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26-08-2011	Nieuw

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Hoofdproject	CARIM	NUTRIM	Hersenen en gedrag	GROW	biomaterialen	Ander UM	Geen UM TUE/[1]
Deelproject	1. 2. 3.	1. 2. 3. 4.	1. 2. 3.	1. 2. 3.			

Financieel beheerder		Budgetnummer	503220

Titel van het onderzoek:

The role of metabolic inflexibility in the development of diabetic cardiomyopathy in mice

startdatum October 2011 einddatum [9] October 2015 *Duur van de proef[10]: 6 weken*

	Naam	Tel (+ Tel privé enkel VO, VVO en VM)	E-mailadres	Bevoegd-heid[5]	Cap. groep /afdeling
1.Verantwoordelijk onderzoeker (VO)				Art.9	
2. Vervanger VO (VVO)				Art.9	
3. Verantwoordelijk medewerker (VM) GGO[7]				Art.9	
4. overige uitvoerenden				Art.12	
5. Principal Investigator (PI)				Art.9	

Diergroep	1	2					
ctrl/exp/sham	ctrl	exp					
Diersoort	muis	muis					
Stam	C57BL/6	C57BL/6					
Construct / mutatie ?	nee	Cpt1b^{E3A} knock-in					
Herkomst (leverancier) *	01	01					
Aantal	11	12					
Geslacht	man	man					
Dieren immuuncompetent ?	ja	ja					
Leeftijd/gewicht	12 weken	12 weken					
Doel van de proef *	33	33					
Belang van de proef *	01	01					
Toxicologisch onderzoek *	01	01					
Bijzondere technieken *	01	01					
Anesthesie *	04	04					
Pijnbestrijding *	01	01					
Mate ongerief *	04	04					
Toestand dier einde exp*	01	01					

* VHI-coderingen zie bijlage

1 Verantwoording

Aanvraag dierproef DEC-UM (kaders zijn licht flexibel, maar het geheel is max. 5 pag. versie 2006)

Titel: The role of metabolic inflexibility in the development of diabetic cardiomyopathy in mice

1. Doel van de proef.

Diabetic cardiomyopathy is a reduced performance of the diabetic heart which occurs independently of any underlying cardiovascular diseases or hypertension. One of the possible causes of diabetic cardiomyopathy is the increased preference of fatty acid oxidation as the main ATP supplier for cardiac work. The increased fatty acid oxidation is also paralleled with the inability to switch to glucose oxidation, a loss of function known as "metabolic inflexibility". Compared to glucose oxidation, fatty acid oxidation produces less ATP per oxygen molecule consumed, so that sustained preference in fatty acid oxidation might be detrimental to the energy status of the heart. As sufficient ATP supply is very crucial for cardiac work, reduced cardiac energy status might eventually lead to cardiac dysfunction, contributing to the development of diabetic cardiomyopathy.

In addition to metabolic inflexibility, excessive supply of fatty acids to the diabetic heart which exceeds the rate of oxidation might induce potentially toxic myocardial lipid accumulation. In the previous study (DEC 2009-124), we have shown that myocardial lipid accumulation is indeed associated with the development of diastolic dysfunction in diabetic db/db mice. However, the contribution of metabolic inflexibility, rather than myocardial lipid accumulation alone, to the development of cardiac dysfunction remains to be elucidated.

In this study, we aim to investigate the role and the contribution of metabolic inflexibility in the development of diabetic cardiomyopathy. The study will be performed in a mouse model of metabolic inflexibility: the mouse. The mouse exhibits increased fatty acid oxidation, however, in contrast to diabetic db/db mice, is not expected to display increased myocardial lipid levels. Using ^{31}P magnetic resonance spectroscopy (MRS), ^{1}H MRS, and magnetic resonance imaging (MRI), we will measure cardiac energy status, myocardial lipid content, and cardiac function, respectively.

2. Maatschappelijke relevantie en/of wetenschappelijk belang

The prevalence of diabetes is expected to double from 171 million in the year 2000 to 366 million in the year 2030 [1]. Diabetic cardiomyopathy is reported to occur in 60% of the diabetic patients, which contributes to 30% of the heart disease population [2]. People with diabetes are shown to have an increased risk for congestive heart failure and myocardial ischemical (MI). The risk of getting an MI for diabetic patients is comparable to that of people without diabetes but with prior MI [3].

