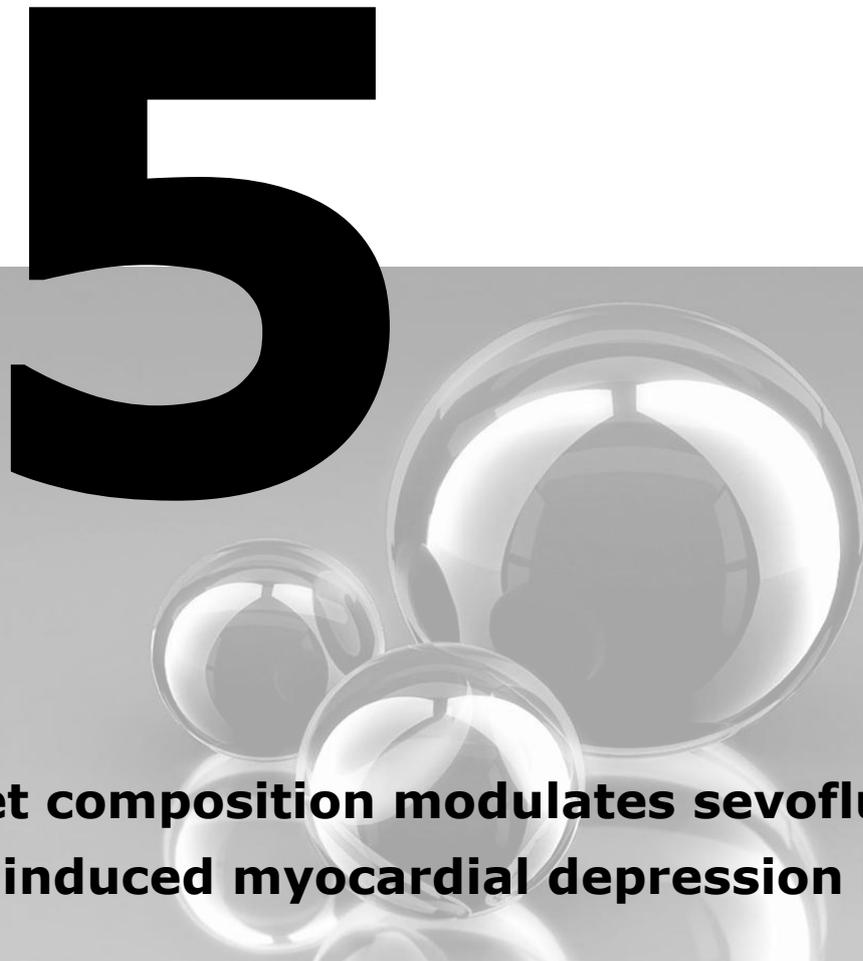


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## **Diet composition modulates sevoflurane-induced myocardial depression in rats**

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*Submitted*



## **Abstract**

### **Introduction**

Cardiometabolic diseases like obesity and/or diabetes mellitus may alter the effects of sevoflurane on the heart, but current evidence is limited to in vitro studies. This study evaluated the influence of western diet-induced obesity with glucose intolerance on the myocardial response to sevoflurane, and further elaborated whether lowering caloric intake can modulate this effect.

### **Methods**

Male Wistar rats were exposed to a western diet (WD) or control diet (CD) for 8 weeks. A third group of WD-fed rats reversed after 4 weeks to CD for 4 consecutive weeks. Study parameters included an oral glucose tolerance test, echocardiography and protein analyses before and after exposure to 2% sevoflurane.

### **Results**

Eight weeks of WD-feeding resulted in a prediabetic phenotype with obesity, glucose intolerance, mild hyperglycemia, hyperinsulinemia, dyslipidemia and reduced myocardial systolic and diastolic function. While sevoflurane did not alter myocardial contractile function in healthy control animals, systolic function in WD-fed rats was further impaired after sevoflurane exposure. Reversion of WD to control diet normalized the prediabetic phenotype, and restored myocardial function during baseline and after sevoflurane exposure to control values. Western diet and diet reversal exerted distinct effects on myocardial calcium handling proteins, while changes in proteins related to substrate metabolism were only minimal.

### **Conclusions**

Sevoflurane is a stronger cardiodepressant in prediabetic than in control rats, which could be restored by lowering caloric intake. These results suggest that normalization of the cardiometabolic profile by dietary changes are of direct influence on the myocardial response to sevoflurane in rats.

## Introduction

Patients with obesity and type 2 diabetes mellitus (T2DM) due to excessive caloric intake may exert a different response to anesthesia and surgery than healthy subjects. In healthy subjects, volatile anesthetics like sevoflurane exhibit negative inotropic effects as shown by depressed myocardial contractility,<sup>1;2</sup> lusitropic effects as reflected by early diastolic dysfunction<sup>1;3</sup> and decreased systemic vascular resistance.<sup>3</sup> While it may be expected that cardiometabolic alterations induced by obesity and/or diabetes mellitus alter the response of the heart to volatile anesthetics, most studies focusing on this relationship are limited by their in vitro nature and the use of models for type 1 diabetes mellitus. Moreover, the results are conflicting, showing either augmentation<sup>4</sup> or suppression<sup>5</sup> of the cardiodepressive effects of volatile anesthetics in papillary muscles of type 1 diabetic rats. A third study suggested that the inotropic effects of several volatile anesthetics were not altered in single ventricular myocytes from type 1 diabetic rats.<sup>6</sup>

These findings warrant further exploration of the effects of obesity and/or diabetes mellitus on the interaction of volatile anesthetics with the heart. Moreover, increasing evidence suggests that preoperative alterations in dietary composition and intake may alter the susceptibility of the cardiovascular system to surgical and anesthetic stress.<sup>7</sup> It has indeed been shown that diet-induced obesity<sup>8-11</sup> and T2DM<sup>12;13</sup> in small rodents is reversible by reducing caloric intake, which resulted in weight loss and improved insulin sensitivity. In obese and T2DM patients, a low caloric diet decreased myocardial fatty acid uptake<sup>14</sup> and improved diastolic function,<sup>15</sup> respectively. Additionally, preoperative dietary restriction exerted favorable effects on surgery-related inflammation, oxidative stress and ischemia.<sup>7;16</sup> It is however unknown whether the interaction of volatile anesthesia with myocardial function can be altered by dietary intake. In the present study we therefore investigated whether western diet-induced obesity with glucose intolerance alters the effects of the volatile anesthetic sevoflurane on myocardial function and calcium handling proteins in rats, and whether changing diet composition can modulate these alterations.

