

Uterine Artery Structural and Functional Changes During Pregnancy in Tissue Kallikrein–Deficient Mice

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Objective—Tissue kallikrein (TK) participates in acute flow-induced dilatation (FID) of large arteries. We investigated whether TK deficiency blunts FID and alters chronic flow-related arterial structural and functional changes in resistance-sized muscular arteries.

Methods and Results—Vasomotor responses and structural parameters were determined in uterine arteries isolated from nonpregnant, 18- to 19-day pregnant, and 7-day postpartum TK^{-/-} and TK^{+/+} littermate mice. In TK^{-/-} mice, values of diameter, medial cross-sectional area (CSA), myogenic tone, and dilatation in response to acetylcholine were comparable to those values in TK^{+/+} mice, but FID (0 to 100 μ L/min) was significantly reduced ($55 \pm 4\%$ versus $85 \pm 4\%$ in TK^{+/+} mice). In both mouse strains, pregnancy resulted in significant increases in diameter and medial CSA and in the *N*^w-nitro-L-arginine methyl ester–sensitive component of FID. By 7 days after pregnancy, uterine arterial diameter and CSA values no longer differed from nonpregnant values, and FID was markedly reduced in TK^{-/-} and TK^{+/+} mice.

Conclusions—These observations (1) confirm at the level of resistance arteries the key role of TK in FID and (2) indicate that TK deficiency does not compromise arterial remodeling and changes in the contribution of NO to FID during and after pregnancy. (*Arterioscler Thromb Vasc Biol.* 2003;23:●●●-●●●.)

Key Words: tissue kallikrein ■ bradykinin ■ uterine artery ■ flow-related remodeling ■ flow-induced vasodilatation ■ pregnancy ■ postpartum period

Tissue kallikrein (TK) is a serine protease that generates kinins, such as bradykinin, by enzymatic cleavage of high and low molecular weight kininogens.^{1,2} Bradykinin modulates vascular tone by stimulating vascular endothelial B₂-receptors and the subsequent release of NO and prostacyclin (PGI₂).^{3,4} Recently, Bergaya et al⁵ demonstrated a reduced flow-induced dilatation (FID) in isolated carotid arteries of TK-deficient (TK^{-/-}) mice, indicating a key role of the local kallikrein-kinin system in vasomotor responses of large arteries to increased shear stress.

Pressure and flow are hemodynamic determinants of circumferential wall stress and wall shear stress (WSS) in the arterial system. On an acute basis, they modulate arterial smooth muscle tone: an increase in transmural pressure triggers a myogenic contractile response,^{6–8} whereas an increase in flow induces an endothelium-dependent FID.^{8–11} Long-term changes in blood flow lead to arterial structural adaptations to normalize WSS.^{12–17} In general, chronic blood flow elevations lead to a widening of lumen diameter, whereas sustained reductions in blood flow lead to a narrowing of lumen diameter. These structural arterial adaptations have been shown to be endothelium dependent.¹⁸

Pregnancy may be considered a model of physiological flow-related remodeling of uterine arteries. It has previously been studied in rats,¹⁹ guinea pigs,²⁰ and ewes.²¹ During pregnancy, blood flow through the uterine circulation increases substantially.^{22,23} To accommodate this increase in uterine blood flow, the uterine vasculature undergoes luminal expansion and an increase in wall mass,¹⁹ a process termed outward hypertrophic remodeling or arteriogenesis. The driving force for this remodeling is increased WSS. Previous studies have demonstrated that chronic elevations in blood flow lead to an upregulation of FID, which is mainly due to an enhanced production of endothelial factors, such as NO and prostaglandins.

The purpose of the present study was to investigate whether TK deficiency alters FID and chronic flow-related arterial structural and functional alterations in resistance-sized muscular arteries. Vasomotor responses and structural parameters were determined in uterine arteries isolated from nonpregnant, late-pregnant, and postpartum TK^{-/-} and TK^{+/+} mice.

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Methods

Mice Lacking TK

TK-null mice were obtained by targeted disruption of the TK *klk1* gene, which was accomplished by replacing 100 bp of exon 4 with the neomycin-resistance gene in embryonic stem cells.²⁴ Breeding of heterozygous mice derived from these embryonic stem cells led to wild-type (TK^{+/+}), heterozygous (TK^{+/-}), and homozygous (TK^{-/-}) littermate mice. The mice had a mixed genetic 129/Sv-C57BL/6 background.

