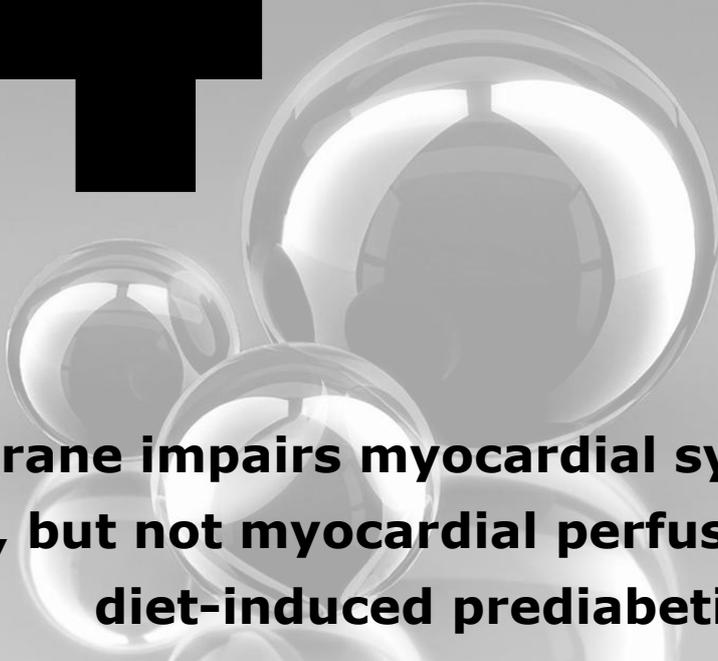# 4

Sevoflurane impairs myocardial systolic function, but not myocardial perfusion in diet-induced prediabetic rats

CE van den Brom, CA Boly, CSE Bulte, RPF van den Akker,
RFJ Kwekkeboom, SA Loer, C Boer, RA Bouwman

Abstract

Introduction

Preservation of myocardial perfusion is particularly important in patients with increased risk for perioperative cardiac complications, such as patients with diabetes. Knowledge regarding regulation of myocardial perfusion and function during anesthesia in subjects with cardiometabolic disease is however limited. In this study we therefore investigated whether the effect of sevoflurane on myocardial perfusion and function is altered in healthy and diet-induced prediabetic rats.

Methods

Male Wistar rats were randomly exposed to a western diet (WD; n=18) or control diet (CD; n=18). After 8 weeks, rats underwent (contrast) echocardiography to determine myocardial perfusion and function during baseline conditions and sevoflurane (2%) exposure. Myocardial perfusion was estimated based on the product of microvascular filling velocity (β) and microvascular blood volume (A), whereas myocardial function was determined by fractional shortening and fractional area change.

Results

Eight weeks of WD feeding resulted in obesity and hyperglycemia compared to CD-feeding. At baseline, WD decreased estimate of perfusion compared to control rats. Systolic function was significantly impaired compared to CD-fed rats. Exposure to sevoflurane increased the microvascular filling velocity in healthy controls, whereas the overall estimate of myocardial perfusion remained unchanged in both diet groups. Moreover, sevoflurane impaired systolic function in both diet groups.

Conclusions

Diet-induced prediabetes is associated with impaired myocardial perfusion and function in rats. While sevoflurane further impaired systolic function, it did not affect myocardial perfusion in prediabetic rats. Our findings suggest that sevoflurane leads to uncoupling of myocardial perfusion and function, irrespective of the metabolic state.

Introduction

Myocardial perfusion in relation with myocardial function determines the balance between myocardial energy supply and demand. During surgery, maintenance of the myocardial oxygen balance is challenged, because extrinsic factors, like anesthetics and surgical stress, and intrinsic factors, like metabolic alterations, affect myocardial oxygen supply and consumption. This altered balance may increase the vulnerability of the heart for an oxygen supply and demand mismatch and consequent ischemia.[1;2]

Sevoflurane has vasodilating properties and is known to reduce coronary vascular resistance[3] and perfusion pressure.[4;5] We recently showed that sevoflurane did not affect myocardial blood flow in healthy patients, while myocardial flow reserve was decreased.[6] Animal studies however showed that sevoflurane, when perfusion pressure remained constant, increased coronary blood flow in hearts of dogs[7] and decreased coronary flow reserve in isolated rat hearts.[5] In contrast, sevoflurane lowered blood pressure and decreased myocardial blood flow in healthy rats,[8] dogs[9] and pigs.[10]