## 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 performed research is in compliance with the modern ARRIVE guidelines on animal research.<sup>17</sup>

The first part of the study was performed in a group of 24 male Wistar rats (baseline body weight:  $264 \pm 5$  g; Charles River Laboratories, France). Rats were exposed to a western diet in combination with sucrose water (20%) (WD,  $n=16$ ) or control diet (CD,  $n=8$ ) for a period of 8 weeks. Four weeks after the start of the diet exposure, WD ( $n=8$ ) exposed rats reversed to CD for 4 consecutive weeks. 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 and caloric intake were determined on a weekly basis. After 4 and 8 weeks of diet exposure, rats underwent an oral glucose tolerance test and echocardiography. After a 6h fasting period, rats were sacrificed by decapitation, trunk blood was collected for plasma determinations and hearts were removed, rinsed in saline, weighted, snap-frozen in liquid nitrogen and stored at -80°C until further analysis.

The second part of the study included 30 male Wistar rats (baseline body weight:  $262 \pm 6$  g; Charles River Laboratories, France) that were either exposed to the control diet (CD,  $n=10$ ), western diet in combination with sucrose water (20%) (WD,  $n=10$ ) or the western diet with reversal to control diet (REV,  $n=10$ ) as described above. After 8 weeks, rats were exposed to sevoflurane (AbbVie, the Netherlands). Rats were sacrificed by decapitation without fasting, trunk blood was collected for plasma determinations and hearts were removed, rinsed in saline, weighted, snap-frozen in liquid nitrogen and stored at -80°C until further analysis.

### *Diets*

Control diet (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 western diet (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.

### *Oral glucose tolerance test*

Awake rats fasted overnight received an oral glucose load (2 g/kg of body weight). Blood glucose was measured from tail bleeds with a Precision Xceed Blood Glucose monitoring system (MediSense, UK) before and 15, 30, 60, 90 and 120 min after glucose ingestion. At similar time points, plasma insulin (LINCO research, St. Charles, Missouri) levels were measured as described previously.<sup>18;19</sup>

### *Echocardiography*

Echocardiography (ALOKA ProSound SSD 4000, Aloka, Tokyo, Japan) using a 13-MHz linear interfaced array transducer was performed after 4 and 8 weeks as described previously.<sup>18-21</sup> Briefly, rats received S-Ketamine (Ketanest®, 75 mg/kg, Pfizer, the Netherlands) and diazepam (4 mg/kg, Centrafarm, the Netherlands) anesthesia intraperitoneally, allowed spontaneous respiration and placed on a underbody heating pad. 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  $FAC = (EDD^2-ESD^2)/EDD^2 \cdot 100$ . Diastolic function was measured in the apical four-chamber view and shown as E flow, E deceleration time and isovolumic relaxation time (IVRT; except 8 weeks data). LV mass was calculated as described previously.<sup>18</sup> All parameters were averaged over at least three cardiac cycles. Analyses were performed off-line (Image-Arena 2.9.1, TomTec Imaging Systems, Unterschleissheim/Munich, Germany).

### *Sevoflurane intervention*

The second group of rats was anesthetized with 125 mg/kg S-ketamine (Ketanest®, Pfizer, the Netherlands) and 4 mg/kg diazepam (Centrafarm, the Netherlands) intraperitoneally and maintenance was performed with 40 mg/kg/h S-ketamine and 1 mg/kg/h diazepam intravenously in the tail vein and allowed spontaneous respiration. Rats (n=6 per group) were exposed to 3x 5' 2% sevoflurane (AbbVie, the Netherlands) in 40% O<sub>2</sub>/60% N<sub>2</sub>, whereas control rats received only 40% O<sub>2</sub>/60% N<sub>2</sub> for the same time period (baseline, n=4). Measurement of systolic function during sevoflurane exposure was started after 5' of 2% sevoflurane and performed as described above.

### *Blood and plasma measurements*

Plasma hematocrit levels were determined using microcentrifugation. Plasma glucose levels (Abcam, Cambridge, MA), plasma insulin (LINCO research, St. Charles, Missouri), plasma free fatty acids (WAKO NEFA-HR, Wako Pure Chemical Industries, Osaka, Japan), plasma triglyceride (Sigma, Saint Louis, Missouri), and plasma HDL and LDL/VLDL cholesterol (Abcam, Cambridge, MA) levels were measured from trunk blood as described previously.<sup>18-21</sup>

### *Protein analysis*

LV tissues were homogenized to obtain cytoplasmic protein fractions for western blot analysis as previously described.<sup>18;20;21</sup> Phosphorylation and/or expression of signaling intermediates were analyzed using the following primary antibodies: Akt, phospho-Akt-Ser473, glycogen synthase kinase 3 $\beta$  (GSK3 $\beta$ ), phospho-GSK3 $\beta$ -Ser9, AMP activated protein kinase  $\alpha$  (AMPK $\alpha$ ), phospho-AMPK $\alpha$ -Thr172 (all Cell signaling Technology, Beverly, MA), pyruvate dehydrogenase kinase 4 (PDK4, Santa Cruz Biotechnology, Santa Cruz, CA), glucose transporter 4 (GLUT4; Abcam, Cambridge, MA), phospholamban (PLB), phospho-PLB-Ser16, (Upstate, Lake Placid, NY), sarcoplasmic reticulum calcium ATPase 2a (SERCA2a)<sup>21</sup> and fatty acid transporter (FAT)/CD36 (MO25).<sup>18;21</sup> All signals were normalized to total protein expression or actin (Sigma, Saint Louis, Missouri).

Myofilament protein phosphorylation was determined using Pro-Q Diamond Phosphoprotein Stain as described previously.<sup>22</sup> LV tissues were separated on gradient gels (Criterion tris-HCl 4-15% gel, BioRad) and proteins were stained with Pro-Q Diamond Phosphoprotein Stain. Subsequently gels were stained overnight with SYPRO Ruby stain (Molecular Probes). Phosphorylation status of myosin binding protein-C (MyBP-C), troponin T (TnT) and cardiac troponin I (cTnI) were expressed relative to SYPRO-stained protein bands to correct for differences in sample loading. Staining was visualized using the LAS-3000 Image Reader (FUJI; 460 nm/605 nm Ex/Em) and immunoblots were quantified by densitometric analysis of films (AIDA, 4.21.033, Raytest, Straubing, Germany).

### *Histology*

Sections (4  $\mu$ m) of left ventricular tissue from the free wall at the level of the papillary muscles were cut in the direction of the fibers and mounted on 3-aminopropyltriethoxysilane-coated slides (Superfrost® Plus, Menzel, Darmstadt, Germany). After deparaffinization and rehydration, cardiomyocyte cross-sectional area was determined in randomly chosen fields of hematoxylin-eosin stained sections for 20-30 cells per heart and normalized to sarcomere length.<sup>18;20;21</sup>

*Statistical analysis*

Data were analyzed using Graphpad Prism 5.0 (La Jolla, USA) and are presented as mean±SD. Statistical analyses were performed using a student t-test after 4 weeks, one-way ANOVA with Bonferroni post-hoc analysis after 8 weeks, two-way ANOVA with repeated measures and Bonferroni post-hoc analysis for the oral glucose tolerance test and two-way ANOVA with Bonferroni post-hoc analysis for data after 8 weeks with sevoflurane intervention.  $p < 0.05$  was considered as statistically significant.

