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**Western diet modulates the susceptibility  
of the heart to ischemic injury and  
sevoflurane-induced cardioprotection in  
rats**

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## Abstract

### Introduction

Sevoflurane is known for its cardioprotective effects during myocardial ischemia and reperfusion, which however seems blunted in the presence of type 2 diabetes mellitus. In this study we investigated whether a reduction in caloric intake reverses the impact of western diet on the cardioprotective potency of sevoflurane in rats subjected to ischemic injury.

### Methods

Male Wistar rats were exposed to a western diet (WD) or control diet (CD). A third group of WD fed rats reversed after four weeks to CD for 4 consecutive weeks. After 8 weeks, rats underwent 40 minutes of coronary occlusion followed by 120 minutes of reperfusion with or without 3x 5 min sevoflurane (2 vol%) preconditioning. An extra group of CD-fed rats underwent myocardial infarction during hyperinsulinemic euglycemic clamping.

### Results

WD feeding resulted in a prediabetic phenotype with obesity and hyperinsulinemia. Sevoflurane exerted cardioprotective effects in control rats, resulting in a reduction of infarct size by 59% ( $p < 0.001$ ). Unexpectedly, WD feeding itself reduced infarct size by 43% ( $p < 0.05$ ) compared to control rats, while sevoflurane even enlarged infarct size in WD fed rats (31%,  $p < 0.05$ ). Reversion of WD to CD resulted in normalization of obesity and hyperinsulinemia, but did not affect infarct size and the protective effect of sevoflurane when compared to WD-fed rats. Interestingly, sevoflurane prior to myocardial infarction tended to increase insulin levels in control rats, while sevoflurane exposure in WD-fed rats undergoing myocardial infarction resulted in a decrease of plasma insulin levels. Our data suggested that the protective effects of diet feeding may be mediated by insulin. Indeed, exposure of rats to a hyperinsulinemic intervention also reduced infarct size after myocardial ischemia.

### Conclusion

Unexpectedly, western diet itself had a protective effect against ischemic injury, and lowering caloric intake did not alter this effect. Sevoflurane anesthesia exerted cardioprotective effects in control rats, but this effect was blunted in prediabetic rats. Our data further suggest that the protective effects of sevoflurane in control rats or western diet are mediated by insulin.

## Introduction

Cardiometabolic alterations due to excessive dietary intake, insulin resistance and type 2 diabetes mellitus (T2DM) increase the risk for perioperative myocardial ischemia.<sup>1</sup> In particular, patients with T2DM are more likely to develop coronary artery disease and myocardial ischemia<sup>2</sup> and have an increased cardiovascular complication rate after major non-cardiac surgery.<sup>3</sup>

The volatile anesthetic sevoflurane exerts protective effects that enhance perioperative preservation of the heart.<sup>4;5</sup> We previously showed that sevoflurane reduced the impact of ischemic myocardial injury.<sup>6;7</sup> However, there is growing evidence that these cardioprotective properties are blunted in case of obesity and/or T2DM. Sevoflurane-induced postconditioning was blocked in hyperglycemic<sup>8</sup> and obese/insulin resistant Zucker rats<sup>9</sup> after myocardial ischemia and reperfusion. Moreover, obesity suppressed sevoflurane preconditioning-induced cardioprotection.<sup>10</sup>

Preoperative lifestyle interventions such as lowering caloric intake to induce weight loss and improve insulin sensitivity may be an attractive approach to improve perioperative myocardial function in obese or diabetic subjects. We previously showed that lowering caloric intake restored the cardiodepressive effects of sevoflurane in western diet-induced prediabetic rats (van den Brom *et al.*, unpublished results). This suggests that the myocardial response to sevoflurane depends on the cardiometabolic state that may be altered by changes in dietary intake. It remains unknown whether these beneficial effects of caloric restriction also affect the cardioprotective effect of sevoflurane in the cardiometabolic altered heart. In this study we investigated whether reversal of western diet-induced prediabetes by caloric restriction restores the cardioprotective potency of sevoflurane in rats subjected to ischemic injury.

## Material and methods

### *Animals and experimental set-up*

All animal experiments were approved by the Institutional Animal Care and Use Committee of the VU University, and were conducted following the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, and the guide for the Care and Use of Laboratory Animals.

The first part of the study was performed in male Wistar rats ( $n=144$ , baseline body weight:  $263\pm 1$  g; Charles River). Rats were exposed to a western diet in combination with sucrose water (20%) (WD,  $n=98$ ) or control diet (CD,  $n=46$ ) for a period of 8 weeks. Four weeks after the start of diet exposure, 48 WD-fed rats reversed to CD for 4 weeks (Figure 6.1A).

Rats were housed in a temperature-controlled room (20-23°C; 40-60% humidity) under a 12/12h light/dark cycle starting at 6.00 am. Body weight was determined on a weekly basis. After 8 weeks of diet exposure, rats underwent myocardial infarction. The second part of the study included 14 male Wistar rats ( $263\pm 3$  g) that were exposed to control diet. After 8 weeks, these rats underwent a myocardial infarction during hyperinsulinemia. After sacrifice, arterial blood was collected for plasma determinations and hearts were removed and stored at -20°C until further analysis.

