# Begeleidingsformulier aanvraag dierproef DEC- UM

*Versie 2006*

**Herziene versie**

**DECNR: 2011-131**

**Ontvangen: 31-10-2011**

| DEC datum goedkeuring# | Type aanvraag [2] |
|---|---|
| 18-11-2011 | Nieuw |

| VROM/GGONR[3] |
|---|
| IG 09-075 |

| LNV/CBDNR[4] |
|---|
| |

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

| Financieel beheerder | | Budgetnummer | 3098.2.263N |
|---|---|---|---|

Titel van het onderzoek:

The role of miR-216 in type 2 diabetes

startdatum November/ 2011 einddatum [9] November / 2014 *Duur van de proef [10]:* *20 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. Verantwoordelijk medewerker (VM) GGO[7] | | | | Art.9 | |
| 4. overige uitvoerenden | | | | Art. 12 | |
| 5. | | | | | |

| Diergroep | 1 | 2 | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ctrl/exp/sham | ctrl | exp | | | | | | |
| Diersoort | Muis (01) | Muis (01) | | | | | | |
| Stam | C57/Bl6 | C57/Bl6 | | | | | | |
| Construct / mutatie ?<br>Bijzonderheid dier | X<br>01 | miR-216<br>KO 02 | | | | | | |
| Herkomst (leverancier) | | | | | | | | |
| Aantal | 234 | 156 | | | | | | |
| Geslacht | Male | Male | | | | | | |
| Dieren immuuncompetent ? | ja | ja | | | | | | |
| Leeftijd/gewicht | 10 weeks | 10 weeks | | | | | | |
| Doel van de proef | 33 | 33 | | | | | | |
| Belang van de proef | 01 | 01 | | | | | | |
| Toxicologisch onderzoek | 01 | 01 | | | | | | |
| Bijzondere technieken | 01 | 01 | | | | | | |
| Anesthesie | 04 | 04 | | | | | | |
| Pijnbestrijding | 04 | 04 | | | | | | |
| Mate ongerief | 05 | 05 | | | | | | |
| Toestand dier einde exp | 01 | 01 | | | | | | |

Verantwoording

*Aanvraag dierproef DEC-UM* **(kaders zijn licht flexibel, maar het geheel is max. 5 pag. feb.'08)**

**Titel: . The role of miR-216 in type 2 diabetes.**

Formatted: English (U.S.)

**1. Doel van de proef.**

It has been shown that adminstration of a high fat diet with associated obesity caused nuclear exclusion and reduced expression of the transcription factor HNF1A in pancreatic beta cells.[1] This resulted in a deficit of GnT-4a glycosyltransferase expression in beta cells and produced signs of type 2 diabetes. Also lacking the GnT-4a glycosyltransferase encoded by the MGAT4A gene has been reported to result in type 2 diabetes.[2] GnT-4a function promotes beta cell surface residency of the SLC2A1- encoded glucose transporter-1 (GLUT1) and SLC2A2-encoded glucose transporter-2 (GLUT2) glycoprotein, needed for glucose uptake.[2] High fat diet-induced deficiency of GLUT1 or GLUT2 has also been reported to be cause in the onset of type 2 diabetes.[1] Recently, we found microRNA-216 to be highly expressed in the pancreas. By using computer analyses, we identified HNF1A, MGAT4A, SLC2A1 and SLC2A2 as predicted targets of miR-216. Thus, increased expression of miR-216 might cause downregulation of HNF1A, MGAT4A, SLCA1 and SLCA2, which has been linked to type 2 diabetes. Furthermore, in diabetic nephropathy it also has been shown that miR216 expression is highly increased.

Downregulation of miR216 could prevent organic and systemic insulin resistance. To test this hypothesis, wild type (WT) and miR-216 knock-out (KO) mice will be fed a high fat diet to induce obesitas and insulin resistance. In these mice, we will determine the role of miR-216 during the onset of type 2 diabetes.

**2. Maatschappelijke relevantie en/of wetenschappelijk belang**

Insulin resistance contributes substantially to the pathophysiology of type 2 diabetes.[3] The identification of molecular pathways by which organ-specific and systemic insulin resistance develops is important for understanding the etiology of type 2 diabetes. Determing the role of miR-216 during the onset of type 2 diabetes could lead to novel therapy in type 2 diabetic patients and could help to prevent the progression from obesitas to insulin resistance and type 2 diabetes.