Almost all of experimental evidence for the metabolic inflexibility of the diabetic heart originates from *ex vivo* data. However, these setups cannot mimic the *in vivo* condition of metabolic inflexibility. Furthermore, very few data on cardiac energy status is available because of the instability of ATP, which makes it almost impossible to measure ATP using traditional biochemical technique. Using our implemented ^{31}P MRS technique, we are able to measure cardiac energy status *in vivo* in a mouse heart. Taken together, the data obtained from this study will contribute to a more complete understanding of the mechanism underlying diabetic cardiomyopathy.

3. Alternatieven

Considering the aim of the study and the type of the experiments, it is not possible to conduct the study in any ways other than using experimental animals. Moreover, in studying diabetic cardiomyopathy, it is important that the patients do not suffer from any cardiovascular complications such as atherosclerosis. While this would be problematic in human, rodents, on the other hand, are resistant to atherosclerosis [4].

4. Ethische afweging

This study will contribute to the elucidation of some important aspects of diabetic cardiomyopathy, one of the main complications in patients suffering from type 2 diabetes. Understanding of the mechanisms underlying such a widely spread disease as type 2 diabetes is highly important, since it ultimately contributes to a better treatment of the disease and the development of new more effective drugs.

5. **Wetenschappelijke onderbouwing**

Type 2 diabetes mellitus is a metabolic disorder characterized by disturbances in glucose homeostasis due to impaired insulin signaling in peripheral tissues such as skeletal and cardiac muscle, adipose tissue, and liver, and impaired insulin production in pancreatic β-cells. Impaired cardiac insulin signaling and related cardiac metabolic changes, due to altered myocardial substrate supply and utilization, can lead to myocardial abnormalities known as diabetic cardiomyopathy [5]. Diabetic cardiomyopathy is characterized by ventricular dilation, cardiomyocyte hypertrophy, interstitial fibrosis, and decreased or preserved systolic function in the presence of diastolic dysfunction.

In the diabetic heart, the availability of free fatty acids and triglycerides is increased. The excessive availability of fatty acids might lead to at least two consequences, which could eventually contribute to the development of diabetic cardiomyopathy: (1) increased dependence on fatty acid oxidation, which is paralleled with "metabolic inflexibility" (i.e. reduced ability to switch between different substrates, e.g. from fatty acid oxidation to glucose oxidation) [6], and (2) myocardial lipid accumulation, when fatty acid supply exceeds the rate of fatty acid oxidation. Myocardial lipid accumulation could be toxic, as it could cause endoplasmic reticulum stress, mitochondrial dysfunction, fibrosis, and apotosis [7].

In the healthy heart, fatty acid oxidation provides 60-70% of cardiac ATP requirement, while 30-40% is supplied by glucose oxidation. In the diabetic heart, the ATP provided by fatty acid oxidation is increased to 90-100% [8]. Compared to glucose metabolism, fatty acid metabolism is oxygen-inefficient, as it needs more oxygen to produce the same amount of ATP. In the long run, this could be detrimental to the cardiac energy status and could compromise cardiac function. Moreover, metabolic inflexibility is causing the heart to be non-adaptive to changes in substrate availability, which makes it prone to, for instance, ischemia/reperfusion injury [8].

The switch between fatty acid oxidation and glucose oxidation in the healthy heart is well-regulated by insulin and glucose through the fatty acid-glucose Randle cycle [9]. High levels of insulin and glucose promote the formation of malonyl-CoA, which is the inhibitor of carnitine palmitoyl transferase-1 (Cpt1) activity. The Cpt1 enzyme plays a role in transporting fatty acid into mitochondria, one of the first important steps in fatty acid oxidation. Therefore, the inhibition of Cpt1 lowers fatty acid oxidation. We hypothesize