## Results

### Animal model characteristics

Western diet (WD) feeding resulted in a prediabetic phenotype with obesity, mild hyperglycemia, hyperinsulinemia, hyperlipidemia and glucose intolerance at 4 and 8 weeks after initiation of the diet (Table 5.1 and Figure 5.1 A-F). Reversion to a control diet (REV) after 4 weeks of western diet feeding resulted in normalization of caloric intake and body weight, and reversed the prediabetic phenotype in western diet-fed rats.

Heart weight and the cross sectional area of individual cardiomyocytes were significantly increased in WD-fed rats compared to controls, and these values normalized after diet reversal. Additionally, diet reversion following western diet feeding resulted in a decrease in liver weight and epididymal and perirenal fat pads (Table 5.1).

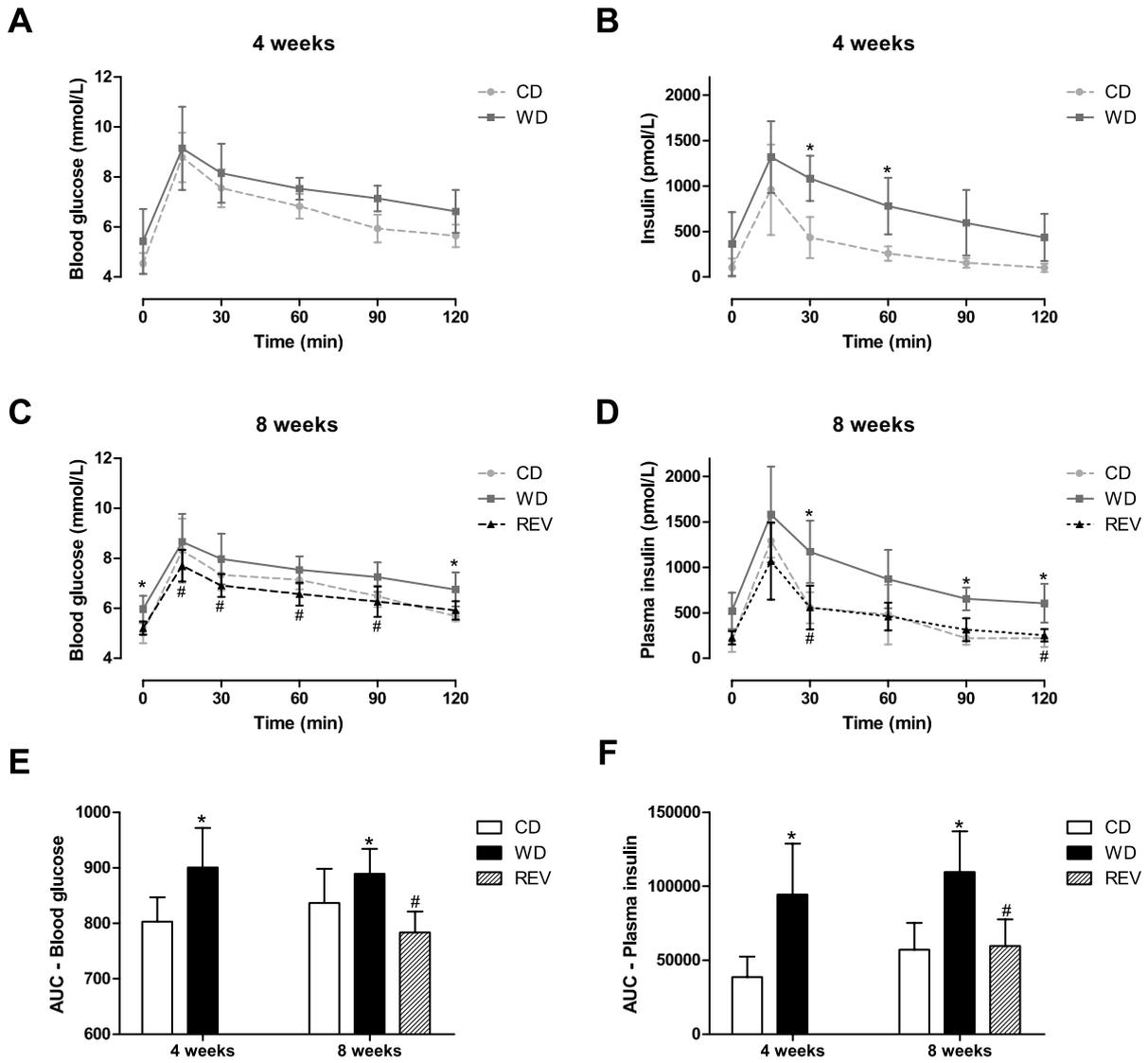
At 4 and 8 weeks, western diet feeding decreased myocardial lumen diameter and increased wall thickness during diastole when compared to control rats (Table 5.2). Moreover, end-systolic lumen diameter was increased at 4 and 8 weeks of western diet feeding, while systolic wall thickness was slightly reduced after 4 weeks of diet exposure. Reversal from western to control diet resulted in normalization of systolic lumen diameter and myocardial diastolic and systolic wall thickness, while the diastolic lumen diameter was not affected by lowering caloric intake (Table 5.2).

Western diet feeding induced a reduction in myocardial fractional shortening (Figure 5.2A) and fractional area change (Figure 5.2B), which could be restored by diet reversal. While E flow was unchanged by WD-feeding, the deceleration time of the E peak and isovolumic relaxation time at 4 weeks were prolonged in rats fed a WD when compared to control rats (Table 5.2). After 8 weeks of diet exposure, there was a trend towards impaired left ventricular relaxation in WD-fed rats. Diet reversal improved diastolic function as shown by a shortening of the E deceleration time when compared to WD-fed rats (Table 5.2).

