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Begeleidingsformulier aanvraag dierproef DEC- UM

Versie 2006

Herziene versie

DECNR: 2011-059

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DEC datum goedkeuring#	Type aanvraag [2]	VROM/GGONR[3]	LNV/CBDNR[4]
28-05-2011	Nieuw / ~~Herz.versie / Pilot~~	IG 02-154	

Hoofdproject	~~CARIM~~	NUTRIM	~~Hersenen en gedrag~~	GROW	~~biomaterialen~~	~~Ander UM~~	~~Geen UM~~
Deelproject		1.					

Financieel beheerder		Budgetnummer	31961830N

Titel van het onderzoek:

Enhancing SIRT3 in skeletal muscle prevents insulin resistance

startdatum May 1 2011 einddatum [9] April 30 2012 *Duur van de proef [10]:* *8 weeks*

	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. Verantwoorde-lijk medewerker (VM) GGO[7]				Art.9	
4. overige uitvoerenden				Art.9	
5. overige uitvoerenden				Art.9	
6. overige uitvoerenden				Art.9	

Diergroep	1: GroupA	2:GroupB	3:GroupC	.	.	.	.
ctrl/exp/sham	pilot	exp	exp				
Diersoort	Rat	Rat	Rat				
Stam	Wistar	Wistar	Wistar				
Construct / mutatie ?	nee	nee	nee				
Herkomst (leverancier) *	02	02	02				
Aantal	6	22	18				
Geslacht	M	M	M				
Dieren immuuncompetent ?	ja	ja	ja	ja/nee[8]	ja/nee[8]	ja/nee[8]	ja/nee[8]
Leeftijd/gewicht	10 week	10 week	10 week				
Doel van de proef *	37	37	37				
Belang van de proef *	01	01	01				
Toxicologisch onderzoek *	01	01	01				
Bijzondere technieken *	01	01	01				
Anesthesie *	04	04	04				
Pijnbestrijding *	04	04	01				
Mate ongerief *	03	04	04				
Toestand dier einde exp*	01	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: Enhancing SIRT3 in skeletal muscle prevents insulin resistance

1. Doel van de proef.

Fat accumulation, mitochondrial dysfunction and oxidative stress in skeletal muscle tissue are all implicated in the development of human insulin resistance and type 2 diabetes mellitus (T2DM). The mitochondrial protein Sirtuin 3 (SIRT3) has recently been demonstrated to have the potential to improve muscle fatty acid oxidation, to enhance mitochondrial biogenesis and to reduce oxidative stress. However, skeletal muscle SIRT3 has not yet been studied in relation to insulin resistance and T2DM. Therefore, the aim of this study is to evaluate the role of SIRT3 in T2DM and its influence on oxidative capacity and oxidative stress. This will be examined by overexpressing skeletal muscle SIRT3 in an insulin resistant rat model using in vivo unilateral gene electroporation and study the subsequent effects on insulin sensitivity, mitochondrial metabolism and oxidative stress. We hypothesize that enhancing muscle SIRT3 may counter several metabolic disturbances leading to insulin resistance, an early hallmark of T2DM. If successful, our experiments may identify SIRT3 as a novel insulin-sensitizing target for (pharmacological) intervention.

2. Maatschappelijke relevantie en/of wetenschappelijk belang

The focus of this project is on skeletal muscle, a metabolically active tissue crucial for insulin-mediated glucose disposal and lipid catabolism. In skeletal muscle, distinct associations between cellular accumulation of lipids and insulin resistance have been observed. More importantly, we as well as other research groups have previously shown that a reduced skeletal muscle mitochondrial capacity and oxidative stress are associated with insulin resistance and that exercise training can improve mitochondrial capacity in parallel with improvements in insulin sensitivity. The mitochondrial protein Sirtuin 3 (SIRT3) has very recently been demonstrated to improve muscle fatty acid oxidation, to enhance mitochondrial biogenesis and to reduce oxidative stress. Furthermore we believe that SIRT3 has the potential to counter all the skeletal muscle disturbances that may lead to insulin resistance.