While sevoflurane exerts contrasting effects on myocardial perfusion in healthy subjects, its vasodilatory impact may be more abundant in patients with cardiometabolic disease, like type 2 diabetes mellitus (T2DM). T2DM patients are more likely to develop coronary artery disease[11] and have an increased cardiovascular complication rate after major non-cardiac surgery.[12] Because myocardial substrate metabolism and myocardial oxygen balance is altered in T2DM,[13] the regulation of myocardial perfusion in these patients is particularly important during normal and intraoperative circumstances. The number of studies focusing on myocardial perfusion during anesthesia in subjects with cardiometabolic disease is however limited. Previously, we found that myocardial perfusion, but not myocardial function, is preserved during hyperemia in glucose intolerant rats,[14] while others showed myocardial perfusion defects in prediabetic insulin resistant patients[15;16] or T2DM patients during the postprandial state.[17;18] The aforementioned data suggest that a cardiometabolic-compromised state may be associated with more severe alterations in myocardial perfusion during anesthesia.

Therefore, the purpose of the present study was to investigate the effect of sevoflurane on myocardial function and perfusion in diet-induced prediabetic rats. While the effects in healthy subjects seem apparently conflicting, we hypothesized that sevoflurane's vasodilatory impact may be more abundant in the presence of cardiometabolic disease and thereby challenging myocardial perfusion regulation.

Methods

Animals and experimental set-up

All 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.

Adult male Wistar rats (n=36; body weight 265±7 g; Charles River Laboratories, France) were fed a western diet (WD) for a period of 8 weeks (n=18). Rats that received a diet low in fat and sugars (control diet; CD) for 8 weeks served as controls (n=18). Animals 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, rats underwent (contrast) echocardiography during baseline conditions and during sevoflurane (AbbVie, the Netherlands) exposure, starting after 5 minutes of sevoflurane.

Diets

CD (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 WD (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_2O; respiratory rate, ~65 breaths/min; tidal volume, ~10 ml/kg) with oxygen-enriched air (40% O_2/60% N_2). Anesthesia was maintained by continuous infusion of 50 mg/kg/h S-Ketamine and 1.3 mg/kg/h diazepam intravenously via the tail vein. Respiratory rate was adjusted to maintain pH and partial pressure of carbon dioxide within physiological limits. Body temperature was maintained stable (36.7±1.2°C) using a warm water underbody heating pad.

A catheter was placed in the right jugular vein for infusion of the contrast agent. The left carotid artery was cannulated for blood sampling, 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·diastolic blood pressure + 1/3·systolic blood pressure. Rate pressure product (RPP) was calculated by the product of heart rate and systolic blood pressure and was used as an estimate of myocardial oxygen demand.

Echocardiography

After surgery, (contrast) echocardiography was performed to determine myocardial function and perfusion during baseline conditions and after 5 minutes of sevoflurane (2%) exposure. Echocardiography (Siemens, ACUSON, Sequoia 512) was performed as previously described.[13] Briefly, left ventricular (LV) dimensions during end-systole (ES) and end-diastole (ED) were determined in the M-(motion) mode of the parasternal short-axis view at the level of the papillary muscles. LV systolic function is represented by fractional shortening (FS) and fractional area change (FAC), which were calculated by the equations: $FS = (EDD-ESD)/EDD \cdot 100)$ and $FAC = (EDD^2-ESD^2)/EDD^2 \cdot 100)$. All parameters were averaged over at least three cardiac contractile cycles.

Preparation of microbubbles

Microbubbles were prepared from perfluorobutane gas and stabilized with a monolayer of distearoyl phosphatidylcholine and PEG stearate. 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC; Avanti Polar Lipids, Alabama, USA) and polyoxyethylene stearate (PEG40; Sigma, St Louis, MO, USA) were dissolved in glycerol (10 mg/ml) and sonicated (Decon FS200, Decon Ultrasonics Ltd, Sussex, UK) at 40 kHz in an atmosphere of perfluorobutane (F2 Chemicals Ltd, Lancashire, UK) and vials were shaken a Vialmix at 4500 rpm (Bristol-Myers Squibb Medical Imaging, Massachusetts, USA). As the gas was dispersed in the aqueous phase, microbubbles were formed, which were stabilized with a self-assembled lipid/surfactant monolayer. Freshly made bubbles were then washed twice to remove excessive DSPC and PEG40 and stored refrigerated in sealed vials in perfluorobutane atmosphere. A Multisizer 3 coulter counter (Beckman Coulter Inc., Miami, FL, USA) was used to measure the particle size distribution as well as the number of particles. The average bubble concentration was $1.578 \cdot 10^9 \pm 0.364 \cdot 10^9$ and the particle range was between 1 and 10 µm. Microbubbles were diluted to a concentration of $200 \cdot 10^6$ with ungassed water.