**Table 5.1: Characteristics after 4 and 8 weeks of diet intervention without sevoflurane exposure**

	4 weeks		8 weeks		
	Control diet	Western diet	Control diet	Western diet	Reversion
Caloric intake (kcal/100gBW)	138±8	155±17 *	124±6	129±7	114±6 #
<b>Blood/plasma characteristics</b>					
6h fasting plasma glucose (mmol/L)	7.1±0.6	7.8±1.3	8.8±0.7	10.7±1.1 *	8.2±1.2 #
6h fasting plasma insulin (pmol/L)	411±135	726±214 *	933±383	1524±353 *	813±369 #
6h fasting plasma free fatty acids (mmol/L)	0.82±0.20	0.96±0.20	0.26±0.10	0.46±0.24 *	0.26±0.11 #
6h fasting plasma triglycerides (mmol/L)	0.92±0.21	2.04±0.72 *	0.68±0.18	3.33±1.19 *	0.78±0.27 #
6h fasting plasma HDL cholesterol (mg/dL)	71.0±3.0	39.8±8.3 *	112.3±11.8	61.9±6.7 *	108.9±11.6 #
6h fasting plasma LDL/VLDL cholesterol (mg/dL)	12.4±2.7	10.2±3.9	15.6±2.2	27.3±7.0 *	17.8±2.7 #
Hematocrit (%)	48.3±2.8	47.4±2.5	50.7±3.0	47.9±2.2 *	49.0±1.3
<b>Body composition</b>					
Bodyweight (g)	362±23	397±16 *	410±27	478±25 *	432±30 #
Heart weight (g)	n.d.	n.d.	1.19±0.06	1.34±0.15 *	1.28±0.06
Liver weight (g)	n.d.	n.d.	10.7±1.0	14.2±1.0 *	11.3±2.0 #
Epididymal fat weight (g)	n.d.	n.d.	6.0±0.8	11.4±2.1 *	7.3±1.5 #
Perirenal fat weight (g)	n.d.	n.d.	8.0±1.5	16.7±3.8 *	9.5±1.8 #
Tibia length (mm)	n.d.	n.d.	42.0±0.5	41.8±1.0	41.4±1.0
<b>Heart composition</b>					
Cross sectional area (µm <sup>2</sup> )	n.d.	n.d.	372±54	625±105 *	389±53 #

Data are mean±SD, n=8-16, 4 weeks: student t-test, \* p<0.05 vs. control diet, 8 weeks: one-way ANOVA with Bonferroni post-hoc test, \* p<0.05 vs. control diet, # p<0.05 vs. western diet. n.d., non determined.



**Figure 5.1: Oral glucose tolerance test after 8 weeks of diet feeding without sevoflurane exposure**

Blood glucose (**A, C**), plasma insulin (**B, D**) and area under the curve (AUC; **E, F**) during an oral glucose tolerance test in rats after 4 and 8 weeks of control diet (CD), western diet (WD) or diet reversion (REV) feeding. Data are mean±SD, n=6, t-test, one- and two-way ANOVA with repeated measures and Bonferroni post-hoc analyses, \* p<0.05 vs. CD, # p<0.05 vs. WD.

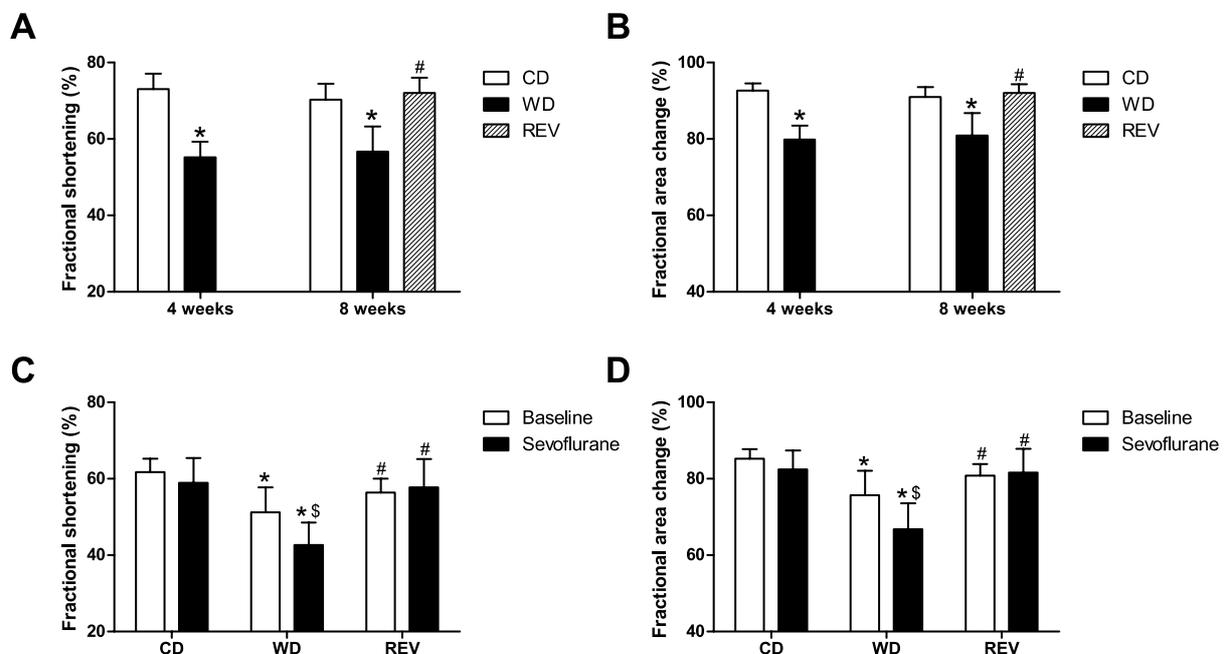
**Table 5.2: Myocardial function after 4 and 8 weeks of diet intervention without sevoflurane exposure**

	4 weeks		8 weeks		
	Control diet	Western diet	Control diet	Western diet	Reversion
Heart rate (bpm)	465±36	488±23	443±31	491±16 *	474±16
LV mass (mg)	786±95	820±57	868±118	955±87	805±62 #
<b>LV dimensions</b>					
Diastolic lumen diameter (mm)	6.6±0.4	5.9±0.5 *	6.7±0.4	6.0±0.5 *	6.1±0.5
Systolic lumen diameter (mm)	1.7±0.2	2.7±0.4 *	2.0±0.3	2.6±0.4 *	1.7±0.3 #
Diastolic wall thickness (mm)	1.7±0.1	2.0±0.2 *	1.7±0.1	2.3±0.2 *	1.9±0.2 #
Systolic wall thickness (mm)	3.7±0.2	3.4±0.2 *	3.7±0.1	3.6±0.1	3.8±0.2 #
<b>LV diastolic function</b>					
E flow (cm/s)	99.8±15.5	109.3±17.0	98.3±17.3	110.9±10.9	115.0±9.5
E deceleration time (ms)	23.9±7.4	31.1±4.2 *	24.5±2.4	29.1±5.1	23.4±6.3 #
Isovolumic relaxation time (ms)	13.8±2.3	20.3±4.2 *	n.d.	n.d.	n.d.

Data are mean±SD, n=6-10, 4 weeks: student t-test, \* p<0.05 vs. control diet, 8 weeks: one-way ANOVA with Bonferroni post-hoc analyses, \* p<0.05 vs. control diet, # p<0.05 vs. western diet. LV, left ventricular.

