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EDITORIALS

# Studying propofol-induced cardioprotection: from mechanism to clinical phenomenon and back again

**Gregory M. T. Hare, MD, PhD**

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The concept that anesthetics could potentially impart protective effects on ischemic myocardium was introduced as early as the 1970s in a landmark study by Bland and Lowenstein.[1] They demonstrated, in a canine model, that the use of halothane avoided electrocardiographic ST segment elevation and reduced myocardial oxygen consumption during acute coronary occlusion. Subsequent research demonstrated that brief exposure to volatile anesthetics prior to an ischemic insult could reduce the size of a myocardial infarction following experimental coronary artery occlusion.[2] Thus, the concept of "anesthesia preconditioning" was born. Four decades after those initial experimental findings, however, scientific studies have yet to demonstrate a clear clinical benefit of "anesthesia preconditioning" in patients undergoing heart surgery.

The majority of published data has focused on the potential of halogenated volatile anesthetics to reduce myocardial injury in patients undergoing cardiac surgery.[3–5] In the current issue of the *Journal*, Ansley *et al.* have pursued a different approach: They report the results of their clinical Phase 2 trial (PRO-TECT II) that assessed the impact of administering a targeted dose of systemic propofol during the ischemia-reperfusion interval in patients undergoing heart surgery.[6] They hypothesized that the antioxidant properties of propofol impart anesthetic preconditioning and protect ischemic myocardium from oxidative injury during cardiac surgery. Their study intervention (i.e., high-dose propofol) was derived from prior experimental studies[7–9] that determined an optimal mean (standard deviation) propofol concentration in the systemic circulation [5.74 (2.50) $\mu g \cdot mL^{-1}$] and blood microplegia [2.04 (1.14) $\mu g \cdot mL^{-1}$] when the propofol was delivered during the ischemia-reperfusion interval. Their protocol assessed both drug concentration and time-dependent effects of propofol for the purpose of defining a therapeutic window for optimal cardioprotection.

As dictated by the scientific method with an emphasis on reductionist mechanistic approaches, the authors proposed a hypothesis based on their, and others', preclinical research. They hypothesized that the antioxidant properties of propofol would decrease oxidative myocardial injury secondary to reactive oxygen species (ROS) generated during ischemia and reperfusion (i.e., a *candidate mechanism*), as reflected by decreased production of the ROS biomarker 15-$F_{2t}$-isoprostane. The predicted impact on the clinical outcome would be a reduction in the incidence of low cardiac output syndrome (LCOS) – i.e., the *clinical phenomenon*. This proposed protective antioxidant mechanism for propofol may be of particular importance in diabetic patients in whom oxidative myocardial injury is thought to be accentuated because of associated mitochondrial dysfunction. The emphasis on the importance of the proposed candidate mechanism over the clinical phenomenon is illustrated by the choice of coronary sinus (CS) 15-$F_{2t}$-isoprostane levels, not LCOS, as the primary outcome of the study. Paradoxically, the authors observed an increase in CS 15-$F_{2t}$-isoprostane levels in the propofol treatment arm. Thus, the primary outcome data did not support the proposed mechanism.

Despite this conclusion, one of the most striking findings of the study was that patients randomized to undergo propofol myocardial preconditioning experienced a lower

G. M. T. Hare, MD, PhD (✉)
Department of Anesthesia and Physiology, St. Michael's Hospital, University of Toronto, 30 Bond Street, Toronto, ON M5B 1W8, Canada
e-mail: hareg@smh.ca

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incidence of LCOS than did the controls who received isoflurane (20.9% *vs* 57.1%, respectively; $P < 0.001$). This effect was more pronounced in patients with type 2 diabetes mellitus compared to that in nondiabetics (17.9% *vs* 70.3%, respectively; $P < 0.001$). These positive clinical outcome data are supported by the findings that CS levels of cardiac troponin I were lower in the propofol group and that protein levels of the anti-apoptotic molecule B-cell lymphoma-2 (BCL-2) were increased in the myocardium of patients in the propofol arm of the study. Thus, the observed clinical phenomenon defied the proposed mechanism, raising a number of interesting questions.

Did the authors choose the best biomarker of oxidative stress? Indeed, the choice of 15-$F_{2t}$-isoprostane as a biomarker of oxidative stress was based on the results of prior basic science studies in experimental animals. Those studies had demonstrated that 15-$F_{2t}$-isoprostane exacerbates myocardial ischemia reperfusion injury, propofol decreases 15-$F_{2t}$-isoprostane released during ischemia-reperfusion, and propofol enhanced ischemia tolerance in rat hearts.[7–9] Thus, there was a good scientific rationale for the choice of 15-$F_{2t}$-isoprostane. Despite the sound preclinical studies, however, the outcome in human subjects was opposite to that observed in animals, with the propofol pretreatment resulting in an increase in 15-$F_{2t}$-isoprostane levels. Interestingly, a prior randomized trial that compared sevoflurane with propofol for preconditioning during off-pump coronary artery bypass graft surgery demonstrated a similar increase in CS isoprostane levels in the propofol group, supporting the results of the current study.[10] These observations suggest that CS isoprostane levels do not adequately reflect propofol's antioxidant capacity, that inter-species differences make translation from animal studies difficult, and that propofol may be exerting its cardioprotective effect via other mechanisms.

Could mechanisms other than oxidative injury be implicated? Indeed, there are a broad number of candidate mechanisms, in addition to ROS, that have been proposed as mediators of anesthesia-induced preconditioning. They include K(ATP) ion channels, G protein-coupled receptor antagonists, protein kinases, heat shock proteins, transcription factors, anti-apoptotic mechanisms, and others.[11] The existence of such a broad group of candidate mechanisms suggests that the biology of anesthetic preconditioning is complex and that multiple cellular mechanisms likely contribute in a synergistic manner. Thus, focusing on any one candidate mechanism may not be sufficient to define the overall clinical phenomenon and might deflect our attention from the primary observation that, under the appropriate conditions, propofol may still have reduced the incidence of LCOS in patients undergoing cardiopulmonary bypass (CPB) and heart surgery, and that this effect may be of greater significance in patients with diabetes mellitus.

Do the results of this study align with those of previous randomized controlled trials? In juxtaposition to Ansley *et al.,* several meta-analyses have demonstrated trends toward more favorable outcomes with inhalation anesthetics.[3–5] Yu and Beattie assessed a heterogeneous group of studies that demonstrated a trend toward decreased mortality with inhalation anesthetics.[3] Sevoflurane and desflurane, but not isoflurane, were associated with a decrease in postoperative troponin levels. Similarly, an analysis of volatile anesthetics by Symons and Myles found that they produced higher cardiac output indices and lowered serum troponin levels. They also reported lower requirements for inotropic support with the use of these volatile anesthetics.[5] In both analyses, more than half of the studies utilized high-dose opioids and/or benzodiazepines, but not propofol, in the intravenous anesthetic arms, potentially weakening any direct comparison between the preconditioning effect of inhalation agent versus propofol. More recent meta-analyses that compared desflurane and sevoflurane with propofol-based anesthesia more directly arrived at a variety of conclusions. Some demonstrated a significant benefit in terms of improved myocardial function and survival with desflurane and sevoflurane versus predominantly propofol-based anesthesia.[4,12] Other analyses compared sevoflurane with propofol directly and showed potential beneficial effects of both treatments, depending on the outcome or subpopulation of patients assessed.[13,14] In one analysis, the authors concluded that "sevoflurane and propofol both possess some, although different, cardioprotective properties."[13] Perhaps this statement summarizes the potential equipoise between treatments and/or inherent limitations of this approach.

A recent randomized trial assessed the impact of preconditioning with 4% sevoflurane administered during the first ten minutes of CPB in which the patients in both study arms received propofol anesthesia. Sevoflurane preconditioning reduced post-CPB increases in biomarkers of myocardial injury (NT-proBNP and cTNT-cystatin T) and the incidence of postoperative cardiac events.[15,16] Because of the small sample size, however, the results should be regarded as hypothesis-generating and require further validation. Although the overall findings of these studies support the hypothesis that volatile anesthetics may have cardioprotective effects relative to intravenous anesthetics, the uniformly small sample sizes of most studies emphasize that further investigation and a properly powered randomized controlled trial (RCT) are required.

What, then, are we to conclude? Should the protocol utilized in this Phase 2 trial be utilized in a Phase 3 trial of

diabetic patients undergoing CPB and heart surgery? Should we forget about the potential mechanism and focus on the phenomenon and design studies based on clinical outcomes such as LCOS, myocardial injury, or survival? Should the comparator group receive a placebo (i.e., Intralipid) as used in the current protocol? Given the prior meta-analyses, should the trial design include a head-to-head comparison of propofol versus sevoflurane preconditioning? After four decades of investigation, regardless of the study design or potential outcome, it seems likely that a large multicentre RCT is required as the next step to answer the question adequately of whether anesthetics can precondition the myocardium and improve clinical outcome.