### *Diets*

Control diet (Teklad 2016, Harlan, Horst, the Netherlands) consisted of 20 %kcal protein, 9 %kcal fat and 74 %kcal carbohydrates (1804 kcal/kg starch, 200 kcal/kg sugars), whereas western diet (D12451, Research Diets, New Brunswick, NJ) consisted of 20 %kcal protein, 45 %kcal fat and 35 %kcal carbohydrates (291 kcal/kg starch, 691 kcal/kg sugars) with 20% sucrose water (800 kcal/kg), totally containing 3300 kcal/kg and 4857 kcal/kg for CD and WD with sucrose water, respectively.

### *Surgery*

After 8 weeks of diet exposure, rats were anesthetized with 125 mg/kg S-ketamine (Ketanest®, Pfizer, the Netherlands) and 4 mg/kg diazepam (Centrafarm, the Netherlands) intraperitoneally and were intubated and mechanically ventilated (UNO, the Netherlands; positive end-expiratory pressure, 1-2 cm H<sub>2</sub>O; respiratory rate, ~65 breaths/min; tidal volume, ~10 ml/kg) with oxygen-enriched air (40% O<sub>2</sub>/60% N<sub>2</sub>). Anesthesia was maintained by continuous infusion of 40 mg/kg/h S-ketamine and 1 mg/kg/h diazepam intravenously via the tail vein or the right jugular vein.

The respiratory rate was adjusted to maintain pH and carbon dioxide within physiological limits. Body temperature was maintained stable ( $37.1\pm 0.05$ °C) by using a warm water underbody heating pad.

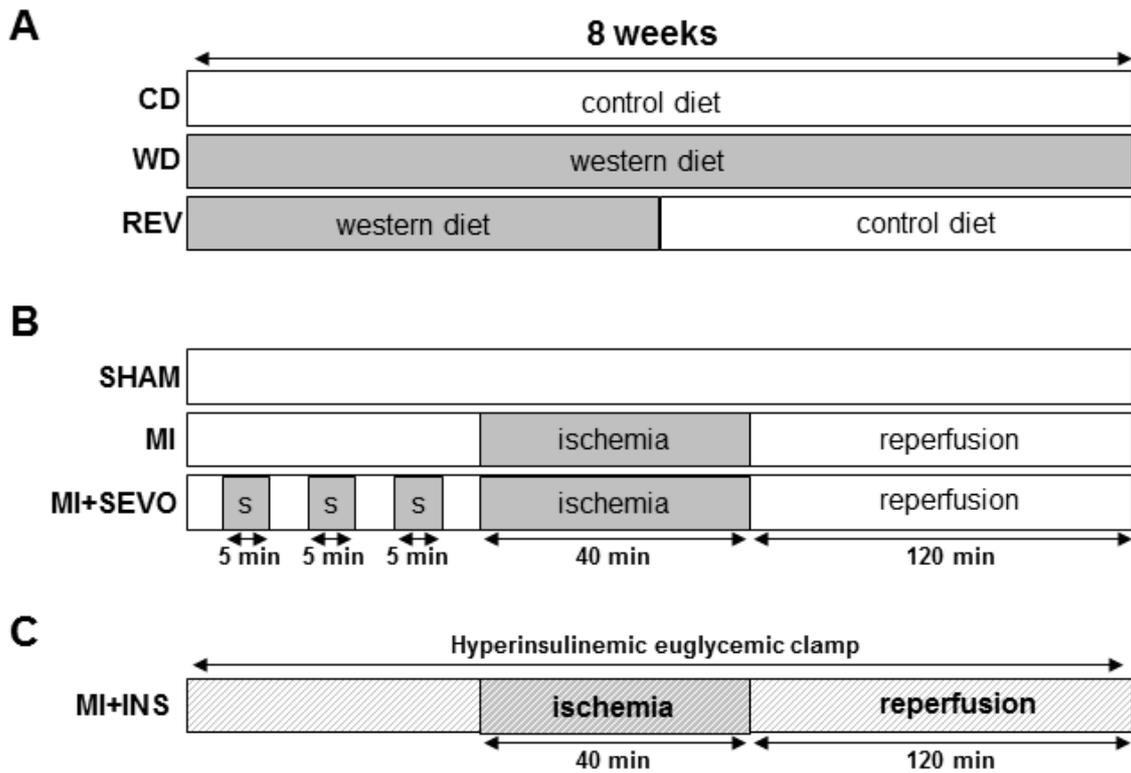
The left carotid artery was cannulated for blood sampling for blood gas analyses (ABL50, radiometer, Copenhagen, Denmark) and for measurements of arterial blood pressure (Safedraw Transducer Blood Sampling Set, Argon Medical Devices, Texas, USA). Arterial blood pressure, ECG and heart rate were continuously recorded using PowerLab software (PowerLab 8/35, Chart 7.0; ADInstruments Pty, Ltd., Castle Hill, Australia). Mean arterial blood pressure was calculated according the following formula:  $2/3 \times \text{diastolic blood pressure} + 1/3 \times \text{systolic blood pressure}$ .

