

AMP-Activated Protein Kinase as a Target for Preconditioning in Transplantation Medicine

Hjalmar R. Bouma,^{1,4} Marlies E. Ketelaar,^{1,2} Benito A. Yard,² Rutger J. Ploeg,³ and Robert H. Henning¹

Graft quality before transplantation is a major factor influencing chronic rejection. Organ preservation and ischemia/reperfusion play an important role in the induction of organ injury. Although both suppression of metabolism by hypothermic preservation and preconditioning before ischemia limit injury, understanding the biochemical signaling pathways will allow us to optimize graft preservation further. Adenosine monophosphate-activated protein kinase (AMPK) is an important enzyme sensing cellular energy balance and regulating downstream signaling pathways, signaling toward an energy-conserving state. In this review, we summarize available literature regarding the protective signaling pathways activated by (hypothermic) ischemia and preconditioning and how they can be activated pharmacologically. Optimizing the graft quality before transplantation improves long-term graft survival. The major factor influencing organ quality is organ preservation, cold storage, currently, being a common practice. Loss of cellular homeostasis, inflammation, and endothelial dysfunction are the major factors inducing injury after cold storage. Adenosine triphosphate depletion and anaerobic metabolism during the cold ischemic period lead to mitochondrial dysfunction, disturbed osmoregulation, and cell death inducing inflammation. Ischemic preconditioning consists of brief periods of ischemia preceding preservation and protects organs against injury because of subsequent ischemia/reperfusion, in which endothelial nitric oxide synthase, nuclear factor- κ B, and adenosine play a major role. After conversion of adenosine to AMP, AMPK can be activated, a central kinase involved in sensing cellular [AMP]:[adenosine triphosphate] levels and signaling toward an energy-conserving state. Pharmacologic activation of AMPK demonstrated its ability to activate endothelial nitric oxide synthase and inhibit nuclear factor- κ B, thereby limiting endothelial dysfunction and inflammation. Further, studies in knock-out mice lacking ENTDP1 and NT5E (enzymes catalyzing formation and degradation of AMP, respectively) demonstrated a clear protective role for AMP in ischemia/reperfusion. AMPK activation before or during organ preservation might be a promising pharmacologic approach to limit organ injury and maintain graft quality before transplantation.

Keywords: Ischemia, Reperfusion, Preservation, AMPK, Adenosine.

(*Transplantation* 2010;90: 353–358)

Organ transplantation has become the preferred treatment for end-stage organ failure. Acute vascular rejection and chronic transplant dysfunction remain the most relevant risk factors for long-term graft survival (1, 2). Data from the Organ Procurement and Transplantation Network show graft survival rates for primary kidney transplants of

92%, 82%, and 72% after 1, 3, and 5 years, respectively (3). Retransplantation after chronic transplant dysfunction, characterized by a relative slow rate of decline in organ function, accounts for about one tenth of all kidney transplantations performed (2). Although the degree of human leukocyte antigen matching influences long-term graft survival, particularly when organs from postmortem donors are used, only a 10% difference in graft survival rates between the best and worst matched human leukocyte antigen pairs is found (4). This suggests that additional factors to immunogenicity are significant for long-term graft survival after transplantation. Pretransplantation injury of organ allografts is now generally considered as an important factor influencing long-term allograft survival and is influenced by both preservation-related factors, for example, ischemia/reperfusion injury, and donor-related factors such as age and type of donor, for example, after living, cardiac, or brain-dead donation (5). In recent years, improvements in preservation methods have led to improved allocation of donor organs. Longer preservation times facilitate the allocation of grafts to recipients in other centers. To date, cold static storage is commonly used to limit organ injury by suppressing cell metabolism under

The first two authors contributed equally to the article.

H.R.B. and M.E.K. participated in collecting literature and writing of the article; and B.A.Y., R.J.P., and R.H.H. revised and reviewed the article.

¹ Department of Clinical Pharmacology, Groningen University Institute of Drug Exploration (GUIDE), University Medical Center Groningen, Groningen, The Netherlands.

² Vth Medical Department, University Hospital Mannheim, Mannheim, Germany.