## L'étude de la cardioprotection induite par le propofol: du mécanisme au phénomène clinique, et vice-versa

Dans les années 1970, une étude phare de Bland et Lowenstein lançait l'idée selon laquelle des agents anesthésiques pouvaient avoir des effets protecteurs sur le myocarde ischémique.[1] Ces auteurs ont montré, en se servant d'un modèle canin, que l'utilisation d'halothane permettait d'éviter l'élévation électrocardiographique du segment ST et de réduire la consommation d'oxygène myocardique pendant une occlusion coronaire aiguë. Par la suite, d'autres recherches ont démontré qu'une brève exposition à des anesthésiques volatils avant une lésion ischémique réduisait la taille de l'infarctus du myocarde après occlusion expérimentale de l'artère coronaire.[2] C'est ainsi que le concept de « préconditionnement anesthésique » a vu le jour. Quarante ans après ces découvertes expérimentales, toutefois, les études scientifiques n'ont pas encore démontré d'avantage clinique clair d'un « préconditionnement anesthésique » des patients subissant une chirurgie cardiaque.

La majorité des données publiées se sont concentrées sur le potentiel des anesthésiques volatils halogénés à réduire la lésion myocardique chez les patients subissant une chirurgie cardiaque.[3–5] Dans ce numéro du *Journal*, Ansley *et coll*. ont adopté une approche différente : ils rapportent les résultats de leur étude clinique de phase 2 (PRO-TECT II) qui avait pour objectif d'évaluer l'impact de l'administration d'une dose ciblée de propofol systémique pendant l'intervalle d'ischémie-reperfusion chez des patients subissant une chirurgie cardiaque.[6] Ils ont émis l'hypothèse que les propriétés antioxydantes du propofol conféraient un préconditionnement anesthésique et protégeaient le myocarde ischémique d'une lésion oxydative pendant une chirurgie cardiaque. L'intervention qu'ils ont testé dans leur étude (soit une forte dose de propofol) dérivait d'études expérimentales antérieures,[7–9] lesquelles avaient déterminé la concentration moyenne optimale (écart type) de propofol dans la circulation systémique [5,74 (2,50) $\mu g \cdot mL^{-1}$] et la microplégie sanguine [2,04 (1,14) $\mu g \cdot mL^{-1}$] lorsque le propofol était diffusé pendant l'intervalle d'ischémie-reperfusion. Leur protocole a évalué la concentration médicamenteuse et les effets en fonction du temps du propofol afin d'identifier la fenêtre thérapeutique offrant la meilleure cardioprotection.

Comme le dictait la méthode scientifique avec une emphase sur des approches mécanistes réductionnistes, les auteurs ont proposé une hypothèse fondée sur leurs recherches précliniques, ainsi que sur celles d'autres avant eux. Ils ont émis l'hypothèse que les propriétés antioxydantes du propofol diminueraient la lésion myocardique oxydative secondaire aux dérivés réactifs de l'oxygène (DRO) générés pendant l'ischémie et la reperfusion (soit un *mécanisme candidat*), telle que reflétée par une production réduite du 15-$F_{2t}$-isoprostane, un marqueur de DRO. L'impact anticipé sur le résultat clinique était une réduction de l'incidence de syndrome de bas débit cardiaque (SBDC) – soit le *phénomène clinique*. Ce mécanisme antioxydant protecteur proposé du propofol pourrait avoir une importance toute particulière pour les patients diabétiques : en effet, on pense qu'une lésion myocardique oxydative pourrait être exacerbée en raison du dysfonctionnement mitochondrial associé au diabète. L'emphase des auteurs sur l'importance du mécanisme candidat proposé plutôt que sur le phénomène clinique est claire par le choix des niveaux de 15-$F_{2t}$-isoprostane au sinus coronaire (SC), et non du SBDC, en tant que critère d'évaluation principal de l'étude. Paradoxalement, les auteurs ont observé une augmentation des niveaux de 15-$F_{2t}$-isoprostane au SC dans le bras de traitement au propofol. Les données concernant le critère d'évaluation principal n'ont donc pas corroboré le mécanisme proposé.

Malgré cette conclusion, l'une des observations les plus étonnantes de l'étude était que les patients randomisés à subir un préconditionnement myocardique au propofol ont manifesté une incidence plus faible de SBDC que les témoins, qui avaient reçu de l'isoflurane (20,9 % *vs* 57,1 %, respectivement; $P < 0{,}001$). Cet effet était plus prononcé chez les patients atteints de diabète de type 2 par rapport aux non-diabétiques (17,9 % *vs* 70,3 %, respectivement; $P < 0{,}001$). Ces résultats cliniques positifs sont corroborés par l'observation que les taux de troponine cardiaque I au SC étaient plus bas dans le groupe

propofol et que les taux protéiques de la molécule anti-apoptotique BCL-2 (lymphome à cellules B 2) étaient plus élevés dans le myocarde des patients du bras propofol de l'étude. Ainsi, le phénomène clinique observé a défié le mécanisme proposé, soulevant plusieurs questions dignes d'intérêt.

Les auteurs ont-ils choisi le meilleur biomarqueur de stress oxydatif? En effet, le choix du 15-$F_{2t}$-isoprostane comme biomarqueur du stress oxydatif est fondé sur les résultats d'études précédentes de science fondamentale réalisées sur des modèles animaux expérimentaux. Ces études avaient démontré que le 15-$F_{2t}$-isoprostane exacerbait la lésion myocardique d'ischémie-reperfusion, que le propofol réduisait la quantité de 15-$F_{2t}$-isoprostane libéré pendant l'ischémie-reperfusion, et que le propofol augmentait la tolérance à l'ischémie dans des cœurs de rats.[7–9] La démarche scientifique justifiant le choix du 15-$F_{2t}$-isoprostane était donc cohérente. Toutefois, malgré ces études précliniques rigoureuses, le résultat chez des sujets humains était à l'opposé de ce qui avait été observé chez les animaux, le prétraitement au propofol entraînant plutôt une augmentation des niveaux de 15-$F_{2t}$-isoprostane. Fait intéressant, une étude randomisée antérieure comparant l'administration de sévoflurane ou de propofol pour réaliser un préconditionnement pendant un pontage aortocoronarien hors pompe a démontré une augmentation semblable des niveaux d'isoprostane dans le SC dans le groupe propofol, ce qui va dans le même sens que les résultats de l'étude en question ici.[10] Ces observations suggèrent que les niveaux d'isoprostane dans le SC ne reflètent pas bien la capacité antioxydante du propofol, que des différences inter-espèces rendent la traduction à partir d'études animales difficile, et que le propofol pourrait exercer son effet cardioprotecteur par le biais d'autres mécanismes.

Est-il alors possible que des mécanismes autres que la lésion oxydative soient impliqués? En fait, de nombreux mécanismes candidats, outre les DRO, ont été proposés comme médiateurs du préconditionnement induit par l'anesthésie. Citons entre autres les canaux ioniques K(ATP), les antagonistes des récepteurs couplés à une protéine G, les protéines kinases, les protéines de choc thermique, les facteurs de transcription, ou encore les mécanismes anti-apoptotiques.[11] L'existence d'un tel éventail de mécanismes candidats laisse à penser que la biologie sous-jacente au préconditionnement anesthésique est complexe et que de nombreux mécanismes cellulaires y contribuent probablement de façon synergique. Ainsi, le fait de se concentrer sur un seul mécanisme candidat pourrait ne pas suffire à définir le phénomène clinique dans son ensemble et détourner notre attention de notre constatation principale : dans les bonnes conditions, le propofol pourrait tout de même avoir réduit l'incidence de SBDC chez les patients subissant une circulation extracorporelle (CEC) et une chirurgie cardiaque, et cet effet pourrait avoir une importance encore plus grande pour les patients diabétiques.

Les résultats de cette étude sont-ils en ligne avec ceux des études randomisées contrôlées précédentes? En juxtaposition à l'étude de Ansley *et coll*., plusieurs méta-analyses ont démontré qu'il existait des tendances vers des pronostics plus favorables lorsqu'on avait recours à des anesthésiques par inhalation.[3–5] Yu et Beattie ont passé en revue un groupe hétérogène d'études qui démontraient une tendance vers une réduction de la mortalité avec les anesthésiques par inhalation.[3] Le sévoflurane et le desflurane, mais non l'isoflurane, ont été associés à une réduction des taux postopératoires de troponine. De la même manière, une analyse des anesthésiques volatils par Symons et Myles a montré qu'ils produisaient des indices de débit cardiaque plus élevés et réduisaient les taux sériques de troponine. Ils ont également rapporté des besoins réduits de soutien inotrope lors de l'utilisation de ces anesthésiques volatils.[5] Dans ces deux analyses, plus de la moitié des études utilisaient de fortes doses d'opioïdes et/ou de benzodiazépines, et non du propofol, dans les bras d'anesthésie intraveineuse, ce qui pourrait potentiellement affaiblir toute comparaison directe entre l'effet de préconditionnement d'un agent par inhalation par rapport au propofol. Des méta-analyses plus récentes comparant le desflurane et le sévoflurane avec une anesthésie à base de propofol sont parvenues à diverses conclusions. Certaines ont démontré un avantage significatif en matière de fonction myocardique améliorée et de survie avec le desflurane et le sévoflurane par rapport à une anesthésie réalisée principalement avec du propofol.[4,12] D'autres analyses ont directement comparé le sévoflurane au propofol et ont montré des effets bénéfiques potentiels pour les deux traitements, selon le critère d'évaluation retenu ou la sous-population de patients évaluée.[13,14] Dans une analyse, les auteurs ont conclu que « le sévoflurane et le propofol possèdent tous deux certaines propriétés cardioprotectrices, bien qu'elles soient différentes ».[13] Peut-être que cette phrase résume bien l'incertitude absolue potentielle entre divers traitements et/ou les limites inhérentes à une telle approche.