### *Myocardial infarction*

Left thoracotomy was performed between the fourth and fifth rib. A ligature (Prolene® 6-0; Ethicon LLC, San Lorenzo, Puerto Rico) was placed around the left anterior descending coronary artery (LAD) with a special ligation device that allowed to pull the suture in one simple movement in order to reduce the chance of preconditioning.<sup>11</sup> Successful coronary occlusion was verified by ECG changes and ischemia was maintained for 40 min and followed by 120 min of reperfusion (MI) (Figure 6.1B). Rats in the control (CON) group received a sham surgery where the LAD was not occluded. Sevoflurane (AbbVie, the Netherlands) intervention was induced before ischemia and reperfusion by three periods of 5 minutes exposure to 2% (v/v) sevoflurane, interspersed with washout periods of 5 minutes (MI+SEVO). In total, n=144 rats were included, from which n=122 rats survived the surgery (84.7 %). Per diet group the survival rate was 87.0 %, 80.0 % and 85.4 % for CD-, WD- and REV-fed rats, respectively.

### *Hyperinsulinemic euglycemic clamp*

Rats in the second part of the study were subjected to the same ischemia and reperfusion protocol during a hyperinsulinemic euglycemic clamp. In total, n=14 rats were included, from which n=11 rats survived the surgery (78.6 %). Surgery was performed similarly as described above; only anesthesia was infused intravenously via the femoral vein due to blood glucose measurements via the tail vein. The hyperinsulinemic euglycemic clamp was initiated by 3 minutes insulin priming at an infusion rate of 120 mU/kg/min (Novorapid®, Novo Nordisk, Denmark), followed by constant infusion of insulin at a rate of 12 mU/kg/min via the jugular vein as described before.<sup>12</sup> Blood glucose was measured every 5 minutes from tail bleeds with a Precision Xceed Blood Glucose monitoring system (MediSense, UK). Simultaneously, a 20% glucose solution was infused at a variable rate to maintain euglycemia. After  $\pm$  90 minutes, glucose disposal rate (*M*-value, mg/kg/min) was calculated as the average glucose infusion rate during 30 minutes. To prove hyperinsulinemia, plasma insulin levels were determined during the steady state, ischemia and reperfusion phase. The M/I index, which is a measure for insulin sensitivity, was calculated by the amount of glucose metabolized per unit of plasma insulin.



**Figure 6.1: Experimental protocol**

**A)** Rats were fed a control diet (CD) or western diet with sucrose water (WD) for 8 weeks. A third group of WD fed rats reversed after 4 weeks to CD for 4 consecutive weeks (REV). **B)** After 8 weeks of diet exposure, animals underwent left coronary artery occlusion for 40 min followed by 120 min of reperfusion without (MI) or with 3x 5 min sevoflurane (s) exposure (MI+SEVO). **C)** After 8 weeks of exposure to CD, animals underwent left coronary artery occlusion for 40 min followed by 120 min of reperfusion during hyperinsulinemia (MI+INS).

### *Evans blue and TTC staining*

After 120 min of reperfusion, the hearts were excised and the aorta was cannulated. The LAD was re-occluded and 0.2 % Evans Blue (Sigma, St. Louis, MO) was infused to stain the non-ischemic myocardium and leaving the area at risk unstained. After rinsing with 0.9% NaCl, hearts were stored at -20°C.

For determination of infarct size, frozen hearts were cut in slices of 1 mm, incubated for 15 min in 2,3,5-triphenyl tetrazolium chloride (TTC; Sigma, St. Louis, MO) solution at 37°C, followed by fixation in 4% formaldehyde (Klinipath, Duiven, the Netherlands). Slices were scanned and the area at risk and infarct size were determined in each slice using ImageJ (1.42q, National Institute of Health). The infarct size is presented as the percentage of the area at risk.

### *Plasma measurements*

Plasma glucose (Abcam, Cambridge, MA), insulin (Millipore, St. Charles, MO), free fatty acid (WAKO NEFA-HR, Wako Pure Chemical Industries, Osaka, Japan), triglyceride (TG; Sigma, St. Louis, MO) and HDL and LDL/VLDL cholesterol (Abcam, Cambridge, MA) levels were measured from arterial blood as described previously.<sup>12-15</sup>

### *Statistical analysis*

Data were analyzed using Graphpad Prism 5.0 (La Jolla, USA) and presented as mean±SD. Statistical analyses were performed using one-way ANOVA with Bonferroni post-hoc analysis and two-way ANOVA with Bonferroni post-hoc analysis (with repeated measurements) for additional interventions, where  $p < 0.05$  was considered as statistically significant.

## Results

### Lowering caloric intake normalized obesity and hyperinsulinemia

After 8 weeks of western diet (WD) feeding, bodyweight and plasma insulin were significantly increased compared to control diet (CD)-fed rats (Table 6.1). Plasma glucose, triglycerides and free fatty acids tended to increase, whereas HDL cholesterol tended to decrease in WD- compared to CD-fed rats (Table 6.1). LDL/VLDL cholesterol remained unchanged (Table 6.1). Diastolic blood pressure, systolic blood pressure, mean arterial pressure and heart rate were similar among groups (Table 6.1). Reversion to control diet resulted in normalized bodyweight and plasma levels of insulin, triglycerides and HDL cholesterol when compared to WD-fed rats (Table 6.1).