At an age of 15 weeks, 4 or 5 TK^{+/+} female virgin mice were placed together in a cage with 1 male TK^{-/-} mouse. Female TK^{-/-} mice were put together with 1 male TK^{+/+} mouse. This allowed us to compare pregnant TK^{-/-} mice with TK^{+/+} mice, each carrying heterozygous fetuses. Males were separated from the females after 24 hours. When mating was successful, the day the male mouse had been placed with the female mouse was considered day 0. For the late pregnant group, pregnant mice were euthanized 18 or 19 days after day 0. For the postpartum group, mothers were euthanized 7 days after delivery of their pups. For the nonpregnant group, age-matched (18-week-old) virgin mice were used.

Preparation of Blood Vessels and Experimental Setup

Mice were euthanized with an overdose of pentobarbital (intraperitoneal). The mesentery and uterus were dissected and pinned out on a Petri dish (coated with a layer of silicon). Adipose and connective tissue were carefully removed from the arteries. From every mouse, 1 segment (≈ 3 mm) of a first-order mesenteric artery and 1 segment (≈ 3 mm) of the uterine arteries at midpoint of the uterine arcade were isolated. Both preparations were mounted in an arteriographic system (Living System Instrumentation), in which wall thickness and lumen diameter could be continuously monitored while intraluminal pressure was controlled. Both ends of the vessels were cannulated on 120- to 150- μ m-wide glass micropipettes (Living System Instrumentation) and tied with two 17- μ m thin nylon threads. The 2 micropipettes were selected such that both had the same resistance to flow. Arterial segments were bathed in a 10-mL organ chamber filled with physiological salt solution of the following composition (mmol/L): NaCl 144, KCl 4.7, CaCl₂ 2.5, MgSO₄ 1.2, KH₂PO₄ 1.2, HEPES 14.9, and glucose 5.5, pH 7.4. The artery was superfused at a rate of 4 mL/min with physiological salt solution warmed at a temperature of 37°C and continuously gassed with 95% O₂ and 5% CO₂. The pressure at both ends of the artery was monitored by using 2 pressure transducers. Intraluminal flow (0 to 100 μ L/min) could be applied without changes in average pressure.

For the in vitro protocols, please see the online data supplement (available at <http://atvb.ahajournals.org>).

Morphological Properties

After reactivity measurements, arteries were pressurized at 80 mm Hg for 1 hour in phosphate-buffered 10% formaldehyde solution. Fixed vessels were embedded in paraffin, and cross sections (4 μ m) were stained with Lawson's solution (Boom) to visualize the internal and external elastic laminae. Video images were made from cross sections by using a Zeiss Axioscope and a standard charge-coupled device camera (Sony). With the use of JAVA software (Sigma Scan, Jandel Scientific), the circumferences of the internal and external elastic laminae were determined, and medial cross-sectional area (CSA) was defined as the area enclosed between both laminae. The average number of medial nuclear profiles per cross section was measured by counting the nuclear profiles in the smooth muscle layers of hematoxylin-eosin-stained cross sections at a magnification of $\times 400$ (3 cross sections were counted for each artery and were averaged).

Drugs

N^v-Nitro-L-arginine methyl ester (L-NAME), indomethacin, HOE-140, and sodium nitroprusside were purchased from Sigma Chemical Co. Other reagents were purchased from Prolabo.

Statistical Analysis

Results are shown as mean \pm SEM. Significance of the differences between the different groups was determined by ANOVA. Means were compared by paired *t* test or the Student-Newman-Keuls test. A value of $P < 0.05$ was considered to denote statistical significance.

Results

General Findings

In TK^{-/-} and TK^{+/+} mice, body weight (29 ± 1 versus 26 ± 1 g, respectively) as well as heart weight (140 ± 6 versus 134 ± 7 mg, respectively) and uterine weight (0.33 ± 0.06 versus 0.33 ± 0.04 g, respectively) did not differ significantly. During pregnancy, in TK^{-/-} and TK^{+/+} mice, body weight (46 ± 2 versus 43 ± 2 g, respectively) and uterine weight (12.13 ± 0.77 versus 11.21 ± 0.98 g, respectively) increased to the same extent. Heart weight was significantly increased by day 18 to 19 of pregnancy in TK^{-/-} mice but not in TK^{+/+} mice (155 ± 3 versus 147 ± 6 mg, respectively). When TK^{-/-} mice were compared with TK^{+/+} mice, the number of fetuses (10 ± 0 versus 8 ± 1 , respectively) and their body weights (0.84 ± 0.04 versus 0.97 ± 0.14 g, respectively) did not differ. At day 7 after pregnancy, in TK^{-/-} and TK^{+/+} mice, body weight (40 ± 1 versus 35 ± 2 g, respectively) and heart weight (200 ± 5 versus 164 ± 9 mg, respectively) remained elevated, but uterine weight (0.19 ± 0.02 versus 0.20 ± 0.03 g, respectively) was decreased to below the original value.