Une étude randomisée récente a évalué l'impact d'un préconditionnement réalisé avec du sévoflurane 4 % administré au cours des dix premières minutes d'une CEC pendant laquelle les patients des deux bras de l'étude ont reçu une anesthésie au propofol. Le préconditionnement au sévoflurane a réduit les augmentations post-CEC des biomarqueurs de lésion myocardique (NT-proBNP et cTNT-cystatine T) et l'incidence de complications cardiaques postopératoires.[15,16] Cependant, en raison de la taille réduite de l'échantillon, les résultats doivent être

considérés comme le fondement d'une hypothèse et nécessitent une validation plus poussée. Bien que les observations globales de ces études appuient l'hypothèse selon laquelle les anesthésiques volatils pourraient avoir des effets cardioprotecteurs par rapport aux anesthésiques administrés par voie intraveineuse, les tailles d'échantillon, restreintes dans la plupart des études, renforcent le fait que des recherches supplémentaires sont nécessaires dans le cadre d'une étude randomisée contrôlée (ERC) bénéficiant d'une puissance suffisante.

Que pouvons-nous conclure? Le protocole utilisé dans cette étude de phase 2 devrait-il être réappliqué dans une phase 3 s'intéressant exclusivement aux patients diabétiques subissant une CEC et une chirurgie cardiaque? Devrions-nous oublier le mécanisme potentiel et nous concentrer sur le phénomène, et donc concevoir des études se basant sur des résultats cliniques tels que le SBDC, la lésion myocardique, ou la survie? Le groupe de comparaison devrait-il recevoir un placebo (soit une solution Intralipid), comme c'est le cas dans ce protocole-ci? Étant donné les méta-analyses précédentes, la conception de l'étude devrait-elle inclure une comparaison directe du préconditionnement au propofol à un préconditionnement au sévoflurane? Après quarante ans de recherche, indépendamment de la méthodologie ou du résultat potentiel, il semble probable qu'une ERC multicentrique d'envergure constitue la prochaine étape logique pour pouvoir bien répondre à la question de savoir si les agents anesthésiques peuvent préconditionner le myocarde et améliorer le résultat clinique.

**Conflicts of interest** None declared.

**Conflit d'intérêt** Aucun.

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REPORTS OF ORIGINAL INVESTIGATIONS

# Propofol cardioprotection for on-pump aortocoronary bypass surgery in patients with type 2 diabetes mellitus (PRO-TECT II): a phase 2 randomized-controlled trial

# La cardioprotection procurée par le propofol pour les chirurgies de pontage aorto-coronarien sous pompe chez les patients atteints de diabète de type 2 (PRO-TECT II): une étude randomisée contrôlée de phase 2

**David M. Ansley, MD · Koen Raedschelders, PhD · Peter T. Choi, MD · Baohua Wang, PhD · Richard C. Cook, MD · David D. Y. Chen, PhD**

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**Abstract**

**Purpose** *The efficacy of myocardial conditioning strategies is compromised in patients with advanced age, diabetes, or low ejection fraction. We conducted a single-centre parallel-arm blinded randomized-controlled trial to determine whether propofol provides perioperative myocardial protection.*

**Methods** *Patients enrolled in this study were scheduled for primary aortocoronary bypass surgery utilizing normothermic cardiopulmonary bypass (CPB) with blood cardioplegia. The participants were stratified by diabetic status and left ventricular ejection fraction and randomly assigned to receive either an elevated dose of propofol – previously associated with experimental cardioprotection– or an isoflurane preconditioning regime. The primary endpoint was the coronary sinus (CS) concentration of 15-$F_{2t}$-isoprostane (isoP). Secondary endpoints included in-hospital low cardiac output syndrome (LCOS) and major adverse cardiac events, 12- and 24-hr CS cardiac troponin I (cTnI) release, and myocardial B-cell lymphoma 2 (Bcl-2) protein expression.*

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D. M. Ansley, MD · P. T. Choi, MD · B. Wang, PhD
Department of Anesthesiology, Pharmacology and Therapeutics, The University of British Columbia, Vancouver, BC, Canada

D. M. Ansley, MD · P. T. Choi, MD
Department of Anesthesiology and Perioperative Care, Vancouver General Hospital, Vancouver, BC, Canada

D. M. Ansley, MD (✉)
Department of Anesthesiology, Pharmacology and Therapeutics, University of British Columbia, Room 3300, 3rd Floor JPP, 910 West 10th Ave, Vancouver, BC V5Z 1M9, Canada
e-mail: david.ansley@vch.ca

K. Raedschelders, PhD
Heart Institute - Cedars Sinai Medical Center, Los Angeles, CA, USA

P. T. Choi, MD
Center for Clinical Epidemiology and Evaluation, Vancouver Coastal Health Research Institute, Vancouver, BC, Canada

R. C. Cook, MD
Department of Surgery (Division of Cardiac Surgery), The University of British Columbia, Vancouver, BC, Canada

D. D. Y. Chen, PhD
Department of Chemistry, The University of British Columbia, Vancouver, BC, Canada

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**Results** *Data were analyzed from 125 of 137 randomized participants. Participants receiving propofol experienced a greater mean (SD) increase from baseline in CS 15-$F_{2t}$-isoP levels compared with those receiving isoflurane [26.9 (10.9) pg·mL$^{-1}$ vs 12.1 (10.4) pg·mL$^{-1}$, respectively; mean difference, 14.8; 95% confidence interval (CI), 11.0 to 18.6; P < 0.001] but a decreased incidence of LCOS (20.9% vs 57.1%, respectively; relative risk [RR],0.37; 95% CI, 0.22 to 0.62; P < 0.001). The incidence of LCOS was similar between groups in participants without type 2 diabetes mellitus (DM2) (P = 0.382) but significantly decreased in the propofol DM2 subgroup compared with the isoflurane DM2 subgroup (17.9% vs 70.3%, respectively; RR, 0.26; 95% CI, 0.13 to 0.52; P < 0.001). Propofol was associated with an increase in myocardial Bcl-2 protein expression (P = 0.005), a lower incidence of a CS cTnI threshold for myocardial infarction (P = 0.014), and fewer heart failure events (P < 0.001).*

**Conclusion** *Propofol may be a preemptive intraoperative cardioprotectant for patients with DM2 under conditions of normothermic CPB and blood cardioplegic arrest. The study is registered at www.clinicaltrials.gov (NCT00734383) and www.controlled-trials.com (ISRCTN70879185).*

**Résumé**

**Objectif** *L'efficacité des stratégies de conditionnement myocardique est compromise chez les patients âgés ainsi que chez ceux atteints de diabète ou présentant une fraction d'éjection faible. Nous avons réalisé une étude randomisée contrôlée unicentrique à bras parallèles et en aveugle afin de déterminer si le propofol procurait une protection myocardique en période périopératoire.*

**Méthode** *Les patients enrôlés dans cette étude devaient subir une chirurgie de pontage aorto-coronarien primaire avec circulation extracorporelle (CEC) normothermique et cardioplégie sanguine. Les participants ont été stratifiés par statut diabétique et fraction d'éjection ventriculaire gauche, puis aléatoirement répartis en deux groupes, dont l'un recevrait une dose élevée de propofol – un agent précédemment associé à une cardioprotection expérimentale – et l'autre un régime de préconditionnement à l'isoflurane. Le critère d'évaluation principal était la concentration dans le sinus coronaire (SC) de 15-$F_{2t}$-isoprostane (isoP). Les critères d'évaluation secondaires comprenaient la survenue d'un syndrome de bas débit cardiaque (SBDC) pendant le séjour hospitalier et les complications cardiaques majeures, la libération de troponine I cardiaque (cTnI) du SC à 12 et 24 h, et l'expression protéinique du lymphome 2 à cellules B (Bcl-2) myocardique.*

**Résultats** *Les données de 125 des 137 patients randomisés ont été analysées. Les participants ayant reçu du propofol ont subi une augmentation moyenne (ÉT) plus importante depuis les valeurs de base en matière de niveaux au SC de 15-$F_{2t}$-isoP par rapport aux patients ayant reçu de l'isoflurane [26,9 (10,9) pg·mL$^{-1}$ vs 12,1 (10,4) pg·mL$^{-1}$, respectivement; différence moyenne, 14,8; intervalle de confiance (IC) 95 %, 11,0 à 18,6; P < 0,001], mais une incidence moindre de SBDC (20,9 % vs 57,1 %, respectivement; risque relatif [RR], 0,37; IC 95 %, 0,22 à 0,62; P < 0,001). L'incidence de SBDC était semblable dans les deux groupes chez les participants qui n'étaient pas atteints de diabète de type 2 (DT2) (P = 0,382), mais significativement réduite dans le sous-groupe DT2 propofol par rapport au sous-groupe DT2 isoflurane (17,9 % vs 70,3 %, respectivement; RR, 0,26; IC 95 %, 0,13 à 0,52; P < 0,001). Le propofol a été associé à une augmentation de l'expression protéinique du Bcl-2 myocardique (P = 0,005), une incidence moindre de seuil de cTnI du SC pour un infarctus du myocarde (P = 0,014), et moins d'épisodes d'insuffisance cardiaque (P < 0,001).*

**Conclusion** *Le propofol pourrait constituer un cardioprotecteur peropératoire préventif pour les patients atteints de DT2 sous CEC normothermique et en arrêt cardioplégique sanguin. Cette étude est enregistrée au www.clinicaltrials.gov (NCT00734383) et au www.controlled-trials.com (ISRCTN70879185).*

Ischemia-reperfusion injury (IRI) is a major factor in the development of myocardial infarction and heart failure events following aortocoronary bypass (ACBP) surgery.[1] Patients with advanced age, diabetes, or low preoperative ventricular ejection fraction are at a particularly high risk for these complications.[1–3] Low cardiac output syndrome (LCOS) secondary to myocardial stunning or necrosis increases postoperative mortality by as much as ten to 17-fold.[3] It is essential to develop a preemptive intraoperative therapeutic strategy to counter these effects.