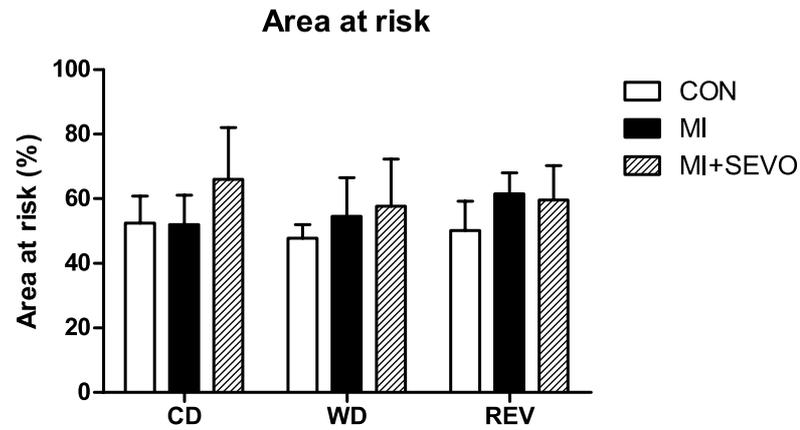
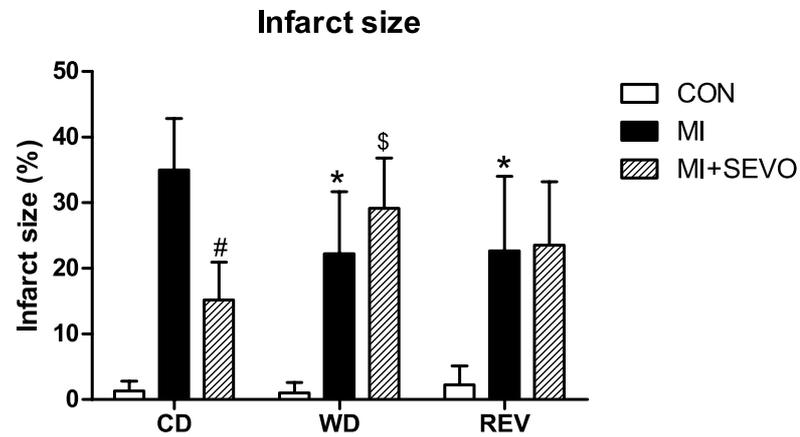
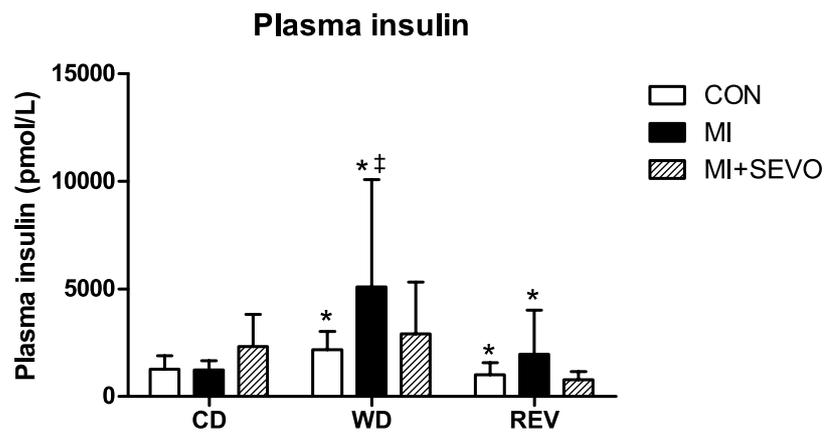
**Table 6.1: Characteristics of rats after 8 weeks of diet feeding**

|                                     | Control diet | Western diet | Reversion   |
|-------------------------------------|--------------|--------------|-------------|
| Bodyweight (g)                      | 421±22       | 450±27 *     | 431±26 #    |
| Plasma insulin (pmol/L)             | 1274±621     | 2165±856 *   | 1006±559 #  |
| Plasma glucose (mmol/L)             | 12.9±5.7     | 16.9±9.9     | 11.0±3.5    |
| Plasma free fatty acids (mmol/L)    | 0.19±0.09    | 0.24±0.19    | 0.18±0.10   |
| Plasma triglycerides (mmol/L)       | 1.76±0.66    | 2.23±0.70    | 1.46±0.44 # |
| Plasma HDL cholesterol (mg/dL)      | 49.4±18.2    | 38.5±10.5    | 63.5±7.7 #  |
| Plasma LDL/VLDL cholesterol (mg/dL) | 29.7±5.6     | 28.0±3.9     | 25.5±4.4    |
| Systolic blood pressure (mmHg)      | 152±44       | 150±22       | 172±27      |
| Diastolic blood pressure (mmHg)     | 110±39       | 111±23       | 130±18      |
| Mean arterial pressure (mmHg)       | 124±40       | 124±22       | 144±21      |
| Heart rate (bpm)                    | 414±32       | 381±29       | 397±39      |