Arterial Structure

The structure of uterine arteries and mesenteric resistance arteries did not differ between TK^{-/-} and TK^{+/+} mice (Figure II, available online at <http://atvb.ahajournals.org>). Neither the arterial lumen diameter, as determined by pressure-diameter curves under passive conditions (Figure 1A), nor the medial CSA and the number of medial cells, determined on cross sections (Table 1), differed between TK^{-/-} and TK^{+/+} mice.

Pregnancy was accompanied by a regionally selective and statistically significant outward hypertrophic remodeling of the uterine artery (Figure II). The increases in arterial structural diameter (Figure 2A and Table 1) and in medial CSA (Table 1) were comparable in TK^{-/-} and TK^{+/+} mice.

By 7 days after pregnancy, most of the structural changes in the uterine artery were reversed (Figure II). In TK^{+/+} mice, the structural lumen diameter and the medial CSA no longer differed from findings before pregnancy (Figure 3A and Table 1). In TK^{-/-} mice, the structural lumen diameter had regressed to normal (Figure 3A), but a significant medial hypertrophy persisted (Table 1). The changes in medial CSA of the uterine artery during and after pregnancy were not accompanied by significant changes in the number of arterial smooth muscle cells per cross section in either TK^{-/-} or TK^{+/+} mice (Table 1). It might be noteworthy that mesenteric resistance arteries, which were not modified during pregnancy, were enlarged in the postpartum period. This was statistically significant for the lumen diameter in TK^{-/-} and TK^{+/+} mice, but the 20% to 30% medial hypertrophy did not reach statistical significance in either strain (Table 1).

Vasoconstrictor Reactivity

Myogenic vasoconstriction (Figure 1B) and constriction induced by 1 μ mol/L phenylephrine (Table I, available online