Therefore, we aim to explore the role of skeletal muscle SIRT3 in insulin resistance/T2DM and how overexpression of SIRT3 may prevent insulin resistance. These experiments may provide a novel target for (pharmacological) intervention.

3. Alternatieven

The current project intends to use dietary interventions in a transient genetically modified rat model. Electro-genetic transfer of genetic material in humans is medically and ethically unacceptable. Furthermore, to assess mitochondrial function in the muscle, several aspects must be evaluated (oxidative capacity, oxidative stress, enzyme assays, etc.) in relationship to whole body insulin sensitivity therefore cell models are inappropriate for this study.

4. Ethische afweging

In the Netherlands, the number of newly diagnosed T2DM patients has dramatically increased over the past 10-15 years, and similar numbers have been reported throughout Europe and worldwide. This current predicament will have devastating effects on our society, mainly in the healthcare sector. Therefore research investigating the causes and consequences of T2DM is critical. To address these concerns we aim to evaluate the role of SIRT3 in insulin resistance.

In a rat model we propose to evaluate insulin resistance under high-fat feeding conditions to mimic the current situation in humans, where excess caloric intake and reduced physical activity are associated with obesity and T2DM. Furthermore, in vivo unilateral SIRT3 gene electroporation will examine the role of SIRT3 in insulin resistance and T2DM. The unilateral gene electroporation is a technique where the same animal serves as its own control, reducing the number of animals required in a study. The SIRT3 vector will be incorporated into the skeletal muscle rat genome in one leg while the contralateral leg is transfected with a control vector. Since this study may identify SIRT3 as a novel target for (pharmacological) intervention of T2DM, we feel it justifies the use of animal models.

5. Wetenschappelijke onderbouwing

Peripheral insulin resistance is an early hallmark in the pathogenesis of type 2 diabetes mellitus (T2DM) and since skeletal muscle is responsible for ~80% of postprandial glucose uptake (1), the muscle is an important target to improve insulin sensitivity. Increased intake of dietary fat in combination with a decreased utilization of fatty acids will contribute to muscle fat accumulation and insulin resistance (2). Further, T2DM patients are characterized by a reduced skeletal muscle mitochondrial function (3) and improving muscle oxidative capacity via exercise training has proven to be a highly successful strategy to improve insulin sensitivity in humans. In fact, a 12-week combined progressive training program in type 2 diabetic patients normalized muscle (mitochondrial) oxidative capacity up to control levels, which was accompanied by a profound increase in insulin sensitivity (4). Although several other adaptations in the skeletal muscle (e.g. an increased capillarisation) contribute to the improvement of muscle oxidative capacity and insulin sensitivity upon exercise training, we showed that an increase in mitochondrial density was the most important mechanism. Why mitochondrial capacity is low in the insulin resistant state is so far not known but the origin of insulin resistance has also been associated with oxidative stress and the excessive production of reactive oxygen species (ROS). During mitochondrial ATP synthesis, ROS formation is an inevitable event that is significantly enhanced by excessive substrate supply or functional impairment of one or more complexes of the respiratory chain (5). Excessive ROS may have detrimental effects on (membrane) lipids, proteins and DNA, thereby resulting in a decrease of cellular - but also mitochondrial - function. In muscle cells, direct exposure to ROS results in insulin resistance (6) while increased anti-oxidant capacity reduces the manifestation of insulin resistance (7). Furthermore, ROS production was identified as the common denominator and causal factor in several distinct cellular models of insulin resistance (8). Finally, it was shown that subjecting mice to a high-fat diet for a prolonged period of time leads to mitochondrial dysfunction, resulting from increased oxidative stress (9).

