

Gut

Leading article

Changes in gut function during hibernation: implications for bowel transplantation and surgery

Organ transplantation has become routine in many centres throughout the world. Although success rates for intestinal transplantation are generally lower than for other organs, it is still high enough to warrant this form of therapy for patients with short bowel syndrome or other untreatable bowel diseases. Much of the success in transplantation is due to the impact of immunosuppressive drugs as well as the development of new surgical techniques and new methods of organ preservation. The intestine has been preserved successfully for about 24 hours, as judged by *in vitro* viability assessment¹ and by survival after transplantation.² However, problems associated with extended cold storage, ischaemia/reperfusion injury, and immunological rejection of grafts still limit the optimal use of intestinal transplantation. The benefit of hypothermic organ preservation is generally attributed to the reduction in oxygen and nutrient demands and in the rates of potentially damaging enzyme catalysed reactions. However, lack of oxygen in cold stored organs gradually leads to a time dependent loss of ATP, with rates of degradative reactions exceeding biosynthesis. In addition, prolonged hypothermia alters cell structure (for example, cytoskeletal proteins), reduces ion pump activity, and slows ATP synthesis. These changes, coupled with oxidative stress secondary to ischaemia-reperfusion injury and immunological factors, eventually lead to loss of metabolic control and critical membrane functions that compromise organ survival on reperfusion. The result for the cold stored transplanted bowel is too often mucosal destruction, loss of absorptive and barrier function, and eventual graft rejection.

Improvements in intestinal preservation could increase organ availability and perhaps more importantly the likelihood that a transplant is successful. To this end, novel insights may be found in animals that naturally experience extreme changes in physiology, particularly hypoxia and hypothermia. Mammalian hibernators offer the unique opportunity to examine what is essentially Nature's version of organ preservation. Indeed, their ability to regularly undergo extended periods of hypothermia and hypometabolism is one of the most remarkable adaptations displayed by mammals. Each winter hibernators such as ground squirrels cease feeding, subsist on fat stores alone, and undergo periodic bouts of torpor that last for a few days to almost a month. Torpor bouts are characterised by depressed metabolism (2–4% of normal) and minimal body temperatures that are very similar to those used for organ preservation (2–4°C). Although hibernators are well adapted and in fact rely on these dramatic changes each

year for survival, several aspects of hibernation could be considered stressful to the gut, including extended fasting, prolonged hypothermia, rapid changes in metabolism, and redistribution of blood flow. Knowledge of how hibernation affects the intestine as well as the gut's response to potential stressors associated with this extreme adaptation may stimulate new approaches to improve success rates of intestinal surgery, including transplantation.

The hibernating gut responds to the long term absence of luminal nutrition during the winter fast by gradual atrophy of the mucosa, due to reductions in villus height and crypt depth.^{3–4} Yet the overall architecture of the epithelium is well preserved with enterocyte microvillus height unchanged and microvillus density slightly increased.⁵ This latter effect may be related to induction of the membrane cytoskeletal linking protein, moesin, in the enterocyte brush border of torpid hibernators. Moesin and the related ezrin-radixin-moesin protein, ezrin, are associated with membrane protrusions and microvilli and are thought to participate in their formation. In adult mammals, including ground squirrels of all activity states, ezrin (but not moesin) is found constitutively in the brush border membrane of enterocytes.⁶ Interestingly, moesin is expressed in the fetal gut of mammals but its levels normally fall at birth when ezrin expression rises.⁷ Thus it is tempting to speculate that expression of moesin in enterocytes during torpor reflects a transient return to the fetal condition, consistent with the extended period of inactivity of the digestive tract during this time.