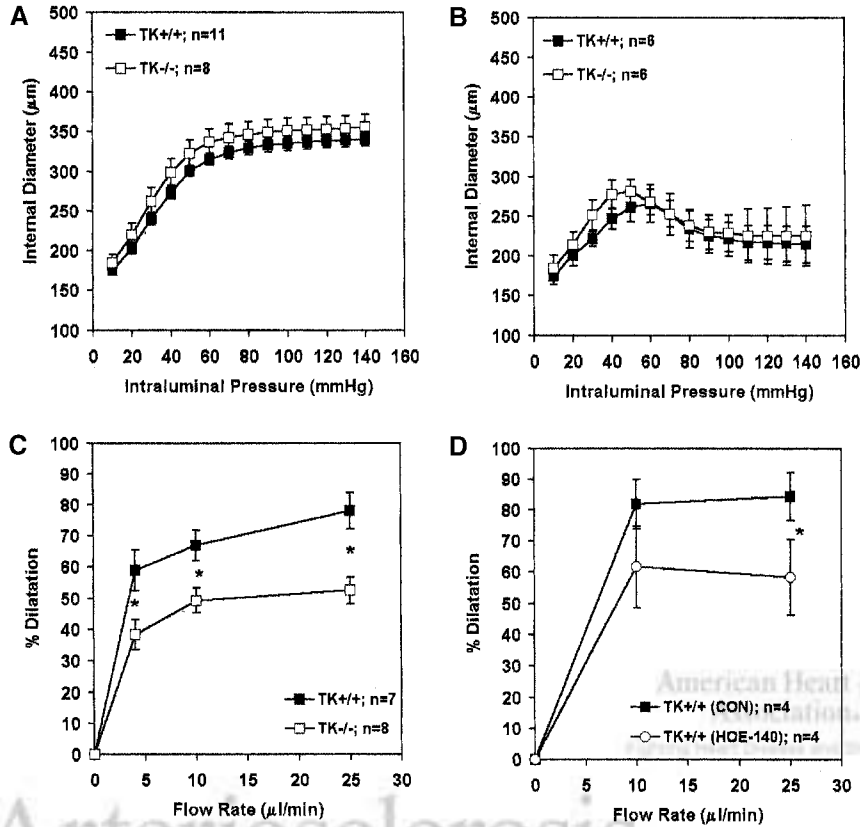


Figure 1. Effects of TK deficiency on structure and reactivity of uterine arteries. A, Pressure-diameter relationships in the absence of calcium for nonpregnant TK^{-/-} (open squares) and TK^{+/+} (solid squares) mice. No significant differences between the 2 groups were observed. B, Pressure-diameter relationships in the presence of calcium. No differences in myogenic tone were observed between the 2 strains. C, FID expressed as percentage dilatation of U46619- and/or myogenic-induced tone. A significantly decreased response to all flow rates was observed in uterine arteries of nonpregnant TK^{-/-} mice (open squares). **P*<0.05 compared with TK^{+/+} mice. D, Effect of 0.1 μmol/L HOE-140 on the FID response in uterine arteries of TK^{+/+} mice. **P*<0.05 compared with control arteries (CON). Data are shown as mean±SEM.

at <http://atvb.ahajournals.org>) did not differ between uterine arteries of TK^{-/-} and TK^{+/+} mice. In the case of myogenic tone, neither the pressure at which the response was induced nor the maximal amplitude of the response differed significantly (Figure 1B).

In uterine arteries of term pregnant mice compared with nonpregnant mice, the myogenic constriction at 100 mm Hg

and the constriction induced by 1 μmol/L phenylephrine were larger when expressed in absolute values (not shown) but comparable when expressed as percentage reduction of the passive diameter (Figure 2B and Table I). This was the case for TK^{-/-} and TK^{+/+} mice. In uterine arteries of late pregnant TK^{+/+} mice, unlike TK^{-/-} mice, myogenic constriction was

TABLE 1. Morphometric Analysis of Uterine and Mesenteric Arteries of Wild-Type and Tissue Kallikrein-Deficient Mice

Measurement	NP		LP		PP	
	TK ^{+/+}	TK ^{-/-}	TK ^{+/+}	TK ^{-/-}	TK ^{+/+}	TK ^{-/-}
Uterine arteries						
Diameter, μm	335±9	357±16	457±8*	466±14*	366±11	399±16
mCSA, ×10 ³ μm ²	4.4±0.6	4.2±0.4	6.3±0.7*	6.2±0.4*	3.8±0.2	5.1±0.5*
No. medial nuclear profiles/cross-section	23±3	25±3	19±2	19±1	22±3	22±1
Mesenteric resistance arteries						
Diameter, μm	239±18	258±14	271±13	289±12	308±19*	310±15*
mCSA, ×10 ³ μm ²	1.9±0.4	2.2±0.4	1.9±0.2	2.6±0.3	2.6±0.3	2.7±0.4

Values are mean±SEM.

Diameters in the absence of smooth muscle tone obtained at an intraluminal pressure of 140 mm Hg, media CSAs (mCSA), and the number of medial nuclear profiles per cross-section of paraffin-embedded uterine arteries and mesenteric resistance arteries of nonpregnant (NP), late pregnant (LP), and postpartum (PP) wild-type (TK^{+/+}) and tissue kallikrein-deficient (TK^{-/-}) mice.