Myocardial contrast echocardiography

Contrast echocardiography was performed using a Siemens (ACUSON, Sequoia 512) equipped with a 14 MHz linear array transducer (Philips Healthcare, Best, The Netherlands).[14] Microbubbles were continuously infused into the jugular vein with a rate of 300 µl/min using a dedicated syringe pump (Vueject, Bracco SA, Switzerland). After two minutes of microbubble infusion, perfusion images were taken from the long axis view of the left ventricle.

Low acoustic power (mechanical index [MI] 0.20) was used for microbubble detection with a dynamic range of 50 dB. A perfusion sequence consisted of about 10 cardiac cycles of low MI imaging, followed by a burst of high acoustic power (MI 1.8) for complete contrast destruction. Subsequently, on average 20 cardiac cycles of low MI images were acquired to allow contrast replenishment in the myocardium. All data were stored for offline analysis.

Myocardial contrast echocardiography analysis

Custom-designed software was used for analysis of the estimate of perfusion (Matlab, 7.10, R2010A, MathWorks Inc. Massachusetts, USA).[6;14] For each cardiac cycle, regions of interest were drawn in the end-systolic frame in the posterior wall in the long axis view of the left ventricle. Myocardial signal intensities from the frames after microbubble destruction were corrected for background noise by subtracting the signal intensity of the first frame after microbubble destruction (Y_0). These intensities were then fitted ($Y = Y_0 + (A-Y_0) \cdot (1-\exp^{(-\beta \cdot x)})$) for calculation of microvascular blood volume *A* and the microvascular filling velocity ß, which corresponds to the capillary blood exchange rate. The estimate of perfusion was calculated as the product of *A* and ß.[19]

Statistical analysis

Data were analyzed using Graphpad Prism 5.0 (La Jolla, USA) and presented as mean±SD. Between group comparisons (CD vs. WD) were performed using a student t-test, whereas the effect of sevoflurane exposure was tested with a two-way ANOVA with Bonferonni as post-hoc test. $p < 0.05$ was considered as statistically significant.

Results

Western diet feeding resulted in obesity and hyperglycemia

After 8 weeks of western diet feeding, bodyweight was significantly increased compared to control rats (Table 4.1), whereas heart rate, systolic blood pressure, diastolic blood pressure and mean arterial pressure remained unchanged (Figure 4.1). After sacrifice, blood glucose levels and left ventricular weights were higher in western diet- compared to control diet-fed rats (Table 4.1).

Table 4.1: Characteristics after 8 weeks of diet intervention

	Control diet	Western diet
Body weight (g)	420±25	450±20 *
Blood glucose after sacrifice (mmol/L)	14.6±5.1	19.3±3.6 *
Tibia length (mm)	42.7±1.0	43.1±0.6
Left ventricular weight (g)	0.53±0.06	0.57±0.08 *

