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● *Original Contribution*

DYNAMIC CONTRAST-ENHANCED ULTRASOUND OF SLAUGHTERHOUSE PORCINE LIVERS IN MACHINE PERFUSION

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Abstract—The aim of this study was to enable investigations into novel imaging and surgical techniques by developing a readily accessible, versatile liver machine perfusion system. Slaughterhouse pig livers were used, and dynamic contrast-enhanced ultrasound was introduced to optimize the procurement process and provide real-time perfusion monitoring. The system comprised a single pump, oxygenator, bubble trap and two flowmeters for pressure-controlled perfusion of the vessels using an off-the-shelf perfusate at room temperature. Successful livers exhibited homogeneous perfusion in both the portal vein and hepatic artery with dynamic contrast-enhanced ultrasound, which correlated with stable oxygen uptake, bile production and hepatic resistance and normal histology at the end of 3 h of perfusion. Dynamic contrast-enhanced ultrasound revealed perfusion abnormalities invisible to the naked eye, thereby providing context to the otherwise systemic biochemical/hemodynamic measurements and focal biopsy findings. The model developed here is a simple, cost-effective approach for stable *ex vivo* whole-organ machine perfusion. (E-mail: Maverk@ucy.ac.cy)

Key Words: **Imaging, Hepatic, Abattoir.**

INTRODUCTION

Machine perfusion (MP) functions as an artificial body with a wholly or partially artificial blood supply to sustain an isolated organ *ex vivo*. Originally an experimental technique to study organ metabolism (Hems et al. 1966; Woods and Krebs 1971), MP has gained a foothold in the clinic, where it is currently being investigated as a donor organ preservation modality that is superior to the gold standard of static cold storage (Fondevila et al. 2011; Groen et al. 2012; Guarrera et al. 2009; Kruger et al. 2013). MP also has the potential to operate as a highly versatile test platform in the medical technology and pharmaceutical industries, because it enables direct access to the organ for targeted diagnostic, imaging and therapeutic investigations (Czymek et al. 2011; Grosse-Siestrup et al. 2004). Access to MP has generally been limited to laboratories with veterinary personnel, operating rooms and housing facilities capable of procuring and handling large animals as a source of human-sized organs (Imber et al. 2002; Schlegel et al. 2013; van der Plaats et al. 2006; Xu et al. 2012). The use of slaughterhouse animals as an alternative source of organs would minimize the regulatory requirements, operational costs and ethical burden associated with live animal surgery, but very limited data are available regarding such models. In particular, the viability of the organ is frequently questioned given the obligatory warm ischemia the organs experience before being isolated from the animals. In 1993, Peter Neuhaus' group compared the isolation of hepatocytes from abattoir porcine livers with that of livers perfused *in situ* (Gerlach et al. 1993). With cell isolation as a quantitative metric of a liver's viable cell content, livers from the abattoir produced cells with significantly higher rates of damage and in lower yields. However, a follow-up article in 1994 indicated a marked improvement in cell yield when procured livers were exposed to 3 h of normothermic (37°C) MP before cell isolation (Schön et al. 1994). In 2001, the same group published a seminal paper

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that reported that unless livers with 60 min of warm ischemic damage were treated with MP, they could not be successfully transplanted (a laboratory animal model was used in this case) (Schön et al. 2001). MP therefore has the capacity to perfuse slaughterhouse organs stably, serving as a platform for whole-organ experimentation. To increase MP accessibility, we scaled up a recently developed sub-normothermic (20°C–30°C) rat liver MP system that was as effective at recovering ischemic damage as normothermic perfusion but simpler to operate (Berendsen et al. 2012; Izamis et al. 2012; Tolboom et al. 2007). A major limitation of this approach, however, is an inability to visually inspect perfusion quality. Erythrocytes, natural but highly variable contrast agents, are unnecessary at sub-normothermic conditions and are excluded from the perfusate, while the decrease in surface area-to-volume ratio in comparing a 10-g rat liver with a 1500-g porcine liver implies less of the liver is exposed to the naked eye. To overcome this visual limitation, we introduced dynamic contrast enhanced ultrasound (DCEUS) into machine perfusion. DCEUS measurements proved to be indispensable in evaluating an organ's structural and functional integrity, as they contextualized systemic perfusate and focal biopsy results. Simply by placing a transducer directly on the organ's capsule, structural variations could be identified and monitored while the extent of perfusion in each of the hepatic artery and portal vein was quantitatively visualized in real time. Here we describe in detail the methodologies of porcine liver procurement from the slaughterhouse, stable organ perfusion with asanguineous room temperature MP and evaluation of perfusion quality with DCEUS.