**P*<0.05 compared with NP.

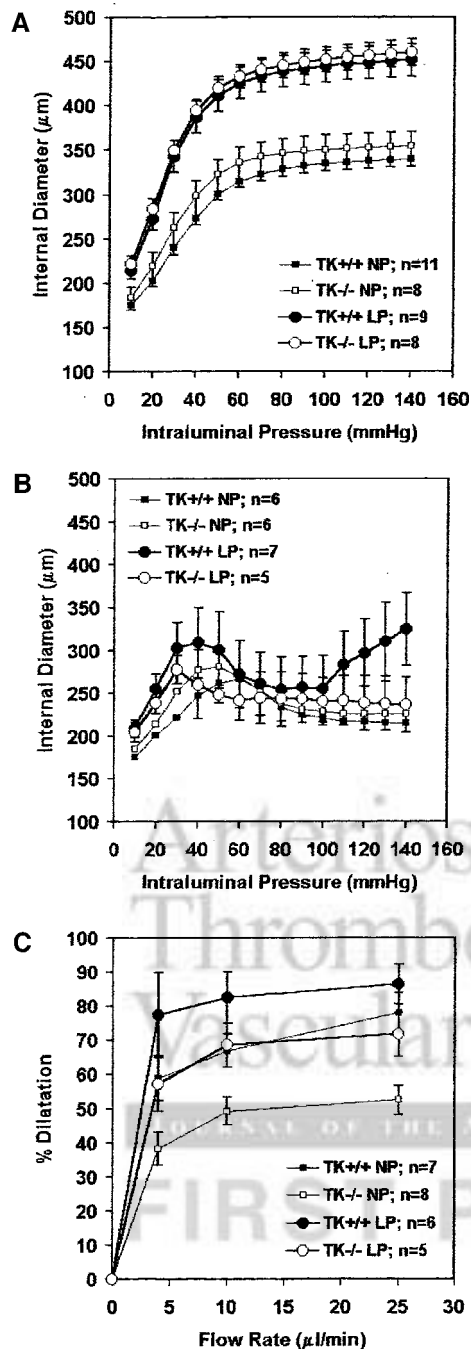


Figure 2. Effects of pregnancy on structure and reactivity of uterine arteries. A, Pressure-diameter relationships in the absence of calcium for uterine arteries isolated from nonpregnant (NP, squares) and late-pregnant (LP, circles) TK^{-/-} (open symbols) and TK^{+/+} (solid symbols) mice. Significantly larger diameters were measured at all pressures for LP mice, indicating an outward remodeling. No differences were observed between the 2 strains. B, Pressure-diameter relationships in the presence of calcium. Myogenic tone measured at 100 mm Hg (see Table 2) tended to be higher in uterine arteries of LP mice. C, FID in uterine arteries of NP (squares) and LP (circles) TK^{-/-} (open symbols) and TK^{+/+} (solid symbols) mice. FID is expressed as percentage dilatation of U46619- and/or myogenic-induced tone. Data are shown as mean±SEM.

TABLE 2. Effects of TK Deficiency and Pregnancy on Flow-Induced Dilatation at 100 μL/min

Condition	NP		LP		PP	
	TK ^{+/+}	TK ^{-/-}	TK ^{+/+}	TK ^{-/-}	TK ^{+/+}	TK ^{-/-}
Control	85±5	55±4‡	94±2†	76±8†‡	38±5†	31±5†
L-NAME	70±4*	47±7‡	48±6*†	44±6*	25±7†	30±6†
L-NAME+ indomethacin	43±5*	27±7*‡	34±8*	36±6*	16±4*†	16±4*†

Values are mean±SEM. Data are expressed as a percentage of the maximal dilatations induced by sodium nitroprusside.

FID (at 100 μL/min) of uterine arteries of nonpregnant (NP), late pregnant (LP), and 7-days postpartum (PP) wild-type (TK^{+/+}) and tissue kallikrein-deficient mice (TK^{-/-}) under control conditions, in the presence of 100 μmol/L L-NAME or 100 μmol/L L-NAME in combination with 10 μmol/L indomethacin.

**P*<0.05 versus control; †*P*<0.05 versus NP; ‡*P*<0.05 versus TK^{+/+}.

not well maintained at high levels of transmural pressure (>100 mm Hg, Figure 2B).

At 7 days after pregnancy, the relative constriction induced by phenylephrine did not differ from observations before and during pregnancy in TK^{-/-} and TK^{+/+} mice (Table I). Sensitivity to myogenic vasoconstriction was increased in the postpartum period compared with the periods before and during pregnancy (Figure 3B). Yet the amplitude of the myogenic constriction at physiological pressures (80 to 100 mm Hg) was comparable to that before pregnancy for TK^{-/-} and TK^{+/+} mice (Table I).

Agonist-Induced Vasodilatation

Dilator responses to 1 μmol/L acetylcholine and intraluminal flow (see below) were evaluated during 40% to 45% constriction induced by a transmural pressure of 80 mm Hg and 1 to 100 nmol/L of the thromboxane A₂ analogue (Table I).

The amplitude of acetylcholine-induced vasodilatation (1 μmol/L) did not differ between TK^{-/-} and TK^{+/+} mice and was not modified in pregnant and postpartum mice (Table I).

