Hemodynamic Response to Volume Stress in Awake Late-Pregnant and Nonpregnant Rats

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ABSTRACT. The present study was designed to determine whether the often observed gestational rise in blood volume alters the hemodynamic response to volume stress relative to the nonpregnant state. Late-pregnant and nonpregnant Wistar rats, prepared with an electromagnetic flow probe around the ascending aorta and/or intravascular catheters were used for this study. One of the following sets of measurements were performed either before and after volume loading or before and after volume depletion (24 h thirst): 1) continuous monitoring of cardiac output, blood pressure, heart rate, and fluid balance (only during volume loading); 2) measurement of cardiac output and regional blood flows (microspheres); and 3) measurement of the inferior vena cava diameter (x-ray). Both rapid (bolus) and slow volume loading (infusion) led to a rise in cardiac output. The generated surplus in cardiac output was distributed to the kidneys, skeletal tissues, and intestines (only in nonpregnant rats), and was paralleled in the pregnant rats by a consistent fall in placental blood flow. Slow volume depletion had neither a consistent effect on cardiac output nor on its distribution, with even a preserved maturational rise in placental blood flow during the 24 h of thirst. Slow volume loading and depletion led to an increase and decrease of the caliber of the inferior vena cava, respectively. The nonuterine hemodynamic response to all forms of volume stress had not changed in pregnancy. The results of this study suggest that the hemodynamic response to volume stress consists of three components: 1) a venous response that dampens with a certain lag time the effects of volume changes on venous return; 2) an instantaneous response of the volume regulatory system, which includes a change in renal perfusion; and 3) an effect on venous return and, with it, cardiac output that only develops when the veins are unable to buffer imposed volume changes. If the latter causes systemic flow to increase in excess of nutritional demands, the surplus flow is shunted through small arteriovenous shunts in skeletal tissues (and intestines in nonpregnancy), to protect the systemic capillary bed. Massive systemic shunting on top of an already congested venous compartment can be expected to raise mean circulatory filling pressure. It is speculated that the latter causes the selective decrease in placental flow after volume loading through a direct negative effect on the peculiar (rat) placental microcirculation. (*Pediatr Res* 30: 479-486, 1991)

Abbreviations

IVC, inferior vena cava
MCFP, mean circulatory filling pressure

BV, blood volume (mL)
CO, cardiac output (mL/min)
HR, heart rate (beats/min)
SV, stroke volume (μ L)
MAP, mean arterial pressure (torr)
SVR, systemic vascular resistance (mm Hg $\cdot$ min/mL)
Hct, hematocrit (vol%)
RBC, red blood cell
P, pregnant
NP, nonpregnant
Th, thirsting
Nonth, nonthirsting
exp, experimental
contr, control
rat_{exp}, experimental rat
rat_{contr}, control rat
A $\leftrightarrow$ P, anterior-posterior

Both maternal CO and BV begin to increase in early human pregnancy to reach a plateau of $\approx 30\%$ above nonpregnant values by the 16th and 34th wk, respectively (1-4). However, since BV and CO have never been measured together throughout pregnancy, their precise interrelation in pregnancy is still obscure. In the nonpregnant state, BV and CO vary as a function of each other, if either one is changed acutely (5-7). In pregnancy, CO and BV increase gradually over a period of weeks to months (1-4), a condition that may be associated with an altered response of the volume regulatory system. Insight in the latter in pregnancy has become particularly important after the introduction in recent years of aggressive volume expansion therapy in the symptomatic management of the preeclamptic patient (8, 9), a development that evolves in spite of still almost complete lack of understanding of the impact on BV regulation of the dynamic state of pregnancy.

The objective of the present study in the rat was to test the hypothesis that the gradually expanding BV compartment in normal pregnancy does not alter the hemodynamic response to an imposed volume load or volume depletion. The impact of pregnancy was determined by comparing the hemodynamic response induced by volume loading/volume depletion in pregnancy with that observed in the NP state. Besides the magnitude, also the rate by which volume stress is imposed can be expected to influence the hemodynamic response (10, 11). Therefore, volume loading was implemented at different rates. Volume depletion was only induced slowly by 24-h thirsting in an attempt to limit the interference with the response of a concomitant rise in sympathetic tone. The awake Wistar rat was considered a suitable model for this study, as BV in late pregnancy in this animal is about 40% higher than in the NP state (12).

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MATERIALS AND METHODS

Validations. The difference in baseline BV between the 19-d P and NP Wistar rats in the present study was determined as follows. Seven P and six NP rats were anesthetized with ether, and polyethylene catheters (PE-10, outer diameter = 0.61 mm, inner diameter = 0.28 mm, heat-sealed to a piece of PE-50 tubing, outer diameter = 0.92 mm, inner diameter = 0.58 mm) were inserted into the abdominal aorta and IVC and advanced from a femoral artery and vein, respectively. The next day the animals received an i.v. bolus injection of 1 μ Ci 25 I-albumin dissolved in 0.5 mL saline. Plasma sp act was calculated on the basis of extrapolation to time zero of the radioactivity determined in 0.2-mL blood samples collected after 5, 10, 15, and 20 min. BV was derived by multiplying plasma volume with $1/(1 - \text{Hct})$.