³ Department of Surgery/Section Transplantation surgery, University Medical Center Groningen, Groningen, The Netherlands.

⁴ Address correspondence to: Hjalmar R. Bouma, B.Sc., Department of Clinical Pharmacology, University Medical Center Groningen, Antonius Deusinglaan 1/FB20, 9713 AV, Groningen, The Netherlands.

E-mail: h.r.bouma@med.umcg.nl

Received 7 January 2010. Revision requested 10 March 2010.

Accepted 12 May 2010.

Copyright © 2010 by Lippincott Williams & Wilkins

ISSN 0041-1337/10/9004-353

DOI: 10.1097/TP.0b013e3181e7a3aa

the ischemic conditions of preservation (4). However, it has been well documented that prolonged cold storage may lead to tissue damage and inferior long-term graft survival after transplantation (6).

The deleterious effects of ischemia and reperfusion on organ function have been well studied in a number of experimental models. Nonetheless, the contribution of hypothermia and rewarming to graft injury are poorly characterized, mostly because of the difficulty to dissect the events of hypothermia, ischemia, and reperfusion in transplantation models. Tissue injury during (cold) ischemia among others is caused by adenosine triphosphate (ATP) depletion, accumulation of hypoxanthine, loss of the $\text{Na}^+ - \text{K}^+$ pump activity, cell swelling, and increases in cytosolic calcium (6). During hypothermic preservation, intracellular ATP concentration rapidly declines. Although ATP is consumed to maintain electrolyte homeostasis, a low temperature decreases ATP synthesis in the absence of oxygen. Moreover, the ischemia induces a switch to anaerobic metabolism, which affects the cellular pH through the accumulation of lactate. This in turn results in lysosomal and mitochondrial instability, the latter contributing to progressive cellular energy depletion (6). The lack of ATP will ultimately result in impairment of the $\text{Na}^+ - \text{K}^+$ ATPase, permitting intracellular accumulation of sodium and water and hence cell swelling. Mitochondrial instability combined with decreased functioning of cellular reactive oxygen species (ROS) scavengers facilitates the accumulation of intracellular ROS (7), which may result in endothelial damage. At the organ level, edema formation, as a consequence of a reduced interstitial pressure during cold storage, severely limits organ perfusion because of the compression of the capillary beds and may induce secondary ischemia during reperfusion (7). These conditions favor an inflammatory reaction in the graft, as reflected by the activation of endothelial adhesion molecules, for example, intracellular cell adhesion molecule (ICAM), vascular cell adhesion molecule (VCAM), and selectins (8). This may explain why recipients of grafts with longer cold ischemia times suffer more often from early acute rejection and are at higher risk for delayed graft function.

Ischemic preconditioning may be used to limit tissue injury after hypothermic organ preservation by preconditioning the graft. The concept of this method is to expose the organ to brief episodes of 3 to 5 min of ischemia before preservation, which primes the allograft to withstand prolonged ischemia, protecting the graft from functional damage and vascular endothelial cell death (9). Although the mechanism of ischemic preconditioning is not fully unraveled yet, it has been shown that ischemic preconditioning induces an energy-saving state, which preserves energy metabolism and reduces inflammation during reperfusion (10). For practical reasons, however, ischemic preconditioning cannot be easily accomplished during transplant surgery, because ischemic preconditioning needs to be applied 24 hr before procurement and preservation to benefit from late phase effects. However, it is unresolved whether early or late phase effects contribute to more important clinical effects of ischemic preconditioning. Therefore, pharmacologic approaches that target adenosine monophosphate-activated protein kinase (AMPK) and endothelial nitric oxide synthase (eNOS) look promising, because they mimic the beneficial effects of ischemic pre-

conditioning. Moreover, in addition to effects induced to conserve cellular homeostasis as described later, AMPK is able to decrease cellular metabolism leading to conservation of cellular energy availability. In this review, we will focus on the central role of AMPK in ischemic preconditioning and its potential use in transplantation medicine.