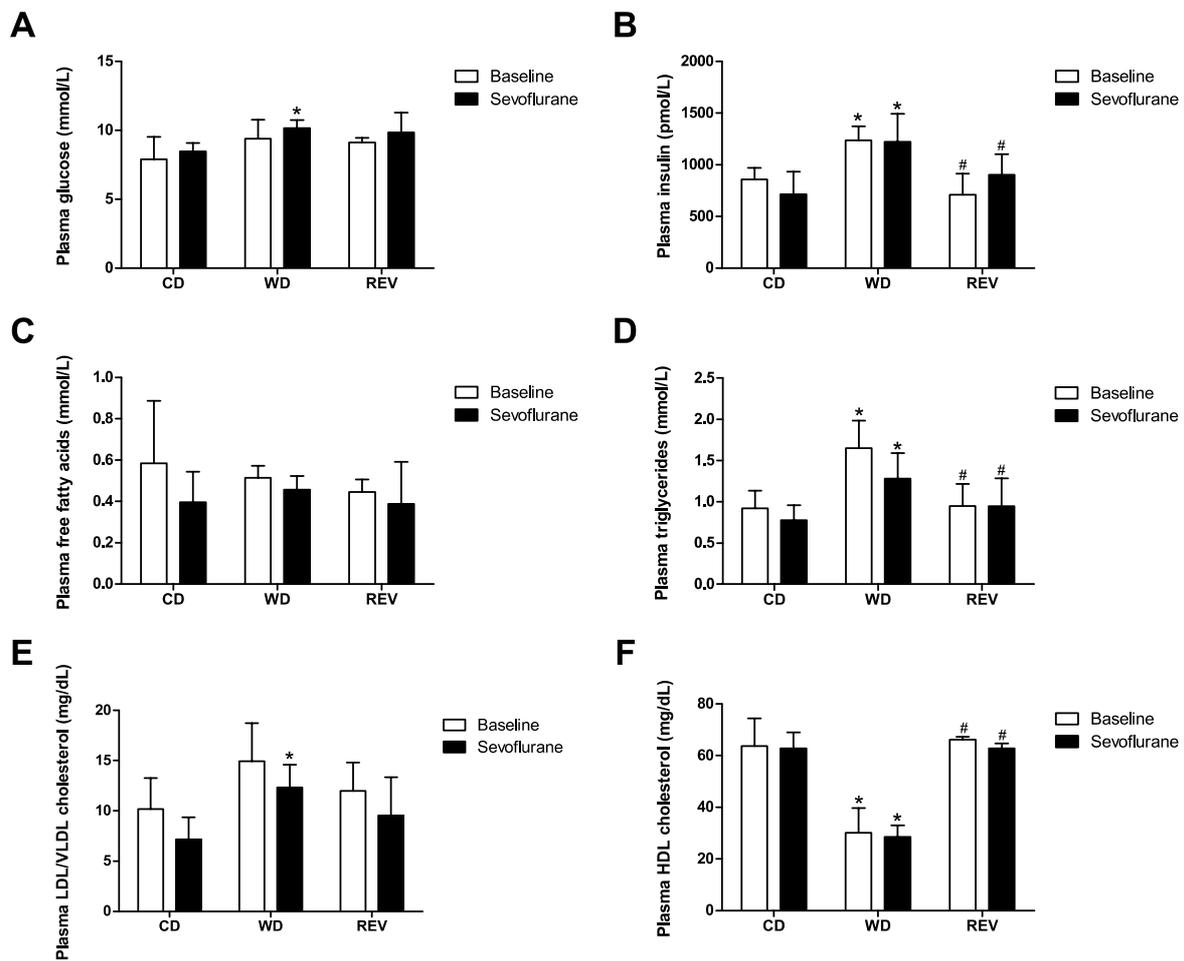
## Sevoflurane-induced cardiodepression is only present in western diet-fed rats

Exposure to sevoflurane did not alter plasma levels of glucose, insulin, free fatty acids, triglycerides, LDL/VLDL cholesterol and HDL cholesterol in all 3 diet groups (Figure 5.3A-F). After 8 weeks of diet exposure, sevoflurane exerted no cardiodepressive effects in control rats as indicated by maintained fractional shortening and fractional area change. However, in rats fed a western diet, sevoflurane induced an additional reduction in myocardial contractility, and reducing caloric intake could restore this (Figure 5.2C and D). These findings suggest that diet composition and/or the cardiometabolic phenotype of the rat modulate the interaction of sevoflurane with myocardial function.



**Figure 5.2: Systolic function after diet feeding with and without sevoflurane exposure**

Fractional shortening (**A**) and fractional area change (**B**) in rats after 4 and 8 weeks of control diet (CD), western diet (WD) or diet reversion (REV) feeding. Fractional shortening (**C**) and fractional area change (**D**) during baseline and sevoflurane in rats after 8 weeks of control diet (CD), western diet (WD) or diet reversion (REV) feeding. Data are mean±SD, n=4-6, two-way ANOVA with Bonferroni post-hoc analyses, \* p<0.05 vs. CD, # p<0.05 vs. WD, § p<0.05 vs. baseline.



**Figure 5.3: Plasma levels after sevoflurane exposure**

Plasma glucose (A), insulin (B), free fatty acid (C), triglyceride (D), LDL/VLDL cholesterol (E) and HDL cholesterol (F) levels during baseline and sevoflurane in rats after 8 weeks of control diet (CD), western diet (WD) or diet reversion (REV) feeding. Data are mean $\pm$ SD, n=4-6, two-way ANOVA with Bonferroni post-hoc analyses, \* p<0.05 vs. CD, # p<0.05 vs. WD.

