

Effect of Sex on Biomechanical Properties of the Proper Hepatic Artery in Pigs and Humans for Liver Xenotransplant

Jing Li,¹ Wen-Chun Li,^{1,2} Jie Song,³ Hong-Mei Zhang,¹ Ming-Hua Yu,¹ Xi-Ju He,¹
Ming-Hua Wang,⁴ Han-Qin Wang,¹ Tie-Zhu Huang¹

Abstract

Objectives: This study investigated the relation between biomechanical properties of the proper hepatic artery and sex in pigs and humans to provide the theoretical basis for selecting suitable donor in pig-to-human liver xenotransplant.

Materials and Methods: The proper hepatic arteries of 32 Chinese Hubei white pigs (8 males, 8 females, 8 castrated males, and 8 ovariectomized females) and 10 deceased donors (5 human men, 5 human women) were obtained. The pressure-diameter relations of the proper hepatic arteries were measured on biomechanical test equipment to calculate the incremental elastic modulus (E_{inc}), pressure-strain elastic modulus (E_p), volume elastic modulus (E_v), and compliance. Each sample was sliced into 5- μ m frozen sections and stained with hematoxylin-eosin.

Results: There were significant differences in E_{inc} ($F=10.24$; $P=.001$), E_p ($F=3.75$; $P=.001$), and E_v ($F=3.41$; $P=.002$) of the proper hepatic arteries of female, male, and gonadectomized pigs; females had the lowest elastic modulus and the gonadectomized group had the highest ($P<.01$). There was a significant difference in compliance of the porcine proper hepatic arteries between the sexes, highest in the female group and lowest in the

gonadectomized group ($P<.01$). No difference in the elastic modulus and compliance of the proper hepatic artery between the male pig and the human man. There was no difference between the female pig and the human woman.

Conclusions: There were differences in the biomechanical properties of the proper hepatic arteries of the female, male, and gonadectomized pigs. The biomechanical properties of the human men/women proper hepatic artery match those of the porcine male/female hepatic artery. The correlation between sex and biomechanical properties of the proper hepatic artery in pigs could imply that a pig of the same sex should be chosen for pig-to-human liver xenotransplant.

Key words: Pig, Sex, Proper hepatic artery, Biomechanics, Xenotransplant

Introduction

The development of clinical allotransplant has extended the lives of many patients with incurable hepatic diseases. However, the shortage of human livers as donors has been increasing. At present, the liver has the second-largest demand for a transplantable organ. To overcome the shortage of donor organs, fundamental research of xenotransplant has become the focus of attention.¹⁻⁴ Many researchers indicate this as the study object of xenotransplant,⁵⁻⁷ the porcine liver is a reasonable and attention-drawing donated organ. The porcine liver and anastomosis of blood vessels are extremely similar to the human's in anatomy and hemodynamics; the research of pig-to-human liver xenotransplant is increasing. During clinical organ transplant, when the donated organ is transplanted

From the ¹Institute of Basic Medical Sciences; the ²Renmin Hospital; the ³Dongfeng Hospital; and the ⁴Taihe Hospital, Hubei University of Medicine, Shiyian, 442000, China.

Acknowledgements: Jing Li and Wen-Chun Li contributed equally to the work and share first authorship. This work was supported by research grants from the Hubei Province Outstanding Scientific Innovation Team Plans (T201008) and Education Department of Hubei Province Foundation (B20122402), China.

Corresponding author: Tie-Zhu Huang, Institute of Basic Medical Sciences, Hubei University of Medicine, Shiyian, 442000, China.

Phone: +86 719 889 1081 Fax: +86 719 889 1080 E-mail: tiezhuhuang@163.com

Experimental and Clinical Transplantation (2012) 4: 356-362

into the receiver, it is crucial that the biomechanical properties of the donor's vessel wall match those of the receiver, and whether the xenotransplanted organ can function normally is closely related to blood vessel reconstruction and patency rate after anastomosis.