In the present study, BV changes were provoked by subjecting awake catheterized NP and P rats to various regimens of volume loading or volume depletion. The induced changes in BV were determined from the observed change in Hct. In our study, this approach was preferred over standard indicator dilution techniques, to avoid the use of radioactivity and to minimize blood sampling. Because this approach is unconventional, it was considered appropriate to validate this procedure beforehand. Four P rats provided with catheters in the femoral artery and vein on the preceding day were subjected to 2 h volume loading with Haemaccel (Hoechst, Germany) at a rate of 6 mL/h. Before and again after volume loading, 0.8 mL of a cocktail containing ^{51}Cr -labeled RBC and ^{125}I -albumin was injected i.v. Red cell volume and plasma volume were calculated as described previously (13). Before the 2nd cocktail injection, an arterial sample was withdrawn to determine background radioactivity. The radioactivity in the 2nd cocktail was three times as high as that in the 1st cocktail. Before the 1st cocktail and again together with the last blood sampling, 0.05 mL extra blood was withdrawn to determine the Hct.

Confirmation of stability in fluid balance after slow volume loading. Hemodynamic steady state including stable fluid balance at the time of measurement was considered a prerequisite for the reliable measurement of organ flows with microspheres in this study. Therefore, we confirmed in a separate group of three previously catheterized 19-d P rats that the stability in HR and MAP after 2 h of slow volume loading applied also to the fluid balance. In these animals, the cumulative urine output and Hct was determined at 30-min intervals, and MAP and HR were monitored continuously throughout 5 h of infusion with Haemaccel at a rate of 6 mL/h.

Experiment 1. Experiment 1 was performed in six 19-d P and six age-matched virgin NP Wistar rats obtained from a local breeder (HSD, Zeist, The Netherlands). The rats (P, 14th d of pregnancy) were anesthetized with ketamin (20 mg) and xylazine (4 mg) intramuscularly, and the trachea was intubated (PE-240). After establishing positive pressure respiration (60 breaths/min, tidal volume 2.5–3 mL), the surgery was carried out under aseptic conditions. The skin and muscle overlying the 3rd right intercostal space were incised and separated. The 3rd and 4th rib were spread and the ascending aorta was freed from its surrounding tissue. An electromagnetic flow probe (outer diameter = 2.3 mm; Skalar, Delft, Holland) was fitted around the ascending aorta just above the heart. The thorax was closed and the probe cable fixed to the ribs. The cable was guided to the neck where the connector was exteriorized. The surgical procedure has been described in detail elsewhere (14). Four d after the probe implantation (in P rats, d 18 of pregnancy) the rats were prepared with an abdominal and an IVC catheter as detailed above. The catheters were tunneled s.c. to the same exit site as the probe cable. After surgery, the rats were placed in a metabolic cage to habituate them to the subsequent experimental environment.

On the next day (P rats, 19th d of pregnancy), the rats were placed in metabolic cages (22 $\times$ 13 $\times$ 13 cm) and connected to the measuring equipment. The flow probe was connected to a

sine-wave electromagnetic flow meter (Skalar), and the arterial catheter, to a pressure transducer. Data were recorded continuously and stored on a hard disk of a real-time data processing system (Dept. of Instrumental Services, Faculty of Medicine, University of Limburg, Maastricht, The Netherlands). This system has virtually no drift because it corrects for baseline before each beat, taking late-diastolic flow as zero flow. Linearity and accuracy of the system were 1% and less than 7%, respectively (14). After a stabilization period of at least 45 min, recording of the hemodynamic variables was started. CO, HR, and MAP were monitored continuously. In NP rats, measurements were continued until 30 min after discontinuation of volume loading. SV and SVR were calculated from CO, HR, and MAP. After the experiments, the animals were killed (overdose of pentobarbital) and, in P rats, the fetuses counted and weighed.

The experiment consisted of an i.v. bolus injection (2 mL) immediately followed by a 2-h infusion with the plasma expander Haemaccel at a rate of 6 mL/h. Haemaccel is an isotonic colloid solution composed of a polymer of urea and polypeptides. It is readily excreted by the kidneys with minimal metabolic breakdown. During infusion, the agent remains within the intravascular compartment with no measurable leakage to the interstitium (15). Haemaccel is known to be well tolerated by the rat with no appreciable effect on MAP, HR, and respiratory rate, even when infused in large amounts (16). It does not alter serum concentrations of electrolytes. Because of the latter and the isotonicity of the solution, the effect of Haemaccel on red cell volume is negligible. The infusion rate of 6 mL/h was chosen on the basis of previous testing, which indicated that a lower infusion rate had an inconsistent effect on BV, whereas a more rapid infusion rate often failed to result in a stable fluid balance within 2 h.