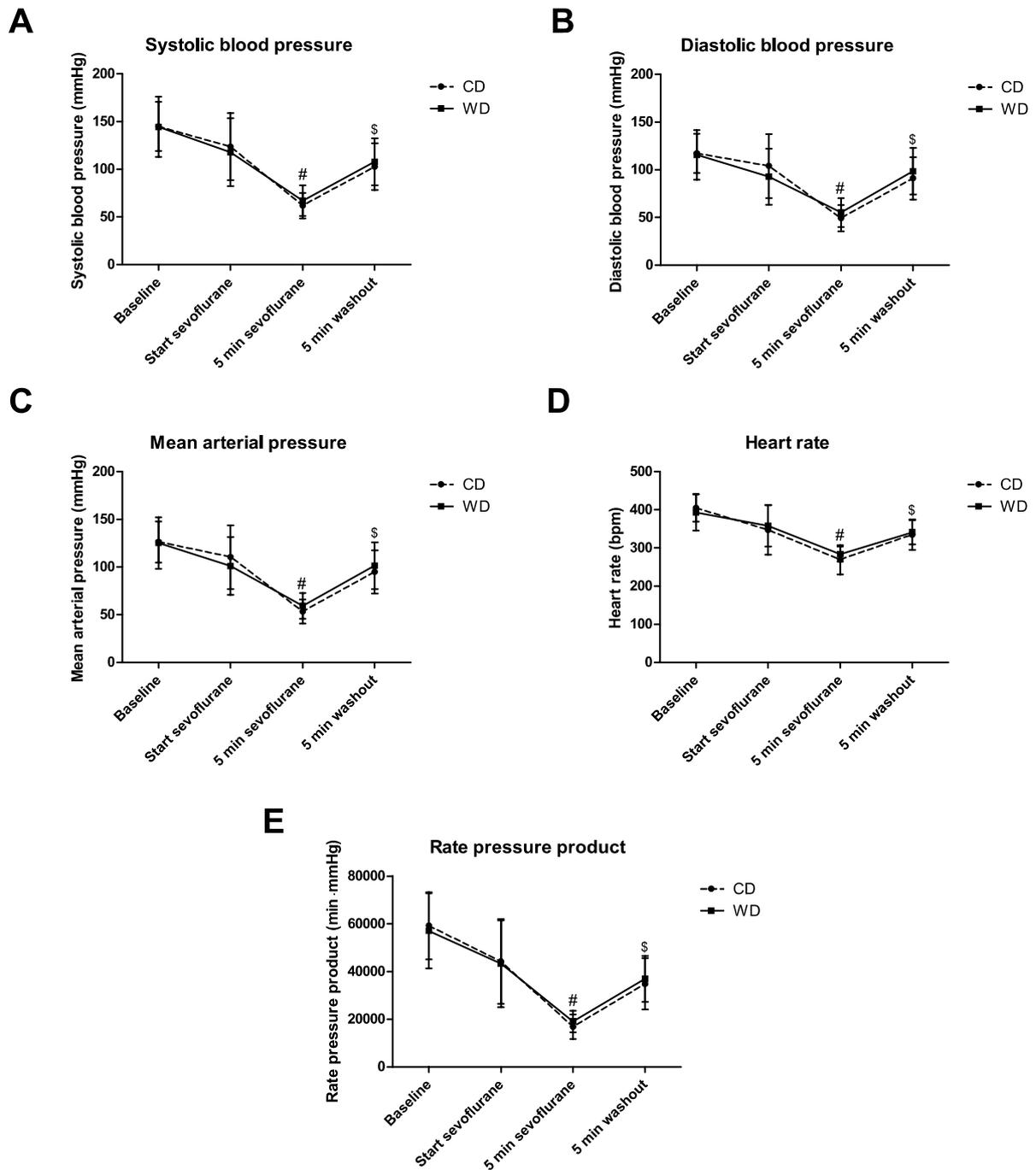Data are mean±SD, n=18, student t-test, * $p < 0.05$ vs. control diet.

Figure 4.1: Hemodynamics during sevoflurane exposure

Systolic blood pressure (**A**), diastolic blood pressure (**B**), mean arterial pressure (**C**), heart rate (**D**) and rate pressure product (**E**) during baseline conditions, before sevoflurane exposure, after 5 minutes of sevoflurane and after 5 minutes of washout period in rats fed a control diet (CD) or western diet (WD) for 8 weeks. Data are mean±SD, n=16-18, two-way ANOVA with repeated measurements and Bonferonni post-hoc analyses, # $p < 0.05$ sevoflurane effect, $ $p < 0.05$ washout effect.

Impaired myocardial perfusion and systolic function in prediabetic rats

Compared to healthy controls, western diet feeding tended to decrease microvascular filling velocity (β) and significantly decreased microvascular blood volume (*A*), which resulted in a significant reduction in the estimate of perfusion (Figure 4.2).

Western diet feeding significantly increased end-systolic lumen diameter and diastolic wall thickness, but did not affect end-diastolic lumen diameter and wall thickness during systole compared to control rats (Table 4.2). Fractional shortening and fractional area change were significantly decreased in western diet-fed rats compared to control animals, suggesting impaired systolic function (Figure 4.3).

Table 4.2: Myocardial function after 8 weeks during baseline and after sevoflurane exposure

	Baseline		Sevoflurane	
	CD	WD	CD	WD
Diastolic lumen diameter (mm)	5.7±0.6	5.5±0.8	5.4±0.8	5.5±0.8
Systolic lumen diameter (mm)	2.0±0.4	2.7±0.7*	2.3±0.5	3.4±0.6*#
Diastolic wall thickness (mm)	1.8±0.1	1.9±0.2*	1.6±0.2	1.9±0.2*
Systolic wall thickness (mm)	3.3±0.3	3.1±0.4	3.0±0.4	2.9±0.2