Steroid hormones have an important effect on the growth and function of target cells in vascular tissues.^{8, 9} It is well known that sex steroids can modulate the growth and function of the blood vessel by genomic and nongenomic mechanisms, change the extracellular matrix of the vascular wall, and subsequently influence vascular biomechanical properties.¹⁰ Many studies have discussed the pig as the organ donor in a transplant and have provided fruitful results, but experimental pigs are mostly gonadectomized. Moreover, some researchers indicate that female, male, and gonadectomized pigs have different hormone levels at different growth stages. But the influence of sex on the biomechanical properties of the porcine proper hepatic artery has not been reported. We proposed to explore the relation between the biomechanical properties of the porcine proper hepatic artery and sex to provide a necessary biomechanical theory for choosing the donated organ in pig-to-human liver xenotransplant.

Materials and Methods

This experiment was carried out at the Laboratory of Medical Biomechanics, Hubei University of Medicine, Shiyan, China. Totally, 32 healthy Chinese Hubei white pigs¹¹ aged 2 months (16 males and 16 females; certification Nos. 00019030, 00019071, 00019073-00019076, and 00019315) were provided by the Experimental Animal Center of Hubei University of Medicine. This investigation conformed to Guide for the Care and Use of Laboratory Animals published by the National Ministry of Science and Technology (2006) 389th document. Prior to proceeding, approval of the protocol was obtained from the Care of Experimental Animals Committee at our institution.

The 32 pigs were housed under standard conditions and randomly divided into 4 groups: 8 males, 8 females, 8 castrated males (castrated by the conventional means at the age of 2 months), and 8 ovariectomized females (ovariectomized by the conventional method at the age of 2 months). When the pigs reached 7 months old, they were killed via

an intramuscular injection of ketamine (5 mg/kg). The average body weight of the pigs at age 7 months in the male group, the female group, and the gonadectomized group was 95.17 ± 10.52 kg, 83.78 ± 8.79 kg, and 85.33 ± 9.34 kg. Before the experiment, blood samples were collected from all groups and centrifuged at $2000 \times g$ for 30 minutes; the serum fraction was pipetted and stored at -20°C until all samples could be assayed simultaneously for estradiol (E_2) and testosterone (T) by radio-immunoassay.

After anatomic isolation and in situ measurement of respective in vivo length, the porcine proper hepatic arteries, from the starting point of common hepatic artery to the bifurcation point of left and right hepatic arteries, were obtained and divided into proximal, middle, and distal segments in the longitudinal direction of the vessel. The porcine proper hepatic arteries were isolated at 10 to 20 minutes after death and immediately shifted into an organ bath with oxygen-saturated Krebs's solution and then put into refrigerators at 4°C . The middle segments were used for the pressure-diameter test, the distal segments were subjected to formaldehyde-fixation for future histologic analysis, and the proximal segments were expected for future measuring of the opening angle of a zero-stress state.

The proper hepatic arteries of humans were obtained from 10 deceased donors (5 human men, 5 human women) without hepatic diseases, who were between 20 and 40 years old. The average body weight in the men and the women was 71.58 ± 7.09 kg and 65.23 ± 6.24 kg. They died by accident and were donated by the Hubei University of Medicine. The Institutional Ethics Committee at our institution approved the project, and all families of the decedents gave informed consent before participation. All protocols conformed to the ethical guidelines of the 1975 Helsinki Declaration.

Pressure-diameter test

Two ends of the proper hepatic arteries of pigs and humans were fixed on a biomechanical test equipment (produced by the Center of Technology, Dongfeng Automobile Co., Ltd. Shiyan, China).¹² One end of the proper hepatic artery was connected to an electronic peristaltic pump (HL-2D; Huxi Analyzes Instrument Factory, Shanghai, China) and a pressure transducer (CY-YB-Y; 0-20 kPa, Jinghua Electric Equipment Factory, Beijing, China) through