Experiment 2. In seven 19-d P and six NP Wistar rats, the distribution of the CO was determined before and after the 2 h slow volume loading as detailed above. Two d before the experiment, the rats were anesthetized with ether, and polyethylene catheters with dimensions as detailed above were inserted into the right carotid artery, a femoral artery, and a femoral vein and advanced into the left ventricle of the heart, the abdominal aorta, and the IVC, respectively. The catheters were tunneled s.c. and exteriorized between the shoulder blades. The day before the experiment, also these rats were allowed to adapt to the metabolic cages in which the experiment was to be performed.

CO and its distribution were measured with 15- μ m radioactive microspheres using the reference sampling technique (17). To this end, approximately 1.2×10^5 microspheres (NEN, Dreieich, Germany), suspended in 0.2 mL saline with Tween 80 (0.05%) added to prevent aggregation, were injected into the left ventricle over a period of 15 s while a reference sample was withdrawn from the abdominal aorta at a rate of 0.5 mL/min. Reference sampling encompassed the period between 10 s before the microspheres injection and 30 s after its completion. The blood withdrawn was replaced by fresh heparinized blood from a donor Wistar rat. After 2 h of slow volume loading, the microspheres experiment was repeated using a different radioactive label.

After the 2nd microspheres experiment, the animals were killed and all organs were dissected and weighed. The radioactivity in each tissue aliquot and reference sample was determined with a sodium crystal scintillation counter (Packard, Delft, The Netherlands). CO was obtained by multiplying the ratio of total cpm in the body and cpm in the reference sample by the reference sampling rate. Organ flows were calculated by multiplying fractional entrapment of microspheres in each organ with the reference sampling rate (18). In all rats mixing of the microspheres was considered adequate on the basis of a difference of less than 5% in fractional entrapment of microspheres by the two kidneys (19). The reference sample always contained 400–700 microspheres.

Experiment 3. Experiment 3 involved the determination of the impact of volume loading on the degree of filling of the IVC.

Six pairs of NP and four pairs of 19-d P Wistar rats were anesthetized with ether and pair-wise positioned in dorsal recumbency on a heated pad. In each rat, a catheter (PE-10) was inserted into a femoral vein and advanced until just below the outlet into the IVC. One rat in each pair was subjected to volume loading (rat_{exp}) and its counterpart served as control (rat_{contr}). After a stabilization period of 20 min (baseline), 1 mL of contrast medium (Hexabrix) was rapidly infused i.v. in both rats simultaneously, using an infusion pump at a constant rate of 6 mL/min, over a period of 10 s. Then, an A↔P and a lateral x-ray were taken of both rats laying next to each other. After completion of the lateral x-ray, the rats were repositioned in dorsal recumbency and a 2-mL bolus injection of Haemaccel was only given to the rat_{exp}. Immediately after the bolus, a second set of x-rays was taken using the same standardized procedure. Then, a steady state infusion with Haemaccel at a rate of 6 mL/h was started in the rat_{exp}. Two h later, the procedure to take x-rays of the rat pair was repeated.

The difference in IVC diameter was determined as follows. On each x-ray the IVC diameter was determined by taking the mean of three measurements at three distinct points in the IVC between the bifurcation and the hepatic venous outlet. In the six NP rats_{exp} and four P rats_{exp}, the IVC diameter after rapid and slow volume loading was compared with the previously measured baseline IVC diameter (intraanimal comparison). The possible contribution of random and/or systematic error to the results was determined in the control rats from the within-animal difference in IVC between the three measurement sessions.

Experiment 4. The placentas from six 19-d P rats were studied morphologically. Three of these rats were subjected to slow volume loading as detailed above and three served as controls. The experimental rats were killed immediately after completion of volume loading. The placentas of all viable fetuses were weighed and incubated for 3 d in buffered 4% formaldehyde, mounted in paraffin, sliced, and stained using standard laboratory techniques. An experienced placentalogist (co-author P.K.) judged the placental slides by light microscopy, without foreknowledge on preceding exposure to volume load.

Experiment 5. Experiment 5 was designed to quantify the changes in BV and urine output in response to 24-h thirsting and was performed on eight 19-d P and eight NP Wistar rats. The induced fall in BV was calculated from the rise in Hct. Hct was measured in duplicate (microcapillary technique) in a blood sample (25 μ L) obtained from the orbital venous plexus before and after 24-h thirsting. Blood sampling was performed while the animal was briefly sedated with ether. The impact of thirsting on the fluid balance was determined by measuring weight loss, water intake, and urine output during a 24-h control period and during the subsequent 24-h thirsting.