METHODS

Choice of livers and abattoir

The studies were approved by the Cyprus National Bioethics Committee, the Cyprus National Veterinary Services and the state abattoir of Kofinou, Larnaca.

Porcine livers were preferred over livers from other species because they are more similar to human livers with respect to physiology and anatomy (Table 1) (Boxenbaum 1980). The method described here was developed with n = 30 routinely slaughtered 150- to 200-d-old castrated males of a variety of porcine strains. The procured livers weighed between 1200 and 1800 g (adult human livers range between 1500 g for females and 1800 g for males) (Price et al. 2003). Porcine livers are reported to receive an average flow of 1–1.5 mL/min/g liver, with approximately 10%–23% of the blood flow being contributed by the hepatic artery (HA). Porcine liver mean arterial pressure is 122 cm H_2O (90 mm Hg), and portal pressure is $<$ 12 cm H_2O (9 mm Hg) (Jakob et al. 2012; Yagi et al. 2012). By comparison, human livers receive approximately 0.8 mL/min/g liver, with 25% deriving from the HA (Upton 2008). Human liver mean arterial pressure is 136 cm H_2O (100 mm Hg), and the average portal pressure is 7 cm H_2O (5 mm Hg). Ruminants, such as goats and sheep, were not considered for this study because their livers are not comparable in size to human livers and have different anatomy and hemodynamics pertaining to the central, middle and left portions of the liver (Brizard et al. 2000).

Procuring and flushing an intact liver

The process of liver procurement did not interfere significantly with the normal abattoir protocol. Briefly, animals were stunned electrically, exsanguinated, cleaned in a water bath, buffed and momentarily singed to remove bristles. The abdomen and thorax were exposed with a longitudinal dermal incision. To minimize the risk of damaging the liver during procurement, we requested that the trachea, lungs, heart, aorta, diaphragm and esophagus in the thorax, along with the stomach, duodenum, pancreas, kidneys and spleen in the abdomen, be removed *en bloc*. The organs were placed into an awaiting container, and the liver was inspected for disqualifying tears/cuts, particularly at the sites of the hepatic ligaments. The liver was isolated from the other organs

Table 1. Comparison of livers from humans and commonly available farm species

	Human	Pigs	Goat	Sheep
Liver weight (g)	1500–1800 (Price et al. 2003)	700–1500 (Soleimani et al. 2012)	600–700 (El-Waziry et al. 2011)	400–700 (Barnes et al. 1983; Upton 2008)
Total flow rate (mL/min/g liver)	0.8 (Upton 2008)	1–1.5 (Nickkholgh et al. 2012; Upton 2008)	1–5 (Sastradipradja et al. 1997)	4–9 (Barnes et al. 1983; Upton 2008)
Hepatic artery flow rate (% of total hepatic flow)	20–25 (Moore and Dalley 2006)	10–23 (Jakob et al. 2012; Nickkholgh et al. 2012)	Not found	1–21 (Burrin et al. 1989)
Mean arterial pressure (mm Hg)	100 (Costanzo 2010)	60–90 (Jakob et al. 2012; Nickkholgh et al. 2012)	95–100 (Brizard et al. 2000; Mabuchi et al. 1989)	97 (Barnes et al. 1983)
Portal pressure (mm Hg)	5 (Vincent et al. 2011)	9 (Jakob et al. 2012; Yagi et al. 2012)	Not found	Not found