Begeleidingsformulier aanvraag dierproef DEC- UM

Versie 2006

Herziene versie

DECNR: 2011-089
Ontvangen: 30-06-2011

DEC datum goedkeuring#	Type aanvraag [2]
13-07-2011	Nieuw

VROM/GGONR[3]

LNV/CBDNR[4]

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

Financieel beheerder		Budgetnummer	503220

Titel van het onderzoek:

The effects of long-term high fat feeding on cardiac function and metabolism in C57BL/6J mice

startdatum July 2011 einddatum [9] July 2014 *Duur van de proef [10]: 20 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	
4. overige uitvoerenden				Art.12	
5. Principal investigator				Art.9	

Diergroep	1	2					
ctrl/exp/sham	ctrl	exp					
Diersoort	muis	muis					
Stam	C57BL/6J	C57BL/6J					
Construct / mutatie ?	nee	nee					
Herkomst (leverancier) *	02	02					
Aantal	12	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 effects of long-term high fat feeding on cardiac function and metabolism in C57BL/6J mice

1. Doel van de proef.

It has been shown that the accumulation of myocardial lipid in diabetes and obesity is associated with the development of diabetic cardiomyopathy [1]. Our previous study in diabetic db/db mice (DEC 2009-124) indeed showed that the high content of myocardial lipid is accompanied by a reduction in cardiac diastolic function. The cardiac dysfunction could be attributed to the toxic effect of myocardial lipid accumulation and the alteration in the cardiac metabolism towards almost exclusive fatty acid oxidation in the diabetic heart [2,3]. In addition, we hypothesized that the alteration in the expression of could also contribute to the development of cardiac dysfunction, because it was shown in an earlier study that high fat feeding affected

In the most recent study (DEC 2010-165), we showed that . However, cardiac function was unaltered, although an increase dependency towards myocardial fatty acid oxidation was observed. This preserved cardiac function, despite the elevation in myocardial lipid, might be due to the short-term high fat diet feeding, and hence short-term exposure to myocardial lipid. In the previous study in db/db mice (DEC 2009-124), we showed that both the concentration of and the exposure time to the elevated myocardial lipid level determine the development of cardiac dysfunction.

Therefore, the aim of this study is to assess the effects of long-term high fat feeding on cardiac function and metabolism. Longitudinal *in vivo* measurements with magnetic resonance imaging (MRI) will be performed after short-term and long-term high fat feeding to assess cardiac function. *In vivo* proton (^{1}H) and phosphorus (^{31}P) magnetic resonance spectroscopy (MRS) will also be performed to assess myocardial lipid content and myocardial energy metabolism, respectively. Intrinsic myocardial mitochondrial function will be measured *in vitro* using high resolution respiratometry. Moreover, to establish the relationship between the *in vivo* cardiac function and metabolism and the composition of contractile apparatus at the molecular levels, the expression of proteins involved in the cardiac muscle contraction (i.e.) will also be determined at mRNA and protein level using quantitative PCR and immunoblotting techniques, 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 [4]. Diabetic cardiomyopathy is reported to occur in 60% of the diabetic patients, which contributes to 30% of the heart disease population [5]. 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 [6].

The relationship between the accumulation of the intramyocardial lipids, impairment of insulin signaling cascade, and development of diabetic cardiomyopathy is extensively studied, showing disturbances in substrate utilization, altered signaling, and stimulation of apoptosis. However, the

studies addressing the relationship between the development of diabetic cardiomyopathy and the expression of cardiac contractile proteins, the direct mediators of cardiac muscle contraction, are scarce. In this study, we will investigate the relationship between and and in a model of high-fat diet induced obesity and insulin resistance. The obtained data will contribute to a more complete understanding of molecular mechanisms underlying diabetic cardiomyopathy and possibly identify new targets for drug development.

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. Performing the experiments in human volunteers will be unethical since it involves long-term high fat consumption, which may have negative effects on the health of the participants. Moreover, the fact that considerable amounts of cardiac tissue are needed for the determination of protein expression excludes the use of human volunteers as the study subjects.

4. Ethische afweging

This study will contribute to elucidation of some important aspects of diabetic cardiomyopathy, one of the main complications in patients suffering from type 2 diabetes. Understanding of the molecular 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. Moreover, the experimental data will be used by partner groups within the Netherlands Consortium of Systems Biology (NCSB) to build a computational model of fat and carbohydrate metabolism, which in the long-term will lead to reduction of animal use.