Experiment 6. Experiment 6 was designed to determine the hemodynamic response to 24-h thirsting in six 19-d P (ThP/exp) and seven NP rats (ThNP/exp). To separate the effects caused by thirsting from those associated with ongoing pregnancy, the hemodynamic changes in the ThP/exp group were compared with those observed in a group of seven 19-d P rats (NonthP/contr) that had unlimited access to water throughout the experimental period. Two d before the experiment, the rats were anesthetized with ether and prepared with a left ventricle and a femoral artery catheter as described for experiment 2. In the postsurgery recovery period, the rats were kept in metabolic cages to habituate them to the actual experimental environment. Then, measurements in baseline (in ThP/exp and NonthP/contr: d 19 of pregnancy) and after 24-h thirsting (ThP/exp) or nonthirsting (NonthP/contr) were performed. MAP and HR were monitored continuously during both measurement sessions until after completion of the blood flow measurement. CO and organ flows were determined with 15- μ m microspheres after a 40-min stabilization period, as described for experiment 2.

Experiment 7. Experiment 7 involved the evaluation of the impact of 24-h thirsting on the diameter of the IVC. Three pairs

of NP and four pairs of 19-d P rats were anesthetized with ether and pair-wise positioned in dorsal recumbency on a heated pad. The day before measurement, the rats were prepared with two femoral vein catheters. One rat in each pair was subjected to 24-h thirsting while the (control) counterpart had free access to water. After a 20-min stabilization period and again the next day, x-rays were taken according to the standardized protocol described above (experiment 3).

Data are presented as means $\pm$ SD throughout the text. Differences between control and experimental observations were evaluated by the Wilcoxon signed rank test. Differences between P and NP groups and differences between the changes in the ThP/exp group and the ThP/contr were evaluated with the Mann-Whitney test. A probability of less than 5% (two-sided) was considered significant.

RESULTS

Validations. BV was about 25% higher in P (23.3 ± 2.2 mL) than in NP rats (18.9 ± 1.8 mL). The induced BV increase in the validation study ranged from 28 to 64%. Despite this large variation, the increase in BV calculated from the fall in Hct and that simultaneously determined with the indicator dilution technique differed by only 1–4% (28 versus 31%; 45 versus 41%, 35 versus 34%, and 64 versus 68%, respectively).

The fluid balance during 5 h of slow volume loading at 6 mL/h is illustrated for one rat in Figure 1. In all rats urine output accelerated within ≈ 30 min and always equilibrated with volume input within 2 h. In steady state the total amount of infused Haemaccel exceeded the cumulative urine output by ≈ 9 mL.

Experiment 1. Maternal weight in the P group (277 ± 31 g) was higher than in the NP group (236 ± 52 g). The litter size was 6 ± 1 with fetuses weighing 1.6 ± 0.2 g. Table 1 lists various central hemodynamic variables in baseline, after rapid and after slow volume loading in P and NP rats. In P rats baseline HR was higher and baseline MAP and SVR lower than in NP rats. Table 2 lists the percentage of change in these parameters after both forms of volume loading. In both P and NP rats, rapid volume load increased CO and SV by $\approx 30\%$ and MAP by $\approx 5\%$. SVR had decreased by $\approx 15\%$, and HR had not changed appreciably. The changes induced by slow volume loading were almost identical, except for the normalization of the MAP and the fall in HR in the P rats only. Figure 2 illustrates for both groups the patterns in these variables throughout slow volume loading. All appeared to have reached steady state after 1 h despite ongoing fluid accumulation in the subsequent hour (Fig. 1). The small (in P) or absent (in NP) change in HR implies that the rise in CO in response to both forms of volume loading was almost entirely accomplished by a rise in SV. Although the mean rise in

Fig. 1. Cumulative volume input and urine output throughout a 5-h period of volume loading at 6 mL/h, in a 19-d P rat.

Table 1. Hemodynamic parameters in baseline, after rapid volume loading (VL), and after slow volume loading in P (n = 6) and NP (n = 6) rats

	P			NP		
	Baseline	Rapid VL	Slow VL	Baseline	Rapid VL	Slow VL
CO (mL/min)	88 ± 11	116 ± 14*	117 ± 12*	78 ± 8	105 ± 8*	113 ± 11
MAP (torr)	90 ± 8	99 ± 12*	94 ± 7	115 ± 12†	121 ± 13*	114 ± 25
HR (bpm)	445 ± 24	433 ± 22	416 ± 31*	383 ± 11†	405 ± 24	423 ± 28
SV (μL)	201 ± 31	268 ± 42*	281 ± 29*	203 ± 20	259 ± 24*	266 ± 19*
SVR (mm Hg·min/mL)	1.1 ± 0.2	0.9 ± 0.2*	0.8 ± 0.2*	1.5 ± 0.2†	1.2 ± 0.2*	1.0 ± 0.2*

* Significant difference from baseline ($p < 0.05$).† Significant difference between groups ($p < 0.05$).