Epithelial function is also well maintained in the hibernating gut. Activities of digestive enzymes are only slightly reduced if at all during hibernation.^{8–9} Although epithelial transport is suppressed during deep torpor, when tissues are warmed *in vitro* to 37°C (simulating arousal) rates of ion and nutrient transport return to summer values.^{3–4, 10} This is likely due in part to maintenance of plasma membrane ion gradients during torpor that are typically lost when cells from non-hibernators are cooled to low temperatures.¹¹ In addition, adenylate pools remain stable throughout torpor-arousal cycles, with minimal or no reduction in ATP levels nor any change in the [ATP]:[ADP] ratio¹² that could compromise active transport when normal body temperature is restored. At the molecular level, protein and mRNA levels of sucrase-isomaltase and the sodium-glucose transporter SGLT1 are preserved throughout hibernation,⁹ and sugar and amino acid absorption (normalised to mucosal protein) is actually

Abbreviations used in this paper: NFκB, nuclear factor κB.

enhanced in the hibernating gut.³ This evolutionary strategy of reducing overall biosynthetic costs through reduction in tissue mass, yet preserving tissue *function*, permits utilisation of any nutrients that may be present in the gut during hibernation (for example, from sloughed enterocytes) as well as the rapid resumption of normal digestive capacity in the spring when hibernation ceases.

Intestinal ischaemia-reperfusion, both for the normothermic and hypothermic intestine, results in characteristic biochemical alterations including oxygen radical formation¹³ and changes in lipid mediator synthesis.^{14,15} What happens to intestinal blood flow during hibernation? As hibernators enter torpor heart rate falls to as low as 4–6 beats/min with corresponding effects on cardiac output. Blood flow to the gut is reduced, remains very low throughout the bout, and then is restored rapidly as animals arouse. Because flow is preferentially shunted to anterior organs (for example, heart, diaphragm, brown fat) both during torpor and arousal, intestinal blood flow is disproportionately low compared with other organs and the gut is one of the last organs to receive normal flow when animals return to normothermia.¹⁶ Because torpid hibernators remain in a state of aerobic metabolism, and overall changes in tissue perfusion generally match the onset of hypothermia, it is unlikely that the intestine experiences true ischaemia during torpor. On the other hand, it is not known whether the shunting of blood flow from the gut during entrance into torpor or the rapid resumption of flow during arousal result in small but significant imbalances between oxygen delivery and oxygen demand. As demonstrated for brain, a regulated arrest of protein synthesis occurs as animals enter torpor which presumably minimises the potential for ischaemic damage due to the profound reduction in cerebral perfusion.¹⁷

Perhaps the most characteristic functional derangement associated with intestinal ischaemia and reperfusion is the large increase in epithelial permeability.¹⁸ In hibernators, ionic permeability of the small intestine, as measured by tissue conductance, is increased by about 1.5-fold when tissues are warmed in vitro to 37°C.^{3,4} Because fasting for as little as two days increases ionic conductance by a similar amount,¹⁹ the change in gut permeability during hibernation is not nearly as large as might be expected based on the length of the winter fast, nor is it of comparable magnitude to the permeability changes induced by ischaemia-reperfusion injury.

Although tissues of hibernators may not experience true ischaemia, there are several indications that the intestinal mucosa is vulnerable to stress, including oxidative stress, during hibernation. These include induction of at least two stress proteins (hsp70 and GRP75), increased levels of conjugated dienes (a measure of lipid peroxidation), and a change in glutathione redox balance (a decrease in GSH/GSSG ratio).^{20,21} These effects are already apparent as active animals enter torpor and are less obvious during arousal when one might expect reperfusion injury to occur. Because the intestine is not overtly damaged during hibernation, we speculate that these changes may reflect a moderate degree of oxidative stress to the gut induced by some aspect of torpor-arousal cycles that subsequently activates defence mechanisms to preserve tissue viability. In keeping with this idea, antioxidant levels are increased in several tissues during hibernation, including brain, liver, and brown adipose tissue, further suggesting that some aspect of torpor-arousal cycles induces oxidative stress that the hibernator senses and responds to.