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 [3]. 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 (FFA) and triglycerides (TG) is increased, causing the heart to rely more on fatty acid oxidation to produce 90-100% of its ATP requirements [3]. When there is an imbalance between FFA/TG supply and maximum rate of utilization, the excessive FFA/TG is stored in the myocardium as TG droplets [7]. This mechanism initially protects the heart from the formation of biologically active fatty acid metabolites, such as ceramides, diacylglycerols, and long chain acyl-CoA esters. However, a longer exposure time to the cardiac lipid overload is potentially toxic, causing endoplasmic reticulum (ER) stress and mitochondrial dysfunction, fibrosis, and apoptosis, which eventually might lead to the development of diabetic cardiomyopathy [2,8]. Besides the exposure time, it has been shown *in vitro* that the saturation degree of the fatty acid is also an important determinant of fatty acid-induced cytotoxicity, with saturated fatty acids being more toxic than unsaturated fatty acids [9]. In the previous study, we observed that a 5-week period of high fat feeding in mice induced more myocardial lipid accumulation in the group fed high fat diet (HFD) containing ,
.) than in the group fed HFD containing (
). Similar trends were also observed on myocardial substrate utilization, which points towards a higher increase in fatty acid oxidation in the animal group fed HFD containing compared to the group fed HFD containing . However, the alteration in myocardial metabolism was not accompanied with cardiac dysfunction, which supports the notion that the exposure time to the elevated myocardial lipid is one of the important factors contributing to the development of lipotoxicity-induced diabetic cardiomyopathy.

In this proposed study, we would like to test the hypothesis that longer term high fat feeding (with HFD containing palm oil only, as it gives the more adverse effect) would further increase the concentration of the myocardial lipid level and its exposure time, which would lead to the development of diabetic cardiomyopathy. We also hypothesize that this long-term high fat feeding would negatively affect the expression profiles of contributing to the development of cardiac dysfunction. In an earlier study in skeletal muscle, we observed a stronger effect of high fat feeding on in than in skeletal muscle fibers (Ciapaite et.al, unpublished), based on which we speculate that the effect of high fat feeding on would also be strong in the exclusively oxidative type of muscles, such as cardiac muscle, although it was not observed during the short-term high fat feeding study. The study will be performed longitudinally after short-term feeding (5 weeks) and long-term feeding (20-weeks) using MRI, ^{1}H MRS, and ^{31}P MRS to assess cardiac function, myocardial lipid accumulation, and myocardial energy metabolism, respectively. After the final MR measurements at 20-week feeding, intrinsic myocardial mitochondrial function will be assessed *in vitro* using high resolution respiratometry. Quantitative PCR and immunoblotting techniques will also be used to determine the expression of and proteins at mRNA and protein level.

Taken together, these experiments will allow us assessing the relationship between *in vivo* cardiac function and metabolism, *in vitro* myocardial mitochondrial function, and the expression of in a mouse model of high-fat diet induced obesity and insulin resistance.

6. Wetenschappelijke beoordeling

The research is within the framework of the -project "In vivo magnetic resonance spectroscopy of lipid dynamics in insulin resistant muscle (number:)". 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 .

5 Proefdier

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

Species en stam:
Wild type C57BL/6J mice will be used. The wild type strain is commonly used in the research of diet-induced obesity. C57BL/6J mice fed a high-fat diet develop obesity, mild to moderate hyperglycemia, and hyperinsulinemia.

Herkomst: C57BL/6J mice will be purchased from Charles River.

Uiteindelijke bestemming: At the start of the study mice will be 12 weeks old. They will be put on a diet for 20 weeks, after which they will be sacrificed and the tissues will be collected for further analyses.

7b. Sexe

In this study we will use male mice. The sex-related differences in the development of obesity and insulin resistance in rodent models as well as humans were reported previously [14]. Male mice are most commonly used in the studies of obesity and insulin resistance. Therefore, the use of male mice will enable the comparison of newly generated data with the bulk of data reported in the literature.

7c. Aantallen

Two experimental groups will be used in this study: one low-fat diet-fed (LFD) control group and one high-fat diet-fed (HFD) group.

The sample size of a diet group 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.

In our previous study there was no loss of animals maintained on the similar diets for 5 weeks. We expect 10% loss for this study, as the diet is given for 20 weeks and the animals will be measured twice instead of once.

Therefore, the number of mice for each group: $(N-0.1*N) = 10.04$ mice; $N = 11.15 = 12$ mice
Total mice = 12 + 12 = 24 mice.

