantly different before and after release of LBNP. Moreover, there was a decrease in elimination rate during the last minutes of LBNP as the concentration curve flattened during that phase. When an exponential curve was fitted to the data obtained before the end of LBNP it was evident that the true rate constant tended to be overestimated by the exponential fit for the last minutes of LBNP (i.e., where the curve was flatter than the fit). This is a possible pitfall of the ICG bolus protocol. Other technical approaches such as the constant infusion protocol would have to be pursued when investigating instantaneous or short-term changes in hepatic blood flow. However, this was not the focus of this study. On the other hand, the process of averaging the change in concentrations for a period of time increases the validity of our conclusion.

The deviations from the exponential (seen as a continuous flattening of the ICG concentrations and a deviation from the straight line in the semilogarithmic plot and also revealed by somewhat lower correlation coefficients) suggest that hepatic clearance continuously declined during the late phases of LBNP and that true hepatic blood flow at the end of LBNP was probably even lower than that estimated from the average decline calculated for the whole observation phase. The deviation therefore rather strengthens our conclusions.

Changes during LBNP. All of the observed effects on plasma mass density, hemodynamic indexes, and ICG clearance that occurred during LBNP were expected and are well known. During LBNP, there is reduction in central blood volume, causing decreased cardiac preload and increased sympathetic activity (5) as well as increased sequestration of blood into lower body regions. Cardiopulmonary receptors mediate LBNP effects (13) with resulting tachycardia and reduced blood flow, like in the forearm and splanchnic region. We found hepatic perfusion reduced by $22 \pm 11\%$ during LBNP.

The stability of arterial blood pressure at this level of LBNP was expected (6) as arterial blood pressure is maintained via both peripheral vascular resistance and heart rate adjustments (23). The LBNP reduction in stroke volume was also expected (2, 24). We observed a 15% decrease in cardiac output following LBNP similar to what has been found by others (3, 6). Plasma volume loss with orthostatic stress occurs primarily in the lower limbs (41) and was 10% in our study, confirming earlier results. Liver blood flow was 22% reduced, from 1.67 ± 0.32 to 1.29 ± 0.26 l/min.

Changes after LBNP. The major finding of this study is clear indication of reactive hyperemia in the liver after LBNP: Liver blood flow was computed as 2.08 ± 0.48 l/min in the 8 min immediately after LBNP, 25% higher than the 1.67 ± 0.32 l/min observed before 15 min of 40 mmHg LBNP. Earlier investigations did not observe increased hepatic perfusion after orthostatic or LBNP stress (1, 10); however, the 40 mmHg LBNP stimulus used in the latter study lasted only 2 min, unlikely to provoke a response (14) as observed by us, where stimulus duration was 15 min. Steiner et al. (37) used a LBNP of longer duration and higher intensity than in our study and found superior mesenteric artery (SMA) perfusion reduced during LBNP to 0.70 l/min, or by 38% together with a significant 37% post-LBNP increase of SMA blood flow above pre-LBNP control (1.55 vs. 1.12 l/min). This demonstrates clear reactive hyperemia after LBNP in the splanchnic region. Indeed, SMA diameter was found significantly increased (+13%) in the post-LBNP recovery period, which is comparable to our calculated 21% increase of liver conductance. Their study did not include measurements of hepatic perfusion, however. Reactive hyperemia (i.e., hepatic arterial conduction above control levels) has been observed after infusion of vasoconstrictive agents in a canine liver model (27); the authors focused on the vasoconstrictive phase and reported, but did not further elaborate on, increased conductance thereafter.

Our study suggests the post- vs. pre-LBNP hepatic perfusion increased by ~0.4 l/min, or 25%. This large effect points toward powerful vasodilatation, causing significant reactive hyperemia. In fact, computed vascular conductance rose as well, from 17.1 to 21.4 units, or by 25%. Larger filling of compliant vessels with blood [as observed by Steiner et al. (37) in the splanchnic circulation] would be a likely consequence, thereby decreasing venous return to the heart and, consequently, cardiac preload. In fact, we found cardiac output decreased by 9% below pre-LBNP control (Table 1). The average proportion of estimated hepatic perfusion relative to cardiac output was 27.3 ± 7.3% before, 25.1 ± 7.0 during, and 36.3 ± 9.1% after LBNP, indicating a considerable increase after LBNP. To see the time frame of restoration to the normal steady state after LBNP, it would have been necessary to conduct the experiment for a longer time period; this was not a focus of the present investigation, however.

Taken together, the data suggest the following hemodynamic pattern: Post-LBNP vasodilatation causes reactive hyperemia in the liver. Blood is being pooled away from the central compartment, reducing cardiac preload. How long these effects would last was not measured, however.

