The use of microvascular anastomosis techniques is an essential part of many surgical procedures, such as free tissue transfer, tissue transplantation, and replantation of amputated limbs.\textsuperscript{1,2} During this process, the vessel walls are manipulated with various types of surgical tools, and repetitive contact occurs between the vessel walls and the chemicals added to the environment to prevent thrombosis and vasoconstriction.\textsuperscript{3}

The arterial wall is composed of 3 distinct layers. The outer layer is a connective tissue layer called adventitia. The muscular layer is located inside the adventitia and has the ability to contract and relax. The inner layer is called endothelium and covers

---

**Background:** Hepatic artery anastomosis is an essential part of live-donor liver transplantation, and during this anastomosis, an unusual contact between bile and vessel ends is observed. In this study, the effects of this non-physiological contact in a rabbit model were evaluated.

**Methods:** The study was designed in 2 steps—in vitro and in vivo. Three groups were established for the in vitro study. In the first group, vessels were incubated in Krebs solution with 5\% bile for 1 minute. In the second group, vessels were kept in Krebs solution with 5\% bile for 5 minutes. Vessels in the control group were kept in Krebs solution without bile. All groups were examined for responses to vasodilator and vasoconstrictor agents in organ bath system. The specimens were evaluated immunohistochemically and histopathologically. In the in vivo step, microvascular anastomosis was performed bilaterally. Right carotid artery was anastomosed during bile contamination as study group, and left carotid artery was anastomosed without bile contamination as control group. Blood flow indexes were measured.

**Results:** The results of the in vitro study revealed decreased responses to contractile and relaxing agents in the first study group compared with that of the control group ($P < 0.0001$). There was no response obtained in the second study group. The Doppler ultrasound results revealed no difference between preoperative and postoperative flow indexes ($P > 0.05$). There was no postoperative spasm in the study group. However, there was significant vasospasm in the control group ($P < 0.05$).

**Conclusions:** Vessels exposed to bile have decreased contractile and relaxing responses, and this effect increases with exposure duration. (Plast Reconstr Surg Glob Open 2015;3:e570; doi: 10.1097/GOX.0000000000000546; Published online 23 November 2015.)
the entire luminal space. The endothelial layer produces and excretes various chemicals that regulate the contraction and relaxation of the muscular layer. The endothelial layer also regulates bleeding and clotting mechanisms by excreting related factors and acting as a physical barrier. Blood vessels provide metabolites for cell oxygenation and nutrition and respond to both physical and chemical stimulants by changing the vessel tonus and permeability.

Plastic surgeons who perform microsurgery are taking an important role in the microvascular anastomosis of the hepatic artery in the live-donor liver transplantation. Therefore, studies about this part of the liver transplantation are particularly important for the plastic surgery armamentarium. A successful liver transplantation depends on a successful microvascular anastomosis, and the anastomosis is a technically challenging part of the procedure. Bile production starts after completing the vena cava and vena porta anastomoses and establishing liver circulation. The bile contacts both vessel ends and the endothelium of the hepatic artery anastomosis because bile duct anastomosis is performed after arterial anastomosis. This contact is physically impossible in the body and is only possible during liver transplantation surgery. The aim of the study is to evaluate the effects of the bile on the vascular tonus and contraction and relaxation responses.

MATERIALS AND METHODS

The experiment was approved by the Local Animal Care and Ethics Committee. The instructions and policies of this committee conform to the Guide for the Care and Use of Laboratory Animals Published by the U.S. National Institutes of Health (NIH Publication No: 85–23, revised 1996).

Bile was obtained from the cholecystectomized bile duct of a healthy female liver donor after written informed consent was obtained from the patient. The bile was stored at +4°C in a sterile medium, and the same bile was used throughout the experiment. Long time storage of the bile in 4°C may be destructive for its proteins. For keeping the bile structurally intact, we performed the in vivo and in vitro studies simultaneously, and the bile was used within 48 hours after its harvest in all experimental groups.

In Vitro Study

Although the carotid artery of the rabbit is not a frequently used model for experimental microsurgery, it is routinely used in studies that use organ bath because its diameter permits manipulation without damaging the endothelium. Additionally, the carotid artery can perform the needed contraction and relaxation functions. Because of these advantages, we chose a rabbit carotid artery model for our study.