Sirtuin 3 (SIRT3) is one of the seven mammalian sirtuins, a protein family of deacetylases that require NAD+ for their enzymatic activity. This implies that sirtuins, through sensing NAD+/NADH ratios, translate the nutritional status of the cell into the appropriate metabolic response via modulation of gene and protein function by deacetylation. Interestingly, SIRT3 is one of the two sirtuins with deacetylase activity that is exclusively localized in mitochondria and that can be found in skeletal muscle (10). Importantly, recent findings in animal models and cell systems specifically link SIRT3 to insulin resistance and T2DM. Thus, SIRT3-deficient mice display a striking hyperacetylation of mitochondrial proteins (11) and exhibit a defective fatty acid oxidation among various tissues, including skeletal muscle (12). On the other hand, SIRT3 overexpression in C2C12 muscle cells stimulated mitochondrial biogenesis (13). Additionally, SIRT3 protein levels are increased upon fasting and exercise while a significant reduction in SIRT3 protein level was observed upon high-fat feeding in mice (14). Finally, a very recent publication reveals that SIRT3 may also have profound effects on ROS-detoxifying enzymes, including glutathione peroxidase-1 and superoxide dismutase 2 (13). In line with this concept, SIRT3 overexpression decreased the basal superoxide level in C2C12 muscle cells while knockdown of this protein caused an increase in cellular ROS production (13). To date, SIRT3 has not yet been examined from the perspective of insulin resistance. Taken together, SIRT3 meets all the criteria to explain the lower mitochondrial capacity and oxidative stress observed in T2DM. Therefore, we here aim to investigate the role of SIRT3 in insulin resistance, (fat) oxidative capacity and oxidative stress.

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6. Wetenschappelijke beoordeling

The proposed research plan has been reviewed and approved by

Furthermore this project is the main experiment in an innovative pilot study, funded by the which was granted after a peer-review process.

5 Proefdier

7. Proefdier keuze

7a. Soort, stam / herkomst / eindbestemming

Rat (Wistar, Charles River), dead at the end of the experiment

7b. Sexe

For the following set of experiments only male rats will be used since female rats tend to maintain insulin sensitivity even on a high fat diet. (Gómez Pérez Y *et al.*, Cell Physiol Biochem. 2008; 22(5-6):539-48).

7.c. Aantallen

The rats will be divided into 3 groups; Group A- pilot phase, Group B- insulin sensitivity measurements and Group C- mitochondrial function measurements. The rats will be placed on a control (low fat) or high fat diet to examine the effects of diet induced insulin resistance. The number of animals required for each group is outlined below.

Group A (pilot): We will have to test the efficiency of the SIRT3 overexpression in vivo to determine the optimal concentration of vector injected and expression level (time after electroporation). In order to obtain maximal SIRT3 overexpression, we will also test 2 electroporation protocols: 1) a single-electroporation and 2) a double-electroporation. For the double-electroporation, rats will undergo 2 electroporation procedures spaced 2 weeks apart. One week after the (last) electroporation, the rats will be sacrificed and tissue harvested determination of SIRT3 levels. Based on our experience from previous studies (DEC 2010-036 and DEC 2006-024), a total of 6 pilot rats should be sufficient to carry out the abovementioned optimization.

Group B (insulin sensitivity): The power calculation is based on blood glucose levels during the clamp for a 5% alpha and 20% beta values. The standard deviation value for the expected increase in glucose infusion rate during the hyperinsulinemic/euglycemic clamp in rats is 11% based on previously reported results. With a minimum difference of 15%, approximately 10 rats per diet group are required. Formula: $n = 15.7 * (\sigma/\delta)^2 = 15,7 * (11/15)^2 = 8.44$). However, due to the cannulation operation we expect a drop-out rate of 20% of the test animals. Taking this into account, the total number of test animals $8.44/0.80 = 10.55$ (rounding up n=11) rats per diet, 22 in total for Group B.