Another indication that the intestine responds to stress signals during hibernation is the marked nuclear translocation of the stress inducible transcription factor nuclear factor κ B (NF κ B), which is only mildly activated in the

mucosa of summer animals.²¹ Significant activation of NF κ B in the gut is evident as animals enter torpor, is high throughout a torpor bout, and is lowest in hibernators arousing from torpor.²¹ Although activation of NF κ B is associated with a variety of enteric diseases including ischaemia-reperfusion injury²² and several inflammatory states,²³ we suspect that NF κ B activation plays a protective role for the gut during hibernation. Many of the gene products induced by NF κ B regulate immune cell trafficking and apoptosis, and there is preliminary evidence that both are altered in the gut during hibernation.²⁰ Enterocyte apoptosis appears to increase during hibernation which suggests that NF κ B activation may be a mechanism to promote enterocyte survival during this time. There is also a dramatic increase in lamina propria and intraepithelial lymphocytes in the mucosa during hibernation²⁰ but little evidence of infiltration of polymorphonuclear leucocytes or other immune elements that are associated with inflammation. It is possible that the change in the mucosal immune system during hibernation reflects an adaptive response that helps preserve gut function during this time, possibly through release of cytokines, growth factors, or other molecules that promote epithelial growth and integrity. Finally, aging may be an additional stress to the intestinal epithelium during hibernation. Enterocyte proliferation and migration to villus tips are virtually zero during torpor, with both processes resuming rapidly during arousal.⁹ However, the duration of typical normothermic periods (less than 24 hours) prevents most cells from reaching the villus tip and cells are arrested in the position they hold when torpor resumes. Full turnover of villi can take as long as three weeks in a hibernating squirrel compared with the typical turnover time of 3–5 days for non-hibernators (including summer squirrels). Thus although “physiologically young”, enterocytes of hibernating mammals are chronologically much older than the typical enterocyte of a non-hibernator.

Are organs from hibernators superior in their tolerance to cold preservation and transplantation compared with organs from non-hibernators? Although there are no data for the gut, work by Green²⁴ has provided provocative preliminary data that kidneys from hibernating ground squirrels are more tolerant to three days of hypothermic storage (as tested by survival after transplantation) compared with organs from normothermic squirrels, rats, or rabbits. Recent work in our laboratory has compared the tolerance of livers from normothermic rats and torpid ground squirrels to hypothermic storage in the University of Wisconsin solution. Rat livers when tested in an isolated perfusion system showed significant cellular damage (that is, enzyme release, depressed bile production) after 48 hours of cold storage. In contrast, ground squirrel livers remained viable after 96 hours of cold storage, and longer storage times have not yet been studied. This is important because it could be argued that organs of hibernators survive extended hypothermia in vivo because of the continuous (albeit greatly reduced) perfusion of tissues with oxygen and nutrients (fatty acids). Demonstration that the hibernator liver ex vivo is still superior to the rat suggests that specific changes, presumably at the molecular level, have occurred prior to hibernation that preserve organ viability in this altered state. Thus evidence is accumulating to suggest that organs of hibernators are more tolerant to conditions used for clinical organ preservation. Elucidation of the mechanism(s) that underlies this increased tolerance, and strategies to adapt this information to organ preservation could have a profound effect on the quality, duration, and availability of human organs, including the gut, for transplantation. Similarly, better understanding of the adaptive basis for specific hibernation patterns should

provide further insights. For example, the significance of periodic arousals to normal body temperature and metabolism during the hibernation season is not known but they appear to be essential because they are characteristic of all mammalian hibernators and they consume a large fraction (~80%) of the hibernator's stored energy (white adipose tissue). It is intriguing to speculate whether periodic arousals constitute a form of "in vivo preconditioning" that induce cellular defence pathways and thereby minimise tissue damage during extended hypothermia and hypometabolism.

H V CAREY

Department of Comparative Biosciences,
School of Veterinary Medicine, University of Wisconsin,
Madison, WI 53706, USA

M J MANGINO
J H SOUTHARD

Division of Transplantation Surgery,
Medical School, University of Wisconsin,
Madison, WI 53706, USA