A pilot study was designed to determine the duration of the contact and the appropriate bile concentration. Initially, the vessels were incubated for 30 minutes with pure bile. This approach yielded no response to agents causing vessel contraction and relaxation. After that, concentration value causing vessel response was investigated by gradually decreasing the bile concentration. However, we could not obtain an appropriate response, and we also tested for vessel responses by decreasing the incubation period until finding the optimal bile concentration and incubation time and used these values for this study.

Ten New Zealand white rabbits (1.4–1.8 kg) were anesthetized with 50 mg/kg intramuscular ketamine and 15 mg/kg xylazine. The bilateral carotid arteries were exposed segmentally and harvested. The vessels were incubated in a modified Krebs–Ringer solution (composition in mM: 118.3 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2 MgSO₄, 1.2 KH₂PO₄, 25.0 NaHCO₃, 0.026 calcium ethylenediaminetetraacetic acid, and 11.1 glucose; pH 7.4) at 4°C. The vessels were then cut into 4-mm ring shaped segments.

The in vitro study was designed with 3 groups, including the control group and 2 study groups.

The vessel ring segments of the control group (n = 5) were kept in the Krebs solution.

In the first in vitro study group, the vessel segments (n = 10) were incubated in 5% bile for 1 minute. In the second group, the vessel segments (n = 10) were incubated in 5% bile for 5 minutes.

The preparations were suspended in 20 mL of modified Krebs–Ringer solution at 37°C under 2 g of resting tension (Fig. 1). The preparations were then rinsed with modified Krebs–Ringer solution at 15-minute intervals for 1 hour until reaching stable resting tension. To achieve maximal tension, 10⁻⁹ to 3 × 10⁻⁵ M dosages of phenylephrine (PNL) were used incrementally. The Krebs solution was changed, and after achieving a basal contractile state, a submaximal (80%) contraction was generated with PNL. The relaxation response was evaluated with 10⁻⁶ to 3 × 10⁻⁵ M dosages of acetylcholine (Ach). The Krebs solution was changed again, and after a basal contractile state was achieved, a submaximal (80%) contraction was generated with PNL. After submaximal contraction, 80 mM KCl was added to the solution, and the contraction responses were evaluated. All of the vessel rings were tested with this standard procedure.

Disclosure: The authors have no financial interest to declare in relation to the content of this article. The Article Processing Charge was paid for by the authors.
Tension was recorded isometrically with a force transducer (COMMAT, Ankara, Turkey), displayed on a Biopac acquisition system (Biopac system Inc., Calif.) and analyzed with the same software. The potency values of the groups were calculated with Prism 3.0 (GraphPad Software, La Jolla, Calif.).

**Histochemical Evaluation**

The vessel rings were also evaluated histopathologically by staining with hematoxylin–eosin before examination using light microscopy.

**Immunohistochemical Evaluation**

The vessel rings were also evaluated immunohistochemically by cutting 5-μm sections from the specimens and applying Ventana iVIEW DAB (Ventana Medical Systems, Tucson, Ariz.) immunohistochemical staining. The sections were then incubated in a 1:250 diluted solution of a smooth muscle actin antibody.

**In Vivo Study**

A rabbit model was chosen for the in vivo study because it has a sufficient carotid artery diameter that permits measurements using Doppler ultrasound (DUS). Additionally, a rabbit model was also used in the in vitro assays.

Ten New Zealand white rabbits (1.4–1.8 kg) were anesthetized with 50 mg/kg intramuscular ketamine and 15 mg/kg xylazine. The left carotid arteries were used as the control group and the right carotid arteries were used as the study group. Before surgery, the blood flow index of each artery was examined with color duplex ultrasonography (Sonoline Elegra 13 mHz linear probe; Siemens, Erlangen, Germany).

In the control group, the left carotid arteries were exposed, and microscope magnification was used to cut the vessels with microscissors and then anastomoses the vessels using 9/0 100 μm and 5 mm ethilon (Ethicon, Wokingham, U.K.) sutures. All of the microsurgical anastomoses were performed by the same surgeon using the standard technique described by Acland. After the anastomosis, the microclamps were released, and clinical observation of the patency was noted. In the study group, the right carotid artery anastomosis was performed by the same technique and the same surgeon. However, the carotid artery endothelium was contaminated with 5% bile during anastomoses (Fig. 2). The vessel patency was then observed (Fig. 3). The study anastomoses were irrigated using 5% bile again. After 30 minutes, the post-anastomosis blood
flow indexes of both groups were examined with color duplex ultrasonography (Sonoline Elegra 13 mHz linear probe; Siemens). The peak systolic flow speed (PS), resistive index (RI), and systolic/diastolic ratio (S/D) values were recorded. After the procedures, the animals were killed using intracardiac KCl injection.

