ORIGINAL ARTICLE

Kidney temperature course during living organ procurement and transplantation

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SUMMARY

Little is known about the actual kidney graft temperature during the 2nd warm ischemia time (WIT2). We aimed to determine the actual temperature course of the WIT2, with emphasis on the 15 °C metabolic threshold. Data of 152 consecutive adult living donor kidney transplantations were collected. The mean WIT2 was 41.3 ± 10.1 (SD) minutes with a temperature of 5.4 °C at baseline which gradually increased to 13.7, 17.4, and 20.2 °C after 10, 20, and 30 min, respectively. The percentage of kidneys with a temperature of 15 °C or higher was 81.2% after 20 min and 97.5% after 30 min. Duration of surgery (95% CI: −0.017 to −0.002, $P = 0.02$), multiple veins (95% CI: 0.0003–2.720, $P = 0.05$) and WIT2 (95% CI: 0.016–0.099, $P = 0.006$) were associated with a rapid temperature increase. No correlation could be determined between a rapid temperature rise and diminished graft function. This study showed a rapid increase in kidney temperature during WIT2, wherein the 15 °C threshold was reached within 20 min in more than 80% of the patients.

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Introduction

During kidney transplantation (KT), a prolonged second warm ischemia time (WIT2), cold ischemia time (CIT), and various donor or recipient variables are considered important risk factors for delayed graft function (DFG) and long-term survival [1,2]. Although WIT2 plays an important role in graft outcome, little is known about the actual kidney temperature variations during WIT2 in living donor kidney transplantation. Generally after kidney procurement, hypothermia by means of cold flushing and static cold storage is applied to reduce metabolism and thus slow down the accumulation of ischemic graft injury [3,4]. Interestingly, the actual temperature course during the WIT2 is largely unknown, as well as contributing factors. Experimental studies show that the metabolic activity in kidneys after cold storage resumes at approximately 15–18 °C [5–7]. Also new data suggest that controlled warming of the graft after cold storage or prolonged hypothermia using hypothermic machine perfusion may improve graft function [8,9]. Recently, a large study in deceased donor kidneys showed that the WIT2 time was an independent factor for DGF, interstitial fibrosis, and tubular atrophy [10]. The actual graft temperature was not mentioned in this study. The aim of this study was to provide a detailed analysis of the graft temperature course during LDKT, with

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emphasis on WIT2 and to identify variables that affect the temperature of the graft.

Materials and methods

Patient and data collection

Between April 2013 and July 2015, a total of 160 consecutive adult LDKTs were evaluated. Donor and recipient characteristics as well as clinical and laboratory data were prospectively collected. Overweight was defined as a body mass index (BMI) of more than 25 kg/m^2. Delayed graft function (DGF) was defined as recipients receiving hemodialysis within 7 days of transplantation. Slow graft function (SGF) was defined as a serum creatinine of ≥3 mg/dl (≥265 mmol/l) at day 5 post-transplantation [3].

Eight cases (5.0%) were excluded due to a deviating perioperative course in which the temperature could not be reliably assessed according to the research protocol, and the patient was considered unsuitable for inclusion [intraoperative venous tear (n = 1), arterial flow limitations due to an intimal flap and/or severe atherosclerosis (n = 2), incomplete case record files (n = 2), and redo of one or two anastomoses (n = 3)]. All transplantations were performed according to the same surgical protocol by four dedicated surgeons. All temperature measurements were made by a team of specially trained medical students under supervision of two authors (TK and RP).

Kidney procurement and transplantation procedure

Following laparoscopic procurement, the kidney was flushed with 500 ml cold University of Wisconsin (UW) solution and placed in cold storage. All recipients were operated under general anesthesia with cefuroxime antibiotic prophylaxis and meropenem when endocarditis prophylaxis was necessary. Induction immunosuppression, consisting of basiliximab (Simulect, 20 mg/50 ml) and methylprednisolone (Solu-Medrol®), was administered, and postoperatively, Mycofenol acid (Myfortic®) and tacrolimus (Advagraf®) were started. An oblique surgical incision from the pubic bone through about 2 cm cranial to the superior anterior iliac spine was used to create exposure of the external iliac vein and artery. The graft was subsequently anastomosed in an end-to-side manner, preferably in the right fossa. Before clamping of the external iliac artery, 5000 IE of heparin was given, if the patient was still preemptive. Mannitol 15% was administered prior to reperfusion. Using the modified Lich–Gregoir method, the ureter-to-bladder anastomosis was performed. During transplantation, the kidney was wrapped in a wet cold sponge wherein the top remained uncovered for the temperature measurements, combined with a nylon tape to hold in place. All recipients received sufficient quantities of lactated Ringer's solution during the procedure. The warm and cold ischemia times (CIT) were documented according to the international standard, in which the first warm ischemia time (WIT1) was defined as the period between clamping of the renal artery during procurement and the start of cold perfusion with UW. The WIT2 was the time between the start of the vascular anastomosis until reperfusion in the recipient.