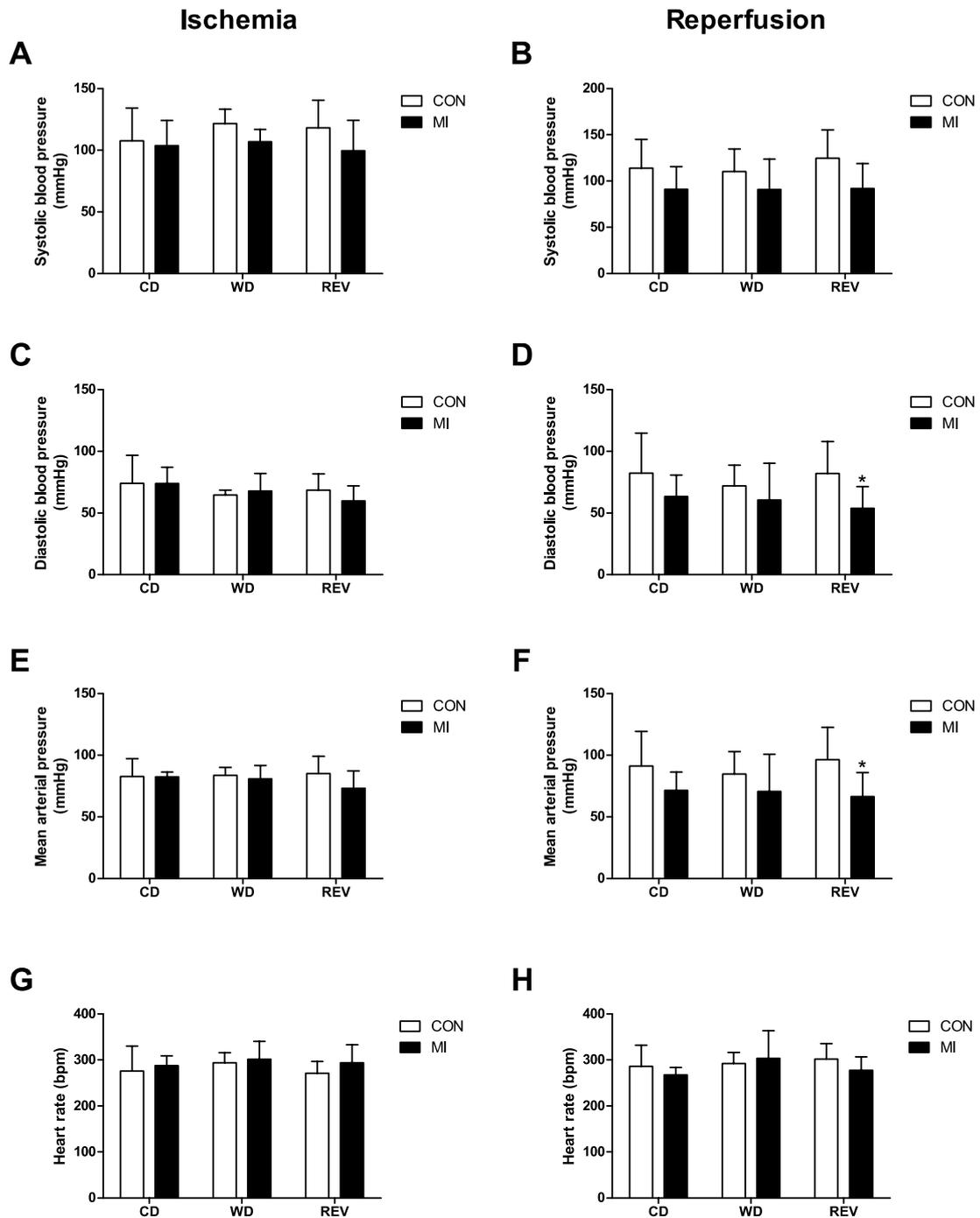
Data are mean±SD, n=5-11, two-way ANOVA with Bonferroni post-hoc analyses, \* p<0.05 vs. control diet, # p<0.05 vs. western diet.

### Western diet feeding reduces myocardial ischemic injury

The area at risk after ischemia and reperfusion did not differ between groups (Figure 6.2A). Unexpectedly, WD-feeding reduced infarct size compared to control rats (Figure 6.2B). Interestingly, these protective effects of western diet feeding on ischemic injury persisted after lowering caloric intake following 4 weeks of WD-feeding (REV group) (Figure 6.2B). Myocardial ischemia did not affect blood pressure and heart rate in all diet groups (Figure 6.3A, C, E, G). During the reperfusion phase, diastolic blood pressure and mean arterial pressure were only significantly reduced in rats that underwent diet reversal (Figure 6.3B, D, F, H).