Group C (mitochondrial function): Based on the variation in respirometry analysis in isolated mouse skeletal muscle mitochondria (to assess mitochondrial functional capacity) from previous experiments (σ=56 nmol O_2/mg/min) and an expected difference of 75 nmol O_2/mg/min in maximal oxygen flux, we estimate that ($\alpha = 0.05$) 9 animals per group are needed to reach a power of 80%. Formula: $n = 15.7 * (\sigma/\delta)^2 = 15,7 * (56/75)^2 = 8.75$ (rounding-up n=9). Since this group will not undergo the cannulation surgery, we do not expect any drop-outs. Therefore, 9 rats per diet, 18 in total are required for Group C.

The total number of test animals is n= 6 (Group A-pilot) + 22 (Group B- insulin sensitivity) + 18 (Group C- mitochondrial function) = 46

8. Experiment

The experimental setup of this DEC is similar to DEC nr: 2006-024 and DEC nr: 2010-036 with minor changes as described below.

The aim is to determine the effects of muscle-specific SIRT3 overexpression on mitochondrial capacity, oxidative stress and insulin sensitivity in insulin resistant rats. This will be evaluated by using a the state of the art in vivo DNA-electroporation technique to obtain specific overexpression in the left anterior tibial (TA) muscle of the rat, while the right TA muscle will serve as sham-electroporated internal control. The rats will be divided up into 3 groups; Group A- a pilot phase, Group B- for insulin sensitivity and Group C- for mitochondrial function measurements. The pilot phase is required to optimize the SIRT3 overexpression by the electroporation procedure.

Pilot (Group A) – Optimization phase

8-week old male Wistar rats purchased from Charles River Laboratories will acclimatize for 2 weeks upon arrival at the CPV. 10-week old rats will animals will undergo either a single- or double-electroporation procedure (SOP 2) to optimize the SIRT3 overexpression. The electroporation procedure introduces exogenous DNA into the skeletal muscle genome by electro-stimulation (200V/cm). According to the scheme below, a single-electroporation procedure will occur at week 5 or 7 of the testing phase while the double-electroporation will be done at both week 5 and 7. Vector concentrations are varied between left and right legs. This approach will generate the necessary information on transfection efficiency, duration of SIRT3 overexpression and optimal vector concentration needed for the main experiment. All rats in this group will be sacrificed at week 4.

Time	Single (1)	Single (2)	Double
Weeks 1-4	-	-	-
Week 5	Electroporation	-	Electroporation
Week 6	-	-	-
Week 7	-	Electroporation	Electroporation
Week 8	Sacrifice	Sacrifice	Sacrifice

Experiments (Group B and C)

8-week old male Wistar rats purchased from Charles River Laboratories will acclimatize for 2 weeks upon arrival at the CPV. 10-week old rats will be placed on either a low fat (LF) chow diet or a high fat (HF) diet for 8 weeks in order to induce insulin resistance. Food intake and rat body weight will be measured weekly. After 6 weeks of diet intervention Group B rats will undergo a cannulation operation (SOP 1) for insulin sensitivity measurements via a hyperinsulinemic-euglycemic clamp, which will be performed after the dietary intervention. The jugular vein and carotid artery are cannulated to sample blood and infuse glucose and insulin during the clamp.

Based on the outcome of the pilot study (Group A), rats will be electroporated at week 5 or 7 for a single-electroporation procedure or at week 5 and 7 for a double-electroporation procedure (SOP2).

One week later after the (final) electroporation, Groups B will under go the hyperinsulinemic-euglycemic clamp (SOP 3) with the simultaneous infusion of labelled deoxyglucose to determine muscle-specific glucose uptake while Group C will be sacrificed and muscle tissue will be

harvested for ex vivo mitochondrial function (by high resolution respirometry), mitochondrial ROS production, oxidative stress and other markers of mitochondrial metabolism.