a 3-way apparatus to a computer. The other end was closed and fixed. The middle part of the sample stained into a black band (width, about 5 mm) with oil-soluble black pigment and was focused with a camera. The baroreceptor was adjusted to zero. The whole experiment was performed in a water bath with Krebs's solution set at a constant flow rate of 0.8 mL/min by an electronic peristaltic pump; the temperature was kept at $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. The circulating compression and decompression ranging between 0 and 25 kPa were performed on the proper hepatic artery 10 times as pretreatment with the pressure increased by 5 kPa each time. Then, the cyclic compression and decompression were performed 4 times on all the samples. We synchronously recorded the pressure-diameter data of the proper hepatic arteries using a computer system. The sampling rate was 15 times per minute. To prevent seepage, the porcine proper hepatic artery was infused and pumped at a constant speed, with intervals shortened as much as possible, when measuring each pressure cycle. The corresponding diameters of the proper hepatic arteries were obtained each time with the gradual increase of the pressure in the proper hepatic arteries. Before the experiment, the distal end of each sample was sliced into 5- μm frozen transverse sections that were rapidly prepared with a cold incubator microtome before treatment with hematoxylin-eosin (H&E) staining for 5 to 10 minutes. The diameters and thickness were measured by a computer image analysis system (Q500IW; Leica, Heidelberg, Germany).¹³

Analysis of biomechanical data

Based on the pressure-diameter data, according to the definition of Cox, Hayashi, and Bergel,¹⁴⁻¹⁶ the identical incremental elastic modulus (E_{inc}), pressure-strain elastic modulus (E_p), volume elastic modulus (E_v), and compliance (C) were calculated according to the following formulas:

$$E_{inc} = 0.75 \cdot \Delta P / \Delta R \cdot R^2 / h \quad (1)$$

$$E_p = \Delta P / \Delta R \cdot R \quad (2)$$

$$E_v = \Delta P / \Delta R \cdot R / 2 \quad (3)$$

$$C = 2\pi R \cdot \Delta R / \Delta P \quad (4)$$

Where P , R , and h are pressure (kPa) in the proper hepatic artery, radius (mm), and thickness (mm); ΔP is pressure change, and ΔR is radius change.

Statistical analyses

The measured data were analyzed statistically by different sex groups. Statistical analyses were performed with SPSS software (SPSS: An IBM Company, version 12.0, IBM Corporation, Armonk, New York, USA). All values were reported as means and standard deviation. Single-factor analysis of variance was performed for comparison among different groups, firstly, and then a significance test (Newman-Keuls method) was performed for one group versus another. The difference was considered significant at $P < .05$.

Results

Change of gonadal hormones in the different sex groups

The change of gonadal hormones of female, male, and gonadectomized pigs is shown in Table 1. Estradiol levels of the female pigs were higher than all other sex groups ($P < .01$). Similarly, testosterone level was the highest in the male pigs compared to all other sex groups ($P < .01$). There was no significant difference between castrated males and ovariectomized female pigs ($P > .05$).

Table 1. Change of gonadal hormones in different sex groups (means $\pm$ SD).

Sex groups	Estradiol (pg/mL)	Testosterone (ng/dL)
Female pigs (n=8)	60.72 $\pm$ 3.80 [†]	0.53 $\pm$ 0.01
Male pigs (n=8)	9.43 $\pm$ 1.29	982.17 $\pm$ 107.31 [‡]
Castrated male pigs (n=8)	2.54 $\pm$ 2.16	4.26 $\pm$ 0.36
Ovariectomized female pigs (n=8)	4.26 $\pm$ 0.36	2.65 $\pm$ 2.06

Single-factor analysis of variance was performed for comparison among different groups, firstly, and then a Newman-Keuls test was performed for one group versus another:

[†] $P < .01$ vs other groups, [‡] $P < .01$ vs other groups.

Relation between elastic modulus of the proper hepatic arteries of pigs and humans and pressure

The E_{inc} , E_p , and E_v of the proper hepatic arteries of female, male, gonadectomized pigs, human women, and human men are shown in Figures 1 to 3. The measured elastic modulus of the proper hepatic arteries of pigs and humans increased with increasing pressure. When the pressure reached 20 kPa and higher, the elastic modulus in all sex groups no longer fluctuated or only fluctuated slightly, and the slopes of the curves became gentle. The changing discipline of E_{inc} , E_p , and E_v with different inside pressures was similar to that of all sex groups.