In this study, we will use mice as a mouse model of metabolic inflexibility. Cpt1b is selectively expressed in cardiac and skeletal muscle, making the effect of the mutant allele relatively tissue-specific. Using ^{31}P MRS, we will measure the PCr-to-ATP ratio, which is the measure of the energy status. We expect that cardiac energy status might be reduced in these mice. Furthermore, we will also perform cardiac cinematic MRI to assess whether reduced energy status is accompanied with cardiac systolic and/or diastolic dysfunction. Previously in diabetic db/db mice, we observed diastolic dysfunction, which was accompanied by myocardial lipid accumulation. In contrast, we do not expect an increase in myocardial lipid content in the mice, which we will confirm using ^{1}H MRS, because whole-body energy balance is not affected in these mice. This characteristic would enable us to further investigate the relative role of metabolic inflexibility to the development of diabetic cardiomyopathy.

6. Wetenschappelijke beoordeling

The research is within the framework of the

The project is evaluated based on its scientific and social merits by an independent committee. This specific project proposed in this proposal has been evaluated and approved by the principal investigator, the PhD promotor of VO and VVO, and the professor of
TU Eindhoven.

7. Proefdier keuze
7a. Soort, stam / herkomst / eindbestemming

Two experimental groups will be used in this study:
Group 1: wild type C67BL/6N as control group
Group 2 mouse group.

All mice will be obtained from
/

The MR measurements will be performed longitudinally at the age of 12-13 and 18-19 weeks. After the final measurement, the animals will be sacrificed, and the tissues will be collected for further analyses.

7b. Sexe

In this study, we will use only male mice because of the sex-related differences in the development of obesity and insulin resistance in rodent models as well as humans [14]. Furthermore, other studies in the , are also performed on male mice, so that the data obtained from this study can complement these other studies.

7c. Aantallen

The sample size of each group required for determination of cardiac function parameters *in vivo* was calculated based on the data from our previous study. The coefficient of variation (σ) was found to be 12%. To be able to detect 15% changes (δ) with a power of 80% and a confidence interval of 95%, the minimum number of animal needed for each group based on Sach's formula is:

$n = [1.96+0.84]^2 * 2 * (12/15)^2 = 10.04$ mice.

We do not expect any loss for the control group. For the knock-in mice, we assume 10% loss.
Therefore, the number of mice for:

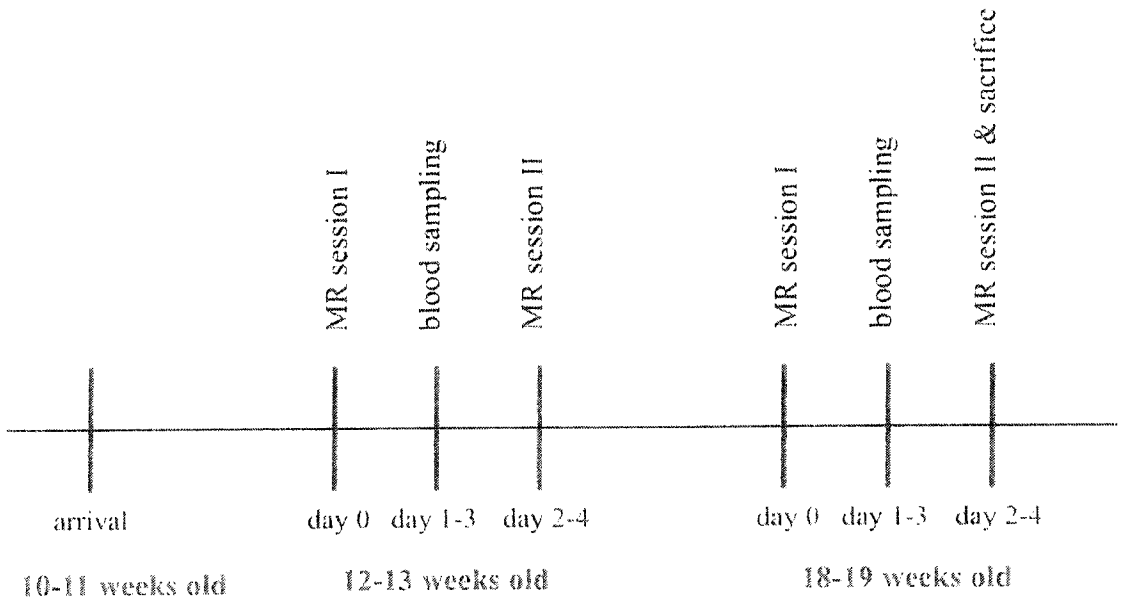
Group 1: n = 10.04 mice = 11 mice.
Group 2: (N-0.1*N) = 10.04 mice; N = 11.15 = 12 mice.
Total mice = 11 + 12 = 23 mice