**3. Alternatieven**

Because type 2 diabetes is a systemic disease, it is only possible to study this disease in a whole body. However, fundamental research already has been performed in several pancreatic cell lines to minimise the amount of mice to test our hypothesis.

***4. Ethische afweging***

**Bij de ethische afweging wordt rekening gehouden met de intrinsieke en instrumentele waarde van de proefdieren. De mate van ongerief en het aantal proefdieren wordt beperkt tot het minimale. Ervaring uit eerdere experimenten speelt hierbij een belangrijke rol. Via de juiste omgang met de muizen en anesthesie wordt de mate van het ongerief beperkt. Gebruik van een combinatie van technieken en van de juiste statistische analyse reduceert het aantal proefdieren.**

Kijkend naar de instrumentele waarde worden de experimenten gedaan met een duidelijk doel.

Diabete patiënten hebben als complicatie vaak hartfalen, dit is tevens de belangrijkste doodsoorzaak bij deze patiëntengroep. Onderzoek naar de moleculaire mechanismen betrokken bij de ontwikkeling van insuline resistentie, diabetes type 2 en hartfalen zal zorgen voor de juiste aangrijpingspunten voor de behandeling van patiënten. Deze behandeling is zowel gericht op preventie als het voorkomen van hartfalen bij diabete patiënten.
Geconcludeerd kan worden dat we verwachten dat het belang van de beoogde resultaten opwegen tegen het ongerief van het proefdier.

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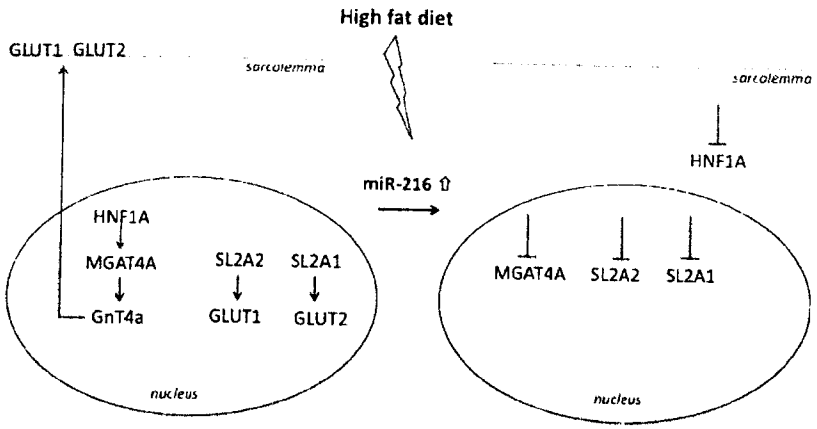
**5. Wetenschappelijke onderbouwing**

Type 2 diabetes affects hundreds of millions of people, and cases are predicted to double within 20 years.[4] This escalation in frequency has been closely associated with a widespread change in the human diet in the presence of obesity. Insulin resitance contributes substantially to the pathophysiology of type 2 diabetes. The identification of molecular pathways by which organ-specific and systemic insulin resistance develop in respons to diet and obesity is important for understanding the etiology of type 2 diabetes and learning how to prevent this disease. However, progress developing new type 2 diabetes therapies has been impaired by an incomplete comprehension of the signaling events underlying this disease.

Recently, it has been shown that adminstration of a high fat diet with associated obesity caused nuclear exclusion and reduced expression of the transcription factor HNF1A in pancreatic beta cells, which resulted in a deficit of GnT-4a glycosyltransferase expression in beta cells and produced signs of type 2 diabetes.[1] Also lacking the GnT-4a glycosyltransferase encoded by the MGAT4A gene has been reported to result in type 2 diabetes.[2] GnT-4a function promotes beta cell surface residency of the Slc2a1- encoded glucose transporter-1 (GLUT1) and Slc2a2-encoded glucose transporter-2 (GLUT2) glycoprotein, needed for glucose uptake. High fat diet-induced deficiency of GLUT1 or GLUT2 has also been reported to be cause in the onset of type 2 diabetes.[1] Recently, we found microRNA-216 to be highly expressed in the pancreas. By using computer analyses, we identified HNF1A, MGAT4A, SLC2A1 and SLC2A2 as predicted targets of miR-216. Thus, increased expression of miR-216 might cause downregulation of HNF1A, MGAT4A, SLCA1 and SLCA2 (see figure).The downregulation of HNF1A, MGAT4A, SLCA1 and SLCA2 previously has been linked to type 2 diabetes.[1] Furthermore, in diabetic nephropathy it also has been shown that miR216 expression is highly increased.[5]




We have designed a study to address the following **hypothesis**: Downregulation of miR216 could prevent organic and systemic insulin resistance and type 2 diabetes.