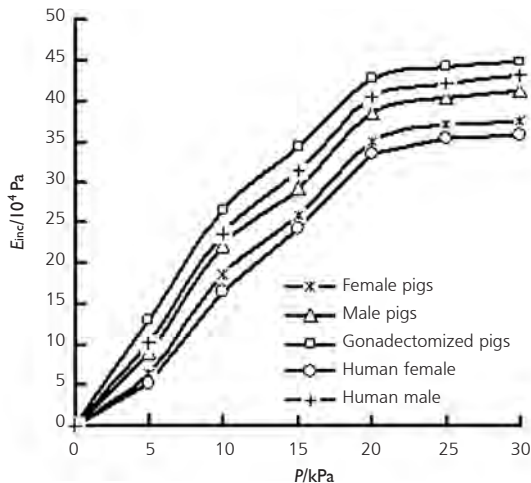


Figure 1. The relation between the incremental elastic modulus (E_{inc}) of the proper hepatic arteries of pigs and humans in different sexes and pressure (P).

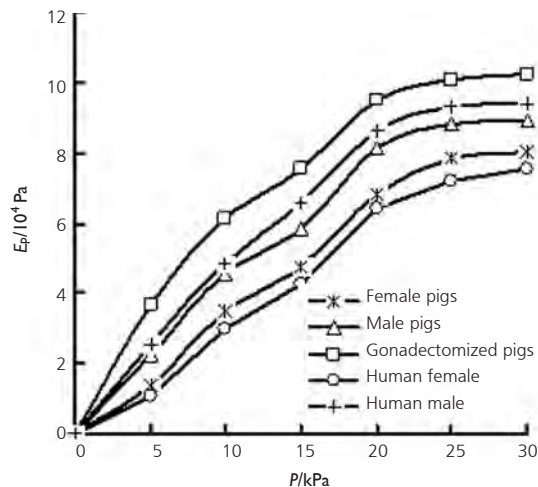


Figure 2. The relation between pressure-strain elastic modulus (E_p) of the proper hepatic arteries of pigs and humans in different sexes and pressure (P).

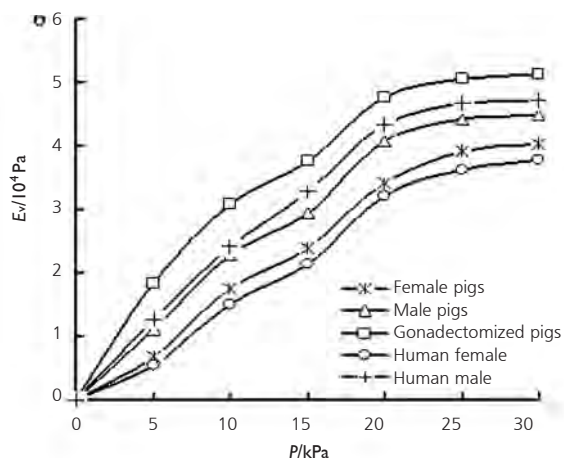


Figure 3. The relation between volume elastic modulus (E_v) of the proper hepatic arteries of pigs and humans in different sexes and pressure (P).

Comparison of elastic modulus of the proper hepatic arteries of pigs and humans of different sexes

The E_{inc} , E_p , and E_v of porcine proper hepatic artery in different sexes obviously increased along with the increase of inside pressure. When the pressure was 15 kPa, the E_{inc} , E_p , and E_v of the porcine proper hepatic artery of different sexes are shown in Table 2. Single-factor analysis of variance showed that there were significantly differences in E_{inc} ($F=10.24$; $P=.001$), E_p ($F=3.75$; $P=.001$), and E_v ($F=3.41$; $P=.002$) of the proper hepatic arteries of female, male, and gonadectomized pigs; the female group had the lowest elastic modulus and the gonadectomized group had the highest ($P<.01$). There was no difference in the elastic modulus of the proper hepatic artery between the male/female pig and the human men/women ($P>.05$).