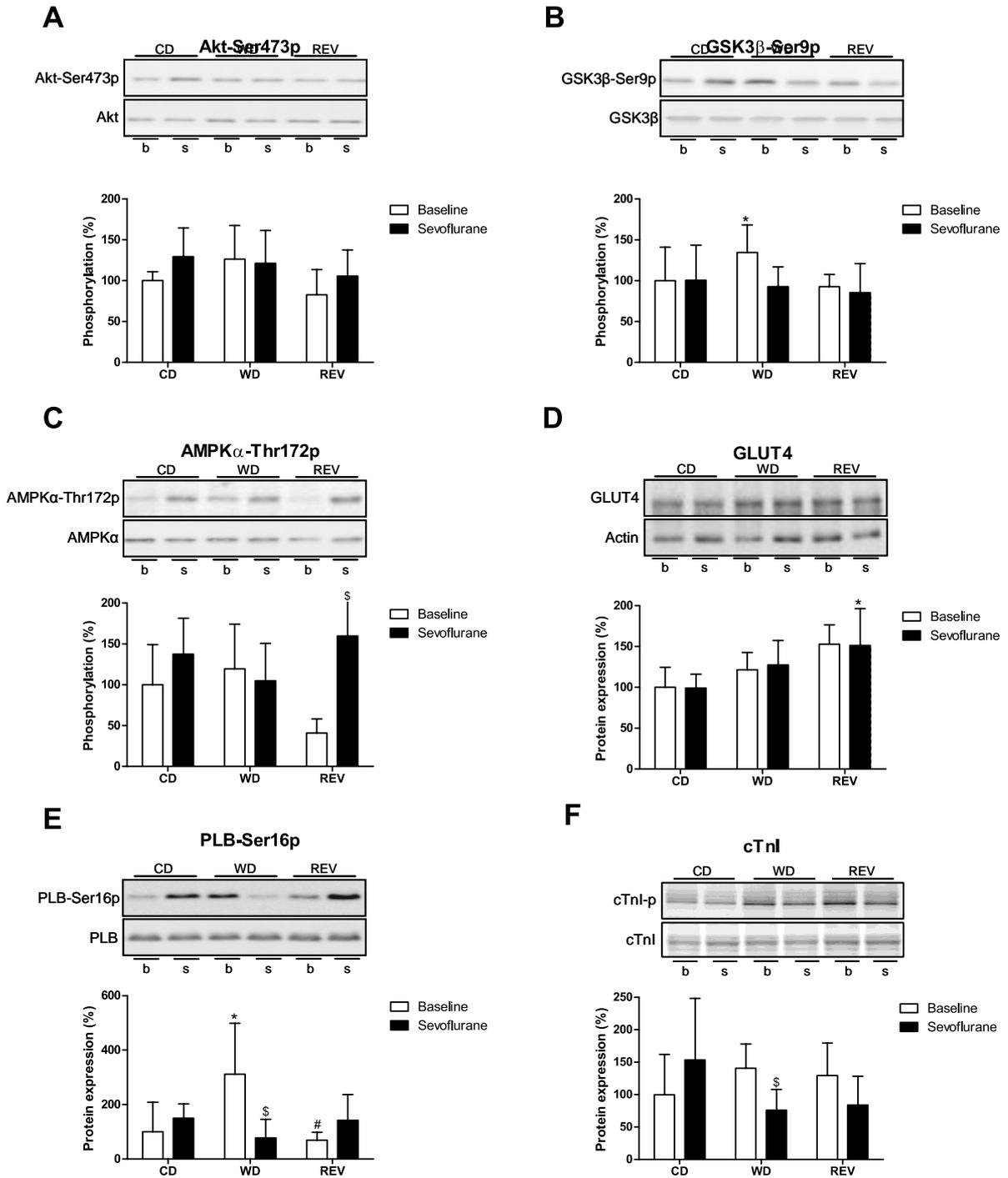
### Sevoflurane induced molecular alterations

Figure 5.4 shows the effects of sevoflurane and type of diet on phosphorylation levels of proteins associated with insulin signaling (Akt (panel A); GSK3 $\beta$  (panel B); AMPK $\alpha$  (panel C)), protein expression levels of the glucose transporter GLUT4 (panel D) and the phosphorylation of two proteins involved in myocardial calcium handling (PLB (panel E) and cTnI (panel F)).

The absent effect of sevoflurane exposure on myocardial function in control animals was associated with maintained levels of phosphorylated GSK3 $\beta$ , while Akt and AMPK $\alpha$  phosphorylation tended to increase by sevoflurane. Sevoflurane did not alter protein expression of GLUT4, phospholamban or cTnI. Sevoflurane did not alter SERCA2a protein expression or phosphorylation of myosin binding protein-C and troponin T (data not shown).

Compared to control animals, western diet increased GSK3 $\beta$  phosphorylation ( $p=0.05$ ), which tended to reduce towards control levels after diet reversion. Akt and AMPK $\alpha$  phosphorylation and GLUT4 protein expression remained unchanged during western diet-feeding. Sevoflurane tended to increase Akt and AMPK $\alpha$  phosphorylation in control rats compared to baseline, which was blunted in western diet-fed rats and restored after diet reversal, however, these alterations failed to reach statistical significance (Figure 5.4A and 4C). Sevoflurane induced a non-significant reduction in GSK3 $\beta$  phosphorylation in western diet-fed rats only.

WD-feeding significantly increased phosphorylation of phospholamban (PLB) and increased phosphorylation of cardiac troponin I (cTnI). PLB phosphorylation was normalized after diet reversal, while cTnI phosphorylation was still high in the diet reversal group. Compared to baseline, sevoflurane significantly reduced PLB phosphorylation in WD-fed rats, which was restored after diet reversal. Sevoflurane also significantly reduced phosphorylation of cTnI in WD-fed rats ( $p=0.05$ ), and a similar pattern was observed in the diet reversion group (Figure 5.4F). Diet or sevoflurane did not alter SERCA2a protein expression or phosphorylation of myosin binding protein-C and troponin T after western diet exposure (data not shown).



**Figure 5.4: Molecular alterations in substrate metabolism and calcium handling after sevoflurane exposure**

Phosphorylation of Akt (**A**), glycogen synthase kinase 3β (GSK3β, **B**), AMP activated protein kinase α (AMPKα, **C**), protein expression of glucose transporter 4 (GLUT4, **D**), phosphorylation of phospholamban (PLB, **E**) and Pro-Q Diamond Phosphoprotein Stain determined cardiac troponin I (cTnI, **F**) during baseline and sevoflurane in rats after 8 weeks of control diet (CD), western diet (WD) or diet reversion (REV) feeding. Data are mean±SD, n=4-6, two-way ANOVA with Bonferroni post-hoc analyses, \* p<0.05 vs. CD, # p<0.05 vs. WD, \$ p<0.05 vs. baseline.

## Discussion

Sevoflurane exerts cardiodepressive properties in healthy subjects, therefore its perioperative use may lead to hemodynamic alterations. Although it has been suggested that the interaction of sevoflurane with the heart is additionally altered by cardiometabolic diseases, most available studies are restricted to *in vitro* observations and experimental models for type I diabetes mellitus. With the growing epidemic of obesity and type 2 diabetes mellitus it might be interesting to understand the influence of this condition on the interaction of sevoflurane with myocardial function.

Unexpectedly, in an experimental rat model we could not show a direct effect of sevoflurane on myocardial contractility. However, in western diet-induced obesity, which was accompanied by glucose intolerance, hyperglycemia, hyperinsulinemia, dyslipidemia and myocardial dysfunction, sevoflurane exerted cardiodepressive effects. Interestingly, lowering caloric intake following western diet exposure could reverse these effects of sevoflurane on myocardial function. These results suggest that sevoflurane has negative inotropic effects on myocardial function in metabolically altered hearts, and that a reduction of caloric intake may improve the effects of sevoflurane on myocardial function. Our results therefore implicate that diet modification may be explored as an intervention to preserve perioperative myocardial function in subjects with cardiometabolic disease.