Since the discovery of ischemic preconditioning by Murry *et al.* nearly 30 years ago, efforts have focused on strategies that increase the tolerance of the myocardium to IRI.[4] Unfortunately, pharmacologic or physical conditioning strategies are not effective in the aged or chronic diabetic heart due to corruption of cardioprotective signalling pathways and mitochondrial dysfunction.[5–11] As oxidative stress is a major factor in the pathophysiology of IRI, propofol, an anesthetic and phenolic antioxidant, could be a therapeutic alternative.[12,13]Nevertheless, meta-analyses of clinical trials show reduced indices of myocardial injury and dysfunction with inhalational agents compared with propofol anesthesia (2-4 μg·mL$^{-1}$) in low-risk ACBP surgery.[14,15] A better approach to achieve cardioprotection might be to apply propofol in

higher concentrations solely during the ischemia-reperfusion interval.[16]

Applying propofol just before, during, and briefly following ischemia-reperfusion in rat heart models decreases release of plasma free 15-$F_{2t}$-isoprostane,[17,18] a biomarker of oxidative stress. It exacerbates myocardial IRI, producing left ventricular dysfunction directly via receptor-induced coronary artery vasoconstriction.[19] Furthermore, we previously found that propofol upregulates anti-apoptotic B-cell lymphoma 2 (Bcl-2) gene and protein expression in cardiomyoblasts, allowing them to withstand subsequent oxidative challenge.[20] B-cell lymphoma 2 sequesters pro-apoptotic BAX, a protein that promotes mitochondrial pore opening. This attenuates free radical release, death sequence activation, and IRI.[21] This could decrease 15-$F_{2t}$-isoprostane generation during reperfusion associated with postoperative ventricular dysfunction in patients.[22]

This report presents the effects of a propofol infusion designed to achieve drug concentrations in patients during cardiopulmonary bypass (CPB) that alleviate experimental oxidative injury.[23] Accordingly, in this study, we investigated the concentration and time-dependent effects of propofol (whole blood concentration 4-12 $\mu g \cdot mL^{-1}$) compared with isoflurane on cyto- and cardioprotective measures of oxidative injury.[16,17] We hypothesized that propofol would decrease 15-$F_{2t}$-isoprostane release and, as a result, reduce the incidence of LCOS. We further explored concomitant myocardial Bcl-2 expression as it may affect or be affected by 15-$F_{2t}$-isoprostane release. Lastly, these endpoints and outcomes were differentially investigated in patients with or without type 2 diabetes mellitus (DM2).

## Methods

PRO-TECT II is a single-centre phase 2 blinded randomized-controlled trial.[24] The study was approved (July 2005) by the University of British Columbia Clinical Research Ethics Board and conforms to the principles outlined in the Declaration of Helsinki.

### Participants

We enrolled adult patients at the Vancouver General Hospital after obtaining their written informed consent. Patients were eligible for participation if they were 18-80 yr of age and scheduled for elective primary ACBP surgery that entailed revascularization of three or more coronary arteries with cardiopulmonary bypass (CPB) and an anticipated aortic cross-clamp (ACC) time of at least 60 min. Patients were ineligible if they had type 1 diabetes mellitus (DM), coexisting valvular heart disease, an acute or evolving myocardial infarction within seven days of surgery, or a history of hypersensitivity to propofol (or its various components). Given the link between inflammation and oxidative stress, patients taking nonsteroidal anti-inflammatory drugs (including acetylsalicylic acid) or antioxidants within five to seven days of surgery were excluded to avoid potential confounding of our primary outcome variable.

### Randomization and blinding

Participants were randomly allocated to either propofol (cardioprotection) or isoflurane (preconditioning) using a computer-generated random number table. Randomization was accomplished with permuted blocks of four or six, stratified by history of DM (no DM or DM2) and by the preoperative left ventricular ejection fraction based on angiography – i.e., normal $\geq 45\%$ or low $< 45\%$.

A study anesthesiologist or fellow initiated the study intervention, leaving the patients, surgeons, investigators, and attending anesthesiologist blinded to the experimental patient therapy. Blinding was facilitated by covering anesthetic vaporizers with opaque drapes, turning off the end-tidal anesthetic agent readout, and mimicking use of propofol in the isoflurane group by infusing Intralipid® 20% lipid emulsion (Frensius Kabi, Bad Homburg, DE, Germany) on the same side as the patient's intravenous catheter into a receptacle concealed from view. During CPB, the study anesthesiologist directed an unblinded perfusionist regarding anesthetic management. Following separation from CPB, draping was removed and the blinded anesthesiologist resumed care. Staff providing postoperative care were unaware of study group allocation.

### Procedures

Intravenous and arterial cannulae were inserted prior to anesthetic induction (fentanyl 10-15 $\mu g \cdot kg^{-1}$ *iv*, midazolam 0.15-0.25 $mg \cdot kg^{-1}$ *iv*, and sodium thiopental 1-2 $mg \cdot kg^{-1}$ *iv*) and muscle relaxation (rocuronium 1-1.5 $mg \cdot kg^{-1}$ *iv*). Anesthesia was maintained with isoflurane 0.5-1.5% (end-tidal) while central venous and pulmonary artery catheterization and transesophageal echocardiography were performed.

Following heparinization, administration of isoflurane to the propofol group was discontinued for ten minutes. At this time, a bolus of propofol 1 $mg \cdot kg^{-1}$ *iv* was administered, followed by an infusion of propofol 120 $\mu g \cdot kg^{-1} \cdot min^{-1}$ *iv* administered prior to aortic cannulation and maintained until 15 min after release of the ACC (i.e., reperfusion) (Fig. 1). Participants allocated to the isoflurane group received 2.5% isoflurane (end-tidal) for

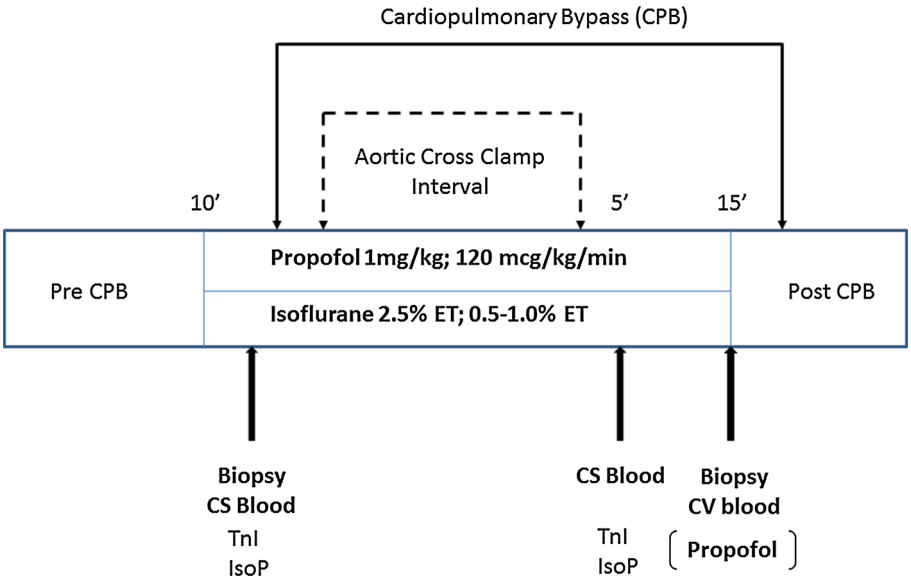



**Fig. 1** Study protocol. CPB= cardiopulmonary bypass; CS = coronary sinus; CV = central venous; ET = end tidal; isoP =15-$F_{2t}$-isoprostane; TnI = troponin I; [ ] Denotes concentration; ' Denotes time in minutes

ten minutes before CPB, followed by isoflurane 0.5-1.0% (end-tidal) during CPB and 15-min reperfusion.

A retrograde coronary sinus (CS) catheter for blood sampling was placed prior to ACC. Nonpulsatile CPB was conducted at 34-37°C after retrograde autologous priming to maintain an intraoperative hematocrit of 0.25- 0.27. Cardioplegia was induced and maintained with intermittent antegrade warm or cold 8:1 and then warm 64:1 blood cardioplegia as per surgeon preference. An arterial conduit was performed to the left anterior descending coronary artery, followed by sequential arterial or saphenous vein grafting as required.