**Statistical Evaluation**

The results of the organ bath studies were evaluated with 2-way analysis of variance. A post hoc Bonferroni test was used to assess statistical significance between the groups. Student t test was used for potency values. The Wilcoxon signed ranks test was used for the RI, S/D, and PS values of the color duplex ultrasonography tests. A $\chi^2$ test was used for statistical analysis of vasospasm in the control group. The level of statistical significance was set at $P < 0.05$ for all of the tests.

**RESULTS**

The results were obtained by comparing the vascular responses of the 2 in vitro groups with the control group.

**First Study Group Contraction Responses after Phenylephrine**

The PNL contraction responses of the first study group were significantly lower than that of the control group ($P < 0.0001$; Fig. 4).

**First Study Group Contraction Responses after KCl Depolarization**

After a single application of KCl, the contractile responses of the first study group were significantly lower than that of the control group ($P < 0.05$; Fig. 5).

**Relaxation Responses after Acetylcholine**

The relaxation responses of the first study group were significantly lower than that of the control group after Ach application ($P < 0.0001$; Fig. 6).

**First Study Group Relaxation Responses after Sodium Nitroprusside**

The relaxation responses of the first study group were significantly lower than that of the control group after sodium nitroprusside application ($P < 0.0001$; Fig. 7).

**Second Study Group Responses**

The vessel segments of the second study group, which were incubated for 5 minutes in a 5% bile solution, showed no response to the contractile and relaxing agents. Thus, statistical analyses could not be performed.
Histochemical and Immunohistochemical Findings of the In Vitro Study

All of the groups were stained with hematoxylin–eosin and smooth muscle actin. The histological specimens were investigated using light microscopy. There was no structural damage detected microscopically. The endothelial layer was intact, and there was no structural deformity in the muscle layer of the vessels (the actin and myosin bands were structurally normal; Figs. 8, 9).

Color Duplex Ultrasonography Findings of the In Vivo Study

Postoperative Flow in the Control and Study Groups

The control and study groups for the in vivo study were postoperatively examined with color duplex ultrasonography. All of the vessels (n = 10) were patent in the study group. There was no flow detected in the control group in the carotid artery of 2 subjects upon first examination. There was no thrombus formation detected in the lumens of these subjects. After warming for 30 minutes (postoperative first hour), the 2 subjects were reexamined, and normal blood flow was detected. This situation was defined as vasospasm. Two vessels of the control group showed vasospasm, whereas no vasospasm was observed in the study group. This result was statistically significant ($P < 0.05$).

The preoperative and postoperative blood flow values of the control and study groups were compared. There were no statistically significant differences between the right and left carotid arteries preoperatively and postoperatively ($P > 0.05$; Figs. 10–12).
DISCUSSION

Bile contains bile acids (60%), phospholipids (30–40%), cholesterol (4%), and bilirubin (2%). Bile acids are amphiphilic molecules and consist of a lipophilic steroid ring with a side chain carrying a carboxyl group that can be differentially conjugated by liver cells. Most bile acids are glycine conjugated or taurine conjugated. Ursodeoxycholic acid is a bile acid that occurs in minor quantities in humans. This bile acid differs from most other bile acids because it is more hydrophilic and can stimulate bile acid excretion by hepatocytes.

The contact between the bile and the endothelium is physiologically impossible. However, this exceptional condition occurs in the recipient patient during live-donor liver transplantation. After performing the hepatic and portal vein anastomoses, the hepatic circulation starts and induces bile production. The bile can freely spread around the hepatic artery anastomosis site and contact the endothelium. The bile duct is sutured to the bowel after completing the hepatic artery anastomosis, so the nonphysiological bile flow ceases.

Blood vessels and their unique structure, including the endothelium and muscular layer, are considered as a separate organ because of their ability to contract, relax, and change permeability in response to systemic and local stimulants instead of functioning as a simple tubular system. Because of the effects of endothelium on vascular tonus, this condition is important in microvascular surgery.

In this study, we only evaluated the effects of the bile on the arteries because of 2 basic reasons. Arteries can give appropriate contraction and relaxation responses in the organ bath system; however, this is not possible in vena. Furthermore, the unusual contact is seen after completing the venous anastomoses and affects the hepatic artery anastomosis.