Data are mean±SD, n=9-18, two-way ANOVA with Bonferroni post-hoc analyses, * $p<0.05$ diet effect, # $p<0.05$ sevoflurane effect. CD, control diet; WD, western diet.
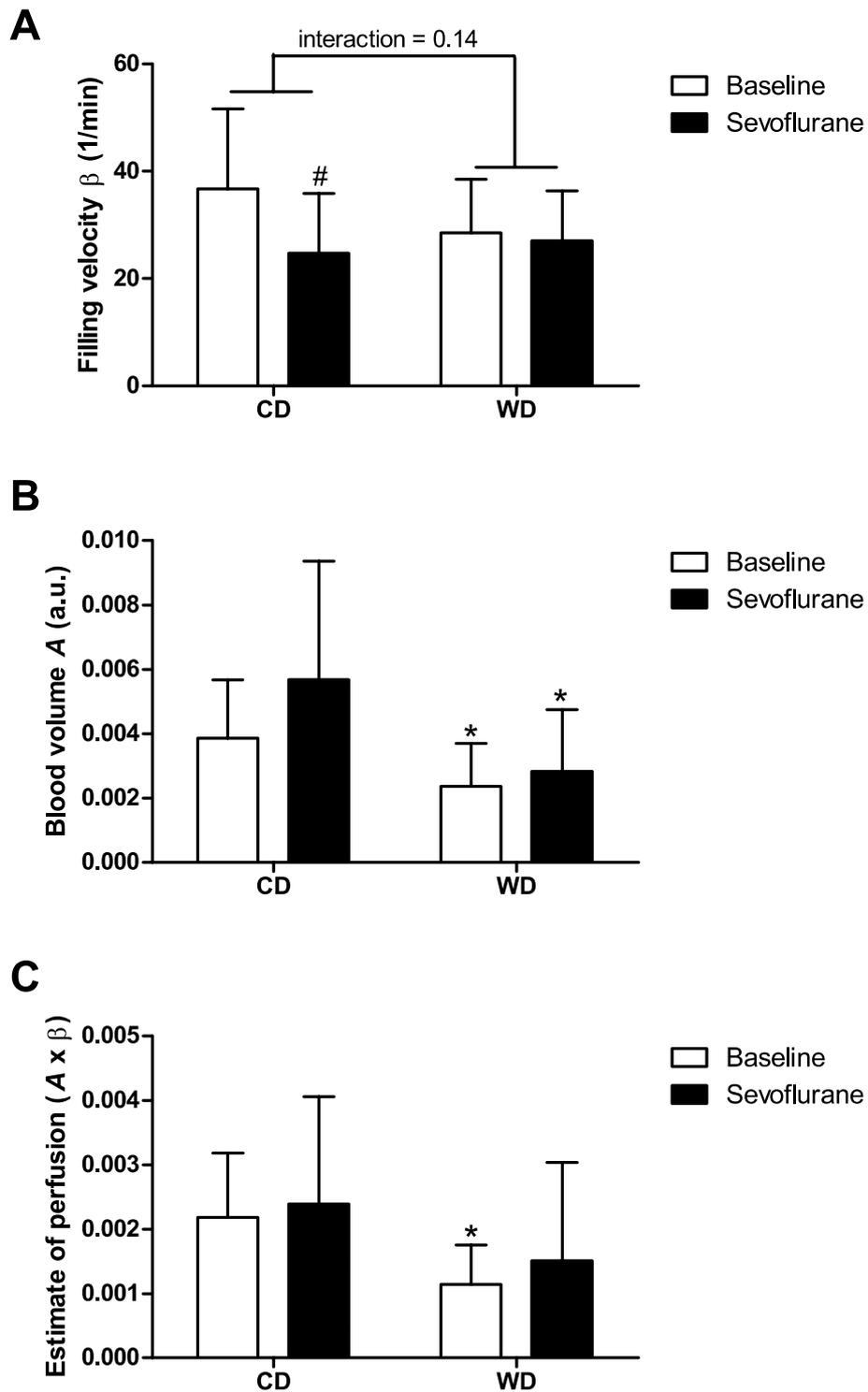
Figure 4.2: Effect of sevoflurane on myocardial perfusion in prediabetic rats
Microvascular blood volume *A* (**A**), microvascular filling velocity β (**B**) and estimate of perfusion (**C**) measured with contrast echocardiography in rats fed a control diet (CD) or western diet (WD) for 8 weeks during baseline conditions and after 5 minutes of sevoflurane exposure. Data are expressed as mean±SD, n=9-13, two-way ANOVA with Bonferonni post-hoc analyses, * p<0.05 diet effect, # p<0.05 sevoflurane effect.

Sevoflurane further impaired systolic function, but not myocardial perfusion

Blood pressure, heart rate and rate pressure product were significantly decreased after 5 minutes of sevoflurane exposure and significantly restored after a 5-minute washout period, without differences among diet groups (Figure 4.1).

Compared to baseline conditions, sevoflurane decreased the microvascular filling velocity (β) and tended to increase microvascular blood volume (*A*) in controls, while this observation was absent in western diet-fed animals. Overall, this resulted in an unchanged estimate of perfusion in both diet groups (Figure 4.2).