8. Experiment

The aim of the experiment is to investigate *in vivo* cardiac energy status, myocardial lipid metabolism, and cardiac function using ^{31}P MRS, ^{1}H MRS, and MRI. The MR measurements will be performed longitudinally at the age of 12-13 weeks and 18-19 weeks. The first MR measurements will be performed at least after one week of the arrival of the mice to our animal facility to allow acclimatization.

The MR measurements at each time point will be performed in two MR sessions on different days because of different setup for MRI/^{1}H MRS and ^{31}P MRS. The length of the measurements will become too long (up to 5 hours) if these two measurements are combined in one session. Blood sampling after 4-hour fasting will also be performed in the same week as the MR measurements to assess the level of plasma glucose, acylcarnitine, and insulin. After the MR measurements at 18-19 weeks, the animals will be euthanized through bleeding from *vena cava caudalis* (SOP 2). This method is chosen because we need to collect as much fresh blood as possible for blood measurement using ^{31}P MRS. Blood measurement is needed to correct the acquired cardiac ^{31}P spectra, which originate not only from the heart but also from blood inside the heart.

The measurement scheme is given in the figure below:



9. Experimentele condities

9a. Anesthesie

The MR measurements are performed under general anesthesia through isoflurane inhalation. For the induction of anesthesia, the mice will be placed in a chamber with a mixture of medical air and isoflurane (3-4% vol). During the measurements, the anesthesia will be maintained using the same anesthetic agent (1-2% vol) using a breathing mask.

9b. Pijnbestrijding

No painkillers will be used.

9c. Euthanasie en Humane eindpunten

Euthanasia method: at the end of experiment, the mice will be sacrificed under general anesthesia by bleeding from *vena cava caudalis*.

Humane endpoint: if there are behavioral changes, weight loss more than 15% or not eating/drinking, a person with WOD art.12 and/or a person with WOD art. 14 will be consulted to decide whether euthanasia is necessary.

10a. Ongerief

Some of the handling steps may cause discomfort to the animals, which we score as follows:

- Handling during weighing of the animal
  - Frequency : weekly
  - Duration : <5 minutes per animal
  - Discomfort : score 01
- 4-hour fasting and handling during blood sampling
  - Frequency : 1x at 12-13 weeks old, and 1x at 18-19 weeks old
  - Duration : <5 minutes per animal
  - Discomfort : score 02
- MR measurements performed under anesthesia
  - Frequency : 2x at 12-13 weeks old, and 2x at 18-19 weeks old
  - Duration : 3 hours per session
  - Discomfort : score 04
- Recovery from anaesthesia after MR measurements
  - Frequency : 2x at 12-13 weeks old, and 1x at 18-19 weeks old
  - Duration : <30 minutes per animal
  - Discomfort : score 04
- Euthanasia under anesthesia after MR measurements
  - Frequency : 1x after the final MR measurements
  - Duration : <5 minutes per animal
  - Discomfort : score 02

Maximal experienced discomfort per experimental group:

- Group 1 – score 04
- Group 2 – score 04

10b. Welzijnsevaluatie:

The animals will be weighed weekly and this data will be used to assess the well-being of the animal. In case of weight loss more than 15%, or not eating/drinking, or any changes in behavior or in physical appearance, a person with WOD art.12 and/or a person with WOD art. 14 will be consulted to decide whether euthanasia is necessary.

11. Verzorging en huisvesting

Mice will be housed in the laboratory animal facility at TU Eindhoven. All experiments described in this DEC protocol will be carried out in the laboratories of Animals will be housed in group in a cage equipped with standard cage enrichment attributes. Animals will receive water and food *ad libitum*. In case of calamities, a person with WOD art. 12 or 14 will be notified.