Flow-Induced Vasodilatation

Increases in flow rate from 0 to 100 μL/min caused flow-dependent increases in diameter in constricted uterine arteries (Figure 1, available online at <http://atvb.ahajournals.org>). FIDs were significantly smaller in TK^{-/-} uterine arteries than in TK^{+/+} uterine arteries at all flow rates tested (Figure 1C). Maximal responses averaged 55±4% (n=8) and 85±4% (n=7) in the TK^{-/-} and TK^{+/+} groups, respectively. In the presence of the B₂-receptor antagonist HOE-140 (0.1 μmol/L), FID of TK^{+/+} arteries was reduced to the level observed in TK^{-/-} arteries (Figure 1D). In the presence of the NO synthase (NOS) inhibitor L-NAME (100 μmol/L), FID was moderately reduced, and the significant difference between both strains persisted (Table 2). In the presence of both L-NAME and the cyclooxygenase inhibitor indomethacin (10 μmol/L), FID was further reduced, and again, the significant difference between both strains persisted (Table 2). In the presence of both inhibitors, 50% of the maximal FID persisted in uterine arteries of TK^{-/-} and TK^{+/+} mice.

Pregnancy was accompanied by increased flow-induced uterine arterial dilatation in TK^{-/-} and TK^{+/+} mice (Figure

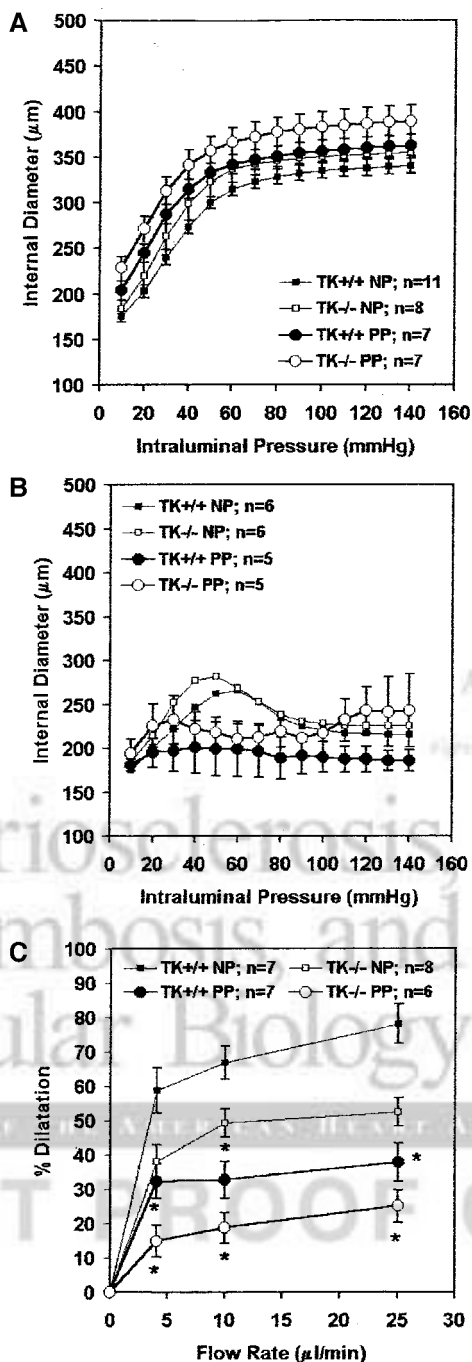


Figure 3. Properties of uterine arteries of TK^{+/+} and TK^{-/-} mice 7 days after pregnancy. A, Pressure-diameter relationships in the absence of calcium for uterine arteries isolated from NP (squares) and postpartum (PP, diamonds) TK^{-/-} (open symbols) and TK^{+/+} (solid symbols) mice. No significant differences were observed, indicating an almost complete remodeling to the original diameters of NP mice. B, Pressure-diameter relationships in the presence of calcium. Myogenic tone was significantly stronger in uterine arteries of PP TK^{+/+} mice compared with NP TK^{+/+} mice (see also Table 2) but was not statistically different between uterine arteries of PP TK^{+/+} and TK^{-/-} mice. C, FID in uterine arteries isolated from NP (squares) and PP (diamond) TK^{-/-} (open symbols) and TK^{+/+} (solid symbols) mice. FID is expressed as percentage dilatation of U46619- and/or myogenic-induced tone. A significantly decreased response to all flow rates was observed in arteries of PP mice compared with NP mice. *P<0.05 vs NP mice. Data are shown as mean±SEM.