To test this hypothesis, miR-216 knockout (KO) mice will be used. To induce insulin resistance and obesity, we will administer these mice a high fat diet. Further, administration of antagomiR-216 (inhibit the effect of this microRNA) will prevent high fat diet-induced type 2 diabetes, while administration of a miR mimic (mimicking the effect of this microRNA) will enhance the development of type 2 diabetes.

Although this proposal has a fundamental character to get a better insight into the molecular pathways that lead to type 2 diabetes, it may have significant therapeutic consequences for the clinical management of diabetic patients.

**6. Wetenschappelijke beoordeling**

This study has been read and approved by ..... (PI of the group).

5 Proefdier

**7. Proefdier keuze**

**7.a. Soort / stam / herkomst / eindbestemming**

Adult (10 weeks of age) c57Bl./6J and miR-216 KO mice, (males) are obtained from [redacted] from the breeding colony of [redacted]. He has imported these animals from [redacted], where the knockouts were generated. miR-216a knockout mice are viable, have a normal life-span, breed normally and produce as much offspring as wildtype SV129/Ola counterparts.

At the end of the study, the animals will be submitted to euthanasia in order to collected tissue for further analysis. Re-use is therefore unfeasible.

**7.b. Sekse**

males (It has been published by using different animal models that females are protected against insulin resistance due to estrogen receptor activation.[6])

**7.c. Aantallen**

**Mouse islet cells will be isolated and prepared for following experiments:**

Experiment 1. Glycoprotein, glycan and transcriptional factor analyses
Experiment 2. Glucose stimulated insulin secretion
Experiment 3. Detection and measurement of RNA transcript abundance using real-time PCR
Experiment 4. Glucose transport assay

**Mouse skeletal muscle cells and adipocytes will be isolated and prepared for following experiments:**

Experiment 5. Glucose transport assay
Experiment 6. western blot analyses to measure insulin-stimulated Akt and IRS-1 phosphorylation

**Pancreatic tissue will be collected for following experiments:**

Experiment 7: histology
Experiment 8: Western blot analyses to measure Akt and IRS-1 phosphorylation (insulin signaling pathway)

The relative effect (difference in experimental parameters) that we can expect in our study is known. A couple of parameters will be measured in this study. For the sample size calculation we adopted the equation proposed by Sachs ($N=2[(Z_{\alpha/2}-Z\Pi)2/(\delta/\sigma)2]$). We will start from a minimal relative effect ($\delta$) of 30%. The alpha for each experiment is based o previous experimentation and literature.[1]

**Experiment 1: the variation coefficient ($\sigma$) is 25% (variability between the animals that undergo the same experimental procedure and are genetically related). It was also adopted a power value of 0.80 ($\Pi$) and a 0.05 for alpha ($\alpha$). The minimal number of animals is 8,18 (or 9 animals per group).**

**Experiment 2: the variation coefficient ($\sigma$) is 30% (variability between the animals that undergo the same experimental procedure and are genetically related). It was also adopted a power value of 0.80 ($\Pi$) and a 0.05 for alpha ($\alpha$). The minimal number of animals is 11,78 (or 12 animals per group).**

Experiment 3: the variation coefficient (σ) is 25% (variability between the animals that undergo the same experimental procedure and are genetically related). It was also adopted a power value of 0.80 (Π) and a 0.05 for alpha (α). The minimal number of animals is **8,18** (or **9** animals per group).

Experiment 4+5: the variation coefficient (σ) is 20% (variability between the animals that undergo the same experimental procedure and are genetically related). It was also adopted a power value of 0.80 (Π) and a 0.05 for alpha (α). The minimal number of animals is **5,23** (or **6** animals per group).

Experiment 6+8: the variation coefficient (σ) is 25% (variability between the animals that undergo the same experimental procedure and are genetically related). It was also adopted a power value of 0.80 (Π) and a 0.05 for alpha (α). The minimal number of animals is **8,18** (or **9** animals per group).