Sevoflurane additionally increased end-systolic lumen diameter in western diet-fed rats compared to baseline conditions (Table 4.2), which resulted in further impaired systolic function in western diet-fed rats compared to control rats (Figure 4.3).
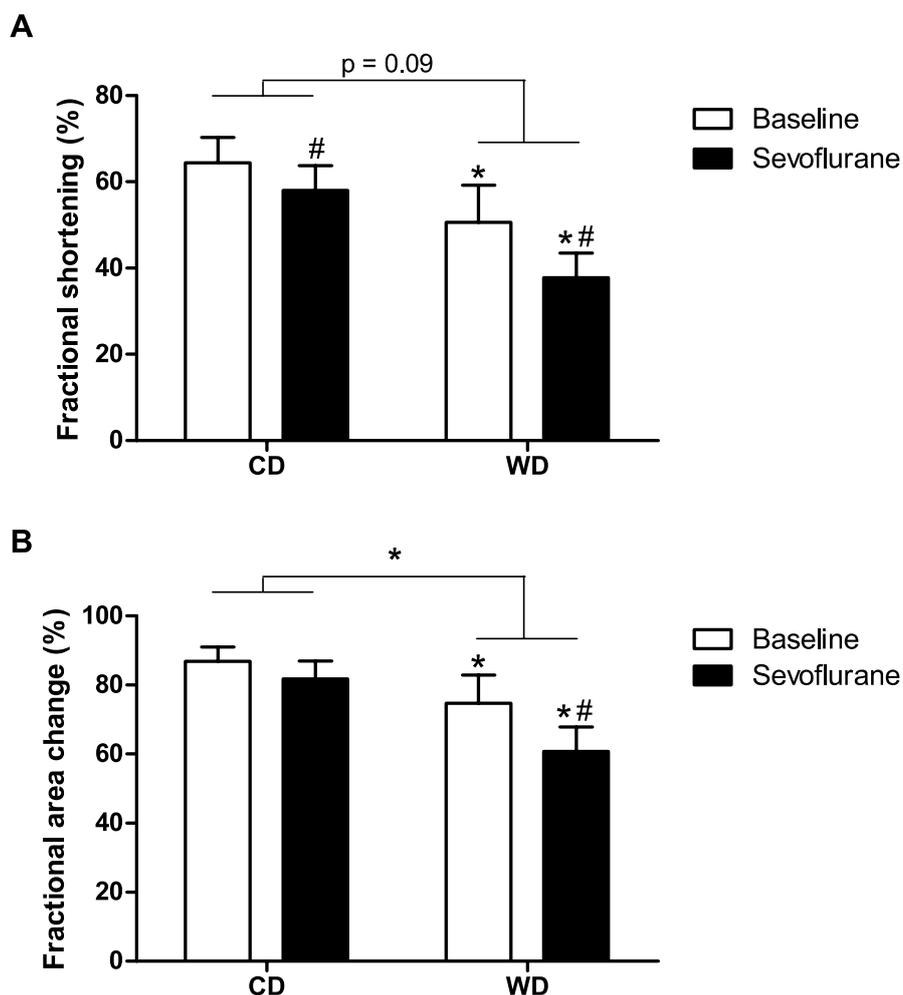

Figure 4.3: Effect of sevoflurane on systolic function in prediabetic rats
Systolic function, as represented by the fractional shortening (**A**) and fractional area change (**B**), measured with echocardiography in rats fed a control diet (CD) or western diet (WD) for 8 weeks during baseline conditions and after 5 minutes of sevoflurane exposure. Data are expressed as mean±SD, n=9-18, two-way ANOVA with Bonferonni post-hoc analyses, * $p < 0.05$ diet effect, # $p < 0.05$ sevoflurane effect.

Discussion

In the present study, we examined the effect of sevoflurane on myocardial perfusion and function in western diet-fed rats. We found that short-term western diet feeding resulted in a prediabetic phenotype characterized by obesity and hyperglycemia. Further, diet-induced prediabetes was associated with impaired myocardial perfusion and systolic dysfunction. Sevoflurane had no overall effect on myocardial perfusion in healthy and prediabetic rats, while systolic function was even further impaired. These results suggest that sevoflurane leads to uncoupling of myocardial perfusion and function, irrespective of the metabolic state.