ISCHEMIC PRECONDITIONING

Recent studies have demonstrated different successful approaches to limit preservation-induced organ injury by preconditioning of the graft. Ischemic preconditioning results in a biphasic protection of cell function in time, that is, within a few minutes (early/acute phase) and from 24 to till 96 hr postpreconditioning (late phase). It is suggested that not only distinctive mechanisms underlie the two phases of preconditioning-induced protection but also protection during the late phase is less effective than in the period shortly after ischemic preconditioning (9). Protection induced in the early phase may rely on preserving energy metabolism in a more direct way, for example, through changes in phosphorylation in signal transduction pathways (10). Conversely, protection during the late phase is believed to be mediated by regulation of gene transcription and translation, particularly in genes involved in the NO^- and apoptotic pathways (9). AMPK represents an important pathway involved in the salutary effect of preconditioning governing both the early and late protective effects. AMPK acts as a low-fuel warning system that is activated by depletion of ATP or, alternatively, by increased levels of AMP. Its activation induces an energy-saving state to prevent lactate accumulation and cell injury (10). Further, activation of AMPK leads to increased levels of eNOS and NO, which are known to limit endothelial dysfunction after cold preservation (11). NO is produced from L-arginine by the enzyme NO-synthase and serves an important role in vascular function and reduces local inflammation through inhibition of thrombocyte aggregation and leukocyte adhesion (12). It has been proposed that NO plays a key role in both the acute and delayed protection offered by ischemic preconditioning. The acute protective effects induced by NO are mostly regulated through direct suppression of cellular energy metabolism, as has been shown in cardiac tissue (13). Cellular energy metabolism can be suppressed by NO through up-regulation of cyclic guanosine monophosphate (cGMP), which in turn up-regulates cGMP-sensitive cyclic adenosine monophosphate (cAMP) phosphodiesterase enzymes leading to increased degradation of intracellular cAMP into AMP. The latter then induces activation of AMPK, which in turn leads to activation of signaling pathways to conserve cellular energy levels (14). Although the acute phase of protection offered by preconditioning is independent of protein synthesis, the delayed phase is dependent on de novo protein production. One of these proteins is inducible nitric oxide synthase (iNOS), which can be produced after activation of the protein kinase C (PKC) and nuclear factor (NF)- κB pathways (15). An increase in iNOS levels leads to increased production of NO, which influences apoptosis, metabolism, and inflammation through cGMP-dependent mechanisms and the $\text{K}^+ - \text{ATP}$ channel (14, 15).

The AMPK pathway can be activated by adenosine (16), whose levels increase under hypoxic conditions. Activa-