Table 2. Percentage of change in central hemodynamic parameters after rapid and slow volume loading (VL)*

	P		NP	
	Rapid VL	Slow VL	Rapid VL	Slow VL
BV		+18 ± 10		+20 ± 2
CO	+32 ± 11	+35 ± 18	+32 ± 12	+47 ± 18
SV	+33 ± 8	+42 ± 20	+29 ± 14	+32 ± 8
HR	NS	-2 ± 1	NS	NS
MAP	5 ± 2	NS	6 ± 2	NS
SVR	-18 ± 4	-23 ± 13	-12 ± 5	-31 ± 18

* All % changes listed were significant ($p < 0.05$, Wilcoxon signed rank test).

BV ($\approx 20\%$) in response to slow volume loading was comparable in P and NP rats, the interanimal variation was larger in the P group (Table 2). Only NP rats were monitored until 30 min after discontinuation of volume loading. In this period CO, SV, and SVR tended to return to baseline (Fig. 2).

Experiment 2. The litter size in the seven P animals varied between 10 and 14 fetuses. Slow volume loading induced a similar rise in BV ($\approx 20\%$) as seen in experiment 1. Systemic shunting (defined as the percentage of microspheres recovered from the lungs) and organ flows before and after slow volume loading are listed in Table 3. Base line systemic shunting and all organ flows except for that to the myometrium (higher in P rats) were comparable in P and NP rats. Renal and carcass flow (skeletal muscle, bone, connective tissue) had increased in both groups. In the NP group, intestinal flow had also increased consistently, whereas in the P group placental blood flow had decreased consistently. The percentage of change in renal, intestinal, carcass, and placental flows in response to slow volume loading is listed in Table 4.

Experiment 3. Neither in P rats_{exp} nor in NP rats_{exp} did the IVC diameter change consistently after rapid volume loading. In contrast, after slow volume loading the IVC diameter on the A↔P picture had increased consistently by $24 \pm 11\%$ and $28 \pm 13\%$, respectively. Meanwhile, the concomitantly measured IVC diameter on the lateral pictures had changed inconsistently. The intrapair comparison for each experimental state yielded almost identical results, although with a higher variation. Figure 3 illustrates for one P rat pair the A↔P IVC outline before and after slow volume loading.

An impression about the measurement error was obtained by comparing the IVC diameter as deduced from the three x-ray pictures in the P rats_{contr} and NP rats_{contr}. The IVC diameters on the 2nd and 3rd picture had changed inconsistently relative to that on the 1st picture. Because the x-ray method combined a high reproducibility with a large measurement error (coefficient of variation = 46%), it was decided to use this method only to obtain qualitative information on induced changes in IVC diameter.

Experiment 4. The placental morphology in baseline and after slow volume loading is illustrated in Figures 4 and 5, respectively. In baseline both maternal and fetal vessels are filled with small

clusters of RBC. After slow volume loading, RBC density had markedly increased in the maternal lacunas causing compression of fetal trabeculae and fetal microvessels.

Experiment 5. Table 5 lists the results obtained in experiment 5 with respect to the effects of 24-h thirsting on body weight, BV, and fluid balance. P rats were heavier and had a lower baseline Hct. In both P and NP rats, thirsting caused a decrease in BV and body weight by 7% and 16–19 g, respectively. P rats showed a higher daily water intake in baseline and a lower urine production during thirsting compared with the NP rats.

Experiment 6. The central hemodynamics before and after thirsting are listed in Table 6. In neither group had CO, MAP, HR, SV, and SVR changed consistently after thirsting (ThNP and ThP/exp) or nonthirsting (NonthP/contr). Table 7 lists for both experimental states the magnitude of systemic shunting and organ flows in the three groups. The lung fraction had decreased by a similar margin in the two ThP groups. Neither in the ThP/exp nor in the NonthP/contr group had the nonuterine organ flows changed appreciably after the 24 h of (non)thirsting. The maturational increase in placental blood flow observed in the NonthP/contr group was not affected by the exposure to 24 h of thirsting (ThP/exp).

Experiment 7. Both P and NP rats responded to thirsting with a variable (10–50%) but consistent decrease in the IVC diameter on the A↔P pictures as illustrated for one NP rat pair (Fig. 6). The concomitant change on the lateral pictures was inconsistent.

DISCUSSION

BV expansion in pregnancy is known to be important for the uneventful development of pregnancy. However, neither purpose of the physiologic BV expansion nor its consequences for volume regulation are well understood. One of the consequences of the physiologic BV expansion may be an altered set point and/or gain of the volume regulatory response. In this study, adaptive changes in the volume regulatory system during pregnancy were explored by comparing the hemodynamic response to volume stress in P rats with that in NP rats. The 19-d P rat was chosen for these studies, as BV at 19 d was found to have increased $\approx 25\%$ relative to NP values.