Sevoflurane did not affect myocardial perfusion, despite of decreased arterial blood pressure, heart frequency and rate pressure product in healthy rats. Previously it has indeed been shown that sevoflurane did not alter myocardial blood flow in healthy rats[20] and healthy subjects[6] compared to the awake condition. In contrast, others however described decreased myocardial blood flow in healthy rats,[8] dogs[9] and pigs.[10] In addition to species variation and the use of different experimental techniques,[19] administration of general anesthetics may explain the contrasting observations, as this may distinctly alter hemodynamics compared to the awake state. While myocardial perfusion remained unchanged, sevoflurane decreased the microvascular filling velocity (β) and increased microvascular blood volume (A). The microvascular filling velocity is a parameter of the capillary exchange rate providing an estimate of the speed of erythrocytes through the capillaries, while microvascular blood volume suggests the surface area for exchange of nutrients and correlates with oxygen consumption. Our observations are in contrast with a previously performed study in healthy subjects by our group, where we showed that sevoflurane decreased myocardial blood volume and increased the microvascular filling velocity.[6] A possible mechanism to explain these differences may be derived by the differences in heart rate among species. We found a decrease in heart rate, while in healthy subjects an increase in heart rate was shown.[6] However, also decreased[8] or unchanged[20] heart rate in rats are found. Taken together, although sevoflurane impaired the microvascular filling velocity, myocardial perfusion was not affected in healthy rats.

Moreover, the present study showed that myocardial perfusion and function were both decreased in prediabetic rats compared to healthy controls. In detail, microvascular blood volume was decreased, which resulted in impairment of myocardial perfusion. Previously, we found that myocardial perfusion was maintained during baseline conditions in high fat diet-induced glucose intolerant rats.[14] The present study used a more severe diet, which resulted in more pronounced disturbances in the cardiometabolic condition of the rats. Independent of glucose tolerance and obesity, myocardial perfusion defects were found in prediabetic insulin resistant patients.[15;16] In T2DM patients, no differences in myocardial blood flow were found during fasting conditions.[17;18] However, during the postprandial state

decreased myocardial blood flow was found in T2DM patients,[17;18] which is in agreement with the present study showing decreased myocardial perfusion in non-fasted prediabetic rats. An underlying mechanism of this impaired myocardial perfusion might be alterations in insulin-mediated recruitment of the capillaries. In healthy subjects, insulin increased basal[21] and adenosine-stimulated[22] myocardial perfusion. Moreover, insulin directly affects myocardial perfusion by enhancing hyperemic myocardial blood flow in a dose-dependent manner in healthy subjects.[23] However, insulin resistance may block insulin-mediated capillary recruitment.[24] In T2DM patients, the use of an insulin analog partially reversed myocardial perfusion abnormalities.[18]

As far as we know, we are the first to study the effect of sevoflurane anesthesia on myocardial perfusion in prediabetic rats. Our results show that sevoflurane has a stronger cardiodepressive effect in prediabetic rats, whereas myocardial perfusion remained unaffected. Under physiological conditions, myocardial blood flow and function are in balance.[25] In our prediabetic rats, myocardial perfusion as well as myocardial function are decreased. However, during sevoflurane myocardial perfusion is maintained, while myocardial function is decreased in healthy and prediabetic rats. This uncoupling of perfusion and function suggests that despite of increased microvascular blood volume and decreased microvascular filling velocity, myocardial function cannot be maintained.

In conclusion, the present study showed impaired myocardial function and perfusion in diet-induced prediabetic rats. Moreover, sevoflurane further impaired systolic function, but myocardial perfusion was maintained. These results suggest that sevoflurane leads to uncoupling of myocardial perfusion and function, irrespective of the metabolic state of the heart.