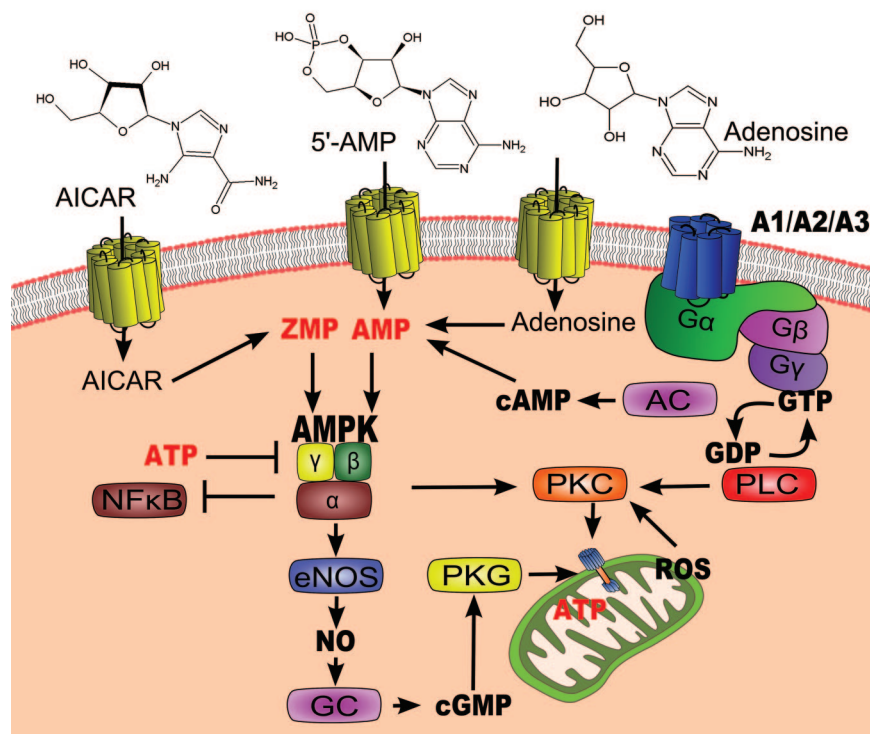


FIGURE 1. Ischemic preconditioning, 5'-AMP and AICAR lead to alterations in the [AMP]:[ATP]-ratio, via depletion of ATP (ischemic preconditioning) or increased availability of AMP (5'-AMP and AICAR). 5'-AMP, AICAR and adenosine (structural formulas shown in the figure) can be transported into the cells via nucleoside transporters. After adenosine has been taken up in the cells, it can be converted into AMP by adenosine kinase. Alterations in the intracellular [AMP]:[ATP]-ratio activates Adenosine Monophosphate Activated Kinase (AMPK). Further, activation of the adenosine-receptor plays a minor role in activation of AMPK. Activation of AMPK induces an energy-conserving state by decreasing anabolic and increasing catabolic pathways, thereby preventing further ATP-depletion and reactive oxygen species (ROS)-accumulation. AMPK activates protein kinase C (PKC), which can also be activated by ROS from mitochondria and indirectly by adenosine via the A1, A2 and A3 adenosine-receptors. Nitric oxide (NO) is produced after activation of eNOS by AMPK and has an important function in maintaining vascular function. In the downstream signaling cascade of AMPK it activates guanylyl cyclase (GC), cyclic guanylyl monophosphate (cGMP) and protein kinase G (PKG). PKC- and PKG-activation affect the K^+ -ATPase on mitochondria, which serves important roles in mitochondrial redox state and osmoregulation. Further, AMPK inhibits nuclear factor kappa B (NF- κ B), which is regarded as a key player in inducing inflammatory reactions and apoptotic pathways.

tion of A1 and A2 adenosine receptors induces activation of an intracellular signaling cascade including AMPK, PKC, and eNOS (Fig. 1). This in turn results in an increased NO production and inhibition of NF- κ B (17). eNOS plays an essential role in downstream signaling of A2-receptor activation, because beneficial effects of adenosine administration is abolished by an eNOS inhibitor (18). High extracellular adenosine levels decrease the expression of the adhesion molecules ICAM, VCAM, and selectins on endothelial cells and inhibit platelet function and ROS production by neutrophils (17). Indirect inhibition of radical production occurs by activation of PKC and tyrosine kinase, which influence the mitochondrial K^+ -ATPase and thus limit mitochondrial redox imbalance (17). The essential role of PKC is demonstrated by that fact that pretreatment with the PKC-specific inhibitor chelerythrine abolishes the beneficial effects of preconditioning (19). On activation, PKC translocates to the nucleus where it phosphorylates substrate proteins to induce ischemic tolerance (20). Down-stream effectors of PKC are mitochondrial K^+ -ATP channels, heat shock proteins, NF- κ B, and iNOS as established in cardiac tissue (21–23). Activation of PKC dur-

ing ischemic preconditioning is believed to occur through increased levels of adenosine leading to activation of adenosine receptors (24). Activation of adenosine receptors increases cellular cAMP (and thus decreases ATP), which will be converted to AMP. Further, extracellular adenosine can be transported into cells by nucleoside transporters, and its phosphorylation leads to increased cellular levels of AMP, which induces activation of AMPK through up-regulation of α 1AMPK (16). Because AMPK plays a major role in conserving energy and preventing loss of cellular homeostasis and is able to activate the NO pathway, PKC, and inhibit NF- κ B (Fig. 1), AMPK seems to be one of the most promising pharmacologic targets to reduce cold storage related organ injury.