Before the actual study, we confirmed that the methods adopted to impose volume load led to stability in hemodynamics and fluid balance at the time of measurements. It was also demonstrated that induced BV changes were reliably quantified from the fall in Hct and that qualitative changes in the venous filling state could be discerned from the changes in IVC outline on x-rays.

To our knowledge, no data have been reported on the use of an electromagnetic CO probe in awake P rats. In the present study, the surgery needed to implant a CO probe was found to be associated with considerable stress to the P rat. This was reflected in a fetal survival rate of only 50%. However, the impact of the stress appeared to be confined to the immediate postsurgical period, inasmuch as, on the 5th postsurgical day, these rats showed not only baseline hemodynamics similar to those in P rats without a CO probe (experiment 1; lower MAP

Fig. 2. Central hemodynamics during rapid and slow volume loading in P and NP rats. In the NP rats, monitoring was continued until 30 min after the end of volume loading.

and SVR), but also unaffected growth of the remaining viable fetuses.

In the present study, the volume regulatory system was challenged by imposing either volume load or volume depletion. Rapid volume loading induced an immediate $\approx 30\%$ rise in CO in both groups, almost entirely accomplished by a rise in SV (Table 2). The 30% rise in CP was reached almost instantaneously after the bolus injection (Fig. 2) and was not accompanied by appreciable venous dilatation. This apparent venous inertia is most likely a consequence of the so-called "stress-relaxation" of the veins (11), which refers to the protracted response of the venous compliance to a rapidly imposed change in venous filling state. It may take as much as 30 min before the adapting venous compliance stabilizes at its new level, depending on basal venous filling state and rate and magnitude of the imposed volume load (7, 10, 11, 20, 21). The gradual rise in venous compliance during the exposure to an increased volume is paralleled by a gradual fall in venous pressure and, with it, normalization of the initially elevated venous return. The immediate 5% rise in MAP after rapid volume loading can be expected to contribute to the normalization of the acutely elevated CO through activation of the baroreceptor, a mechanism supported by previous studies (22).

Rapid and slow volume loading induced a comparable rise in CO in P rats (Table 2), despite a twice as large rise in BV in the latter (18% versus 2/23.3 mL or $\approx 9\%$). Assuming also comparable excess CO after both forms of volume loading, this result indicates that the venous dilatation after slow volume loading appears to allow about half of the 18% extra BV to be accommodated in the veins. Interestingly, in the NP rats, CO had increased by 32 and 47% after rapid and slow volume loading, respectively, also with a twice as large increase in BV after the latter (20 versus 2/18.9 mL or $\approx 11\%$). The larger rise in CO in NP rats after slow volume loading could indicate a lower capacity of the venous compliance to increase in response to slow volume loading in the NP state than in the P state. However, it is not possible to draw any conclusions on this matter on the basis of the (x-ray) methodology used in the present study.

Our values on baseline CO, CO distribution, and organ flows in the NP rats agreed well with reported (microspheres) flow data in rat (19). Comparable data from normal and awake P rats are scarce. A study in hypertensive rats confirmed our observation that CO in P rats is higher than in NP rats (23). The extra CO generated by slow volume loading was directed toward kidneys, carcass, and, in NP rats, also toward intestines (Table 4). The

Table 3. Central hemodynamics, systemic shunting (fraction of injected microspheres recovered from the lungs), and regional blood flows (mL/min) at baseline and after slow volume loading (VL) as observed in experiment 2

	P		NP	
	Baseline	Slow VL	Baseline	Slow VL
CO (mL/min)	109 ± 20	155 ± 38*	99 ± 19	141 ± 31*
MAP (mm Hg)	77 ± 12	71 ± 17	102 ± 24	98 ± 27*
HR (bpm)	404 ± 40	374 ± 51	356 ± 47	346 ± 33
Systemic shunting (%)	4.0 ± 1.6	2.6 ± 0.9	2.5 ± 2.4	1.6 ± 1.2
Blood flows (mL/min) to				
Brain	2.4 ± 0.6	3.0 ± 1.1	2.7 ± 0.7	4.5 ± 1.2
Kidneys	12 ± 3	20 ± 7*	13 ± 5	23 ± 9*
Carcass	27 ± 10	41 ± 13*	33 ± 10	41 ± 9*
Intestines	28 ± 6	37 ± 15	28 ± 10	38 ± 13*
Skin	9 ± 3	10 ± 4	8 ± 3	8 ± 3
Myometrium	2.3 ± 0.7	1.7 ± 0.6	0.3 ± 0.1†	0.3 ± 0.1
Placental blood flow	2.2 ± 1.0	1.2 ± 0.6*		

* $p < 0.05$ (Wilcoxon signed rank test).

† $p < 0.05$ (Mann-Whitney).