An intravenous infusion of insulin was administered to achieve perioperative glucose levels $< 10$ mmol·$L^{-1}$ according to hospital guidelines. Following the study intervention, anesthesia maintenance, sedation, and analgesia were conducted according to routine clinical practice. Echocardiography and pulmonary artery catheter measurements guided the use of fluids and vasoactive drugs for separation from CPB and in the intensive care unit.

## Sampling and analysis

Arterial and CS blood samples were drawn prior to ACC and at five minutes after reperfusion. A central venous blood specimen was collected at 15 min after reperfusion. In a small subset of patients ($n$ = 8), cardioplegia samples were drawn from delivery tubing at the midpoint of surgery to determine if the study intervention produced blood cardioplegia enriched with propofol. Right atrial biopsies taken after aortic cannulation and at 15 min after reperfusion were flash frozen in liquid nitrogen and stored along with the blood specimens at -80°C for a subsequent analysis of plasma free 15-$F_{2t}$-isoprostane (i.e., the primary study outcome), troponin I (TnI), propofol concentration, and myocardial Bcl-2 protein expression.

Plasma free 15-$F_{2t}$-isoprostane (hereafter denoted as "15-$F_{2t}$-isoprostane") was quantitatively analyzed using immunoaffinity purification followed by liquid chromatography-mass spectrometry analysis.[25] A prior *in vitro* study from our laboratory determined that the normothermic heart was a major source of 15-$F_{2t}$-isoprostane.[17,18,26] Mean (SD) *arterial* 15-$F_{2t}$-isoprostane measurements were lower than concurrent CS measurements [30.1 (18.9) *vs* 94.0 (32.1) pg.$mL^{-1}$, respectively] in samples from the first ten participants that were analyzed without unmasking the allocation. Based on these results, the primary endpoint was modified with subsequent analyses based on only CS 15-$F_{2t}$-isoprostane levels.

Troponin I concentration in plasma was measured ten minutes prior to CPB and five minutes, 12 hr, and 24 hr after reperfusion by our central hospital laboratory (Siemens Vista analyzer, Siemens Healthcare Diagnostics Ltd, Camberley, UK). Whole blood propofol concentrations in cardioplegia and in central venous blood were determined by capillary electrophoresis as previously reported.[27] Myocardial Bcl-2 protein expression in the atrial biopsies was determined by immunoblotting as previously described.[28]

## Clinical outcomes

Participants were diagnosed to have LCOS if they required dopamine or dobutamine $>$ 4 μg·$kg^{-1}$·$min^{-1}$ *iv*, epinephrine or norepinephrine $>$ 0.04 μg·$kg^{-1}$·$min^{-1}$ *iv*, or milrinone $\geq$ 0.125 μg·$kg^{-1}$·$min^{-1}$ *iv* and/or intra-aortic balloon pump for $>$ 30 min within the first six hours of

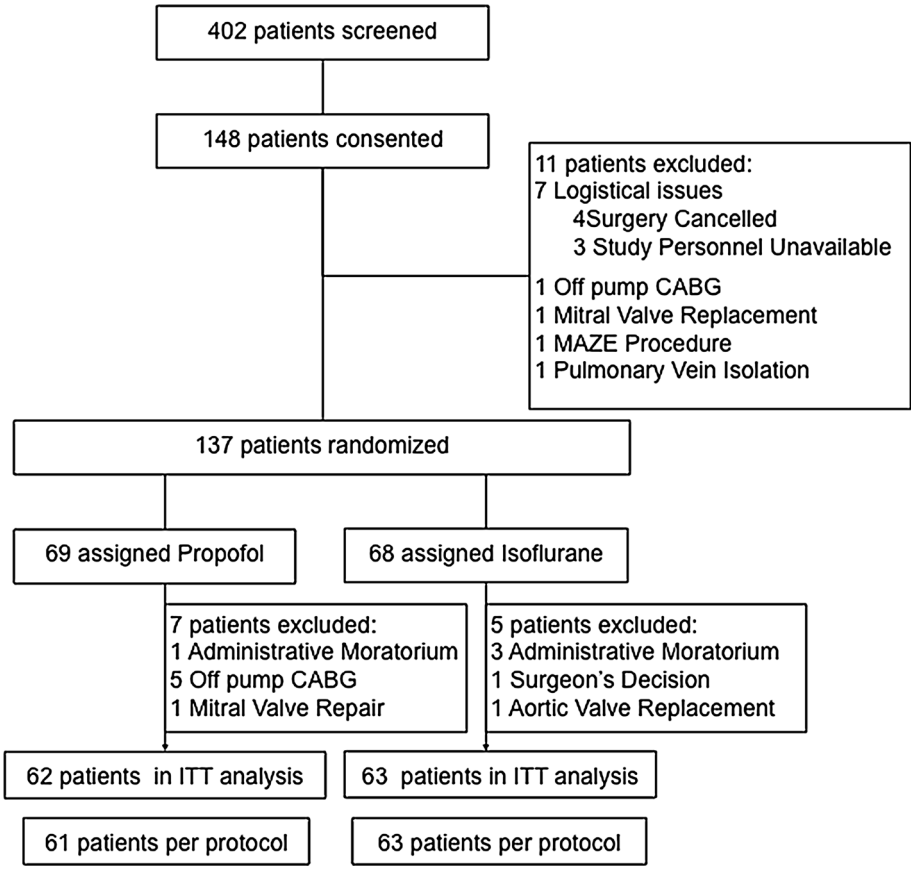



**Fig. 2** Flow diagram of patient enrolment, allocation, follow-up, and analysis. The administrative moratorium refers to a period of institutional review when collection of human specimens was suspended for all clinical trials at the study site. CABG = coronary artery bypass graft surgery; ITT = intention-to-treat

reperfusion. These treatments help to maintain systolic blood pressure > 90 mmHg and cardiac index > 2.1 $L{\cdot}min^{-1}{\cdot}m^{-2}$ following optimization of heart rate, preload, and afterload.[3] The diagnosis was excluded when norepinephrine was used to treat low systemic vascular resistance in the presence of a normal or elevated cardiac index or when echocardiography identified non-cardiac causes of hemodynamic instability.[3]

Major adverse cardiac events (MACE) were documented as defined by the 2014 American College of Cardiology/American Heart Association cardiovascular endpoints for clinical studies: death, myocardial infarction, unstable angina, transient ischemic attack or stroke, heart failure event (i.e., prolonged LCOS > 24 hr or postoperative congestive heart failure), percutaneous or peripheral vascular intervention.[29]

## Statistical methods

Using prior results for arterial 15-$F_{2t}$-isoprostane measured by enzyme-linked immunoassay, assuming a mean (SD) 15-$F_{2t}$-isoprostane of 150 (120) $pg{\cdot}mL^{-1}$, a two-sided type I error rate of 0.05, and power of 0.80, 144 participants (36 per stratum - i.e., type 2 DM or no DM, normal or low left ventricular ejection fraction per group) were needed to detect an anticipated relative difference of 50% between groups.[24]

Data analysis followed the intention-to-treat principle. Categorical data were summarized as counts and percentages. Normal and skewed continuous data were summarized as mean (SD) or median (interquartile range [IQR]), respectively. The Mann-Whitney *U* test was used for intergroup comparisons of 15-$F_{2t}$-isoprostane levels. Fisher's exact test was used for intergroup comparisons, and the relative risk (RR), absolute risk reduction, and their respective 95% confidence intervals (CI) were determined. Subgroup analyses of LCOS episodes based on diabetic status were planned *a priori*. Coronary sinus cardiac troponin I (CS cTnI) levels were analyzed with the Kruskal-Wallis test. We excluded spurious TnI values from participants without LCOS or MACE indicative of rapid CS sampling or associated with surgical manipulation, cardiac tamponade, or pericarditis. Results were explored in comparison with prognostic thresholds for intraoperative myocardial damage (CS cTnI $\geq$ 0.5 $\mu g{\cdot}L^{-1}$) and infarct-related events (CS cTnI $\geq$ 0.94 $\mu g{\cdot}L^{-1}$ and systemic cTnI > 6.93 $\mu g{\cdot}L^{-1}$ in 24 hr).[30–32] Post/Pre-CPB Bcl-2 ratios were analyzed using the unpaired two-tailed Student's *t* test. A two-sided $P < 0.025$ was considered statistically significant. Analyses were performed with

SPSS® program for Windows Version 15 (SPSS Inc, Chicago, IL, USA) or GraphPad Prism Version 6 (GraphPad Software, San Diego, CA, USA).

## Results

During August 2005 to June 2011, 402 patients were screened for eligibility, 148 patients consented to participate in the study, and 137 participants were randomized to propofol cardioprotection ($n$ = 69) or isoflurane preconditioning ($n$ = 68) (Fig. 2). The study was discontinued before the planned target enrolment was reached due to local recruitment for a competing study as well as the unavailability of thiopental. Twelve participants (seven in the propofol group and five in the isoflurane group) were excluded due to a temporary moratorium on institutional research or an unanticipated change of surgery. One hundred twenty-five participants – propofol ($n$ = 62) or isoflurane ($n$ = 63) – were included in the intention-to-treat analysis. One participant in the propofol group developed prolonged pre-bypass ischemia and instability and received only isoflurane (Fig. 2).