AMPK AS A PRIME PHARMACOLOGICAL TARGET IN PRECONDITIONING

To date, studies that have dealt with pharmacologic pretreatment to improve organ functioning after cold storage have primarily focused on influencing cellular metabolism or energy status and the reduction of inflammation. Effects of

most pharmacologic approaches are induced by or involve activation of AMPK. Thus, AMPK may be considered as an important prime pharmacologic target to limit organ injury during preservation. AMPK is the central component of a protein kinase cascade that represents a key regulator of energy production. AMPK is a heterotrimeric complex that consists of a catalytic subunit (α) and two regulatory subunits (β and γ) and is activated allosterically by binding of AMP to the γ subunit, allowing for phosphorylation at the α subunit by the upstream serine/threonine kinase 11 (LKB1) (25). High levels of ATP competitively antagonize AMP binding to AMPK (25). Thus, AMPK is a key sensor of cellular energy levels, as small changes in the [AMP]:[ATP] ratio induce changes in AMPK activity. Serine/threonine kinase 11 (or LKB1) is an upstream activator of AMPK and has strong similarities with the mammalian calcium-/calmodulin-dependent protein kinase subgroup. This suggests that it may play a role in the intracellular calcium “danger” pathways. Much less is known about the β -subunit, but studies suggest a role in cellular glycogen energy pathways, as radiolabeled glycogen has been found to bind to the β -unit (26). Once AMPK is activated, it initiates a series of responses that aim at restoration of the intracellular energy balance. Anabolic, ATP-consuming, pathways such as fatty acid synthesis and protein synthesis are suppressed, whereas catabolic, ATP-generating, pathways such as fatty acid oxidation and glycolysis are activated (26).

Downstream effects of AMPK are mediated by phosphorylation of regulatory proteins and subsequent effects on gene expression. AMPK activation inhibits *de novo* protein production (an energy-consuming process) by repression of the elongation step in translation and down-regulating the rapamycin pathway, which normally initiates translation (27). Also, AMPK down-regulates important anabolic pathways through acute inhibition of lipid biosynthesis by phosphorylation and inactivation of key metabolic enzymes such as ACC1 (fatty acid synthesis), glycerol phosphate acyl transferase (triacylglycerol synthesis), and HMGCoA reductase (cholesterol/isoprenoid biosynthesis). Further, AMPK activates important catabolic pathways by stimulation of fatty acid oxidation through phosphorylation of ACC2 and by stimulation of glucose uptake through activation of glucose transporter 1 and glucose transporter 4 (25, 27). Other downstream effectors of AMPK are eNOS, NO, and NF- κ B (Fig. 1) (12, 26, 28, 29). Activation of AMPK induces activation of eNOS, leading to an increased NO level. In human aortic endothelial cells, AMPK activation induces eNOS phosphorylation, which increases NO production. It has been proposed that the eNOS-mediated effects of AMPK are of main importance in the delayed protective effects of ischemic precondition (29). Other studies have focused on the immunologic modulation by AMPK and have demonstrated that AMPK prevents tumor necrosis factor- α -induced activation of NF- κ B in the human umbilical vein endothelial cells (28). Downstream mediators in the NF- κ B pathway are also decreased by activation of AMPK. The expression of the chemokine monocyte chemoattractant protein-1, E-selectin, and adhesion molecules such as VCAM-1 and ICAM-1 are reduced by AMPK activation (28), resulting in decreased rolling or adhesion of leukocytes after ischemia/reperfusion (29). Thus, in addition to its important role in cellular energy ho-

meostasis, AMPK conserves vascular function and reduces the inflammatory state. These characteristics make AMPK a promising pharmacologic target to prevent or at least reduce injury during organ preservation.