Timeline:

Week 1:
10-week old male Wistar rats begin LF or HF diet (Group B and C)
↓
Week 5:
Electroporation procedure (Group B and C) *depending on pilot results
↓
Week 6:
Cannulation operation (Group B)
↓
Week 7:
Electroporation procedure (Group B and C) *depending on pilot results
↓
Week 8:
Clamp and sacrifice (Group B)
Sacrifice (Group C)

9. Experimentele condities

9a. Anesthesie

During the cannulation operation and during the electroporation anesthesia the rats will be sedated. The anesthesia (initially 4% isoflurane, then reduced to 3% isoflurane will be set at a flowrate of 500 mL/min) is administered with air and the depth of the anesthesia is monitored by checking reflexes and breathing. At the end of the experiment, Group C rats will be sedated with a CO_2 and O_2 (67:33%) mixture, followed by immediate cervical dislocation.

9b. Pijnbestrijding

Pre-operative Rimadyl will be used (4 mg/kg body weight), with a second dose administered the next day post-surgery.

9c. Euthanasie en Humane eindpunten

At the end of the experiment Group A and B rats will be sacrificed by an overdose injection of pentobarbital (200mg/kg IV or 1/10 diluted IP). Group C rats will be sedated with a CO_2 and O_2 (67:33%) mixture, followed by immediate cervical dislocation. Anesthesia may have adverse affects on skeletal muscle mitochondrial function, and therefore are not recommended for use in this rat Group (C).

If a rat has a 15% reduction in body weight due to the surgery or intervention, it will be sacrificed upon discussion and consultation with the CPV staff and veterinarian. If a rat experiences complications during or post cannulation surgery (such as severe bleeding, infection, blocked cannulation lines or other issues), or if complications arise during the electroporation procedure, it will be sacrificed in consultation with CPV staff and veterinarian. If the CPV staff or we detect signs of hypoglycemia during the intervention or clamp, we will immediately stop the protocol and consult the CPV veterinarian. Also if the animal facility personnel or we encounter animals that seem ill, we will consult the veterinarian and decide whether or not to sacrifice the animal. Ill rats

will be sacrificed with an overdose of pentobarbital (200 mg/kg IV or 1/10 diluted IP).

Zorg

10a. Ongerief

Group A rats: These rats will only undergo a 10-minute electroporation procedure (SOP2) (intramuscular injection with simultaneous transcutane electro-stimulation) under general anesthesia. The rats will undergo the electroporation either one or two times as indicated in the pilot experiment plan. The electroporation protocol is estimated to cause a moderate discomfort (category 03).

Group B rats: The degree of discomfort experienced by the rats for the electroporation (SOP2) (intramuscular injection with simultaneous transcutane electro-stimulation) is estimated to be moderate (category 03). Depending on the pilot results, the animals will participate in a 10-minute electroporation procedure either one or two times. These animals will be cannulated under general anesthesia (SOP 1). The surgery takes approximately 2 hours and is estimated to have a moderate discomforted level (category 03). The rats must also be housed individually to prevent other rats from biting/breaking the cannulation tubing and will be handled on a daily basis to ensure patency of the canulation lines (this increases the discomfort to category 04). Then the rats will undergo a 3-hour clamp procedure. The estimated discomfort for the clamp (SOP3) is assessed as mild/moderate (category 02), based on our experience from human clamps. Finally, at the end of the clamp procedure, the rats are sacrificed (under anesthesia) and muscle tissue is harvested. Therefore the overall discomfort level is category 04.

Group C rats: These rats will only undergo the electroporation procedure (SOP2) estimated to be a moderate discomfort (category 03), however since they will be housed individually for food intake analysis the discomfort level is increased (category 04). Depending on the pilot results, the animals will participate in a 10-minute electroporation procedure either one or two times.

10b. Welzijnsevaluatie

The overall discomfort levels described above were obtained from DEC nr: 2006-024 and DEC nr: 2010-036.

11. Verzorging en huisvesting

Group A rats: Can be housed together.