Temperature measurement

At set time intervals (after procurement, UW flushing, cold storage, and backtable preparation, at the start of WIT2, after 10, 20, and 30 min, at reperfusion, and 10 min afterward), kidney temperature was measured using a noncontact infrared thermometer (Voltcraft®, IR 800-20D Thermometer, Conrad Electronic, Sweden) [11]. The thermometer has a double laser with sharp-point optics for target localization with a pickup time of <150 ms and a basic accuracy of 2%. During measurement, the exposed kidney cortex was scanned, resulting in multiple and continuous measurements, after which the mean temperature (°C) was automatically calculated on an optical display. This was repeated in the case an incorrect measurement was suspected to correct or confirm the result.

Ethical considerations

During the study period, data were prospectively collected and stored in an electronic database. For this study, the Medical Ethical Committee granted dispensation for the Dutch law regarding patient-based medical research (WMO) obligation (METc registration no. 2013424). Patient data were processed and electronically stored according to the Declaration of Helsinki—ethical principles for medical research involving human subjects.

Statistical analysis

Summary statistics were obtained using conventional methods and presented as percentages or means with standard deviation. To examine the course of temperate in this study, generalized estimating equations

(GEE) analysis with an exchangeable correlations structure was used. Such a model takes into account the correlations between the repeated measurements of temperature within the individual. The analyses were adjusted for potentially important variables [CCI, age (years), gender recipient, BMI recipient (continuous), preoperative creatinine (μmol/l), preemptive dialysis, length kidney (cm), number of arteries, number of veins, kidney side, blood loss (ml), duration of surgery (min), CIT (min), WIT2 (min)] using backward selection, that is starting with a full model (containing all the variables) and then eliminating least significant variables step-by-step until all *P*-values were <0.2. Estimates of the effects were reported with corresponding 95% confidence intervals. We estimated the same model after categorizing the continuous variables (age, BMI recipient, preoperative creatinine, length of kidney, blood loss, duration of surgery, CIT and WIT2). All statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS 22.0, SPSS, Chicago, IL, USA, 2013). Figures were made with GRAPHPAD PRISM (GraphPad Software, version 6.00, La Jolla, CA, USA).

Results

Baseline characteristics

Baseline patient and graft characteristics are presented in Tables 1 and 2. Donor and recipient ages were similar. The mean BMI of the recipients was 26.0 ± 4.5 (range 18.0–40.3), and 73 patients were transplanted preemptively (48%). The mean kidney length was 11.4 ± 0.86 centimeters (9.3–14.0). The majority of procured kidneys were left-sided (69.7%). Nearly all kidneys had immediate graft function in terms of intra- or postoperative urine production (98.7%). Twelve kidneys (7.9%) developed an impaired early graft function (DGF 0.7%, SGF 7.3%).

Temperature course during transplantation

The mean temperature of the donor and recipient during induction were, respectively, 36.3 ± 0.6 °C (range 33.6–39.9) and 35.9 ± 0.4 °C (range 35.0–37.1). Directly after procurement, the mean kidney temperature was 33.4 ± 1.4 °C (27.5–35.5) (WIT1 3.74 ± 1.2 min, Table 3). After flushing with UW perfusion fluid (4.1 ± 1.6 °C), the temperature dropped to 15.2 ± 5.1 °C (range 5.8–28.8). The mean CIT was 157.5 ± 33.7 min (70–321 min). After cold storage, the kidney temperature decreased to 1.6 ± 1.5 °C (range −0.7 to 8.4). Prior to transplantation, after backtable preparations, the temperature increased to 4.2 ± 2.8 °C (range 0.4–13.8). After 10, 20, and 30 min during the transplantation, the temperature increased to, respectively, 13.2 ± 3.1 °C (6.5–22.0), 17.1 ± 2.9 °C (10.2–27.3), and 19.8 ± 3.3 °C (12.9–35.8). The mean WIT2 was 41.32 ± 10.1 min (17–76; Fig. 1).