**A****B****C****Figure 6.2: Myocardial ischemia and reperfusion injury**

Area at risk (**A**), infarct size (**B**) and plasma insulin levels (**C**) after sham (CON), myocardial infarction (MI) and myocardial infarction with sevoflurane (MI+SEVO) in rats after 8 weeks of control diet (CD), western diet (WD) or reversion diet (REV) feeding. Data are mean±SD, n=5-11, two-way ANOVA with Bonferroni post-hoc analyses, \* p<0.05 diet effect, # p<0.05 sevoflurane effect, \$ p<0.05 diet and sevoflurane effect, ‡ p< 0.05 MI effect.

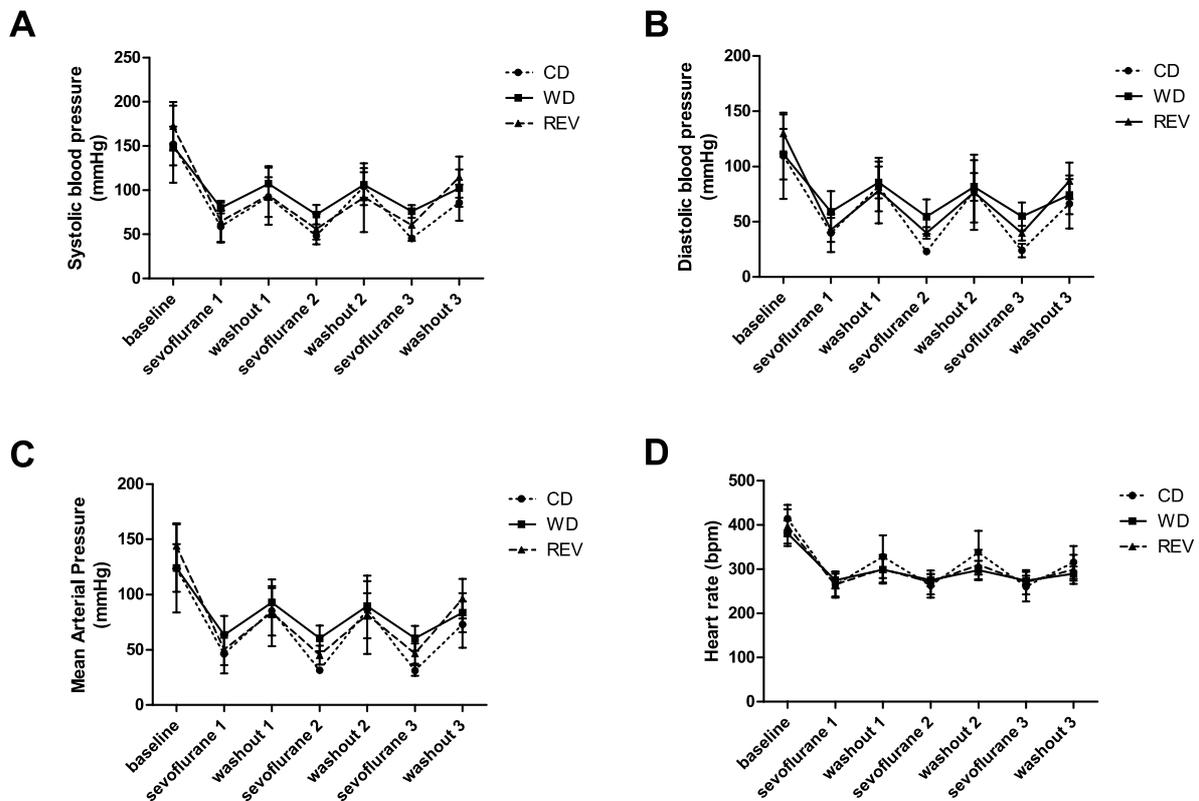


**Figure 6.3: Hemodynamics during myocardial ischemia and reperfusion**

Systolic blood pressure (**A,B**), diastolic blood pressure (**C, D**), mean arterial pressure (**E, F**) and heart rate (**G, H**) after 40 minutes of ischemia (**A, C, E, G**) or 120 minutes of reperfusion (**B, D, F, H**) in rats fed a control diet (CD), western diet (WD) or reversion diet (REV) for 8 weeks. Data are mean $\pm$ SD, one-way ANOVA with Bonferroni post-hoc analyses, \*  $p < 0.05$  MI effect. CON, sham; MI, myocardial infarction.

### Sevoflurane reduces ischemic injury in control rats

Sevoflurane-induced preconditioning reduced infarct size in CD-fed rats, but offered no additional protection in WD- and REV-fed rats (Figure 6.2B). In particular, infarct size was even higher in WD-fed rats compared to control rats after sevoflurane exposure. Blood pressure and heart rate decreased during sevoflurane exposure, but were partly restored during the washout periods without differences among groups (Figure 6.4A-D).



**Figure 6.4: Blood pressure and heart rate during sevoflurane exposure**

Systolic blood pressure (**A**), diastolic blood pressure (**B**), mean arterial pressure (**C**) and heart rate (**D**) in rats after 8 weeks of control diet (CD), western diet (WD) or reversion diet (REV) feeding. Data are mean $\pm$ SD, n=5-11.