Table 4. Percentage change in organ flows after slow volume loading

	P	NP
Kidneys	+76	+87
Carcass	+81	+28
Intestines	NS	+37
Placentas	-40

Fig. 3. The IVC outline as visualized by A↔P x-ray imaging in a 19-d P rat pair. The rats are positioned in dorsal recumbency. Left panel, baseline; right panel, after 2 h of slow volume loading.

higher renal blood flow complies with the increased requirement for fluid dissipation. However, it is obscure why the blood flow toward skeletal tissues (carcass) and, in NP rats, also intestines had increased. These tissues are tightly autoregulated, and on the basis of the animals' behavior during slow volume loading (quietly sitting, little or no food intake), it seemed unlikely that nutritional flow requirements of these tissues had increased. Furthermore, it is unlikely that the observed fall in Hct had triggered a higher muscle (and in NP rats, intestinal) flow, inasmuch as studies in guinea pig have shown that isovolemic hemodilution does not increase the perfusion of these tissues (24). On the other hand, half of the resting blood flow to rabbit

Fig. 4. Placental histology of 19-d P rat in baseline condition (×160). Small clusters of RBC in both fetal and maternal vessels alternative with cell-free areas.

Fig. 5. Placental histology of 19-d P rat after 2 h of slow volume loading at 6 mL/h (×160). Maternal lacunas show marked RBC crowding causing compression of fetal trabeculae.

Table 5. Hct, body weight, water intake, and urine production in basal conditions and after 24-h thirsting and weight loss and change in BV caused by thirst period

	ThP/exp		ThNP/exp	
	Baseline	24-h thirst	Baseline	24-h thirst
Hct (vol%)	43.6 ± 1.6	46.6 ± 1.9*	48.8 ± 1.9†	52.2 ± 1.6*
Weight (g)	305 ± 40	290 ± 43*	254 ± 14†	238 ± 16*
Water intake (mL/d)	37 ± 7	0	26 ± 5†	0
Urine output (mL/d)	18 ± 7	4 ± 2*	14 ± 5	10 ± 3
Weight loss (g)		-16 ± 7		-19 ± 3
BV change (%)		-7 ± 1		-7 ± 2

* $p < 0.05$ (Wilcoxon signed rank test).

† $p < 0.05$ (Mann-Whitney).

tenuissimus muscle was found to be shunted through 5 to 11-μm arteriovenous shunts, and this "reserve" flow is redirected to the capillary bed during exercise (25, 26). In the intestinal bed, comparable functional arteriovenous shunts have been demonstrated (27).

Assuming unchanged nutritional flow in our study, it is well possible that the observed fall in vascular resistance in these

Table 6. *Hemodynamics in baseline and in volume-depleted (V-depleted) state**

	ThP/exp		NonthP/contr		ThNP/exp	
	Baseline	V-depleted	Baseline	Sham-V-depleted	Baseline	V-depleted
CO (mL/min)	105 ± 34	96 ± 21	104 ± 27	109 ± 19	97 ± 24	94 ± 29
MAP (torr)	107 ± 13	96 ± 23	90 ± 7	89 ± 18	104 ± 13	103 ± 19
SVR (mm Hg·min/mL)	1.1 ± 0.3	1.0 ± 0.3	1.1 ± 0.5	0.9 ± 0.3	1.3 ± 0.4	1.2 ± 0.4
HR (bpm)	413 ± 29	411 ± 26	400 ± 51	450 ± 22	403 ± 33	411 ± 34
SV (μL)	259 ± 95	236 ± 61	265 ± 109	241 ± 35	242 ± 62	234 ± 91

* None of the variables listed in this table changed significantly in response to thirsting as determined by the Wilcoxon signed rank test.

Table 7. *Systemic shunting (defined as % microspheres recovered from lungs) and regional blood flows (mL/min) in baseline and in volume-depleted state (V-depleted)*

	ThP/exp		nonthP/contr		ThNP/exp	
	Baseline	V-depleted	Baseline	V-depleted	Baseline	V-depleted
Systemic shunting	5.9 ± 2.8	2.7 ± 0.7*	5.8 ± 2.3	1.7 ± 1.6*	2.5 ± 2.1	1.4 ± 0.7
Organ flows						
Brain	3 ± 1	3 ± 1	3 ± 1	3 ± 1	3 ± 1	3 ± 2
Kidneys	11 ± 4	11 ± 2	13 ± 5	12 ± 4	16 ± 5	13 ± 4
Intestines	26 ± 9	20 ± 3	24 ± 10	24 ± 8	26 ± 6	17 ± 7
Carcass	31 ± 10	32 ± 14	23 ± 9	32 ± 8	30 ± 10	42 ± 15
Skin	11 ± 4	8 ±	11 ± 2	11 ± 3	8 ± 3	7 ± 3
Myometrium	2.4 ± 1.3	1.7 ± 0.4	2.3 ± 1.1	1.9 ± 0.8	0.3 ± 0.1	0.3 ± 0.2
Placental flow	1.4 ± 1.1	4.0 ± 1.8*	0.7 ± 0.5	4.4 ± 1.1*		

* $p < 0.05$ (Wilcoxon signed rank test).