The present study showed that western diet feeding resulted in a prediabetic phenotype accompanied by myocardial dysfunction. Our results confirm previous findings showing impaired systolic function after 8 weeks of diet feeding.<sup>18</sup> Interestingly, the present study showed that lowering caloric intake completely normalized diet-induced T2DM. In small rodents, reducing dietary intake has been shown to reverse diet-induced obesity<sup>8-11</sup> and T2DM.<sup>12;13</sup> However, it is also shown that diet-induced obesity was not reversed by withdrawal of an energy dense diet.<sup>23;24</sup> Importantly, our study showed that lowering caloric intake also improved diet-induced systolic as well as diastolic dysfunction, which is in agreement with the clinical observation that in obese and T2DM patients a low caloric diet decreased myocardial fatty acid uptake<sup>14</sup> and improved diastolic function,<sup>15</sup> respectively. Taken together, lowering caloric intake normalized the prediabetic phenotype induced by short-term western diet feeding and improved myocardial function.

Exposure to sevoflurane further impaired systolic function in diet-induced prediabetes, but not in healthy rats. In contrast, sevoflurane caused a reduction in left ventricular systolic function at concentrations of 2% and higher compared to isoflurane in healthy mice.<sup>25</sup> An important difference with previous findings is that in the present study all rats were primary sedated with S-ketamine and diazepam. As S-ketamine and diazepam may have intrinsic cardiodepressive effects,<sup>26;27</sup> this might

have blurred the additional effect of sevoflurane on the heart in control animals. Limited data are available on the effects of sevoflurane in metabolic altered hearts. In papillary muscles from type 1 diabetic rats, decreased sensitivity to the negative inotropic interaction of halothane, enflurane and isoflurane was found,<sup>5</sup> whereas sevoflurane resulted in greater negative inotropic effects compared to controls.<sup>4</sup> In contrast, it has been shown that in single ventricular myocytes from type 1 diabetic rats the inotropic effects of halothane, isoflurane, desflurane and sevoflurane were not altered compared to controls.<sup>6</sup> As far as we know we are the first to show the negative inotropic effects of sevoflurane on hearts of diet-induced prediabetic rats.

A suggested underlying mechanism in the effect of sevoflurane on myocardial function is substrate availability and metabolism,<sup>28</sup> which is known to be altered by T2DM.<sup>18;20;21</sup> Unexpectedly, sevoflurane did not affect plasma glucose, insulin and lipid levels. In humans, there is indirect evidence that volatile anesthesia increases blood glucose; however, these observations may be biased by confounding factors such as surgical stress.<sup>29</sup> Compared to non-anesthetized rats without surgical stress, sevoflurane increased plasma glucose levels, but not plasma insulin levels.<sup>30</sup> It is possible that glucose levels did not increase in the present study due to short duration and low concentration of sevoflurane used, but also differences in nutritional status may exist. Moreover, in the present study rats were sedated with S-ketamine and diazepam. Ketamine<sup>31</sup> and diazepam<sup>32</sup> are known to increase blood glucose levels, which could have abolished the additional effect of sevoflurane on blood glucose levels.

Sevoflurane may also affect proteins related to myocardial substrate metabolism. Akt, which regulates translocation of glucose transporter 4 (GLUT4) to the sarcolemma for glucose uptake, is increased during sevoflurane in the isolated ischemic rat heart.<sup>33</sup> Moreover, sevoflurane enhances GLUT4 expression in lipid rafts, increases glucose oxidation and decreases fatty acid oxidation after ischemia and reperfusion injury in isolated working rat hearts.<sup>34</sup> Although not in ischemic and reperfused hearts, the present study showed no significant differences in proteins related to myocardial substrate metabolism.

Another possible mechanism is altered calcium handling, which also seems a common target in T2DM and sevoflurane. Sevoflurane reduces myocardial calcium availability, but increases sarcoplasmic reticulum calcium content.<sup>35</sup> In contrast, sevoflurane decreased fractional calcium release, but maintained sarcoplasmic reticulum calcium content.<sup>36</sup> In papillary muscles of type 1 diabetic rats, sevoflurane decreased myofilament calcium sensitivity to a greater extent than control rats.<sup>4</sup> In the present study, western diet feeding blunted phosphorylation of phospholamban and cardiac troponin I during sevoflurane, but not SERCA2a protein expression. In preconditioned hearts, phosphorylation of phospholamban and SERCA2a protein expression were unaltered after ischemia and reperfusion injury;<sup>37</sup> however, no data were reported for non-ischemic hearts. Our data indicate that phospholamban and

cardiac troponin I might play a role in impaired systolic function in prediabetic rats during sevoflurane.

In conclusion, sevoflurane induced cardiodepression in prediabetic rats, whereas lowering caloric intake restored myocardial function during sevoflurane. These results suggest that a change in dietary intake could be a potential intervention to support the preservation of myocardial function during sevoflurane in subjects with obesity and type 2 diabetes mellitus.