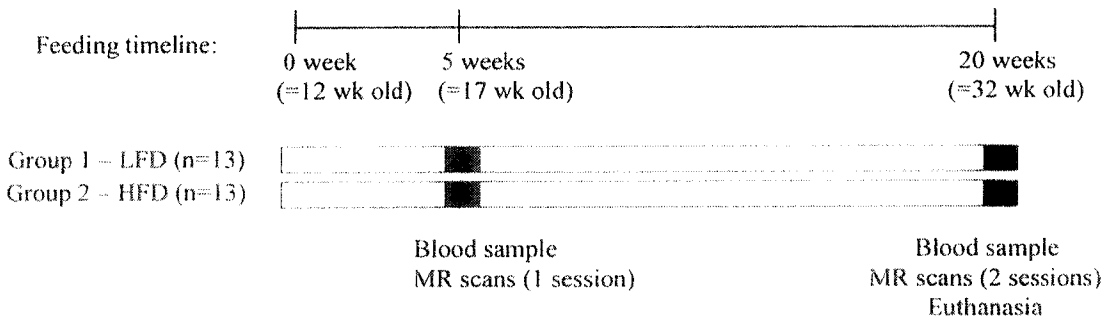
6 Dierproef

8. Experiment

The aim of the experiment is to investigate *in vivo* cardiac function and metabolism using MRI, ^{1}H MRS, and ^{31}P MRS. The experiments will be performed at least after one week of the arrival of the mice to our animal facility, to allow acclimatization. After acclimatization, at the age of 12 weeks, mice will be randomly divided into 2 groups:
Group 1 (n=11) will receive low-fat diet (10 kcal% from fat; fat from palm oil)*.
Group 2 (n=11) will receive high-fat diet (45 kcal% from fat; fat from palm oil)*.
**The diets will be purchased from Research Diet Services B.V. (Hoge Maat 10, 3961 NC Wijk bij Duurstede).*

The animals will be fed the diets for 20 weeks *ad libitum*. The animal weight and food intake will be measured once a week to assess the effect of the diet on the body weight. The MR measurements (SOP 1) will be performed longitudinally after 5 weeks (short-term feeding) and 20 weeks (long-term feeding) of diet. The measurements at 5 week-feeding will be performed in one session per mouse, only for ^{31}P MRS measurements, because the MRI and ^{1}H MRS data have been acquired during the study in DEC 2010-165. The measurements at 20 week-feeding 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 after 5 and 20 weeks of high-fat feeding to assess the levels of plasma glucose, free fatty acid, and triglycerides. After the MR measurements at 20 weeks, the animals will be euthanized through cervical dislocation (SOP 2). The heart and skeletal muscle (soleus and extensor digitorum longus) will be collected for HRR measurements and for further determination of the expression profile of at the mRNA and protein level.

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

At the end of experiment, the mice will be sacrificed under general anesthesia by cervical dislocation.

The animals are expected to get obese. From the previous study of 5-week high fat feeding (DEC 2010-165), the animals were 30% heavier than controls. The rate of weight gain was higher in the first few weeks but it was flattened out at 5 weeks. In case of deterioration in physical appearance due to high fat feeding (e.g. condition of the fur and skin), which leads to a change in the behavior of the animal indicating elevated discomfort, a person with WOD art.12 and/or a person with WOD art. 14 will be consulted to decide whether euthanasia is necessary.

In another case, 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.

Zorg

10a. Ongerief

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

- Feeding of high-fat diet (group 2)
  - Frequency : daily
  - Discomfort : score 01
- Handling during weighing of the animal (all animals)
  - Frequency : weekly
  - Duration : <5 minutes per animal
  - Discomfort : score 01
- 4-hour fasting and handling during blood sampling (all animals)
  - Frequency : 1x after 5 weeks of feeding, and 1x after 20 weeks of feeding
  - Duration : <5 minutes per animal
  - Discomfort : score 02
- MR measurements performed under anesthesia (all animals)
  - Frequency : 1x after 5 weeks of feeding, and 2x after 20 weeks of feeding
  - Duration : 3 hours per session
  - Discomfort : score 04
- Recovery from anaesthesia after MR measurements (all animals)
  - Frequency : 1x after 5 weeks of feeding, and 1x after 20 weeks of feeding
  - Duration : <30 minutes per animal
  - Discomfort : score 04
- Euthanasia under anesthesia after MR measurements (all animals)
  - Frequency : 1x after the final MR measurements
  - Duration : <5 minutes per animal
  - Discomfort : score 02