PHARMACOLOGIC AMPK ACTIVATORS

Because of its role in energy metabolism, AMPK has gained attention as a drug target in metabolic disorders such as type 2 diabetes mellitus and obesity (30). Surprisingly, only few activators of AMPK have been identified and characterized pharmacologically. Several agents currently under investigation in the field of metabolic disorders might also have beneficial effects by limiting injury induced by preservation, because activation of AMPK by aminoimidazole carboxamide ribonucleotide (AICAR), metformin, curcumin, biguanides, and thiazolidinediones has been shown (31–35). Currently, the most investigated is AICAR. After its uptake into cells by nucleoside transporters, it is converted by adenosine kinase to the monophosphorylated nucleotide AICAR monophosphate (commonly denoted as AICAR monophosphate [ZMP]), which acts as an analogue of AMP. ZMP allosterically activates both AMPK (similar to AMP) and the upstream AMPK kinase that causes further activation of AMPK (30). Interestingly, in transplantation models, AICAR treatment induces a similar level of protection as found in ischemic preconditioning, including specific antiinflammatory effects (29, 30). However, these effects may not be entirely specific for AMPK, as ZMP also regulates additional cAMP-sensitive enzymes such as fructose-1,6-bisphosphatase and muscle glycogen phosphorylase (30). Whether effects on these proteins may partially govern the protective effect of AICAR/ZMP in addition to the AMPK route remains to be determined.

A second well-known molecule is adenosine, which can induce activation of AMPK. Because effects of adenosine are inhibited by treating cells with 5'-iodotubercidine, an inhibitor of adenosine kinase, the beneficial effect of adenosine seems dependent on its phosphorylation and thus on AMP production (16). Endogenous 5'-nucleotidase (CD73 or NT5E) catalyzes the degradation of AMP into adenosine, and it has been shown that knock-out mice lacking 5'-nucleotidase are less prone to ischemia/reperfusion-induced injury (36). Further, knock-out mice lacking ectonucleoside triphosphate diphosphohydrolase 1 (ENTPD1, CD39, or apyrase), which catalyzes the hydrolysis of ATP to AMP, are more prone to injury after ischemia/reperfusion, which can be reversed by addition of soluble apyrase (36). Together, these results imply that AMP is the principal nucleoside generating the protective effects of adenosine and that inhibition of AMP formation increases injury after ischemia/reperfusion. Although studies investigating AMPK activity in transplantation models are lacking, these beneficial effects of modifying the activity of enzymes involved in the formation or breakdown of AMP (i.e., adenosine kinase, CD73, and CD39) suggest that pharmacologic activation of AMPK might be beneficial to limit organ injury during preservation.

The University of Wisconsin preservation solution contains relatively high concentrations of adenosine compared with intracellular concentrations. As described earlier, beneficial effects of adenosine have been shown to depend for an important part on activation of AMPK, and thus, addition of exogenous AMP or increasing endogenous levels of AMP

by altering activity of ENTPD1 and NT5E in organs might be the most beneficial strategy to limit organ injury after organ preservation. Addition of exogenous AMP to organs will lead to uptake in the cells by the nucleoside transporters and increase the [AMP]:[ATP] ratio without depleting ATP levels. Naturally occurring AMP is a mixture of the molecules 3'-AMP and 5'-AMP derived from cAMP, whereas the synthetically produced agent used mostly in research only consists of 5'-AMP. Although there are isoform-specific enzymes, it is not known whether they have a different potency in activating AMPK. Although studies that investigate the beneficial effects of 5'-AMP in organ preservation have not been conducted yet, indirect evidence demonstrating its potential to limit organ injury by suppressing metabolism can be found in nature. Metabolic stress (i.e., a negative energy balance because of food shortage or increased workload) can induce a hibernation-like state in mice, in which an increase of 5'-AMP in serum is dissected as being a key mediator (37). Further, injection of 5'-AMP in mice suppresses metabolism and induces the same hibernation-like behavior as induced by metabolic stress (37). In addition, in hibernating species as the ground squirrel (38), and also in freeze-tolerant frogs (39), activity of AMPK increases in several tissues, suggesting that activation of AMPK represents a natural way to cope with metabolic stressful situation related to hypothermia. Because 5'-AMP suppresses metabolism in vivo, it is reasonable to presume that 5'-AMP may also have beneficial effects in transplantation medicine through suppression of cellular metabolism during preservation and activation of AMPK. Thus, pretreatment of organs by agents that are able to activate AMPK seems to be a promising novel strategy to limit preservation-induced organ injury.