Fig. 6. The IVC outline as visualized by A↔P x-ray in an NP rat pair. The rat on the left had free access to water before imaging and served as control for the rat on the right, which was thirsted during the preceding 24 h.

tissues was caused by massive opening of these small shunts, a response that remains undetected by the microsphere method as applied in this study. Assuming that shunt recruitment occurs as part of the hemodynamic response to volume load, the sequence of events triggered by slow volume loading may be as follows: Gradually accumulating excess volume in the vascular bed immediately activates volume regulatory receptors in the venous system and atria, thus triggering the rise in renal blood flow and accelerated diuresis. The large capacitance vessels dilate gradually, accommodating as much excess volume as possible. If the capacity of the large veins to dilate is exceeded, MCFP increases and causes a rise in venous return (20, 21). As a consequence, a rise in CO in excess of nutritional demands is generated. This excess systemic flow is subsequently shunted across the systemic circulation through small arteriovenous shunts in skeletal tissues and, in NP rats, intestines. Massive arteriovenous shunting, possibly in combination with altered arteriolar vasomotion activity (28), may represent an efficient mechanism to protect the Starling forces in the capillary bed.

In P rats, slow volume loading caused a consistent decrease in placental blood flow. If the CO rise in excess of nutritional flow demands is assumed to be caused by a rise in MCFP, it is conceivable that the flow decrease, which seems to occur selectively in the placentas, is a direct consequence of the unique microarchitecture of the rat placenta. The hemotrichorial placenta of this species differs from all other vascular beds by its lack of lymphatic drainage, absent venules, and peculiar rectangular branching pattern in the microcirculation without possibility for capillary and/or shunt opening or closure. Particularly the latter feature implies that a fall in perfusion pressure associated with a rise in MCFP can be expected to reduce local shear forces. A further reduction of already low shear forces will compromise rheologic conditions, causing red cell sludging and accelerated formation of red cell aggregates (Fig. 5).

Support for this concept was expected to come from the subsequent studies on the hemodynamic response to slow volume depletion. In these studies the finding of a hemodynamic response opposite to the one observed after slow volume loading would provide support for the proposed mechanisms delineated above. In an attempt to minimize the activation of the sympathetic nervous system, volume depletion was induced both gradually and mildly by 24-h thirsting. The weight loss of 16–19 g in the ThP/exp and ThNP/exp groups was probably only partly caused by loss of body water, inasmuch as the rats also tended to eat less during the period of thirsting. In this context, it should be emphasized that the absolute weight loss in the ThP/exp rats was probably larger because their normal daily weight gain in late pregnancy (≈ 20 g) was reversed. P rats also drink more than NP rats (Table 5), and the observed 7% volume depletion develops while the P rat is in the process of expanding its BV. Therefore, these results confirm previous data (29) that indicate that 24-h thirsting has a larger impact on the P rat than on the NP rat. In the ThP/exp and ThNP/exp groups, none of the hemodynamic parameters changed significantly, despite a 7% decrease in BV. CO and all organ flows were maintained, which indicates that the observed venoconstriction (Fig. 6) was apparently adequate to preserve venous return. Placental blood flow had increased in the ThP/exp group. Comparison of the post-thirst value in ThP/exp with the second blood flow value in the ThP/control group indicated that the rise in placental blood flow

was entirely explained by the advancement in pregnancy. Unfortunately, the decrease in the size of the venous compartment could not be quantified with the method used. The similarity in CO rise in P rats after rapid and slow volume loading, despite a twice as large increase in BV after the latter, suggested that the venous compartment could accommodate about 10% extra volume. If the venous compartment also has the capacity to neutralize volume reductions of up to 10%, the 7% BV depletion observed after 24-h thirsting may have been too small to elicit a "complete" hemodynamic response, which theoretically would include, besides venoconstriction, a fall in renal blood flow and CO.

With respect to possible clinical implications, one should realize that 24-h thirsting induces volume depletion characterized by a certain rate, magnitude, and duration. The magnitude of the volume depletion induced in the present study (7%) is likely to be smaller than that occurring in preeclamptic pregnancies, where BV may be reduced by as much as 30% (30, 31). In pregnancies in which volume depletion has developed to such an extent, venoconstriction may be inadequate to maintain venous return. One may speculate that the resulting inadequacy of CO has led to an increased sympathetic tone and, thus, to a redistribution of CO at the expense of kidneys and possibly also placenta. However, this concept assumes a yet unproved causal role of volume depletion in the pathogenesis of this disease.

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