PNL is a $\alpha_1$ receptor agonist and causes intracellular Ca$^{2+}$ entrance that leads to contraction by an endothelium dependent mechanism. KCl is a non-endothelium-dependent depolarizing agent for smooth muscles in vessels. It also causes intracellular Ca$^{2+}$ entry and is required for contraction by opening voltage-dependent Ca$^{2+}$ channels located on the sarcolemmal membrane. Sodium nitroprusside is a direct vascular relaxing agent independent of endothelium. It includes nitrous oxide (NO), which is released by a reduction reaction catalyzed in tissue. NO directly activates the cytosolic fraction of intracellular guanylate cyclase in vessel smooth muscle. Vasodilatation occurs because of an elevation of cyclic guanosine monophosphate levels, which is an endogenous vasodilator. Ach causes vasodilatation in an indirect fashion. Ach binds muscarinic receptors located on the endothelium and causes the release of NO, which is synthesized by endothelial nitric oxide synthase. The results of the in vitro study demonstrate statistically significant decreases in responses to various contracting and relaxing agents.

Bile acids are present in bile and may affect vessel function. Bile acids are important components of bile, and their effects on the cardiovascular system are well established. These studies demonstrate that bile acids decrease the myogenic tonus leading to vasodilatation and bradycardia. Several studies explain the mechanism of effect in different ways. The mechanisms include acting on endothelial receptors, NO production, or alteration of the intracellular Ca$^{2+}$ levels. Although bile acids have some

Fig. 8. Hematoxylin—eosin staining (original magnification, ×200) of the second in vitro study group. Endothelial layer of the vessels are intact, and there is no structural deformation of the vessels.

Fig. 9. Second in vitro study group smooth muscle actin staining (original magnification, ×1000). There is no structural deformity of the vessel muscle layer.
**Fig. 10.** The graphic showing the preoperative and postoperative peak systolic flow speeds (PS) of the carotid arteries in the control and study groups. No statistically significant difference could be encountered ($P > 0.05$). C, control; S, study; Pre, preoperative; Post, postoperative.

**Fig. 11.** The graphic showing the preoperative and postoperative resistive index (RI) values of the carotid arteries in the control and study groups. No statistically significant difference could be encountered ($P > 0.05$). C, control; S, study; Pre, preoperative; Post, postoperative.
other mechanisms causing vessel responses such as the blockade of specific receptors, their major mechanism of action is the impairment of smooth muscle function by decreasing intracellular calcium levels.

A bile acid-specific G-protein–coupled receptor, TGR5 (also known as GPBAR1, M-BAR, and BG37), was recently identified.24 This receptor is associated with the immunomodulatory properties of bile acids24 and some hepatic functions.25 In endothelial cells, TGR5 regulates nitric oxide production via cyclic adenosine monophosphate-dependent activation of endothelial nitric oxide synthase.26 However, the presence of TGR5 in cardiomyocytes has not been investigated. Several other reports have suggested that bile acids might affect muscarinic cholinergic receptor signaling.27 One study evaluated the effects and mechanism of the conjugated bile acid deoxycholylglycine on the vascular tonus of rat mesenteric arteries. Bile acids were found to decrease the tonus, and this effect was related to decreased intracellular Ca\(^{2+}\) levels and not to NO production, dose-dependent muscarinic receptors, or potassium channels.28,29 Furthermore, it was shown that the bile acid taurocholate reduces Ca\(^{2+}\) release in the sarcoplasmic reticulum and the contraction amplitude in a rat cardiomyocyte model.22 In this study, both endothelium dependent and independent contraction and relaxation responses were reduced. These results indicate that there is a disruption in the intracellular mechanism of smooth muscle cells independent from the endothelium. The result is supported by studies showing decreased intracellular Ca\(^{2+}\) levels and disruption in sarcolemmal Ca\(^{2+}\) release.28 There were no responses obtained in the second group, which suggests that the effect increases with dose and that this result is consistent with the current literature.22