Patient characteristics and medication use were similar between groups (Table 1). Procedural complexity was comparable except for increased use of right internal mammary artery ($P$ = 0.024) or bilateral internal mammary artery grafting ($P$ = 0.009) in the propofol group (Table 1). No 15-$F_{2t}$-isoprostane data were lacking. Cardiac troponin I data were excluded or missing in 10/125 patients (8%) at reperfusion, 16/125 patients (12.8%) at 12 hr, and 32/125 patients (25.6%) at 24 hr due to early discharge from the intensive care unit. B-cell lymphoma 2 data were lacking in 19/125 patients (15%). No data were lacking in patients experiencing LCOS or MACE.

At baseline, i.e., prior to CPB, the median [IQR] CS 15-$F_{2t}$-isoprostane was 60.6 [39.7-94.0] $pg{\cdot}mL^{-1}$ in the propofol group *vs* 53.6 [37.6-76.9] $pg{\cdot}mL^{-1}$ in the isoflurane group ($P$ = 0.218) (Fig. 3). The median [IQR] CS 15-$F_{2t}$-isoprostane level at reperfusion increased to 77.7 [56.3-124.4] $pg{\cdot}mL^{-1}$ in the propofol group ($P$ = 0.004) *vs* 62.7 [42.46-104.4] $pg{\cdot}mL^{-1}$ in the isoflurane group ($P$ = 0.084). Participants receiving propofol experienced a greater mean (SD) increase from baseline in CS 15-$F_{2t}$-isoprostane levels compared with those receiving isoflurane group (26.9 (10.9) $pg{\cdot}mL^{-1}$ *vs* 12.1 (10.4) $pg{\cdot}mL^{-1}$, respectively; mean difference, 14.8; 95% CI, 11.0 to 18.6; $P < 0.001$).

Low cardiac output syndrome occurred in 49/125 (39.2%) participants, attributable to stunning (CS cTnI < 0.5 $\mu g{\cdot}L^{-1}$) in 24/49 (49%) episodes or intraoperative myocardial damage (CS cTnI $\geq$ 0.5 $\mu g{\cdot}L^{-1}$) in 25/49 (51%) episodes.[30,31] Compared with the isoflurane group, the incidence of LCOS was 1) lower overall in the propofol group (20.9% propofol *vs* 57.1% isoflurane; RR, 0.37; 95% CI, 0.22 to 0.62; $P < 0.001$); 2) similar between groups for participants without diabetes (26.1% propofol *vs* 38.4% isoflurane; RR, 0.68; 95% CI, 0.29 to 1.57; $P$ = 0.382); and 3) decreased in the DM2 propofol subgroup (17.9% DM2 propofol *vs* 70.3% isoflurane; RR, 0.26; 95% CI, 0.13 to 0.52; $P < 0.001$; interaction $P$ value = 0.08) (Table 2). Median [IQR] duration of inotropic support was 3.6 [2.0-6.9] hr in the propofol group *vs* 11.2 [4.7-18.0] hr in the isoflurane group (difference in the medians, 7.6 hr; 95% CI, 1.8 to 13.5; $P$ = 0.004). Intra-aortic balloon pump counterpulsation was not required. Major adverse cardiac events (MACE) occurred in three participants in the propofol group *vs* 17 participants in the isoflurane group (RR, 0.26; 95% CI, 0.09 to 0.77; $P$ = 0.001). Heart failure events decreased with propofol compared with isoflurane [0/62 (0%) *vs* 14/63 (22.2%), respectively; RR, 0.34; 95% CI, 0.19 to 0.59; $P < 0.001$] (Table 3).

Median [IQR] cTnI values were similar at baseline, i.e., pre-CPB, but increased similarly in both groups at reperfusion, 12 hr, and 24 hr ($P < 0.001$) (Fig. 4). Sixteen participants had CS cTnI $\geq$ 0.94 $\mu g{\cdot}L^{-1}$ at reperfusion (three participants in the propofol group *vs* 13 in the isoflurane group, $P$ = 0.014), and nine participants had systemic cTnI $\geq$ 6.93 $\mu g{\cdot}L^{-1}$ in 24 hr (none in the propofol group *vs* nine in the isoflurane group, $P$ = 0.003).[31,32]

The mean (SD) relative protein expression of myocardial Bcl-2 at reperfusion was 1.43 (0.94) in the propofol group *vs* 0.77 (0.52) in the isoflurane group (mean difference, 0.65; 95% CI, 0.29 to 1.01; $P$ = 0.005) (Fig. 5).

The mean (SD) propofol concentration was 5.74 (2.50) $\mu g{\cdot}mL^{-1}$ in systemic blood and 2.04 (1.14) $\mu g{\cdot}mL^{-1}$ in blood microplegia.

## Discussion

In this study, our results did not confirm our hypothesis that propofol would decrease 15-$F_{2t}$-isoprostane release. We showed that, compared with isoflurane, participants receiving propofol had higher CS levels of this marker of oxidative stress at reperfusion following CPB. Interestingly, the incidence, duration, and risk of LCOS were decreased in patients with DM2. The incidence of MACE, primarily heart failure events, was lowest in patients with DM2 treated with propofol cardioprotection. This may be attributable in part to a decreased incidence of severe postoperative cardiac injury. B-cell lymphoma 2, a mitochondrial protectant, increased in myocardium at reperfusion in participants receiving propofol. Propofol could mediate a pro-oxidant mechanism of cardioprotection.

**Table 1** Patient characteristics and procedural complexity

| Variable | Propofol (*n*=62) | Isoflurane (*n*=63) |
|---|---|---|
| Age (yr), mean (SD) | 63.6 (9) | 64.5 (8) |
| Female sex, *n* (%) | 8 (13%) | 10 (16%) |
| Body mass index ($kg \cdot m^{-2}$), mean (SD) | 29.1 (5) | 28.7 (5) |
| Diabetes, *n* (%) | 39 (63%) | 37 (59%) |
| Hemoglobin A1C (%), mean (SD) | 7.3 (1.5) | 7.3 (1.6) |
| Fasting blood sugar ($mmol \cdot L^{-2}$), mean (SD) | 8.2 (3) | 8.1 (2) |
| Creatinine ($\mu mol \cdot L^{-2}$), mean (SD) | 91.5 (19) | 94.1 (19) |
| Glomerular filtration rate ($mL \cdot min^{-2}$), mean (SD) | 73.9 (16) | 70.0 (16) |
| Unstable angina, *n* (%) | 22 (35%) | 19 (30%) |
| Recent non-ST-elevation myocardial infarction, *n* (%) | 22 (36%) | 32 (51%) |
| Current smoker, *n* (%) | 15 (24%) | 15 (24%) |
| Left ventricular ejection fraction (%), mean (SD) | 51.2 (14) | 49.4 (12) |
| > 45% *n* (%) | 40 (65%) | 41 (65%) |
| 35-45% *n* (%) | 15 (24%) | 13 (21%) |
| 25-35% *n* (%) | 4 (6%) | 9 (14%) |
| < 25% *n* (%) | 3 (5%) | 0 (0%) |
| Left ventricular end-diastolic pressure (mmHg), mean (SD) | 17 (8) | 17 (6) |
| > 15 mmHg *n* (%) | 31 (55%) | 36 (65%) |
| Coronary artery disease | | |
| 2 vessel disease, *n* (%) | 12 (19%) | 15 (24%) |
| 3 vessel disease, *n* (%) | 50 (81%) | 44 (70%) |
| Left main > 50% stenosis, *n* (%) | 12 (19%) | 19 (30%) |
| Total coronary occlusion in major artery, *n* (%) | 22 (36%) | 26 (41%) |
| *Preoperative medications* | | |
| Beta-blocker, *n* (%) | 55 (89%) | 53 (86%) |
| Angiotensin-converting enzyme inhibitor / angiotensin receptor blocker, *n* (%) | 40 (65%) | 42 (68%) |
| Calcium-channel blocker, *n* (%) | 17 (27%) | 17 (27%) |
| Diuretic, *n* (%) | 16 (25%) | 11 (18%) |
| Statin, *n* (%) | 53 (86%) | 55 (87%) |
| Oral hypoglycemic agent, *n* (%) | 33 (53%) | 30 (48%) |
| Insulin, *n* (%) | 6 (10%) | 11 (17%) |
| Digoxin, *n* (%) | 1 (1.6%) | 2 (3.2%) |
| *Surgical details* | | |
| Total number of grafts, mean (SD) | 3.8 (1.1) | 3.7 (1.0) |
| Left internal mammary artery, *n* (%) | 51 (82%) | 58 (92%) |
| Right internal mammary artery, *n* (%) | 17 (27%) | 7 (11%)* |
| Bilateral internal mammary artery, *n* (%) | 23 (37%) | 10 (16%) ** |
| Free radial artery graft, *n* (%) | 13 (21%) | 19 (30%) |
| Aortic cross-clamp duration (min), mean (SD) | 87 (32) | 86 (32) |
| Cardiopulmonary bypass duration (min), mean (SD) | 116 (42) | 119 (47) |
| Cardiopulmonary bypass volume (mL), mean (SD) | 4,834 (2,543) | 4,963 (2,897) |
| Hematocrit on bypass, mean (SD) | 0.27 (0.04) | 0.27 (0.04) |
| *Insulin treatment and glycometrics* | | |
| Insulin in OR ($units \cdot hr^{-1}$), mean (SD) | 2.0 (3.0) | 2.7 (3.7) |
| Insulin in ICU ($units \cdot hr^{-1}$), mean (SD) | 4.6 (7.1) | 4.0 (2.8) |
| Total insulin in 24 hr, mean (SD) | 48.4 (41) | 55.6 (47) |
| Mean intraoperative glucose ($mmol \cdot L^{-1}$), mean (SD) | 8.2 (2) | 8.6 (2) |