CONCLUSION

AMPK plays a central role in the induction of the protective phenotype by ischemic preconditioning of organs before transplantation. AMPK not only acts as an important regulator of cellular energy metabolism but also connects to other downstream targets of ischemic preconditioning, thus influencing endothelial function and inflammation. Inducing the biochemical signaling pathway downstream of AMPK can occur efficiently using AMP and might thus lead to improved organ preservation. However, hypothermia during preservation might influence drug delivery. Further research is needed to test novel agonists that can efficiently induce activation of AMPK (e.g., AICAR) and the effects of hypothermia on the pharmacokinetics and dynamics of these agents. Taken together, AMPK stands out as a pharmacologic target to prevent preservation-induced organ injury in transplantation medicine, and the challenge is to find new effectors to modulate this pathway.

ACKNOWLEDGMENT

The authors thank N. van Velzen for his assistance in producing the graphics.

REFERENCES

- McDonald S, Russ G, Campbell S, et al. Kidney transplant rejection in Australia and New Zealand: Relationships between rejection and graft outcome. *Am J Transplant* 2007; 7: 1201.
- Barocci S, Valente U, Fontana I, et al. Long-term outcome on kidney retransplantation: A review of 100 cases from a single center. *Transplant Proc* 2009; 41: 1156.
- Organ Procurement and Transplantation Network. Available at: <http://www.unos.org>. Accessed January 4, 2010.
- Terasaki PI, Gjertson DW, Cecka JM, et al. Fit and match hypothesis for kidney transplantation. *Transplantation* 1996; 62: 441.
- Bouma HR, Ploeg RJ, Schuur TA. Signal transduction pathways involved in brain death-induced renal injury. *Am J Transplant* 2009; 9: 989.
- Salahudeen AK. Consequences of cold ischemic injury of kidneys in clinical transplantation. *J Investig Med* 2004; 52: 296.
- Brinkkoetter PT, Song H, Losel R, et al. Hypothermic injury: The mitochondrial calcium, ATP and ROS love-hate triangle out of balance. *Cell Physiol Biochem* 2008; 22: 195.
- Jassem W, Koo DD, Cerundolo L, et al. Cadaveric versus living-donor livers: Differences in inflammatory markers after transplantation. *Transplantation* 2003; 76: 1599.
- Kloner RA, Jennings RB. Consequences of brief ischemia: Stunning, preconditioning, and their clinical implications: Part 2. *Circulation* 2001; 104: 3158.
- Peralta C, Bartrons R, Serafin A, et al. Adenosine monophosphate-activated protein kinase mediates the protective effects of ischemic preconditioning on hepatic ischemia-reperfusion injury in the rat. *Hepatology* 2001; 34: 1164.
- Sola A, De OJ, Gonzalez R, et al. Protective effect of ischemic preconditioning on cold preservation and reperfusion injury associated with rat intestinal transplantation. *Ann Surg* 2001; 234: 98.
- Morrow VA, Fougelle F, Connell JM, et al. Direct activation of AMP-activated protein kinase stimulates nitric-oxide synthesis in human aortic endothelial cells. *J Biol Chem* 2003; 278: 31629.
- Rakhit RD, Edwards RJ, Marber MS. Nitric oxide, nitrates and ischaemic preconditioning. *Cardiovasc Res* 1999; 43: 621.
- Lochner A, Marais E, Du TE, et al. Nitric oxide triggers classic ischemic preconditioning. *Ann N Y Acad Sci* 2002; 962: 402.
- Nandagopal K, Dawson TM, Dawson VL. Critical role for nitric oxide signaling in cardiac and neuronal ischemic preconditioning and tolerance. *J Pharmacol Exp Ther* 2001; 297: 474.
- Aymerich I, Fougelle F, Ferre P, et al. Extracellular adenosine activates AMP-dependent protein kinase (AMPK). *J Cell Sci* 2006; 119: 1612.
- Bouma MG, van den Wildenberg FA, Buurman WA. The anti-inflammatory potential of adenosine in ischemia-reperfusion injury: Established and putative beneficial actions of a retaliatory metabolite. *Shock* 1997; 8: 313.
- Peralta C, Hotter G, Closa D, et al. Protective effect of preconditioning on the injury associated to hepatic ischemia-reperfusion in the rat: Role of nitric oxide and adenosine. *Hepatology* 1997; 25: 934.
- Ricciardi R, Meyers WC, Schaffer BK, et al. Protein kinase C inhibition abrogates hepatic ischemic preconditioning responses. *J Surg Res* 2001; 97: 144.
- Nakano A, Cohen MV, Downey JM. Ischemic preconditioning: From basic mechanisms to clinical applications. *Pharmacol Ther* 2000; 86: 263.
- Baines CP, Liu GS, Birincioglu M, et al. Ischemic preconditioning depends on interaction between mitochondrial KATP channels and actin cytoskeleton. *Am J Physiol* 1999; 276: H1361.
- Morgan EN, Boyle EM Jr, Yun W, et al. An essential role for NF-kappaB in the cardioadaptive response to ischemia. *Ann Thorac Surg* 1999; 68: 377.
- Xuan YT, Tang XL, Qiu Y, et al. Biphasic response of cardiac NO synthase isoforms to ischemic preconditioning in conscious rabbits. *Am J Physiol Heart Circ Physiol* 2000; 279: H2360.
- Heemann UW, Azuma H, Tullius SG, et al. The contribution of reduced functioning mass to chronic kidney allograft dysfunction in rats. *Transplantation* 1994; 58: 1317.
- Hardie DG. The AMP-activated protein kinase pathway—New players upstream and downstream. *J Cell Sci* 2004; 117: 5479.
- Carling D. AMP-activated protein kinase: Balancing the scales. *Biochimie* 2005; 87: 87.
- Hardie DG. Minireview: The AMP-activated protein kinase cascade: The key sensor of cellular energy status. *Endocrinology* 2003; 144: 5179.
- Hattori Y, Suzuki K, Hattori S, et al. Metformin inhibits cytokine-induced nuclear factor kappaB activation via AMP-activated protein kinase activation in vascular endothelial cells. *Hypertension* 2006; 47: 1183.
- Gaskin FS, Kamada K, Yusof M, et al. 5'-AMP-activated protein kinase activation prevents postischemic leukocyte-endothelial cell adhesive interactions. *Am J Physiol Heart Circ Physiol* 2007; 292: H326.