Table 2. Comparison of elastic modulus of the proper hepatic arteries of pigs and humans in different sex groups (means $\pm$ SD, $\times 10^4$ Pa).

Sex groups	E_{inc}	E_p	E_v
Female pigs (n=8)	$28.04 \pm 0.70^{1, \dagger}$	$5.26 \pm 0.05^{1, 2}$	$2.74 \pm 0.70^{1, \dagger}$
Male pigs (n=8)	30.35 ± 0.67	5.84 ± 0.04	2.85 ± 0.67
Gonadectomized pigs (n=16)	32.37 ± 0.22	6.22 ± 0.03	3.67 ± 0.22
Human women (n=5)	$27.54 \pm 0.48^{\S}$	$4.82 \pm 0.07^{\S}$	$2.14 \pm 0.55^{\S}$
Human men (n=5)	$31.15 \pm 0.43^{\P}$	$5.98 \pm 0.06^{\P}$	$3.05 \pm 0.38^{\P}$

Abbreviations: E_{inc} , identical incremental elastic modulus; E_p , pressure-strain elastic modulus; E_v , volume elastic modulus

Single-factor analysis of variance was performed for comparison among different groups, firstly: E_{inc} ($F=10.24$; $P=.001$), E_p ($F=3.75$; $P=.001$), and E_v ($F=3.41$; $P=.002$), and then a Newman-Keuls test was performed for one group versus another.

$^{\dagger}P<.05$ vs male pigs, $^{\S}P<.01$ vs gonadectomized pigs, $^{\P}P>.05$ vs female pigs, $^{\P}P>.05$ vs male pigs.

Comparison of compliance of the proper hepatic arteries of pigs and humans in different sexes

Figure 4 illustrates the relation between compliance of proper hepatic arteries of pigs and humans and sex with the pressure of 5 to 25 kPa. It indicates that the compliance of porcine proper hepatic artery decreased with the pressure. There was a significant difference in compliance ($F=11.63$; $P=.001$) of the porcine proper hepatic arteries of different sexes at the same pressure—highest in the female group and lowest in the gonadectomized group ($P<.01$). There was no difference in the compliance of the proper hepatic artery between the male/female pigs and the human men/women ($P>.05$).

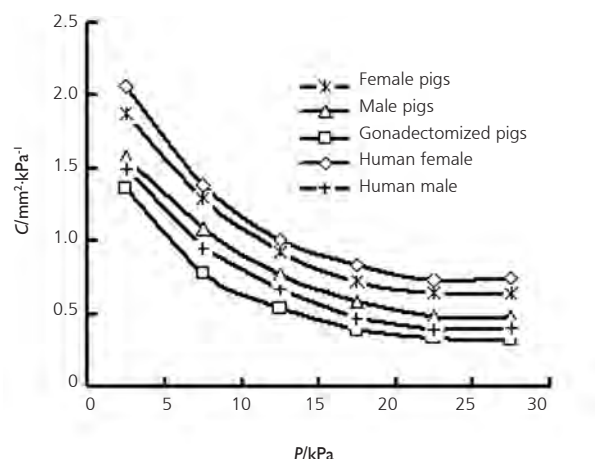


Figure 4. The relation between compliance (C) of the proper hepatic arteries of pigs and humans in different sexes and pressure (P).

Discussion

Treatment of acute and chronic liver failure remains a challenge despite modern therapeutic innovations. While liver transplant can restore liver function and improve patient survival, donor shortages limit this treatment to a few patients. Organ xenotransplant has emerged as an alternative for treating liver failure, and xenotransplant using pig organs could solve the shortage of donor organs for allotransplant. Many researchers have found that the pig is the best source of xenotransplant.¹⁷ Chinese Hubei white pigs adopted in this experiment be highly homologous with the American inbred National Institutes of Health miniature swine of the porcine major histocompatibility complex class I molecules. The analysis of major histocompatibility complex genetic heredity level shows that the swine leukocyte antigen of Chinese Hubei white pigs is highly genetically compatible with human leukocyte antigen,¹¹ so Chinese Hubei white pigs are the ideal basic research model for xenotransplant. In this study, the difference in biomechanical properties of the porcine proper hepatic artery between female, male, and gonadectomized pigs is discussed biomechanically, in the hope of providing the theoretical basis for obtaining a compatible donor organ for pig-to-human liver xenotransplant.