The percentage of kidneys that crossed the metabolic threshold of 15 °C during WIT2 was 25.9% after 10 min, 87.3% after 20 min, and 96.0% after 30 min. At the time of reperfusion, the temperature was 25.5 ± 5.0 °C (15.6–36.7), which quickly increased to 36.1 ± 0.7 °C (26.4–35.5) 10 min after reperfusion (Fig. 2).

Factors associated with temperature rise

The unadjusted analyses are shown in Table 3. The adjusted analysis (Table 4) showed that WIT2 (95% CI: 0.016–0.099, $P = 0.006$), duration of surgery (95% CI: −0.017 to −0.002, $P = 0.015$), and number of veins (95% CI: 0.0003–2.720, $P = 0.05$) were statistically significant factors associated with the course of the donor kidney temperature during transplantation. Other important confounders, with a *P*-value of <0.2, were preemptive dialysis (95% CI: −0.030 to 1.336, $P = 0.06$), length of the donor kidney (95% CI: −0.723 to 0.18, $P = 0.06$), BMI of the recipient (95% CI: −0.004 to 0.136, $P = 0.06$), age of the recipient (95% CI: −0.006 to 0.046, $P = 0.06$), and number of arteries (95% CI: −0.159 to 1.321, $P = 0.124$). In Table 5, the results of the same analysis are shown after categorizing the continuous variables age, BMI, preemptive dialysis, length of the donor kidney, blood loss, operation time, CIT, and WIT2. This yielded practically the same results except for adding preemptive dialysis (95% CI: 0.101–1.441, $P = 0.024$) and recipient BMI > 30 (95% CI: 0.255–2.262, $P = 0.014$) as factors associated with the course of the donor kidney temperature during transplantation.

Discussion

To our knowledge, this is the first study providing a detailed representation of the actual graft temperature during LDKT. Our results show that during the WIT2, the temperature increases rapidly after start of the implantation and in 80% of the grafts, the metabolic threshold of >15 °C was reached within 20 min after cold storage.

Table 1. Baseline characteristics.

Parameters	Number or mean ± SD	Percentage or range
Number of patients	152	
Age (years)		
Recipient	50.3 ± 13.4	19–74
Donor	54.9 ± 11.1	24–78
Recipient gender		
Male	83	54.6%
Female	69	45.4%
BMI recipient	26.0 ± 4.5	18.0–40.3
Pretransplant dialysis	73	48.0%
Comorbidity (CCI)	3.8 ± 1.4	2–9
ASA score, ≥3	117	77.0%
Origin renal graft		
Right side	46	30.3%
Left side	106	69.7%
Diuresis directly postoperative	150	98.7%
Slow graft function (SGF)	11	7.3%
Delayed graft function (DGF)	1	0.7%
Temperature during induction		
Recipient core temperature	35.9 ± 0.40	35.0–37.1 °C
Donor core temperature	36.3 ± 0.57	33.6–39.9 °C
Kidney procurement side		
Right side	46	30.3%
Left side	106	69.7%
Renal length	11.4 ± 0.86	9.3–14.0
One	11.47 ± 0.89	9.3–14.0
Two	11.2 ± 0.76	9.6–12.5
Renal arteries		
Right	125	82.3%
Left	27	17.7%
Renal veins		
Right	142	93.4%
Left	10	6.6%

BMI, Body Mass Index (body mass (kg)/(height (m)^2)); CCI, Charlson Comorbidity Index [Predicts 1-year mortality based on age and the patients' comorbidities; (0–10)]; ASA, American Society of Anesthesiologists score (classification system for assessing the fitness of patients prior to surgery; range 1–5); SGF, slow graft function was defined as a serum creatinine level of ≥3 mg/dl (≥265 mmol/l) at day 5 post-transplantation; DGF, delayed graft function was defined as recipients as the need for dialysis in the first week after transplantation.