Experiment 7: the variation coefficient (σ) is 25% (variability between the animals that undergo the same experimental procedure and are genetically related). It was also adopted a power value of 0.80 (Π) and a 0.05 for alpha (α). The minimal number of animals is **8,18** (or **9** animals per group).

Experiment 1 and 2 can be combined and experiment 3 and 4 too. For Experiment 5 and 6 we don't need extra mice.
Thus, in total we will do 4 separated experiments Thus, we need 12+9+9+9= 39 mice per group. We have 6 WT groups, so we will need 234 WT mice. We also have 4 miR-216 KO groups, so we need 156 KO mice in total.

**Groep 1**: WT mice on a low fat diet
**Groep 2**: WT mice on a low fat diet, treated with antogomiR-216
**Groep 3**: WT mice on a low fat diet, treated with miR-216 mimic
**Groep 4**: WT mice on a high fat diet
**Groep 5**: WT mice on a high fat diet, treated with antogomiR-216
**Groep 6**: WT mice on a high fat diet, treated with miR-216 mimic
**Groep 7**: miR-216 KO mice on a low fat diet
**Groep 8**: miR-216 KO mice on a low fat diet, treated with miR-216 mimic
**Groep 9**: miR-216 KO mice on a high fat diet
**Groep 10**: miR-216 KO mice on a high fat diet, treated with miR-216 mimic

8 Dierproef

**8. Experiment**

**10 groups of mice (all around 10-12 weeks of age) will be included in the experiment:**

**Groep 1**: WT mice on a low fat diet
**Groep 2**: WT mice on a low fat diet, treated with antogomiR-216
**Groep 3**: WT mice on a low fat diet, treated with miR-216 mimic

**Groep 4**: WT mice on a high fat diet
**Groep 5**: WT mice on a high fat diet, treated with antogomiR-216
**Groep 6**: WT mice on a high fat diet, treated with miR-216 mimic

**Groep 7**: miR-216 KO mice on a low fat diet
**Groep 8**: miR-216 KO mice on a low fat diet, treated with miR-216 mimic

**Groep 9**: miR-216 KO mice on a high fat diet
**Groep 10**: miR-216 KO mice on a high fat diet, treated with miR-216 mimic

The experiment will take 20 weeks in total. In week 8, 12, and 16 we will measure blood glucose, insulin, fatty acids and cholesterol levels,. To measure this, we will collect blood from the tail vein (2 capillaires/mouse). In week 19, we will perform a glucose tolerance test (GTT), and in week 20 an insulin tolerance test. Additionally, following experiments will be performed:

**Mouse islet cells will be isolated and prepared for following experiments:**
Experiment 1. Glycoprotein, glycan and transcriptional factor analyses
Experiment 2. Glucose stimulated insulin secretion
Experiment 3. Detection and measurement of RNA transcript abundance using real-time PCR
Experiment 4. Glucose transport assay

**Mouse skeletal muscle cells and adipocytes will be isolated and prepared for following experiments:**
Experiment 5. Glucose transport assay
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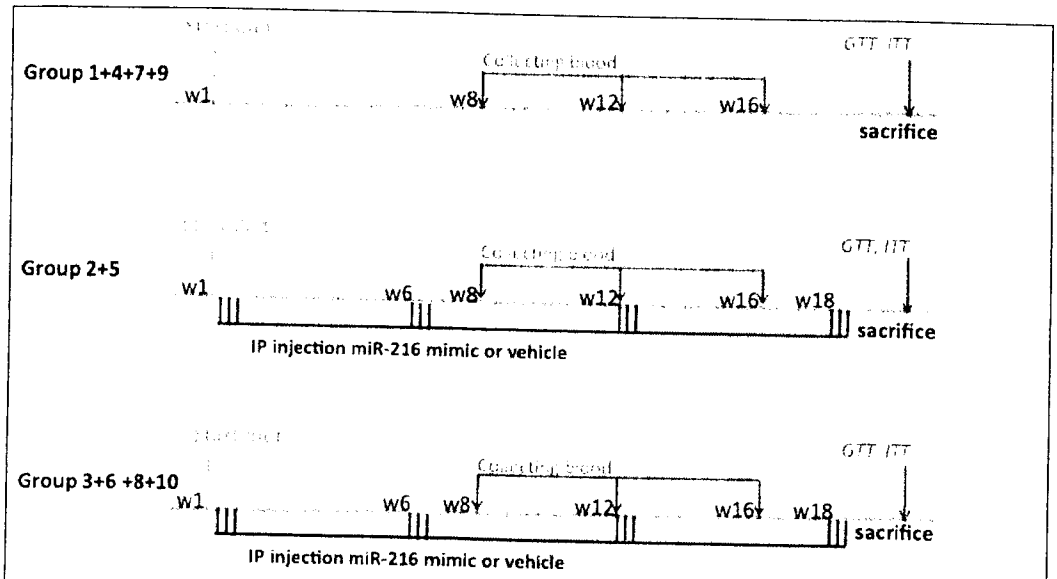
**Pancreatic tissue will be collected for following experiments:**
Experiment 7: histology
Experiment 8: Western blot analyses to measure Akt and IRS-1 phosphorylation (insulin signaling pathway)