Comparisons on biomechanical parameters of the proper hepatic artery including the elastic modulus and compliance between female, male, gonadectomized pigs, human women, and human men have not been reported. The elastic modulus is the mathematical description of an object or

substance's tendency to be deformed elastically (ie, nonpermanently) when a force is applied to it and is a mechanical parameter that reflects the relation between stress and strain of materials. It represents hardness of materials; that is, their ability to resist distortion effects from loads. Therefore, the elastic characteristics of materials are reflected by the calculated elastic modulus.

Vascular compliance, which accommodates blood pressure changes without rupture under pressure or force, is an important marker that reflects the mechanical characteristics of a blood vessel. As a result, we can study the flexibility characteristics of blood vessels by computing the elastic modulus and the compliance.¹⁸ In this study, the curves of relations between E_{inc} , E_p , and E_v of the proper hepatic arteries of female, male, and gonadectomized pigs, and the pressure indicated that the elastic modulus increased along with increased pressure. When the pressure reached 20 kPa and higher, the elastic modulus of all sex groups no longer fluctuated (or only fluctuated slightly), and the slopes of the curves became gentle. This showed that under the same inside pressure, changes of the elastic modulus of all sex groups represented the vessel had certain flexibility and sustained certain hardness. Because the artery is flexible and rigid to some extent, the elastic modulus would not increase indefinitely as the pressure increased; thus; it maintained the elastic characteristic of the artery; that is, expandability is inversely proportional to the elastic modulus: The higher the elastic modulus, the smaller the expansibility and the poorer the elasticity. Our experiment showed that the female group had the smallest elastic modulus value, the male group had a greater value, and the gonadectomized group had the greatest value; which suggests that the proper hepatic artery of the female group had best expandability and elasticity. This may be closely associated with the microstructural components of the vessel in all sexes.

It is well known that the collagen and elastin are largely responsible for the passive mechanical properties of arteries, collagen being relatively stiff, whereas elastin is quite extensible. Collagen fiber can prevent the blood vessel from undue inflation, and elastic fiber has good extensibility, but its elastic modulus is obviously smaller than that of collagen fiber—approximately 1:500.¹⁹ Recent evidence has revealed that sex hormones have an effect on the

accumulation of vascular connective tissue and alter the proportions of collagen and elastin.⁸

Compliance of the porcine proper hepatic artery decreases with increasing pressure; this indicates that the proper hepatic artery is anisotropic, nonlinear viscoelastic material. There was a difference in compliance of the porcine proper hepatic artery in all sexes (highest in the female group and lowest in the gonadectomized group) probably owing to the effect of sex hormones on vascular connective tissue.

Many studies have demonstrated that sex differences in vascular function are entirely due to differences in sex hormones. Differences in compliance of the abdominal aorta in women and men may be attributed to a combination of differences in hormonal influence, and/or genetic structural factors.²⁰ Sex differences in arterial stiffness may be both intrinsic and influenced by sex steroids. Changes in the sex steroid profile associated with menopause have been linked with higher arterial stiffness in postmenopausal women. Together, these data suggest that sex differences in large artery stiffness are intrinsic but are modulated by both male and female sex steroids.²¹ Our data show the importance of differentiating between female, male, and gonadectomized pigs.

The compliance differences in the proper hepatic artery between the sexes could be the result of several factors. The structural variables include the vessel wall content and the wall thickness. The normal aging process of the vessel wall includes a decrease in elastin concentration, a progressive fraying of elastic lamellae, an increase in collagen content, and vessel wall thickening. Along with these degenerative changes, the distensibility of the arteries decreases with age. Thus, the compliance curves could imply that these degenerative changes are delayed in females.