The relationship between the WIT2 and graft outcome is extensively discussed in the literature. A prolonged WIT2 is associated with a worse graft outcome in which every minute counts [10,12–14]. In general, the graft is thought to be protected from warm ischemia when temperatures are below 15 °C. The length of WIT2 may therefore be only relevant when temperatures are above the metabolic threshold. The actual temperature of the graft may therefore be more important. Until now, only one other study was published which focussed on kidney temperature course in deceased donor kidney transplantation (DDKT) [15]. They concluded that the (*in vivo*) temperature increased during WIT2 in a logarithmic curve, ending above the metabolic threshold of 15 °C. In our living donor kidney cohort, a similar pattern was observed in which 87.3% of the grafts reach this critical level within 20 min. Despite the similarities with the aforementioned study, our DGF rate was remarkably lower, which is explained by the healthy living donors and the shorter CIT. Interestingly, the size and weight of the kidneys was an important factor in temperature increase in both the previous study and ours in which a larger kidney warmed less quickly (CI 95% −0.723 to 0.18, $P = 0.06$).

In the literature, a positive correlation between WIT2 and obesity has been reported, resulting in more DGF and a worse graft survival [2,10,16,17]. In patients with obesity, the technical aspects of the transplantation (especially for right sided kidneys) are much more challenging due to a deeper fossa with more exposure to and transfer of recipient body warmth, potentially leading to a longer WIT2 and a more rapid warming. In our study, recipient weight, although not statistically significant, was an important confounder (95% CI: −0.004 to 0.136, $P = 0.06$) for a rapid temperature rise during the WIT2.

The 15 °C metabolic threshold is considered the temperature at which an imbalance in metabolic requirement and necessity arises within an ischemic kidney and results in tissue hypoxia and microvascular dysfunction. This affects the proximal tubules resulting in decreased kidney function and eventually acute tubules necrosis (ATN) [18,19]. In addition, fats solidify and lipoproteins become unstable after which lipid denaturation occurs at about 15 °C, causing capillary blockage with a rise of the perfusion pressure and endothelial shear stress [5,20]. In combination with the release of reactive oxygen species (ROS) during rapid warming in the oxygen-deprived graft, an inflammatory response is formed which in turn leads to ATN and subsequently a diminished graft function and survival. Over the years, several techniques have attempted unsuccessfully to prevent rapid warming of the graft and preserve graft function during transplantation. The ice bag technique, wrapping the graft in an ice-packed bag, has been tested most extensively, and contradictory results regarding

Table 2. Ischemia times.

Parameters	Minutes ± SD	Range in minutes	Mean temperature end of ischemia time (°C ± SD)
WIT1	3.74 ± 1.2	2–9	33.4 ± 1.4 (27.5 to 35.5)
CIT	157.5 ± 33.7	70–321	4.2 ± 2.8 (−0.4 to 13.8)
WIT2	41.3 ± 10.1	17–76	25.5 ± 5.0 (15.6 to 36.7)
Right fossa	40.9 ± 10.2	17–76	25.5 ± 4.9 (16.6 to 36.7)
Left fossa	43.3 ± 9.7	28–64	25.3 ± 5.0 (15.6 to 35.7)
BMI ≤ 30 (n = 129)*	40.9 ± 9.6	22–76	25.5 ± 4.9 (15.6 to 36.7)
BMI > 30 (n = 22)*	43.6 ± 12.6	19–73	25.8 ± 5.2 (17.4 to 33.3)

WIT1, 1^{e} warm ischemia time; CIT, cold ischemia time; WIT2, 2^{e} warm ischemia time; BMI, body mass index.
*WIT2 subdivided by BMI.

Table 3. Univariate analyses.

Parameters	*B*	95% CI	*P*-value
CCI			
≤3	Reference		
≥4	0.539	−0.099 to 1.178	0.098
Age recipient (years)	0.027	0.005–0.050	0.018
Gender recipient			
Male	Reference		
Female	−0.369	−1.024 to 0.286	0.270
BMI recipient	0.047	−0.026 to 0.120	0.206
Creatinine preoperative (μmol/l)	0.00016	−0.001 to 0.001	0.810
Preemptive dialysis			
No	Reference		
Yes	0.200	−0.438 to 0.839	0.538
Length kidney (cm)	−0.426	−0.785 to −0.067	0.020
Number arteries			
1	Reference		
2	0.504	−0.246 to 1.254	0.188
Number veins			
1	Reference		
2	0.979	−0.357 to 2.315	0.151
Kidney side			
Right	Reference		
Left	0.270	−0.676 to 1.216	0.576
Blood loss (ml)	0.00009	−0.001 to 0.001	0.894
Duration of surgery (min)	−0.001	−0.010 to 0.008	0.872
CIT (min)	0.003	−0.009 to 0.015	0.625
WIT2 (min)	0.048	0.012 to 0.085	0.010

CCI, Charlson comorbidity index; BMI, body mass index; CIT, cold ischemia time; WIT2, 2^{e} warm ischemia time.

graft outcome and survival have been reported [19,21]. However, extended criteria kidneys, which are regarded as the most vulnerable grafts, are underrepresented in these studies. New cooling techniques may provide new insights and possible better results. In pancreas grafts, a combination of topical cooling with intraductal infusion was very effective in rapidly reducing the temperature [22]. In kidneys, renal hypothermia can be achieved by transurethral cold saline perfusion [23]. During transplantation, the added effect may help in maintaining the graft temperature under 15 °C, although this has yet to be demonstrated.