Correspondence to: Dr H V Carey.
careyh@svm.vetmed.wisc.edu

- 1 Kawashima Y, Takeyoshi I, Furukawa H, *et al.* Cold preservation of the human colon and ileum with University of Wisconsin solution. *Clin Transpl* 1999;13:420–5.
- 2 Takeyoshi I, Zhang S, Nomoto M, *et al.* Mucosal damage and recovery of the intestine after prolonged preservation and transplantation in dogs. *Transplantation* 2001;71:1–7.
- 3 Carey HV. Seasonal changes in mucosal structure and function in ground squirrel intestine. *Am J Physiol Regul Integr Comp Physiol* 1990;259:R385–92.
- 4 Carey HV. Effects of fasting and hibernation on ion secretion in ground squirrel intestine. *Am J Physiol Regul Integr Comp Physiol* 1992;263:R1202–8.
- 5 Carey HV, Sills NS. Hibernation enhances D-glucose transport by intestinal brush border membrane vesicles in ground squirrels. *J Comp Physiol (B)* 1996;166:254–61.
- 6 Gorham DA, Bretscher A, Carey HV. Hibernation induces expression of moesin in intestinal epithelial cells. *Cryobiology* 1998;37:146–54.
- 7 Barilà D, Murgia C, Nobili F, *et al.* Transcriptional regulation of the ezrin gene during rat intestinal development and epithelial differentiation. *Biochim Biophys Acta* 1995;1263:133–40.
- 8 Galluser M, Raul F, Canguilhem B. Adaptation of intestinal enzymes to seasonal and dietary changes in a hibernator: the European hamster (*Cricetus cricetus*). *J Comp Physiol (B)* 1988;158:143–9.
- 9 Carey HV, Martin SL. Preservation of intestinal gene expression during hibernation. *Am J Physiol Gastrointest Liver Physiol* 1996;271:G805–13.
- 10 Carey HV, Cooke HJ. Intestinal secretion after jejunal bypass in the ground squirrel. *Am J Physiol Regul Integr Comp Physiol* 1992;263:R1209–14.
- 11 Willis JS. Hibernation: Cellular aspects. *Ann Rev Physiol* 1979;41:275–86.
- 12 Staples JF, Hochachka PW. Liver energy metabolism during hibernation in the golden-mantled ground squirrel, *Spermophilus lateralis*. *Can J Zool* 1997;75:1059–65.
- 13 Carden DL, Granger DN. Pathophysiology of ischaemia-reperfusion injury. *J Pathol* 2000;190:255–66.
- 14 Kurose I, Argenbright LW, Wolf R, *et al.* Ischemia/reperfusion-induced microvascular dysfunction: role of oxidants and lipid mediators. *Am J Physiol* 1997;272:H2976–82.
- 15 Mangino MJ, Mangino JE, Murphy MK, *et al.* Arachidonic acid metabolism in intestinal hypothermic preservation injury. *Cryobiology* 1996;33:404–12.
- 16 Bullard RW, Funkhouser GE. Estimated regional blood flow by rubidium 86 distribution during arousal from hibernation. *Am J Physiol* 1962;203:266–70.
- 17 Frerichs KU, Smith CB, Brenner M, *et al.* Suppression of protein synthesis in brain during hibernation involves inhibition of protein initiation and elongation. *Proc Natl Acad Sci USA* 1998;95:14511–16.
- 18 Mangino JE, Kotadia B, Mangino MJ. Characterization of hypothermic intestinal ischemia-reperfusion injury in dogs. Effects of glycine. *Transplantation* 1996;62:173–8.
- 19 Ferraris RP, Carey HV. Intestinal transport during fasting and malnutrition. *Ann Rev Nutr* 2000;20:195–219.
- 20 Carey HV, Frank CL, Aw TY. Cellular response to metabolic stress in hibernating mammals. In: Heldmaier G, Klaus S, Klingenspor M, eds. *Life in the cold: 11th International Hibernation Symposium*. Berlin: Springer, 2000:339–46.
- 21 Carey HV, Frank CL, Seifert J. Hibernation induces oxidative stress and activation of NF- κ B in ground squirrel intestine. *J Comp Physiol (B)* 2000;170:551–9.
- 22 Yeh KY, Yeh M, Glass J, *et al.* Rapid activation of NF- κ B and AP-1 and target gene expression in posts ischemic rat intestine. *Gastroenterology* 2000;118:525–34.
- 23 Neurath MF, Becker C, Barbutescu K. Role of NF- κ B in immune and inflammatory responses in the gut. *Gut* 1998;43:856–60.
- 24 Green C. Mammalian hibernation: Lessons for organ preservation? *Cryo Lett* 2000;21:91–8.