Group B and C rats: During the diet intervention period the rats will be housed individually to obtain food intake information. For Group B, the cannulation operation is performed in room [redacted]; the rats will recover from the surgery there before returning to the CPV. The rats will remain in individual cages to prevent other rats from biting/breaking the cannulation tubing.

Both the researchers involved as well as the CPV technicians and veterinarian will monitor the care and well being of all study rats.

12. Deskundigheid

The cannulation operations will be performed by a CPV biotechnician, who is certified and experienced with these surgeries. The electroporation and clamps will be performed by certified and experienced researchers as listed below.

	Electroporation	**Clamp**
	Certified and experienced	Certified and experienced
	Certified and experienced	Certified and experienced
	Certified and experienced	Certified and experienced
	Certified	Certified
	Certified	Certified
	Certified	Certified

13. Standard Operating Procedures (SOP) (in Dutch)

SOP 1: Canulatie
De rat wordt d.m.v. Isofluraan (4%) onder narcose gebracht.
De narcose wordt daarna onderhouden d.m.v. IsoFlo 2-3%.

Desinfecteer verrichtingsgebied
Het operatiegebied (nek en hals) wordt geschoren, verwijderen haren met VEET en gedesinfecteerd.
De rat wordt gepositioneerd op een warmteplaat (37 'C).
Oogzalf aanbrengen.

Analgesie preoperatief.
Pre-operatief Caprofen (Rimadyl) subcutaan als pijnbestrijding toedienen (4 mg/kg lichaamsgewicht). Dit kun je verdunnen in Nacl zodat je wat meer volume hebt om in te spuiten.

Rat in rugligging. Nek ondersteunen met kussentje.

Incisie in hals 1-1,5 cm t.p.v. vene jugularis r en art carotis l.
Canules vullen met fysiologisch zout incl. Heparine (1 ul/ml) en afsluiten met stopje gemaakt van een blauwe naald.
Zowel vene jugularis r. als arterie carotis l. vrij prepareren.

Veneus !!
2 ligatuurtjes aanbrengen. Vat distaal afbinden.
Canule bevochtigen met NaCl. Gaatje knippen en mbv canuleerhaakje (oranje naald) canule met pincet inbrengen.

Canule tot verdikking (versmelting of siliconenpropje) opvoeren en fixeren.
Canule testen / flushen.

Arterie !!
3 ligatuurtjes aanbrengen. Vat distaal afbinden.
Canule bevochtigen met NaCl. Gaatje knippen en mbv canuleerhaakje (oranje naald) canule met pincet inbrengen. Je schuift de tip voorbij het middelste touwtje (stropje) tot 3e touwtje. Je fixeert canule lekdicht met middelste touwtje. Vervolgens haal je met pincet 3e touwtje los en je schuift mbv pincet de canule door.
Canule tot verdikking (versmelting) opvoeren en fixeren.
Canule testen / flushen.

Rat in buikligging.
Incisie in nek 1 cm.
Doorvoerder (kocher) richting hals onderhuids opvoeren. Canules doorvoeren vanaf hals.
Incisies in de hals sluiten (tevens subcutus). Canules in nek fixeren.
Incisie hechten en hesje plaatsen.

(Inhalatieanaesthesie uitschakelen en rat laten bijkomen mbv extra O2).
De volgende dag zal een herhalingsdosis Caprofen (Rimadyl) gegeven worden (4 mg/kg lichaamsgewicht). Indien nodig gebruiksantibiotica om besmetting na cannulatie te verhinderen.