New data on graft temperature and function became apparent from the study by Mahboub *et al.* [24] in which

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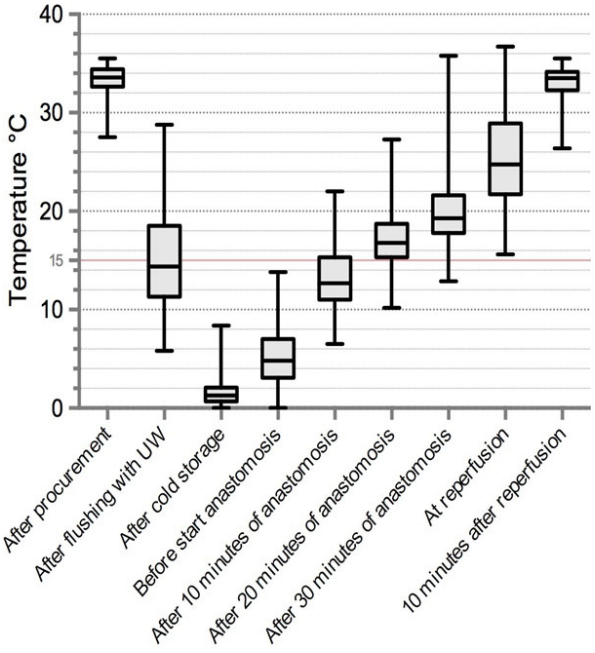

Figure 1 Temperature course during kidney procurement and transplantation. Box plots of all time intervals (min). The red line marks the 15 °C metabolic threshold.

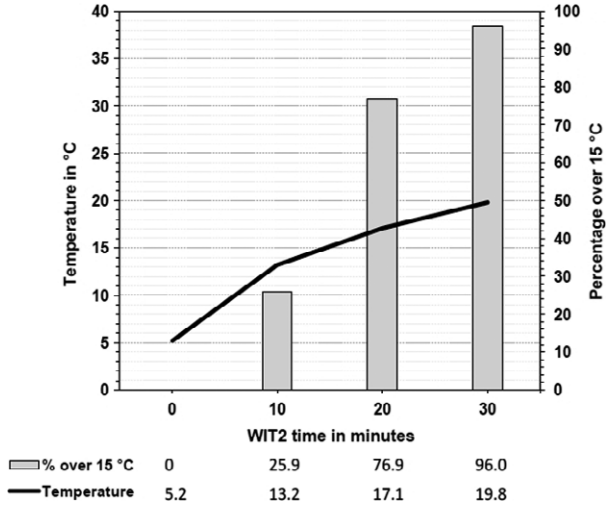

Figure 2 Temperature course during the second warm ischemia time. Relationship between WIT2 (min), kidney temperature (°C), and percentage crossing the 15 °C metabolic threshold.

they experimentally slowly rewarmed kidneys after cold storage with a gradual increase in perfusion pressure. It proved to be beneficial to gradually increase temperature, in 90 min from 4 °C toward 38 °C, compared to sudden reperfusion at body temperature with regard to decreasing reperfusion injury induced by ROS. This data further support the idea that the temperature increase is more important than the actual length of the WIT2.