**Mimic/ antagomir**
10-week-old C57BL/6 mice will receive either antagomir against miR-216, or a miR-216 mimic at a dose of 80 mg/kg body weight or a comparable volume of saline through i.p. injection.
Injections will be given once a day, for 3 days in a row and this will be repeated every 4 weeks.




*in week 1, all mice are 10 weeks of age

**9. Experimentele condities**

**9a. Anesthesie**

To collect tissue and to perform a Hyperinsulinemische euglycemic clamp, mice will be anaesthetized with 3-4% Isoflurane and maintained with 1.5-2.5% of Isoflurane during the procedure via an endotracheal tube procedure.

**9b. Pijnbestrijding**

Before performing a Hyperinsulinemische euglycemic clamp: Mice will be submitted to a pre-operative administration of temgesic (buprenorphine,0,05 mg/kg i.e 0,05 mL/10g) to reduce the pain.

**9c. Euthanasie en Humane eindpunten**

A humane end point will be carried out when animals show:
changes in physical appearance (e.g., coat texture; hair soiled with urine or faeces);
changes in clinical signs (e.g., dyspnea; posture; diarrhea; >10% of body weight loss;
changes in food and water consumption; although we don't expect tumors, presence of > 1cm3 tumours; changes in unprovoked behaviour (e.g., self mutilation;
compulsive behaviour; posture; grooming patterns; activity levels); behavioural changes in response to external stimuli (e.g., excitability; righting reflex). If euthanasia is necessary, a person with WOD art.12 and/or a person with WOD art. 14 will be consulted to determine the euthanasia. Also the VO and VVO will be contacted via email before euthanasia will be performed. Euthanasia will be perfomed by putting the animal in a container with first a low $CO_2$ concentration followed by a 100% $CO_2$ concentration.

Zorg

**10a. Ongerief**

Intraperitoneale injection en verwijdering van weefsel na anesthesie: gering/matig
Alle dieren ontvangen een dieet gedurende 20 weken (leeftijd: week 10-30). Dieren krijgen of een hoog vet-dieet, of het controle laag vet-dieet.

- Measuring body weight every week. Estimated degree of discomfort: 1
- Euthanasia. Estimated degree of discomfort: 2
- Glucose tolerance test:1 time in week19. Estimated degree of discomfort: 5
- Insulin tolerance test: 1 time in week 19. Estimated degree of discomfort: 5
- Antagomir/mimic/vehicle injection:i.p. Week 1, 6, 12 and 18: 3 times each week. Estimated degree of discomfort: 4
- s.c. Temgesic: Estimated degree of discomfort: 1
- Collecting blood from tail vein: In week 8, 12, and 16. Estimated discomfort: 3

**10b. Welzijnsevaluatie**

The body weight (measured weekly) will be used to assess the well being of the mice.
Animals will also be checked (any hunching) to see if they experience discomfort.
A person with WOD art.12 and/or a person with WOD art. 14 will be consulted to determine if euthanasia is necessary in the case that animals display discomfort.

**11. Verzorging en huisvesting**

The Animal Facility (CPV) of the University of Maastricht will be responsible for the housing and caring of the animals during the protocol. Animals will be housed with 3-4 animals in 1 cage.

Diet:
D12329 control diet (10,5 % fat, 16,4 % proteins, 73,1 % carbohydrates) en D112331 diet (58% fat, 16.4% proteins, 25.5% carbohydrates) from 'Research Diets'.
Diet will be administered **ad libitum**.