Physiological and clinical significance. High-pressure, well-oxygenated blood derived from the hepatic artery (25–30% of the total flow) mixes with low-pressure, poorly oxygenated blood from the portal venous system (70–75%) within the hepatic sinusoids. These have been shown to respond in a graded and reversible manner to specific mediators (25, 28, 32). Locally controlled constriction is modulated by dilators that are also generated within the sinusoids. During a cardiovascular stress situation like LBNP, vasoconstriction is part of a general reflex pattern aiming at stabilizing blood pressure.

The relative ischemia of the resulting blood flow decrease during the cardiovascular stress seems to elicit vasodilatation after LBNP that causes liver perfusion to exceed its pre-LBNP control level. Local mediators like adenosine, nitric oxide, carbon monoxide, or prostaglandins are likely candidates for exerting a reactive hyperemia effect by either modulating vasoconstrictors or directly relaxing vascular smooth muscle (25, 28, 32). The question of which mechanism(s) exert a hyperemic effect after LBNP was not addressed in this study. Hepatic perfusion is not constant; even with mechanisms (hepatic arterial buffer response, HABR) that can keep oxygen delivery unaltered vis-à-vis altered hepatic perfusion, this does not fully compensate for changing portal flow. In other words, LBNP-induced changes of hepatic perfusion are not likely to be prevented by HABR.

If indeed modest central hypovolemia, as caused by real (upright standing) or simulated orthostasis (LBNP) is a stimulus sufficient to induce considerable hepatic hyperemia as our data suggest, then it is conceivable to attribute physiological significance to that mechanism. According to our data, LBNP reduced supine control hepatic perfusion by ~22%, whereas reactive hyperemia brought a 25% increase above that value. Furthermore, the percentage of estimated hepatic perfusion relative to cardiac output increased to 35%, compared with 26% before LBNP. The LBNP stimulus, equivalent in its major hemodynamic consequences to quiet upright standing (14) for 15 min, seems to transiently increase blood flow through the human liver. The exact time course, and how long this effect prevails, remains to be investigated.

The data lead us to speculate that repetitive mild central hypovolemia (as caused by, e.g., frequent postural changes or an according LBNP protocol) might result in a time-averaged hepatic blood flow approximately equal to the steady-state value that would be observed with constant supine positioning (lying in bed). It appears possible, therefore, that everyday frequent postural changes do not, in average, reduce liver perfusion as would be expected without reactive hyperemia, a hypothesis to be tested in consecutive studies. On the basis of this assumption, it seems debatable whether orthostatic stimuli (i.e., getting up) do present a disadvantage in terms of hepatic perfusion compared with strict adherence to bed rest in liver patients.

Limitations. Echo-Doppler measurements of portal venous blood flow and ICG clearance methods compare favorably (7) so it would be reasonable to expect similar results from vena porta ultrasonographic measurements. We do not have such data, and it would be interesting to collect independent evidence of post-LBNP hepatic hyperemia from sonographic studies.

Furthermore, hemodynamic data were obtained with the Finapres system. Finapres measurements correlate well with intra-arterial measurements of blood pressure (22) and have been validated in subjects undergoing stress situations (30). Hemodynamic monitoring with the Finapres system has its own limitations since it may be affected by bias and drift and,

therefore, relative changes in blood pressure were analyzed rather than absolute values (26). To avoid any drift, we calibrated to absolute values from conventional cuff measurements on the contralateral upper arm every 15 min.

Lastly, ICG elimination is an indirect measure, but there is ample evidence to suggest that it indicates liver blood flow with sufficient reliability. The method has been repeatedly used for this purpose (e.g., Refs. 4, 6, 33). We interpret our results as indirect indication of reactive hyperemia; using an invasive design with liver catheterization would allow for more precise blood flow as well as hepatic oxygen saturation determinations.

In conclusion, a moderate cardiovascular challenge that simulates brief orthostatic stress (i.e., 15 min of 40 mmHg LBNP) and causes vasoconstriction in peripheral vessels including the hepatic circulation is followed, after stress release, by reactive hyperemia in the liver. Compared with hepatic perfusion before the LBNP stimulus, our data suggest liver blood flow to be increased by ~25% during an 8-min post-LBNP observation period. During that time, cardiac output was reduced below pre-LBNP control values. We believe to be first to report hepatic hyperemia after a moderate central hypovolemic stimulus in healthy humans. This effect might have practical significance in terms of patient positioning requirements, particularly in those with acute and chronic liver diseases. Further studies are needed to reveal the mechanisms driving this effect.

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