One critical finding was that the endothelium and smooth muscle integrity were intact in immunohistochemical and histochemical analyses performed on the vessels segments in vivo. This finding supports the hypothesis that vessel changes occur in the receptor and intracellular messengers after contact with bile. It is also important that the endothelium is not damaged after contact with bile considering its importance in the coagulation cascade. In experiments conducted during the design of this study, direct bile to vessel contact was used. The hepatic artery thrombosis rate was found to be 2% in a study evaluating 150 cases of liver transplantation.7 According to these and other data on the thrombosis rates of hepatic artery in liver transplantation, we conclude that bile is not a thrombogenic agent.30 In our previous study investigating the effect of bile on the rat femoral artery, we found that bile does not cause any thrombogenic effects and does not cause
latent damage to the endothelium.30 In the immunohistopathological examination performed in the in vivo group, we found that the endothelium structures and smooth muscles were intact in vessels incubated in a 5% bile solution. This finding indicates that despite having a structure like detergent, bile does not cause any destruction of vessel tissue. These results are consistent with the current literature.30

DUS is a method for evaluating blood flow changes, and various different indexes are used for the evaluation of flow speed. If a restriction occurs in the vessel diameter, it increases the resistance against blood flow, which increases flow speed. Thus, the RI, S/D, and PS increase.31,32 There was no difference observed in intraoperative DUS measurements, which indicates that there is no difference in the basal flows of rabbit carotid arteries. There was no difference observed in postoperative DUS measurements, and this finding indicates that bile does not have any contractile effect. Additionally, there was no vasospasm observed in the vessels of the study group. However, there was a statistically significant vasospasm in the vessels of the control group. These data support the results of the in vitro study and are in agreement with studies showing decreased myogenic response in vessels incubated with bile acids. Actually, the initial design of our study did not involve a proper vasospasm model; however, during the study, we observed spontaneous vasospasm. Nevertheless, we measured and added important data, results, and statistics about this vessel contraction. However, it should be stated that the effects of the bile on vasospasm should be further evaluated.

CONCLUSIONS

In this study, the effects of the unusual contact between bile and the hepatic artery were seen in the live-donor liver transplantation on the arterial contraction and relaxation responses were investigated in vivo and in vitro fashion. The results of the study revealed that the arterial wall incubated with bile shows decreased contraction and relaxation responses, and this effect was found to be increased with an increased exposure time. According to these data, it is not expected that the above-mentioned unusual contact can cause a vasospastic response impairing the circulation of the liver.

Gökhan Temiz, MD
Department of Plastic Reconstructive and Aesthetic Surgery
Dr. Lütfi Kirdar Kartal Training and Research Hospital
Şemsi Denizer Cad. E-5 Karayolu Cevzili Mevki Kartal
İstanbul 34890, Turkey
E-mail: drgokhantemiz@yahoo.com