ICU = intensive care unit; OR = operating room; SD = standard deviation. Data are mean (SD) or number (%). * $P = 0.024$; ** $P = 0.009$

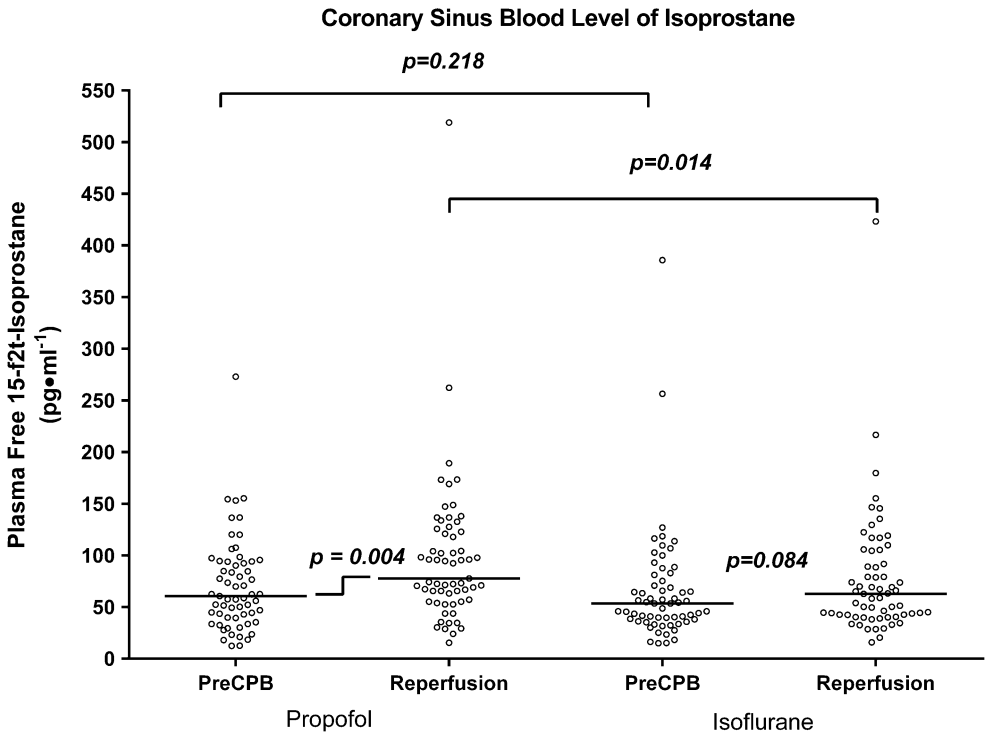



**Fig. 3** Plasma free 15-$F_{2t}$-isoprostane concentration measured in coronary sinus blood. The dots denote the individual measurements; the horizontal lines denote the medians in each group. Pre-CPB = before cardiopulmonary bypass; reperfusion = after release of aortic cross-clamp

**Table 2** Incidence, relative risk, and risk difference of low cardiac output syndrome

| Low Cardiac Output Syndrome | In-hospital incidence *n* (%) Propofol | Isoflurane | *P* value | RR (95% CI) | ARR (95% CI) |
|---|---|---|---|---|---|
| Overall | 13/62 (20.9) | 36/63 (57.1) | <0.0001 | 0.37 (0.22 to 0.62) | −0.36 (−0.52 to −0.20) |
| Participants without diabetes | 6/23 (26.1) | 10/26 (38.4) | 0.36 | 0.68 (0.29 to 1.57) | −0.12 (−0.38 to −0.14) |
| Participants with diabetes | 7/39 (17.9) | 26/37 (70.3) | <0.0001 | 0.26 (0.13 to 0.52) | −0.52 (−0.71 to −0.33) |

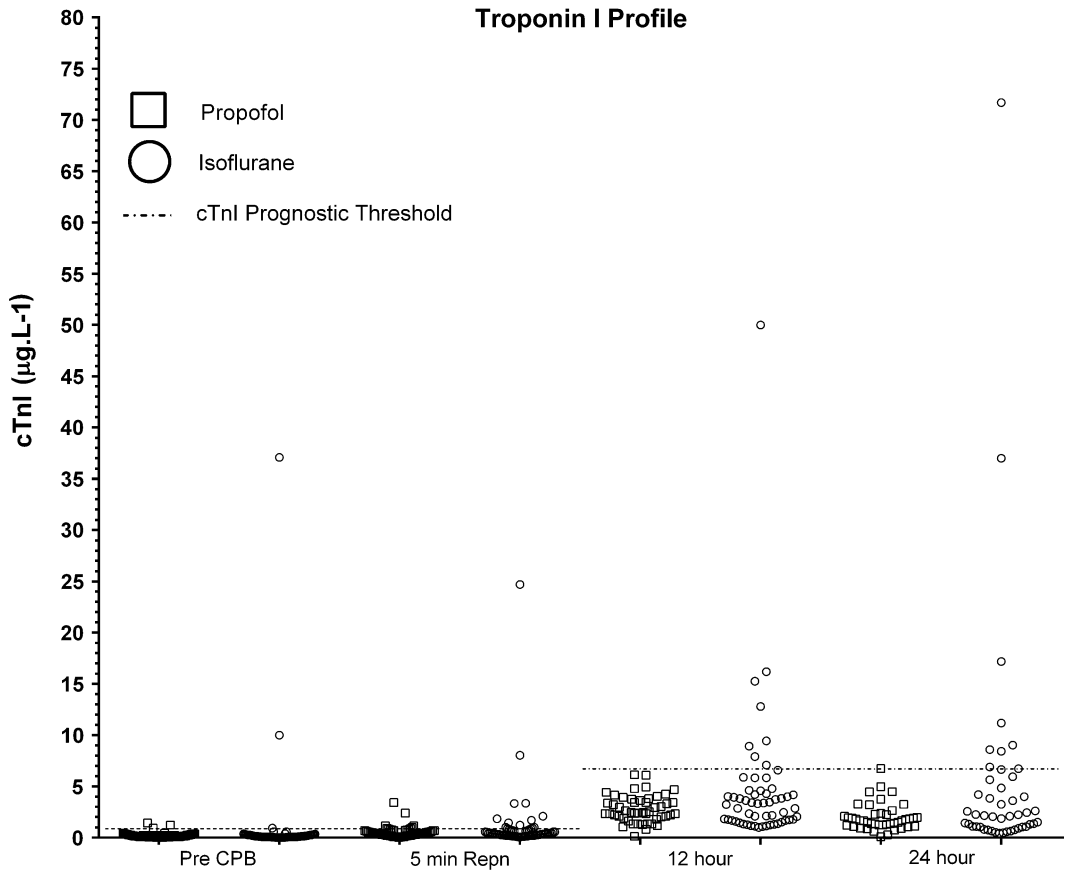
Interaction *P* value = 0.08

ARR = absolute risk reduction; CI = confidence interval; RR = relative risk

**Table 3** Major adverse cardiac events

| | Propofol | Isoflurane | *P* value |
|---|---|---|---|
| In-hospital incidence, *n (%)* | 3/62 (4.8) | 17/63 (26.9) | 0.001 |
| Death | 0 | 1 (1.6) | 1.00 |
| Myocardial Infarction | 0 | 4 (6.3) | 0.12 |
| Stroke | 1 (1.6) | 3 (4.7) | 0.62 |
| Graft Revision/PCI | 0 | 3 (4.7) | 0.11 |
| Unstable Angina | 3 (4.8) | 3 (4.7) | 1.00 |
| Heart Failure Events | 0 | 14 (22.2) | < 0.0001 |
| Prolonged LCOS | 0 | 9 (14.3) | 0.003 |
| CHF | 0 | 5 (7.9) | 0.06 |

CHF = congestive heart failure; LCOS = low cardiac output syndrome; PCI = percutaneous coronary intervention




| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Median | 0.10 | 0.12 | 0.41 | 0.44 | 2.47 | 3.33 | 1.67 | 2.17 |
| IQR | 0.06 – 0.25 | 0.07 – 0.25 | 0.21 - 0.69 | 0.21 - 0.79 | 1.97 - 3.69 | 1.69 - 4.61 | 1.21 - 2.29 | 1.04 - 5.67 |

**Fig. 4** Perioperative troponin I profiles. The squares denote individual measurements in the propofol group; the dots denote individual measurements in the isoflurane group; hashed lines denote coronary sinus or postoperative prognostic cTnI thresholds for infarct-related events. cTnI = cardiac troponin I; IQR = interquartile range; Pre-CPB = before cardiopulmonary bypass; repn = reperfusion after release of aortic cross clamp