2C). The sensitivity and the maximal effect were increased. The latter increased from 85±4% to 94±2% in TK^{+/+} mice and from 55±4% to 76±8% in TK^{-/-} mice. During pregnancy, FID remained significantly smaller in TK^{-/-} mice than in TK^{+/+} mice. As judged from findings with L-NAME, the pregnancy-related increase in flow-induced vasodilatation

was primarily due to a larger contribution of NO to the process. In uterine arteries of pregnant TK^{+/+} mice, L-NAME inhibited 50% of the flow-induced response, whereas this inhibition was only 18% before pregnancy (Table 2). Also in TK^{-/-} mice, the effect of L-NAME was larger during pregnancy (43% inhibition) than before pregnancy (15%

inhibition). The L-NAME and indomethacin-resistant part of FID was not significantly modified by pregnancy. However, in uterine arteries of pregnant mice, FID that could not be attributed to NO or prostaglandins no longer differed significantly between TK^{-/-} and TK^{+/+} mice (Table 2).

After pregnancy, FID was reduced to below prepregnancy levels (Figure 3C). Maximal FID averaged 31±5% and 38±5% in TK^{-/-} and TK^{+/+} uterine arteries, respectively (Table 2). Although all components seemed to be affected, primarily the L-NAME-sensitive part of the FID was blunted or even abolished. In uterine arteries obtained at 7 days after pregnancy, differences between TK^{-/-} and TK^{+/+} mice were no longer statistically significant, but this might be due to the small and variable nature of the responses at this time point.

Discussion

In the present study, we observed in uterine arteries of the mouse that (1) FID is markedly reduced in the absence of TK, (2) pregnancy is accompanied by rapidly reversible outward remodeling and upregulation of FID, and (3) these pregnancy-related arterial structural and functional changes are comparable in the absence and presence of TK.

In large conduit arteries, a reduced FID was previously observed in mice lacking TK, pointing out the importance of locally produced kinins in regulating vasomotor responses to shear stress in these vessels.^{5,24} The objectives of the present study were to determine (1) whether a blunted FID was also present in resistance-sized arteries of TK^{-/-} mice and (2) whether blunted acute responses to shear stress hamper arterial adaptations to chronically altered blood flow. We have chosen pregnancy as a physiological model representing an increased blood flow during gestation and reduced blood flow after birth (7 days after pregnancy) in the uterine vasculature. In many species, normal pregnancy is associated with a marked increase in uterine blood flow.^{25,26} Uterine blood flow has not yet been measured in mice, but cardiac output has been demonstrated to be 64% higher in late-pregnant mice compared with nonpregnant mice.²⁷

Uterine arteries of TK^{+/+} and TK^{-/-} mice had similar responses to acetylcholine and sodium nitroprusside, indicating that the release of NO, prostaglandins, and endothelium-derived hyperpolarizing factor by acetylcholine is unaffected by the lack of TK. We have not addressed the direct vasodilatation induced by bradykinin. However, exogenous bradykinin elicits similar responses in TK^{+/+} and TK^{-/-} carotid arteries.⁵

Uterine arteries of nonpregnant mice responded to intraluminal pressure by developing myogenic tone and responded to flow by vasodilatation. Myogenic tone developed at subphysiological pressures (50 to 60 mm Hg), at which no further increases in diameter were recorded, and subsequent decreases in diameter were followed at higher pressures until a stable tone was maintained over a wide pressure range. Myogenic tone was similar in arteries of TK^{+/+} and TK^{-/-} mice, suggesting no contribution for the kallikrein-kinin system, which is in line with the endothelium-independent nature of the process.⁸

FID was markedly reduced in the uterine arteries of TK^{-/-} mice and was reduced by B₂-receptor blockade in the uterine

arteries of TK^{+/+} mice. This demonstrates the participation of the kallikrein-kinin system in FID of not only conduit⁵ but also resistance arteries. FID depended to a large extent on the production of prostaglandins, inasmuch as the NOS blocker L-NAME only marginally suppressed the response to flow, whereas incubation with both L-NAME and indomethacin induced a 50% reduction in the response to flow in both strains. There was no significant difference in the relative contribution of NO, PGI₂, and the non-NO/non-PGI₂ compound(s) to the FID response between TK^{+/+} and TK^{-/-} arteries. This rules out any compensation by other mediators for the lack of the kallikrein-kinin system and argues for a role of bradykinin as an autocrine mediator during FID.