References

1. Dole WP: Autoregulation of the coronary circulation. Prog Cardiovasc Dis 1987, 29:293-323.
2. Hoffman JI, Spaan JA: Pressure-flow relations in coronary circulation. Physiol Rev 1990, 70:331-390.
3. Malan TP, Jr., DiNardo JA, Isner RJ, Frink EJ, Jr., Goldberg M, Fenster PE, Brown EA, Depa R, Hammond LC, Mata H: Cardiovascular effects of sevoflurane compared with those of isoflurane in volunteers. Anesthesiology 1995, 83:918-928.
4. Park KW: Cardiovascular effects of inhalational anesthetics. Int Anesthesiol Clin 2002, 40:1-14.
5. Larach DR, Schuler HG: Direct vasodilation by sevoflurane, isoflurane, and halothane alters coronary flow reserve in the isolated rat heart. Anesthesiology 1991, 75:268-278.
6. Bulte CS, Slikkerveer J, Kamp O, Heymans MW, Loer SA, de Marchi SF, Vogel R, Boer C, Bouwman RA: General anesthesia with sevoflurane decreases myocardial blood volume and hyperemic blood flow in healthy humans. Anesth Analg 2013, 116:767-774.
7. Crystal GJ, Zhou X, Gurevicius J, Czinn EA, Salem MR, Alam S, Piotrowski A, Hu G: Direct coronary vasomotor effects of sevoflurane and desflurane in in situ canine hearts. Anesthesiology 2000, 92:1103-1113.
8. Conzen PF, Vollmar B, Habazettl H, Frink EJ, Peter K, Messmer K: Systemic and regional hemodynamics of isoflurane and sevoflurane in rats. Anesth Analg 1992, 74:79-88.
9. Hirano M, Fujigaki T, Shibata O, Sumikawa K: A comparison of coronary hemodynamics during isoflurane and sevoflurane anesthesia in dogs. Anesth Analg 1995, 80:651-656.
10. Manohar M, Parks CM: Porcine systemic and regional organ blood flow during 1.0 and 1.5 minimum alveolar concentrations of sevoflurane anesthesia without and with 50% nitrous oxide. J Pharmacol Exp Ther 1984, 231:640-648.
11. Preis SR, Pencina MJ, Hwang SJ, D'Agostino RB, Sr., Savage PJ, Levy D, Fox CS: Trends in cardiovascular disease risk factors in individuals with and without diabetes mellitus in the Framingham Heart Study. Circulation 2009, 120:212-220.
12. Lee TH, Marcantonio ER, Mangione CM, Thomas EJ, Polanczyk CA, Cook EF, Sugarbaker DJ, Donaldson MC, Poss R, Ho KK, Ludwig LE, Pedan A, Goldman L: Derivation and prospective validation of a simple index for prediction of cardiac risk of major noncardiac surgery. Circulation 1999, 100:1043-1049.
13. van den Brom CE, Huisman MC, Vlasblom R, Boontje NM, Duijst S, Lubberink M, Molthoff CF, Lammertsma AA, Van der Velden J, Boer C, Ouwens DM, Diamant M: Altered myocardial substrate metabolism is associated with myocardial dysfunction in early diabetic cardiomyopathy in rats: studies using positron emission tomography. Cardiovasc Diabetol 2009, 8:39.
14. van den Brom CE, Bulte CS, Kloeze BM, Loer SA, Boer C, Bouwman RA: High fat diet-induced glucose intolerance impairs myocardial function, but not myocardial perfusion during hyperaemia: a pilot study. Cardiovasc Diabetol 2012, 11:74.
15. Nasr G, Sliem H: Silent myocardial ischemia in prediabetics in relation to insulin resistance. J Cardiovasc Dis Res 2010, 1:116-121.
16. Nasr G, Sliem H: Silent ischemia in relation to insulin resistance in normotensive prediabetic adults: early detection by single photon emission computed tomography (SPECT). Int J Cardiovasc Imaging 2011, 27:335-341.
17. Scognamiglio R, Negut C, De Kreutzenberg SV, Tiengo A, Avogaro A: Postprandial myocardial perfusion in healthy subjects and in type 2 diabetic patients. Circulation 2005, 112:179-184.

18. Scognamiglio R, Negut C, De Kreutzenberg SV, Tiengo A, Avogaro A: Effects of different insulin regimes on postprandial myocardial perfusion defects in type 2 diabetic patients. Diabetes Care 2006, 29:95-100.
19. Bulte CS, Slikkerveer J, Meijer RI, Gort D, Kamp O, Loer SA, de Marchi SF, Vogel R, Boer C, Bouwman RA: Contrast-enhanced ultrasound for myocardial perfusion imaging. Anesth Analg 2012, 114:938-945.
20. Crawford MW, Lerman J, Saldivia V, Carmichael FJ: Hemodynamic and organ blood flow responses to halothane and sevoflurane anesthesia during spontaneous ventilation. Anesth Analg 1992, 75:1000-1006.
21. Liu Z: Insulin at physiological concentrations increases microvascular perfusion in human myocardium. Am J Physiol Endocrinol Metab 2007, 293:E1250-E1255.
22. Laine H, Nuutila P, Luotolahti M, Meyer C, Elomaa T, Koskinen P, Ronnemaa T, Knuuti J: Insulin-induced increment of coronary flow reserve is not abolished by dexamethasone in healthy young men. J Clin Endocrinol Metab 2000, 85:1868-1873.
23. Sundell J, Nuutila P, Laine H, Luotolahti M, Kalliokoski K, Raitakari O, Knuuti J: Dose-dependent vasodilating effects of insulin on adenosine-stimulated myocardial blood flow. Diabetes 2002, 51:1125-1130.
24. Zhang L, Vincent MA, Richards SM, Clerk LH, Rattigan S, Clark MG, Barrett EJ: Insulin sensitivity of muscle capillary recruitment in vivo. Diabetes 2004, 53:447-453.
25. Heusch G, Schulz R: The relation of contractile function to myocardial perfusion. Perfusion-contraction match and mismatch. Herz 1999, 24:509-514.