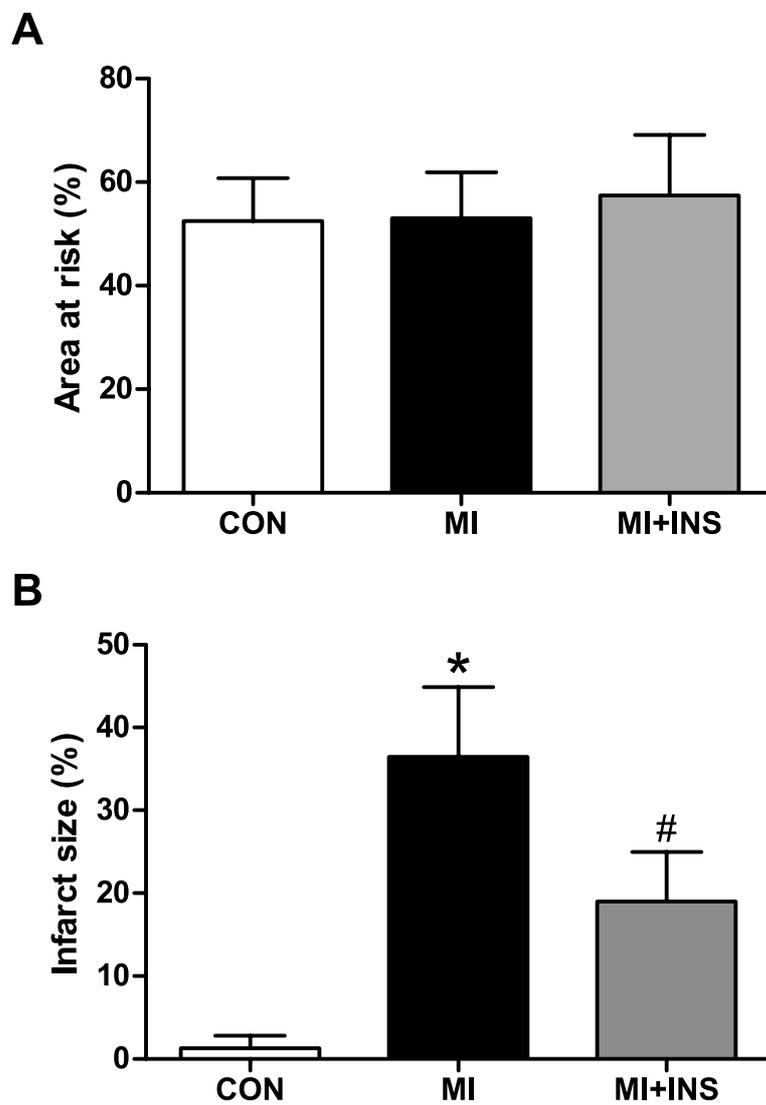
### **Plasma insulin levels after surgery**

Western diet feeding increased plasma insulin levels when compared to control rats, while this hyperinsulinemic effect was absent in rats subjected to diet reversal. While myocardial infarction had no effect on insulin levels in control rats, WD-fed rats showed a hyperinsulinemic response upon cardiac ischemia and reperfusion. This effect remained present in the diet reversal group, although to a lesser extent. Sevoflurane prior to myocardial infarction tended to increase insulin levels in control rats, while sevoflurane exposure in WD-fed rats undergoing myocardial infarction decreased plasma insulin levels. In rats exposed to diet reversal the rise in insulin levels during myocardial infarction was abolished after sevoflurane exposure (Figure 6.2C).

Myocardial infarction, but not MI+SEVO, significantly increased HDL cholesterol in CD-fed rats, which was absent in WD- and REV-fed rats. MI and MI+SEVO had no effect on plasma glucose, free fatty acids, triglycerides and LDL/VLDL cholesterol (data not shown).

### **Hyperinsulinemia protects against ischemic injury in healthy rats**

From the findings as shown in figure 6.2C it was hypothesized that the protective effects of western diet feeding against myocardial ischemia may be the result of increases in plasma insulin levels. In order to study the direct effects of hyperinsulinemia on the cardioprotective effects of western diet, control diet-fed rats were subjected to a hyperinsulinemic euglycemic clamp. Steady-state blood glucose levels during hyperinsulinemic euglycemic clamping were  $5.43 \pm 0.05$  mmol/L, whereas the glucose disposal rate ( $M$ -value) was  $9.83 \pm 1.34$  mg/kg/min in healthy CD-fed rats. Plasma insulin levels were  $9027 \pm 679$  pmol/L and the insulin sensitivity ( $M/I$ -index) was  $1.19 \pm 0.22$  in CD-fed rats during the steady state of the clamp. Hyperinsulinemia combined with euglycemia resulted in a similar area at risk when compared to control rats, but itself protected the heart against myocardial ischemia (Figure 6.5A and B).



**Figure 6.5: Myocardial ischemia and reperfusion injury during hyperinsulinemia**

Area at risk (A) and infarct size (B) after sham (CON), myocardial infarction (MI) and myocardial infarction during hyperinsulinemia (MI+INS) in rats after 8 weeks of control diet (CD) feeding. Data are mean $\pm$ SD, n=5-11, one-way ANOVA with Bonferroni post-hoc analyses, \* p<0.05 vs. CON, # p<0.05 vs. MI.