Gering	= 01
Gering/matig	= 02
Matig	= 03
matig/ernstig	= 04
Ernstig	= 05
Zeer ernstig	= 06

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. The physical appearance of the mice will be monitored on the daily basis in order to prevent the development of skin problems due to high-fat diet feeding. From our earlier experience, five weeks of high-fat diet (similar composition as in this proposal) feeding did not cause noticeable health impairment. The high-fat diet fed C57BL/6J mice were 30% heavier after 5 weeks of experiment compared to low-fat diet fed controls and none of the animals developed skin problems.

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 . 2 animals will be housed per 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 cervical dislocation.

Relevante literatuur

1. Rijzewijk et al. (2008) J Am Coll Cardiol, 52:1793.
2. Unger and Orci. (2002) Biochim Biophys Acta, 1585:202.
3. Taegtmeyer et al. (2002) Circulation 105:1727.
4. Wild et al. (2004) Diabetes Care. 27:1047.
5. Redfield et al. (2003) JAMA. 289:194.
6. Solang. (1999) Eur Heart J. 20:789.
7. Schaffer. (2003) Curr Opin Lipidol 14:281.
8. Brindley et al. (2010) Am J Physiol Endocrinol Metab 298:E897.
9. Dimopoulos et al. (2006) Biochem J. 399:473-81.

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% 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 ( ) at the of the Eindhoven University of Technology ( ).

End of experiment:

- The mouse is sacrificed by cervical dislocation under general anesthesia (SOP2).

SOP2: Euthanasia through cervical dislocation

- The mouse, lying on the abdomen, is maintained under general anesthesia through breathing mask using isoflurane (see SOP1).
- The thumb and index finger is placed on either side of the neck or at the base of the skull, or alternatively, a rod is pressed at the base of the skull.
- With the other hand, the base of the tail is quickly pulled, causing separation of the cervical vertebra from the skull.
- The separation is confirmed by palpation of the cervical region.

University Maastricht

Faculty of Health, Medicine and Life Sciences

Dierexperimenten Commissie

DEC

voorzitter
p/a Secretariaat DEC-UM
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Aan: TUE/

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5600 MB Eindhoven

Uw referentie:

Onze referentie:

Maastricht, 29-06-2011

Geachte Onderzoeker,

Uw projectaanvraag: "*The effects of long-term high fat feeding on cardiac function and metabolism in C57BL/6J mice*", is op de DEC vergadering van 24 juni 2011 besproken.

De DEC heeft een aantal vragen en opmerkingen:

- Bij punt 7c merkt de DEC op dat er bij de berekening van de uitval niet tussentijds afgerond mag worden. De DEC verzoekt de aantallen op het voorblad en bij punt 7c aan te passen.
- Bij punt 10a verzoekt de DEC ook het 2x bijkomen uit anesthesie (groep 2) te vermelden.

Gelieve eventuele vragen te beantwoorden in een brief en indien noodzakelijk Uw project aan te passen en duidelijk de aanpassingen grijs te markeren.
Uw project staat bij de DEC geregistreerd onder nummer 2011-089, gelieve dit nummer in verdere correspondentie te vermelden.

Hoogachtend,

Voorzitter DEC-UM

From:
Sent: donderdag 30 juni 2011 17:54
To: Dec Secretariaat (
Subject: revision: DEC 2011-089
Attachments: Voorblad DEC 2011-089 revised.doc; DEC 2011-089 revised.doc

Dear Sir/Madam,

Following the advice from the DEC committee, attached are the revisions of our research proposal (DEC 2011-089). The revision is marked with grey as requested, and the changes are:

- In point 7c: the number of animals is calculated correctly. The cover also now states the correct number of animals.
- In point 10a: the discomfort level for the recovery from anaesthesia is included.

Please let me know if this is received in a good order, and looking forward to hearing from you.

Kind regards

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Ons kenmerk	*Doorkiesnummer*	*Maastricht*
	043-	13-07-2011

Project: *The effects of long-term high fat feeding on cardiac function and metabolism in C57BL/6J mice.*

Verantwoordelijk onderzoeker (VO):

Hierbij 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:	2011-089
Diersoort:	muis
Aantal dieren:	24
Einddatum:	13-07-2015

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
t
p/a secretariaat DEC-UM

Secretariaat DEC-UM
t (043)

Bezoekadres

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