At late pregnancy, FID of the uterine arteries of both strains of mice was significantly elevated. Predominantly NO was responsible for this upregulation in FID, inasmuch as the contribution of the L-NAME-sensitive compound was significantly increased in TK^{+/+} and TK^{-/-} mice. Because NO has been reported to inhibit the production of other endothelium-derived factors,^{28,29} the observed upregulation of the role of NO in FID during pregnancy might be accompanied by a drop in the relative importance of prostaglandin and non-prostaglandin/non-NO mediators. Pregnancy increases uterine arterial NO-mediated vasodilatation via increased basal NO production (NOS) in rats³⁰ and humans.³¹ Expression of endothelial NOS (eNOS) mRNA and eNOS protein is increased in ovine pregnancy,³² which likely follows estrogen-induced upregulation of eNOS,³³ causing an increase in uterine blood flow.³⁴ Because of this increase in WSS, the uterine artery undergoes a structural increase in diameter, termed outward remodeling. A limitation of the present study is the absence of a quantitative uterine blood flow measurement. However, we have reasons to speculate that blood flow increases substantially in late-pregnant mice. First, uterine blood flow has been shown to increase dramatically (≈40-fold) at term in rats.²⁶ Second, cardiac output increases 64% during murine pregnancy.²⁷ Because uterine weight and the number and weight of the fetuses are not statistically different between TK^{+/+} and TK^{-/-} late-pregnant mice, we believe that the uterine blood flow rise is comparable in both strains.

In previous studies, chronic elevations and reductions in blood flow have been reported to induce outward and inward arterial remodeling, respectively.^{13–20} This adaptive response allows a normalization of WSS and might be accompanied by changes in arterial smooth muscle cell number, size, and phenotype to restore circumferential wall stress.^{35,36} The increase in uterine arterial wall mass during murine pregnancy was due to vascular smooth muscle cell hypertrophy rather than hyperplasia. The outward remodeling and increase in medial CSA were similar in TK^{+/+} and TK^{-/-} mice, despite a significantly different acute response to flow before and during pregnancy. This indicates that TK deficiency does not compromise arterial structural and functional changes in response to long-term blood flow elevations.

TK and bradykinin B₂-receptor mRNA and protein are expressed in several tissues of the uterus and myometrial blood vessels during human pregnancy.³⁷ However, lack of TK does not cause any noticeable complications in murine

pregnancy, as far as uterine arterial remodeling, fetus weight, and the number of pups delivered are considered. However, more subtle consequences cannot be excluded. To this end, cardiovascular and renal function will have to be critically compared in the heterozygous offspring of TK^{-/-} and TK^{+/+} mothers.

Body weight remained elevated in postpartum mice; this situation was possibly due to increased food intake to facilitate lactation. Heart weight also remained increased compared with heart weight of nonpregnant mice. The cardiac hypertrophy most likely resulted from the increased cardiac output and heart rate at late pregnancy and at 3 days after pregnancy, as measured by Wong et al.²⁷ Myogenic tone was stronger in uterine arteries of 7-day postpartum mice compared with virgin mice. The FID response was markedly reduced in uterine arteries of 7-day postpartum mice, suggesting a reduced shear stress sensing of the endothelium, despite normal acetylcholine-mediated vasorelaxation. We currently have no explanation for this phenomenon. An increased myogenic tone and a decreased FID lead to an increased contractility, possibly reducing the risk of excessive blood loss after delivery.

As observed from the passive pressure-diameter curves of uterine arteries of postpartum mice, diameters no longer differed from the nonpregnant values. Medial CSA values were similar in the uterine arteries of 7-day postpartum mice and nonpregnant mice, reflecting an inward hypotrophic remodeling compared with the uterine arteries of mice near term. Again, the blunted acute responses to flow are not sufficient to disturb structural arterial adaptations in response to long-term changes in blood flow.

A surprising finding was the outward remodeling observed in mesenteric resistance arteries of 7-day postpartum mice. Feeding of the pups requires a higher food intake than normal, which is likely to increase blood flow through the mesentery, leading to this increase in diameter. In rats with streptozotocin-induced diabetes, blood flow to the small intestine is markedly increased because of marked hyperphagia.³⁸ Our group previously showed that this rise in mesenteric blood flow resulted in a larger diameter of the mesenteric resistance artery of diabetic rats compared with control rats.³⁹

In conclusion, we confirmed the role of the kallikrein-kinin system in acute FID via B₂-receptors at the level of resistance arteries. Absence of this kallikrein-kinin system seems not to be compensated by other mediators of FID in TK^{-/-} arteries. Furthermore, we demonstrated the dynamic nature of physiological arterial remodeling and arteriogenesis during and after pregnancy, and we have shown that TK deficiency does not compromise arterial structural and functional changes, which are accompanied by marked changes in local blood flow, during and after pregnancy. Bradykinin produced by TK seems to play different roles in acute and chronic arterial responses to altered blood flow.

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