Xenotransplant can provide a solution to the problem of organ shortage, but it is limited to the experimental stage rather than the clinical realm, because hyperacute rejection is difficult to control. The cloning of a pig, in August 2000, demonstrated that with genetic engineering, it is possible for pigs to become an organ source for xenografts without hyperacute rejection.²² After rejection has been avoided, another important problem is the physiological compatibility; namely, whether the pig liver can function adequately in the new recipient. In future studies, more attention must be concentrated

on identifying physiologic differences, including biomechanical properties.

With development of liver transplant in recent years, skilled surgery, highly developed anesthesia, and continually rising surgical success rates have brought hope to end-stage liver patients. But complications after the operation are still important factors that affect short-term and long-term curative effects of liver transplant. In particular, hepatic artery thrombosis and hepatic artery stenosis are the most serious complications, which are keys to successful liver transplant. Whether a pig-to-human liver xenotransplant can exert normal functions is closely related to the blood vessel reconstruction and the patency rate. The patency rate is commonly used as a criterion to evaluate the success or failure of a blood vessel reconstruction.

Some studies²³ have indicated that anastomosis, pleriosis, and transplant of the blood vessel are involved in alterations in the biomechanical properties of blood vessel walls. Therefore, the biomechanical properties either directly or indirectly affect the patency rate of a reconstructed hepatic artery. Because hepatic arterial complications of liver transplant might be related with the compatibility of the biomechanical properties of the hepatic artery between the donor and the receiver, the choice of the hepatic artery with the matched biomechanical properties for reconstruction might also be one vital factor for successful liver transplant.

The difference in biomechanical properties between the donor and the receiver may cause a dysfunction or incomplete compensation of transplants, so successful anastomosis and reconstruction of the hepatic artery are crucial for enhancing the survival rate of the transplanted liver and ensuring normal function. If the biomechanical properties of the hepatic arteries do not match, it may even cause transplant failure.

Sex hormones interact with the organs in the whole body. According to Fung's "stress-growth" law,²⁴ the biomechanical properties of the porcine proper hepatic artery of different sexes should be different. They adjust the growth and function of the blood vessel through the genetic and nongenetic mechanisms, change the contents and structures of connective tissues of the vessel wall, and subsequently cause the difference in the biomechanical properties of the blood vessel.

This study indicates that there were differences in the biomechanical properties of the proper hepatic arteries of female, male, and gonadectomized pigs, with the female group having the highest compliance and the gonadectomized group the lowest. The biomechanical properties of the human male/female proper hepatic artery were similar to those of the porcine males/females.

Researchers²⁵ in Switzerland found that the matched sexes of donors and recipients in kidney transplant would provide for better graft survival and clinical effects. This suggests that consideration of sex should be integrated into future prospective analyses and decisions about organ allocation. So, the pig of the matched sex should be chosen as the donor so that the biomechanical properties of the proper hepatic artery between the donor and the receiver were possibly similar to reduce hepatic arterial complications and enhance the survival of the transplanted liver in a pig-to-human liver xenotransplant.

Our results suggest that there were differences in the biomechanical properties of the proper hepatic arteries of female, male, and gonadectomized pigs. The biomechanical properties of the human male/female proper hepatic artery match those of the porcine male/female ones. The correlation between sex and biomechanical properties of the proper hepatic artery in pigs could imply that a pig of the same sex should be chosen for pig-to-human liver xenotransplant.