Oxidative stress is a major factor in the pathophysiology of myocardial IRI (Fig. 6).[12,13,21] During ischemia, the mitochondrial electron transport chain (ETC) is damaged. B-cell lymphoma 2, a mitochondrial protein, is depleted.[21] During reperfusion, reactive oxygen species (ROS) generated by the damaged ETC induce opening of the mitochondrial permeability transition pore (mPTP), a non-specific channel of the inner mitochondrial membrane. Permeation is enhanced by translocation of the proapoptotic effector protein, BAX, from cytosol to mitochondria. Oxidative phosphorylation becomes uncoupled, increasing ROS production and release of cell death activators that induce mitochondrial rupture.[12] While the effects of ROS are largely compartmentalized within cells, they trigger generation of stable diffusible lipid peroxidation products like 15-$F_{2t}$-isoprostane, a coronary artery vasoconstrictor implicated in the development of postoperative ventricular dysfunction.[19,26]

We attribute our findings to patient characteristics, surgical conditions, and study interventions that differ from prior studies. We studied higher risk patients undergoing normothermic CPB and blood cardioplegic arrest. We included patients prescribed oral hypoglycemic agents or statins known to inhibit pre/postconditioning. Our study intervention was derived from prior *in vitro* and *in vivo* studies of the concentration and time-dependent effects of propofol which define a therapeutic window that we correlate with cyto- and cardioprotection (4.2-8.4 $\mu g \cdot mL^{-1}$).[16,17,23] These concentrations were achieved in the systemic circulation at reperfusion in 87% of participants where aortic cross-clamp intervals exceeded 60 min. Our technique enriches blood cardioplegia. Propofol 2 $\mu g \cdot mL^{-1}$ was delivered in 300 $mL \cdot min^{-1}$ boluses every 15-20 min during global ischemia. Compared with isoflurane, this regimen appears to decrease transient LCOS episodes and acute heart failure events secondary to myocardial stunning or injury.

In contrast, myocardial injury increased in low-risk patients following propofol anesthesia for ACBP surgery conducted with hypothermic CPB and cold crystalloid or blood cardiopreservation.[14,33] Membrane fluidity decreases

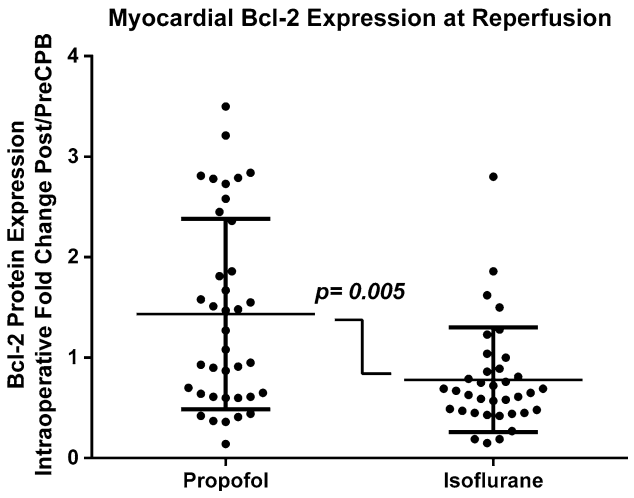



**Fig. 5** Myocardial Bcl-2 protein expression at reperfusion. The dots denote the individual measurements determined by Western blotting. The long horizontal lines and the vertical lines with cross bars denote the means and standard deviations, respectively, in each group. Values greater than one denote upregulation or gain. Values less than one denote downregulation or loss. Bcl-2 = myocardial B-cell lymphoma 2

in the presence of propofol at 25°C.[34] This could disrupt pharmacologic recruitment of cardioprotective signal transduction.[35,36] Detoxification of ROS by phenolic antioxidants involves conversion to peroxide and a phenoxyl radical that stimulates DNA fragmentation.[12] Furthermore, propofol induced transcriptional changes in fatty acid metabolism and DNA damage signalling, correlating with release of N- terminal pro-brain natriuretic peptide (NT-proBNP), a biomarker of cardiac

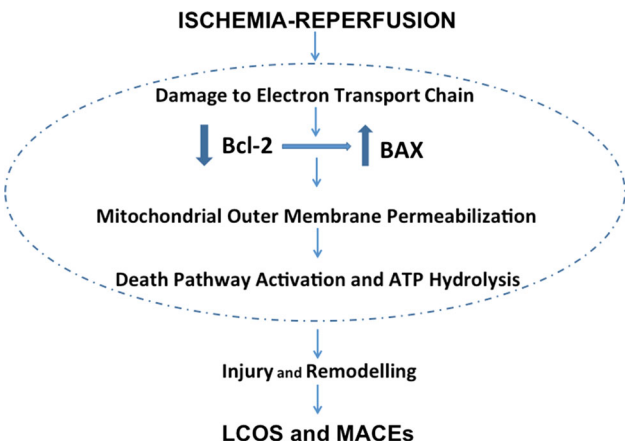



**Fig. 6** Proposed mechanism of injury from ischemia-reperfusion. Loss of Bcl-2 secondary to ischemic damage to the electron transport chain predisposes activation of the mitochondrial death pathway, alteration of cardiac energetics, and development of cellular acidosis resulting in cardiac injury and dysfunction. Bcl-2 = myocardial B-cell lymphoma 2; LCOS = low cardiac output syndrome; MACE = major adverse cardiac events

dysfunction.[37] Propofol concentrations were not measured and were likely suboptimal. Interestingly, a recent randomized multicentre trial concluded that inhalational anesthesia was not superior to total intravenous anesthesia in patients undergoing high-risk cardiac surgery.[8] The authors suggested that the benefits of inhalational anesthesia may be limited to low-risk surgical settings.

Similar 15-$F_{2t}$-isoprostane results were reported following off-pump coronary artery bypass graft surgery.[38] Although counterintuitive, there are two potential explanations. First, inhalational anesthesia may protect by suppressing burst of free radicals at reperfusion, inhibiting the translocation of BAX from cytosol to mitochondria.[39]Nevertheless, Bcl-2 levels are decreased in the diabetic or aged heart and subject to ischemic degradation during surgery.[21,28] If the buffering capacity of Bcl-2 is exhausted, BAX would initiate mitochondrial permeability transition, and cell injury would ensue.[21] Second, instead of scavenging ROS, propofol may initiate ROS-mediated signalling events whereby cardiomyocytes opt for cell survival.[20,21] Sublethal oxidative stress could trigger adaptive stress resistance via the upregulation of proteins like Bcl-2 to control mPTP opening.[40] Unlike inhalational anesthesia, the mechanism may involve transcription factor STAT3, which doesn't require a second messenger system for signal transduction.[41] Release of ROS would subsequently be attenuated, and 15-$F_{2t}$-isoprostane generation would decline. This protective response, termed mitochondrial hormesis (i.e., mitohormesis), could prevent injury and remodelling of the ischemic-reperfused heart.[42,43]

In mitohormesis, mitochondria generate ROS as part of a cellular adaptation involving downregulation of the cellular respiration.[44] This preserves Bcl-2 and inhibits the mPTP.[45] B-cell lymphoma 2 prevents the decline in pH and cardiac adenosine triphosphate (ATP) stores during ischemia.[46] Metabolic regulation shifts from glucose to fatty acid oxidation to restore cellular homeostasis.[44] Concern has been raised over the potential negative impact of propofol-mediated insulin resistance and fatty acid oxidation on the heart.[47]Nevertheless, fatty acid oxidation reverses an energy deficit and improves function of the post-ischemic insulin-resistant heart.[48] This could explain discrepancies in the incidence of LCOS episodes and events of heart failure seen in our study. Given our results, the areas of ongoing research by our laboratory include the molecular mechanism, functional significance, and therapeutic impact of propofol cardioprotection in patients with type 2 diabetes.

Our findings should be considered hypothesis-generating, not confirmatory, and should be interpreted with caution. Our phase 2 study design was based on the pharmacology of propofol. Our approach to CPB and

cardioplegia protection may not reflect the practices of other institutions. The increase in 15-$F_{2t}$-isoprostane and decrease in LCOS in response to propofol may be incidental and not reflective of a cardioprotective mechanism. Cardiac events in the isoflurane group could have arisen by chance or could be secondary to other factors, including microembolization, inadequate revascularization, or other mediators of cardiac injury.[1,6] In patients without diabetes, we cannot differentiate between the nonexistence of effect and the lack of power to detect it. Our clinical observations from the diabetes subgroup require further validation. Also, we cannot exclude a synergistic mechanism of protection involving propofol and insulin by which to explain our results.[49,50] Finally, our analysis of cTnI was exploratory. We did not incorporate sophisticated measures of cardiac injury and areas at risk in this study.

In summary, compared with isoflurane, continuous, systemic, and intermittent delivery of propofol-enriched microplegia during ischemia-reperfusion elicited a prooxidant response associated with decreased LCOS episodes and heart failure events following primary ACBP surgery. Propofol may be a preemptive intraoperative cardioprotectant for patients with DM2 under conditions of normothermic bypass and blood cardioplegic arrest. Further mechanistic studies and larger phase 3 clinical trials are needed to acquire better clarification regarding this possible effect.

**Author contributions:** *David Ansley*, *Koen Raedschelders*, *David Chen,* and *Peter Choi* designed the study. *David Ansley, Peter Choi*, *Koen Raedschelders,* and *Baohua Wang* coordinated the study. *David Ansley*, *Baohua Wang*, and *Koen Raedschelders* analyzed the data. *Koen Raedschelders* and *David Chen* conducted drug measurements and blood bioanalysis. *Richard Cook* helped with data acquisition. *David Ansley* was the principal investigator. *Baohua Wang* conducted tissue analysis. All authors contributed to critical revision of article content.

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