## Discussion

The present study showed that short-term western diet feeding in rats resulted in a prediabetic phenotype, and exerted an unexpected cardioprotective effect during myocardial infarction, which was not abolished by lowering caloric intake. Sevoflurane preconditioning protected the heart against myocardial ischemia in healthy control rats. However, in diet-induced prediabetic rats, sevoflurane exposure paradoxically increased infarct size. Sevoflurane prior to myocardial infarction tended to increase insulin levels in control rats, while sevoflurane exposure in western diet-fed rats undergoing myocardial infarction decreased plasma insulin levels. By mimicking this hyperinsulinemic cardioprotective response using a hyperinsulinemic, euglycemic clamp in control rats, we suspect a pivotal role for insulin underlying the protective effects of sevoflurane or western diet against myocardial ischemia.

The present study demonstrated a protective effect of western diet feeding during myocardial ischemia and reperfusion. We are not unique with respect to this unexpected finding, as other groups also showed that high caloric diet feeding may exert cardioprotective properties during ischemia and reperfusion.<sup>16-18</sup> In contrast, there are also reports available that show no effect<sup>10;19;20</sup> or aggravation of ischemic injury after diet feeding in rats.<sup>21-24</sup> These conflicting findings may be due to the type and severity of metabolic disease,<sup>25</sup> the used ischemia and reperfusion protocol, the duration of diet feeding and the composition of the diet.

Several mechanisms may explain diet-mediated cardioprotection, such as the presence of a high percentage of carbohydrates.<sup>16-18</sup> The present diet consisted mainly of saturated fatty acids and a high percentage of sucrose. Jordan et al. showed that 3 days or 4 weeks of fructose diet feeding both reduced infarct size, while only the 4-week fructose intervention was associated with insulin resistance. These findings suggest a protective effect of fructose.<sup>17</sup> It is suggested that carbohydrates, such as fructose or sucrose, may protect the heart against ischemia and reperfusion injury via antioxidant mechanisms<sup>26,27</sup> or via the response of the heart to injury by increasing myocardial glucose metabolism to improve its energetic efficiency.<sup>28,29</sup> Glycolysis becomes an important source of energy due to its ATP-generating ability in the absence of oxygen and is reported to be cardioprotective during ischemia and reperfusion.<sup>30</sup> Sucrose feeding may further stimulate myocardial substrate metabolism. On the other hand, the high fat content of diets may also be cardioprotective.<sup>31</sup>

Another explanatory mechanism is the presence of metabolic alterations, such as hyperinsulinemia, during ischemic stress. Several studies showed a cardioprotective effect of insulin in healthy rats during myocardial ischemia and reperfusion<sup>32-35</sup> and positive effects glucose, insulin and potassium (GIK) treatment in patients with myocardial infarction.<sup>36;37</sup> Moreover, infarct size was decreased in diet-induced obese

compared to healthy rat hearts in absence of insulin, whereas the presence of insulin in the coronary perfusate was cardioprotective in healthy and obese hearts, suggesting the importance of insulin.<sup>22</sup> In accordance with the literature, we showed a cardioprotective effect of insulin during myocardial ischemia and reperfusion using a hyperinsulinemic euglycemic clamp. Moreover, two independent observations in our study provide further support for the protective role of insulin. First, sevoflurane exposure of control rats tended to increase insulin levels, while this was associated with myocardial protection against ischemic injury. Secondly, while western diet-associated cardioprotection was paralleled by hyperinsulinemia, the absent cardioprotective effect of sevoflurane exposure in western diet fed rats undergoing myocardial infarction resulted in a decrease of plasma insulin levels. However, further research is needed to discriminate the underlying cardioprotective mechanism in our diet-induced prediabetic rat model.

Beside the unexpected cardioprotective effect of western diet, the present study also demonstrated that sevoflurane preconditioning reduced infarct size in healthy rats, but did not have any additive cardioprotective effect in western diet-induced prediabetic rats nor was affected by lowering caloric intake. Previously, it is shown that hyperglycemia<sup>8</sup> and obesity/insulin resistance<sup>9</sup> abolished the cardioprotective effect of sevoflurane. However, the generalizability of the prediabetic phenotype in these rat models was limited.<sup>8;9</sup> Moreover, Song *et al.* demonstrated in high fat diet-induced obese rats that sevoflurane preconditioning-induced cardioprotection is suppressed.<sup>10</sup> Interestingly, our study shows that sevoflurane even increases infarct size in prediabetic rats when compared to control rats, suggesting that western diet feeding has negative effects on sevoflurane preconditioning.

In conclusion, western diet feeding resulted in an unexpected cardioprotective effect during myocardial infarction, which was not affected by lowering caloric intake. Sevoflurane preconditioning had no additive cardioprotective effect in prediabetic rats nor was affected by lowering caloric intake. However, the combination of diet and sevoflurane was less cardioprotective. Moreover, hyperinsulinemia was cardioprotective in healthy rats, which might be an explanatory mechanism of the cardioprotective effect of the diet. Future studies will discriminate between the possible cardioprotective mechanisms of the diet and sevoflurane anesthesia.

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