References

- Cooper DK, Teuteberg JJ. Pig heart xenotransplantation as a bridge to allotransplantation. *J Heart Lung Transplant*. 2010;29(8):838-840.
- Rocha H, Eliziário LF, Wafae GC, Silva NC, Ruiz CR, Wafae N. Anatomy of the septomarginal trabecula in Landrace pig hearts. *Morphologie*. 2010;94(305):26-29.
- Diswall M, Angström J, Karlsson H, et al. Structural characterization of alpha1,3-galactosyltransferase knockout pig heart and kidney glycolipids and their reactivity with human and baboon antibodies. *Xenotransplantation*. 2010;17(1):48-60.
- Li WC, Yu MH, Zhang HM, et al. Biomechanical properties of ascending aorta and pulmonary trunk in pigs and humans. *Xenotransplantation*. 2008;15(6):384-389.
- Wang PJ, He F, Liao DH, et al. The impact of age on incremental elastic modulus and incremental compliance of pig hepatic portal vein for liver xenotransplantation. *Xenotransplantation*. 2009;16(1):5-10.
- Hara H, Gridelli B, Lin YJ, Marcos A, Cooper DK. Liver xenografts for the treatment of acute liver failure: clinical and experimental experience and remaining immunologic barriers. *Liver Transpl*. 2008;14(4):425-434.
- Lunney JK. Advances in swine biomedical model genomics. *Int J Biol Sci*. 2007;3(3):179-184.
- Natoli AK, Medley TL, Ahimastos AA, et al. Sex steroids modulate human aortic smooth muscle cell matrix protein deposition and matrix metalloproteinase expression. *Hypertension*. 2005;46(5):1129-1134.
- Bronson RE, Calaman SD, Traish AM, Kagan HM. Stimulation of lysyl oxidase (EC 1.4.3.13) activity by testosterone and characterization of androgen receptors in cultured calf aorta smooth-muscle cells. *Biochem J*. 1987;244(2):317-323.
- Acs N, Székács B, Nádas GL, et al. Effects of combined sex hormone replacement therapy on small artery biomechanics in pharmacologically ovariectomized rats. *Maturitas*. 2000;34(1):83-92.
- Wu Q, Xiong P, Gong FL, Liu J, Chen S. Different results in polymorphism analyses of SLA class I molecules between two xenotransplantation models. *Transplant Proc*. 2003;35(1):536-537.
- Li WC, Ruan XZ, Zhang HM, Zeng YJ. Biomechanical properties of different segments of human umbilical cord vein and its value for clinical application. *J Biomed Mater Res B Appl Biomater*. 2006;76(1):93-97.
- Li WC, Zhang HM, Wang PJ, et al. Quantitative analysis of the microstructure of human umbilical vein for assessing feasibility as vessel substitute. *Ann Vasc Surg*. 2008;22(3):417-424.
- Cox RH. Passive mechanics and connective tissue composition of canine arteries. *Am J Physiol*. 1978;234(5):H533-H541.
- Hayashi K, Handa H, Nagasawa S, Okumura A, Moritake K. Stiffness and elastic behavior of human intracranial and extracranial arteries. *J Biomech*. 1980;13(2):175-184.
- Bergel DH. The static elastic properties of the arterial wall. *J Physiol*. 1961;156(3):445-457.
- Ekser B, Rigotti P, Gridelli B, Cooper DK. Xenotransplantation of solid organs in the pig-to-primate model. *Transpl Immunol*. 2009;21(2):87-92.
- Yu Q, Zhou J, Fung YC. Neutral axis location in bending and Young's modulus of different layers of arterial wall. *Am J Physiol*. 1993;265(1 Pt 2):H52-H60.
- Wan W, Yanagisawa H, Gleason RL Jr. Biomechanical and microstructural properties of common carotid arteries from fibulin-5 null mice. *Ann Biomed Eng*. 2010;38(12):3605-3617.
- Sonesson B, Länne T, Vernerström E, Hansen F. Sex difference in the mechanical properties of the abdominal aorta in human beings. *J Vasc Surg*. 1994;20(6):959-969.
- Ahimastos AA, Formosa M, Dart AM, Kingwell BA. Gender differences in large artery stiffness pre- and post puberty. *J Clin Endocrinol Metab*. 2003;88(11):5375-5380.
- Butcher J. Scientists propose two ways to clone a pig. *Lancet*. 2000;356(9230):657.
- Haruguchi H, Teraoka S. Intimal hyperplasia and hemodynamic factors in arterial bypass and arteriovenous grafts: a review. *J Artif Organs*. 2003;6(4):227-235.
- Fung YC. What are the residual stresses doing in our blood vessels? *Ann Biomed Eng*. 1991;19(3):237-249.
- Gratwohl A, Döhler B, Stern M, Opelz G. H-Y as a minor histocompatibility antigen in kidney transplantation: a retrospective cohort study. *Lancet*. 2008;372(9632):49-53.