The study presented here has a few limitations that need to be addressed. First, we used a topical infrared technique for measuring graft temperature. Even though this is a validated technique, it provides no information about the core temperature. However, as the glomeruli are located in the cortex, we feel that this is an appropriate reflection of the 'functional' temperature. Secondly, the temperature during backtable preparations depended on various surgical factors (e.g., reconstruction of multiple renal arteries). Between cold storage and the start of the vascular anastomosis, the kidney temperature increased from 3.4 °C to a mean of 4.5 °C at the start of the WIT2. Thirdly, our anastomosis time is on the high end compared to the international literature [10]. This can be explained by the added technical difficulty of transplanting a living donor kidney compared to a deceased donor graft wherein the vessels are considerably shorter. In addition, senior residents and fellows participate in our transplant program which leads to a longer duration of surgery and subsequently WIT2. Fourthly, because of the high-quality grafts and the low incidence of DGF, compared to deceased donor kidneys, we were unable to detect a correlation between temperature increase and a diminished graft outcome. In hindsight, urinary biomarker analysis (NGAL, NAG, or KIM-1) might have been better to detect subtle differences in damage. However, our data show a detailed representation of the rapid temperature rise kidney grafts experience. In view of the study by Feuillu *et al.*, who demonstrated a significant association between temperature increase and graft outcome after heart-beating kidney donation, marginal or extended criteria kidneys are perhaps the most vulnerable to the effects of rapid warming. Particularly, these kidneys could potentially benefit from either extra topical or central cooling or a gradually controlled (machine) rewarming prior to transplantation instead of rapid reperfusion [25]. However, this is an assumption that cannot be inferred from these data and future studies are needed to prove this supposition. Overall, we believe that the reported WIT2 should perhaps not be equal to anastomosis time, but defined in terms of the time after which graft temperature reaches a certain level, until reperfusion, or even graft temperature at time of reperfusion. This is much more consistent with the pathophysiological processes. So, instead of rushing the vascular anastomosis to obtain a shorter WIT2, effort should probably be directed at keeping the organ cooler during this period.

In conclusion, this study shows that the temperature course increases more rapidly during WIT2 than initially thought wherein a larger donor kidney (and

Table 4. Multivariate analysis.

Parameters	*B*	95% CI	*P*-value
WIT2	0.058	0.016 to 0.099	0.006
Duration of surgery (min)	−0.010	−0.017 to −0.002	0.015
Number veins			
1	Reference		
2	1.360	0.0003 to 2.720	0.050
Preemptive dialysis			
No	Reference		
Yes	0.653	−0.030 to 1.336	0.061
Length kidney (cm)	−0.353	−0.723 to 0.18	0.062
BMI recipient	0.066	−0.004 to 0.136	0.066
Age recipient (years)	0.020	−0.006 to 0.046	0.131
Number arteries			
1	Reference		
2	0.581	−0.159 to 1.321	0.124

BMI, body mass index; WIT2, 2^e warm ischemia time.

Table 5. Multivariate analysis, continuous variables categorized.

Parameters	*B*	95% CI	*P*-value
WIT2 (min)			
≤30	Reference		
30–40	0.763	−0.470 to 1.996	0.225
40–50	1.763	0.519 to 3.006	0.005
>50	1.427	−0.046 to 2.808	0.043
Duration of surgery (min)			
≤190	Reference		
190–215	−0.940	−2.119 to 0.238	0.118
215–245	−0.990	−2.113 to 0.132	0.084
>245	−1.595	−2.832 to −0.358	0.012
Number veins			
1	Reference		
2	1.190	−0.098 to 2.478	0.070
Preemptive dialysis			
No	Reference		
Yes	0.771	0.101 to 1.441	0.024
BMI recipient			
≤30	Reference		
>30	1.258	0.255 to 2.262	0.014
Age recipient (years)			
≤40	Reference		
40–50	0.751	−0.436 to 1.938	0.215
50–60	1.102	0.093 to 2.111	0.032
>60	0.774	−0.222 to 1.770	0.128

BMI, body mass index; WIT2, 2^e warm ischemia time.

therefore mass) is protective against temperature increase. Whether extensive topical or central cooling or gradual rewarming is better, with regard to early or late graft function, has to be proven in new studies. Even though our study was unable to detect an independent relationship between graft temperature and outcome, we would still like to advocate reporting the graft temperature prior to reperfusion in addition to the WIT2 as an indicator for the potential risk of DGF.

Authorship

TGJK: acquired the data and was involved in data analysis, interpretation and contributed to the final adjustments of the manuscript after critically revising it for intellectual content. JH and MEM: involved in data analysis, interpretation and contributed to the final adjustments of the manuscript after critically revising it for intellectual content. CK and JWH: acquired the data and contributed to the final adjustments of the manuscript after critically revising it for intellectual content. SPB and HGL: involved in data interpretation and contributed to the final adjustments of the manuscript after critically revising it for intellectual content. RAP: conceived the study and its design, acquired the data and was involved in data analysis and interpretation and writing the manuscript.

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Conflict of interest

None declared.

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