12. Deskundigheid

The experiments will be carried out by a responsible investigator who has WOD art. 9. If necessary, assistance will be provided by biotechnicians available at the department, who both have WOD art. 12. The investigator has experience in all methods employed in the experiments proposed in this DEC protocol.

13. **Standard Operation Procedures (SOP) (see enclosures)**

1. SOP 1: In vivo MR scans.
2. SOP 2: Euthanasia through vena cava caudalis.

Relevante literatuur

1. Wild et al. (2004) Diabetes Care. 27:1047.
2. Redfield et al. (2003) JAMA. 289:194.
3. Solang. (1999) Eur Heart J. 20:789.
4. Severson. (2004) Can J Physiol Pharmacol. 82, 813-823.
5. Taegtmeyer et al. (2002) Circulation 105:1727.
6. Taegtmeyer et. al. (2004) Ann NY Acad Sci 1015:202.
7. Unger and Orci. (2002) Biochim Biophys Acta, 1585:202.
8. Lopaschuk. (2002) Heart Fail Rev. 7, 149.
9. Randle. (1998) Diabetes Metab Rev. 14, 263.

Enclosures: Standard Operation Procedures (SOP)

SOP1: *In vivo* MR scans

Anesthesia

- Anesthesia is induced using inhaled anesthesia (Isoflurane mixed with medical air 0.4 L/min) in a plexiglass chamber. Induction: 3-4% vol, maintenance: 1-2%.
- The depth of anesthesia, for the induction, is controlled by testing the reflex of eyelid and toe. During maintenance, the depth of anesthesia is monitored by breathing rate (through a small balloon placed on the abdomen) and heart rate (through ECG electrodes attached to the front feet).

Preparation and positioning:

- The mouse is placed in a specially designed coil with integrated anesthesia delivery. Warming bed and eye salve are also prepared.
- The mouse is fixed lightly on the warming bed. A small balloon is placed on the abdomen to monitor the breathing signal, in order to assess the anesthetic effect. The body temperature (through a rectal temperature probe) and the temperature of the warming bed are also monitored.
- The front feet are put on the gel using tweezers.
- The front feet are then attached to ECG electrodes using a small piece of tape. The ECG is used to monitor the heart rate, to assess the anesthetic effect and to synchronize the MR measurements with the heart rate.
- The coil with the mouse is put inside the scanner.

Scanning:

- The MR scanner is set up. Then, the measurement is run for a maximum of 3 hours. The MR scans are carried out in the
  of the Eindhoven University of Technology

End of experiment:

- The mouse is sacrificed through bleeding via *vena cava caudalis* under general anesthesia (SOP2).

SOP2: Euthanasia through bleeding via *vena cava caudalis*

- The mouse is brought under anesthesia using isoflurane.
- The abdomen and thorax are shaved thoroughly.
- The skin is incised from lower abdomen to the throat.
- The abdomen is opened so that the diaphragm is visible.
- A small hole in the diaphragm is created with pointed scissors below the *xiphoid*.
- The diaphragm is then cut free from the ribcage on both sides.
- A needle is inserted to the *vena cava caudalis* above the liver, and the blood is emptied.

Maastricht University

Faculty of Health, Medicine and Life Sciences

Aan:

Ons kenmerk	*Doorkiesnummer*	*Maastricht*
		30-08-2011

Project: *The role of metabolic inflexibility in the development of diabetic cardiomyopathy in mice.*

Verantwoordelijk onderzoeker (VO):

Namens de Vergunninghouder van de DEC-UM, delen wij u mede dat voornoemd project aan de ethische toetsingscriteria voor proefdiergebruik voldoet.
De DEC maakt geen bezwaar tegen uitvoering van dit project zoals aangevraagd en geeft een positief advies.

Projectnummer:	2011-117
Diersoort:	muis
Aantal dieren:	23
Einddatum:	26-08-2015

Uw project staat bij de DEC en CPV geregistreerd onder bovenstaand nummer. Gelieve dieren, die voor dit project bestemd zijn, ook onder dit nummer aan te vragen.

Voorzitter DEC-UM

Vicevoorzitter DEC-UM

DEC-UM
Voorzitter DEC-UM

p/a secretariaat DEC-UM

Secretariaat DEC-UM

Bezoekadres

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