REFERENCES

1. Ramirez AE, Lao WW, Wang YL, et al. Two-stage face transplantation: a new concept in vascularized composite allotransplantation. Microsurgery 2015;35:218–226.
2. Temiz G, Bilkay U, Tiftücióğlu YO, et al. The evaluation of flap growth and long-term results of pediatric mandible reconstructions using free fibular flaps. Microsurgery 2015;35:253–261.
3. Acland R. Prevention of thrombosis in microvascular surgery by the use of magnesium sulphate. Br J Plast Surg 1972;25:292–299.
4. Eroschenko VP. DiFiori’s Atlas of Histology with Functional Correlations. 11th ed. Baltimore: Lippincott Williams & Wilkins; 2008:171–179.
5. Förstermann U, Sessa WC. Nitric oxide synthases: regulation and function. Eur Heart J. 2012;33:829–837, 837a.
6. Bruno RM, Gori T, Ghiadoni L. Endothelial function testing and cardiovascular disease: focus on peripheral arterial tonometry. Vasc Health Risk Manag. 2014;10:577–584.
7. Alper M, Gundogan H, Tokat C, et al. Microsurgical reconstruction of hepatic artery during living donor liver transplantation. Microsurgery 2005;25:378–383.
8. Tanaka K, Uemoto S, Tokunaga Y, et al. Surgical techniques and innovations in living related liver transplantation. Ann Surg. 1995;217:82–91.
9. Geuken E, Visse D, Kuipers F, et al. Rapid increase of bile salt secretion is associated with bile duct injury after human liver transplantation. J Hepatol. 2004;41:1017–1025.
10. Seaman DS. Adult living donor liver transplantation: current status. J Clin Gastroenterol. 2001;33:97–106.
11. Evans GR, Gherardini G, Gürlek A, et al. Drug-induced vasodilation in an in vitro and in vivo study: the effects of nicardipine, papaverine, and lidocaine on the rabbit carotid artery. Plast Reconstr Surg. 1997;100:1475–1481.
12. Pflueger A, Croatt AJ, Peterson TE, et al. The hyperbilirubinemic Gunn rat is resistant to the pressor effects of angiotensin II. Am J Physiol Renal Physiol. 2005;288:F552–558.
13. D’Uscio LV, Baker TA, Mantilla CB, et al. Mechanism of endothelial dysfunction in apolipoprotein E-deficient mice. Arterioscler Thromb Vasc Biol. 2001;21:1017–1022.
14. Harvey RA. Lipid metabolism. In: Lippincott’s Biochemistry, 5th ed. Baltimore: Lippincott Williams & Wilkins; 2011: 230–235.
15. Bigaud M, Julou-Schaeffer G, Parratt JR, et al. Endotoxin-induced impairment of vascular smooth muscle contractions elicited by different mechanisms. Eur J Pharmacol. 1990;190:185–192.
16. Ghatta S, Tunstall R, Kareem S, et al. Sirolimus causes relaxation of human vascular smooth muscle: a novel action of sirolimus mediated via ATP-sensitive potassium channels. J Pharmacol Exp Ther. 2007;320:1204–1208.
17. Palmer RM. The discovery of nitric oxide in the vessel wall. A unifying concept in the pathogenesis of sepsis. Arch Surg. 1993;128:396–401.
18. Gürlek A, Gherardini G, Cromeens D, et al. Drug-induced vasodilation: the effects of sodium nitroprusside, hydralazine, and cromakalin on the rabbit carotid artery: in vitro and in vivo study. J Reconstr Microsurg. 1997;13:415–421.
19. Palmer RM. The discovery of nitric oxide in the vessel wall. A unifying concept in the pathogenesis of sepsis. Arch Surg. 1993;128:396–401.
20. Furchtgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 1980;288:373–376.
21. Pak JM, Lee SS. Vasoactive effects of bile salts in cirrhotic rats: in vivo and in vitro studies. Hepatology 1993;18:1175–1181.

22. Gorelik J, Harding SE, Shevchuk AI, et al. Taurocholate induces changes in rat cardiomyocyte contraction and calcium dynamics. Clin Sci (Lond). 2002;103:191–200.

23. Sheikh Abdul Kadir SH, Miragoli M, Abu-Hayyeh S, et al. Bile acid-induced arrhythmia is mediated by muscarinic M2 receptors in neonatal rat cardiomyocytes. PLoS One 2010;5:e9689.

24. Kawamata Y, Fujii R, Hosoya M, et al. A G protein-coupled receptor responsive to bile acids. J Biol Chem. 2003;278:9435–9440.

25. Vassileva G, Golovko A, Markowitz L, et al. Targeted deletion of Gpbar1 protects mice from cholesterol gallstone formation. Biochem J. 2006;398:423–430.

26. Keitel V, Reinehr R, Gatsios P, et al. The G protein-coupled bile salt receptor TGR5 is expressed in liver sinusoidal endothelial cells. Hepatology 2007;45:695–704.

27. Raufman JP. Activation of muscarinic receptor signaling by bile acids: physiological and medical implications. Dig Dis Sci. 2003;48:1431–1444.

28. Khurana S, Raina H, Pappas V, et al. Effects of deoxycholyglycine, a conjugated secondary bile acid, on myogenic tone and agonist-induced contraction in rat resistance arteries. PLoS One 2012;7:e32006.

29. Khurana S, Raina H, Pappas V, et al. Effects of deoxycholyglycine, a conjugated secondary bile acid, on myogenic tone and agonist-induced contraction in rat resistance arteries. PLoS One 2012;7:e32006.

30. Klıç K, Temiz G, Zeytunlu M, et al. Effects of bile on arterial anastomosis in a rat model. Arch Clin Exp Surg 2015;4:135–141.

31. Gosling R, King D. Ultrasonic angiography. In: Hascus AW, Adamson L, eds. Arteries and Veins. Edinburgh: Churchill Livingstone; 1975:61–98.

32. Bluth EI. Doppler US velocity measurements. Radiology 2002;223:882.

33. Rainer PP, Primessnig U, Harenkamp S, et al. Bile acids induce arrhythmias in human atrial myocardium—implications for altered serum bile acid composition in patients with atrial fibrillation. Heart 2013;99:1685–1692.