## References

1. Harkin CP, Pagel PS, Kersten JR, Hettrick DA, Warltier DC: Direct negative inotropic and lusitropic effects of sevoflurane. *Anesthesiology* 1994, 81:156-167.
2. Pagel PS, Kampine JP, Schmeling WT, Warltier DC: Influence of volatile anesthetics on myocardial contractility in vivo: desflurane versus isoflurane. *Anesthesiology* 1991, 74:900-907.
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. David JS, Tavernier B, Amour J, Vivien B, Coriat P, Riou B: Myocardial effects of halothane and sevoflurane in diabetic rats. *Anesthesiology* 2004, 100:1179-1187.
5. Hattori Y, Azuma M, Gotoh Y, Kanno M: Negative inotropic effects of halothane, enflurane, and isoflurane in papillary muscles from diabetic rats. *Anesth Analg* 1987, 66:23-28.
6. Graham M, Qureshi A, Noueihed R, Harrison S, Howarth FC: Effects of halothane, isoflurane, sevoflurane and desflurane on contraction of ventricular myocytes from streptozotocin-induced diabetic rats. *Mol Cell Biochem* 2004, 261:209-215.
7. Mitchell JR, Beckman JA, Nguyen LL, Ozaki CK: Reducing elective vascular surgery perioperative risk with brief preoperative dietary restriction. *Surgery* 2013, 153:594-598.
8. Hill JO, Dorton J, Sykes MN, DiGirolamo M: Reversal of dietary obesity is influenced by its duration and severity. *Int J Obes* 1989, 13:711-722.
9. Rogers PJ: Returning 'cafeteria-fed' rats to a chow diet: negative contrast and effects of obesity on feeding behaviour. *Physiol Behav* 1985, 35:493-499.
10. Bartness TJ, Polk DR, McGriff WR, Youngstrom TG, DiGirolamo M: Reversal of high-fat diet-induced obesity in female rats. *Am J Physiol* 1992, 263:R790-R797.
11. Walks D, Lavau M, Presta E, Yang MU, Bjorntorp P: Refeeding after fasting in the rat: effects of dietary-induced obesity on energy balance regulation. *Am J Clin Nutr* 1983, 37:387-395.
12. Parekh PI, Petro AE, Tiller JM, Feinglos MN, Surwit RS: Reversal of diet-induced obesity and diabetes in C57BL/6J mice. *Metabolism* 1998, 47:1089-1096.
13. Harris RB, Martin RJ: Changes in lipogenesis and lipolysis associated with recovery from reversible obesity in mature female rats. *Proc Soc Exp Biol Med* 1989, 191:82-89.
14. Viljanen AP, Karmi A, Borra R, Parkka JP, Lepomaki V, Parkkola R, Lautamaki R, Jarvisalo M, Taittonen M, Ronnema T, Iozzo P, Knuuti J, Nuutila P, Raitakari OT: Effect of caloric restriction on myocardial fatty acid uptake, left ventricular mass, and cardiac work in obese adults. *Am J Cardiol* 2009, 103:1721-1726.
15. Hammer S, Snel M, Lamb HJ, Jazet IM, van der Meer RW, Pijl H, Meinders EA, Romijn JA, de Roos A, Smit JW: Prolonged caloric restriction in obese patients with type 2 diabetes mellitus decreases myocardial triglyceride content and improves myocardial function. *J Am Coll Cardiol* 2008, 52:1006-1012.
16. Mitchell JR, Verweij M, Brand K, van d, V, Goemaere N, van den Engel S, Chu T, Forrer F, Muller C, de JM, van IW, IJzermans JN, Hoeijmakers JH, de Bruin RW: Short-term dietary restriction and fasting precondition against ischemia reperfusion injury in mice. *Aging Cell* 2010, 9:40-53.
17. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG: Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. *PLoS Biol* 2010, 8:e1000412.
18. Ouwens DM, Diamant M, Fodor M, Habets DD, Pelsers MM, El Hasnaoui M, Dang ZC, van den Brom CE, Vlasblom R, Rietdijk A, Boer C, Coort SL, Glatz JF, Luiken JJ: Cardiac contractile dysfunction in insulin-resistant rats fed a high-fat diet is associated with elevated CD36-mediated fatty acid uptake and esterification. *Diabetologia* 2007, 50:1938-1948.

19. 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.
20. van den Brom CE, Bosmans JW, Vlasblom R, Handoko ML, Huisman MC, Lubberink M, Molthoff CF, Lammertsma AA, Ouwens DM, Diamant M, Boer C: Diabetic cardiomyopathy in Zucker diabetic fatty rats: the forgotten right ventricle. *Cardiovasc Diabetol* 2010, 9:25.
21. 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.
22. Zaremba R, Merkus D, Hamdani N, Lamers JML, Paulus WJ, Remedios C, Duncker DJ, Stienen GJM, Van der Velden J: Quantitative analysis of myofilament protein phosphorylation in small cardiac biopsies. *proteomics Clin Appl* 2007, 1:1285-1290.
23. Llado I, Proenza AM, Serra F, Palou A, Pons A: Dietary-induced permanent changes in brown and white adipose tissue composition in rats. *Int J Obes* 1991, 15:415-419.
24. Rolls BJ, Rowe EA, Turner RC: Persistent obesity in rats following a period of consumption of a mixed, high energy diet. *J Physiol* 1980, 298:415-427.
25. Gentry-Smetana S, Redford D, Moore D, Larson DF: Direct effects of volatile anesthetics on cardiac function. *Perfusion* 2008, 23:43-47.
26. Plante E, Lachance D, Roussel E, Drolet MC, Arsenault M, Couet J: Impact of anesthesia on echocardiographic evaluation of systolic and diastolic function in rats. *J Am Soc Echocardiogr* 2006, 19:1520-1525.
27. Stein AB, Tiwari S, Thomas P, Hunt G, Levent C, Stoddard MF, Tang XL, Bolli R, Dawn B: Effects of anesthesia on echocardiographic assessment of left ventricular structure and function in rats. *Basic Res Cardiol* 2007, 102:28-41.
28. van den Brom CE, Bulte CSE, Loer SA, Bouwman RA, Boer C: Diabetes, perioperative ischaemia and volatile anaesthetics: Consequences of derangements in myocardial substrate metabolism. *Cardiovasc Diabetol* 2013, In press.
29. Lattermann R, Schrickler T, Wachter U, Georgieff M, Goertz A: Understanding the mechanisms by which isoflurane modifies the hyperglycemic response to surgery. *Anesth Analg* 2001, 93:121-127.
30. Zuurbier CJ, Keijzers PJ, Koeman A, Van Wezel HB, Hollmann MW: Anesthesia's effects on plasma glucose and insulin and cardiac hexokinase at similar hemodynamics and without major surgical stress in fed rats. *Anesth Analg* 2008, 106:135-42.
31. Saha JK, Xia J, Grondin JM, Engle SK, Jakubowski JA: Acute hyperglycemia induced by ketamine/xylazine anesthesia in rats: mechanisms and implications for preclinical models. *Exp Biol Med (Maywood )* 2005, 230:777-784.
32. Yamada J, Sugimoto Y, Noma T: Involvement of adrenaline in diazepam-induced hyperglycemia in mice. *Life Sci* 2000, 66:1213-1221.
33. Yao Y, Li L, Li L, Gao C, Shi C: Sevoflurane postconditioning protects chronically-infarcted rat hearts against ischemia-reperfusion injury by activation of pro-survival kinases and inhibition of mitochondrial permeability transition pore opening upon reperfusion. *Biol Pharm Bull* 2009, 32:1854-1861.
34. Lucchinetti E, Wang L, Ko KW, Troxler H, Hersberger M, Zhang L, Omar MA, Lopaschuk GD, Clanachan AS, Zaugg M: Enhanced glucose uptake via GLUT4 fuels recovery from calcium overload after ischaemia-reperfusion injury in sevoflurane- but not propofol-treated hearts. *Br J Anaesth* 2011, 106:792-800.

35. Hannon JD, Cody MJ: Effects of volatile anesthetics on sarcolemmal calcium transport and sarcoplasmic reticulum calcium content in isolated myocytes. *Anesthesiology* 2002, 96:1457-1464.
36. Davies LA, Gibson CN, Boyett MR, Hopkins PM, Harrison SM: Effects of isoflurane, sevoflurane, and halothane on myofilament Ca<sup>2+</sup> sensitivity and sarcoplasmic reticulum Ca<sup>2+</sup> release in rat ventricular myocytes. *Anesthesiology* 2000, 93:1034-1044.
37. An J, Rhodes SS, Jiang MT, Bosnjak ZJ, Tian M, Stowe DF: Anesthetic preconditioning enhances Ca<sup>2+</sup> handling and mechanical and metabolic function elicited by Na<sup>+</sup>-Ca<sup>2+</sup> exchange inhibition in isolated hearts. *Anesthesiology* 2006, 105:541-549.