SOP 2: Electroporatie

- Anesthesie: isofluraan
- Scheren van de achterpoten
- Eenzijdige intramusculaire injectie met genconstruct in de tibialis anterior. In andere poot sham construct injecteren en electroporeren.
- In beginsel zal gestart worden met de volgende electroporatie condities: elektrostimulatie met 200V/cm (8 pulsen van 20msec/puls) volgens de techniek van Mir et al. (PNAS 1999;96:4262-4267).
- Na elke variatie in de SOP volgens de parameters in het protocol beschreven zal geëvalueerd worden of de veranderingen een te meten verschil in eiwit expressie tussen de behandelde en onbehandelde spier teweeg brengen en de transfectie efficiëntie dus in orde is.
- De electrostimulatie wordt uitgevoerd door het plaatsen van platina plaat electroden op de huid die de musculus tibialis anterior omgeeft. Er wordt electrode gel tussen de electrode en het contactoppervlak wordt geplaatst teneinde schroeiing van de huid te voorkomen.

SOP 3: Hyperinsulinemische euglycemische clamp

- De eerder geprepareerde ratten staan gedurende 4 uur voor aanvang van het protocol nuchter
- Het arteriële infuussysteem wordt gevuld met 30% glucose oplossing met een infusie snelheid van 500µl/uur.
- Via dezelfde infuuslijn wordt het insuline infuus gestart met en snelheid van ongeveer 10 uL/min (afhankelijk van het gewicht van de rat) en wordt gedurende de clamp de respons van de bloedglucose gemonitord.

- Vervolgens worden insuline en glucose infusie snelheid zo op elkaar afgesteld dat er een euglycemisch plateau van 5 mmol/l in het bloedglucose ontstaat zodat de insuline gevoeligheid kan worden berekend
- Telkenmale zal er via de veneuze lijn bloed gesampled worden (om de 10 min 25 µl) om de euglycemie te waarborgen, tevens 3 extra grote bloedafnames (400uL) voor additionele bloed bepalingen.
- Na afloop van de clamp (na ongeveer 1,5 a 2 uur) zal onder anesthesie weefseldissectie plaatsvinden waarna de ratten onmiddellijk zullen worden opgeofferd middels een overdosis pentobarbital via de veneuze lijn.

University Maastricht

Faculty of Health, Medicine and Life Sciences

Dierexperimenten Commissie

DEC

oorzitter
p/a Secretariaat DEC-UM
Postbus 616
NL-6200 MD Maastricht
Telefoon: 043

Aan:

Uw referentie:

Onze referentie:

Maastricht, 03-05-2011

Geachte Onderzoeker,

Uw projectaanvraag: "*Enhancing SIRT3 in skeletal muscle prevents insulin resistance*", is op de DEC vergadering van 29 april 2011 besproken.

De DEC heeft één enkele vraag/opmerking:

- Bij punt 7c merkt de DEC op niet tussentijds af te ronden bij de uitval. Dit heeft volgens de DEC consequenties voor de aantallen.

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 **2011-059**, gelieve dit nummer in verdere correspondentie te vermelden.

Hoogachtend,

Voorzitter DEC-UM

Dear DEC-UM committee members,

Upon review of DEC 2011-059 "*Enhancing SIRT3 in skeletal muscle prevents insulin resistance*", you asked for the following revision to be made.

De DEC heeft één enkele vraag/opmerking:

- Bij punt 7c merkt de DEC op niet tussentijds af te ronden bij de uitval. Dit heeft volgens de DEC consequenties voor de aantallen.

This correction has been made to the DEC and is now highligthed in grey.

Thank you for your input.

Sincerely,

Maastricht University
PO BOX 616
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The Netherlands

Maastricht University

Faculty of Health, Medicine and Life Sciences

Aan:

Ons kenmerk	*Doorkiesnummer*	*Maastricht*
	043-	30-05-2011

Project: *Enhancing SIRT3 in skeletal muscle prevents insulin resistance.*

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:	**2011-059**
Diersoort:	**rat**
Aantal dieren:	46
Einddatum:	28-05-2015

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

Vice-Voorzitter DEC-UM

DEC-UM
Voorzitter DEC-UM

p/a secretariaat DEC-UM

Secretariaat DEC-UM
T (043)

Bezoekadres

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