30. Hardie DG. AMP-activated protein kinase as a drug target. *Annu Rev Pharmacol Toxicol* 2007; 47: 185.
31. Yu LF, Qiu BY, Nan FJ, et al. AMPK activators as novel therapeutics for type 2 diabetes. *Curr Top Med Chem* Feb 25, 2010 [Epub ahead of print].
32. Guh JH, Chang WL, Yang J, et al. Development of novel adenosine monophosphate-activated protein kinase activators. *J Med Chem* 2010; 53: 2552.
33. Fogarty S, Hardie DG. Development of protein kinase activators: AMPK as a target in metabolic disorders and cancer. *Biochim Biophys Acta* 2010; 1804: 581.
34. Kim T, Davis J, Zhang AJ, et al. Curcumin activates AMPK and suppresses gluconeogenic gene expression in hepatoma cells. *Biochem Biophys Res Commun* 2009; 388: 377.
35. Gruzman A, Babai G, Sasson S. Adenosine monophosphate-activated protein kinase (AMPK) as a new target for antidiabetic drugs: A review on metabolic, pharmacological and chemical considerations. *Rev Diabet Stud* 2009; 6: 13.
36. Lu B, Rajakumar SV, Robson SC, et al. The impact of purinergic signaling on renal ischemia-reperfusion injury. *Transplantation* 2008; 86: 1707.
37. Zhang J, Kaasik K, Blackburn MR, et al. Constant darkness is a circadian metabolic signal in mammals. *Nature* 2006; 439: 340.
38. Horman S, Hussain N, Dilworth SM, et al. Evaluation of the role of AMP-activated protein kinase and its downstream targets in mammalian hibernation. *Comp Biochem Physiol B Biochem Mol Biol* 2005; 142: 374.
39. Rider MH, Hussain N, Horman S, et al. Stress-induced activation of the AMP-activated protein kinase in the freeze-tolerant frog *Rana sylvatica*. *Cryobiology* 2006; 53: 297.