**12. Deskundigheid**

Verantwoordelijke onderzoekers en medewerker zijn volgens art.9 of art.12 bevoegd voor het uitvoeren en ontwerpen van dierproeven. Bekwaamheid blijkt uit de ervaring opgedaan uit vergelijkbare dierproeven van afgelopen jaren.

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

*SOP2 Glucose Tolerance Test Protocol (GTT)*

Put mice without food for 6 hours
Measure blood glucose with a glucometer by taken a droplet of blood form the tail
inject the mice with glucose solution following the steps below:
-fill the syringe with the glucose solution:1 unit to 1 gram of weight□24.5 grams = 24.5 units of glucose, inject IP
-as soon as you inject the mouse start your timer counting up from 0:00 and record the time and blood glucose
-exactly 30 minutes from the time of injection, take the second blood glucose
-wait 60 minutes from the time of injection to take the third blood glucose
-wait 120 minutes from the time of injection to take the forte blood glucose

*SOP3 Insulin tolerance test (ITT)*

Weigh the animals the afternoon before the test.
Place each of the animals in a separate fresh cage with food.
Prepare the insulin solution shortly before the test (for details see below).
Calculate the amount of insulin solution to be injected: inject 3 x BW in µl of the insulin solution described below (0.25 U/ml) or for a 30 g mouse 90 µl.
Use random fed animals and perform the test in the afternoon (between 2-5 pm).
Determine a basal glucose level in a drop of blood obtained from the cut tail vein.
Insulin injection intraperitoneal: inject 0.75 U insulin/kg body weight. . Remove the needle and let the mouse go back into the cage.
Measure the blood glucose after 15, 30,45 and 60 min.

Evaluation of ITT:
For each mouse the blood glucose levels at each time point are calculated as percentage of its blood glucose levels at 0 min.
Mean values +/-SEM for each strain are plotted versus time.

**Relevante literatuur**


1. *Ohtsubo K, Chen MZ, Olefsky JM, Marth JD. Pathway to diabetes through attenuation of pancreatic beta cell glycosylation and glucose transport. Nat Med. 2011.*
2. *Ohtsubo K, Takamatsu S, Minowa MT, Yoshida A, Takeuchi M, Marth JD. Dietary and genetic control of glucose transporter 2 glycosylation promotes insulin secretion in suppressing diabetes. Cell. 2005;123(7):1307-1321.*
3. *Schenk S, Saberi M, Olefsky JM. Insulin sensitivity: modulation by nutrients and inflammation. J Clin Invest. 2008;118(9):2992-3002.*
4. *Smyth S, Heron A. Diabetes and obesity: the twin epidemics. Nat Med. 2006;12(1):75-80.*
5. *Kato M, Putta S, Wang M, Yuan H, Lanting L, Nair I, Gunn A, Nakagawa Y, Shimano H, Todorov I, Rossi JJ, Natarajan R. TGF-beta activates Akt kinase through a microRNA-dependent amplifying circuit targeting PTEN. Nat Cell Biol. 2009;11(7):881-889.*
6. *Tiano JP, Delghingaro-Augusto V, Le May C, Liu S, Kaw MK, Khuder SS, Latour MG, Bhatt SA, Korach KS, Najjar SM, Prentki M, Mauvais-Jarvis F. Estrogen receptor activation reduces lipid synthesis in pancreatic islets and prevents beta cell failure in rodent models of type 2 diabetes. J Clin Invest. 2011;121(8):3331-3342.*

University Maastricht

Faculty of Health, Medicine and Life Sciences

Dierexperimenten Commissie

DEC

Aan:

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

Uw referentie: Onze referentie Maastricht, 26-10-2011

Geachte Onderzoeker,

Uw projectaanvraag: "*The role of miR-216 in type 2 diabetes*", is op de DEC vergadering van 21 oktober 2011 besproken.

De DEC heeft een aantal vragen en opmerkingen:

- De DEC merkt op dat de ethische afweging (punt 4), geen afweging is en verzoekt dit aan te vullen.
- De DEC verzoekt bij punt 7 de namen te verwijderen. Het is niet de bedoeling dat er verwezen wordt naar personen in de aanvraag in verband met de Wet Openbaarheid van Bestuur.
- Punt 7c- De powerberekening ten behoeve van bepaling van de groepsgroottes, berust op de variatie coëfficiënt van 25%.
  Echter in de 8 genoemde "experimenten" worden tenminste 6 verschillende primaire parameters bepaald. De DEC acht het onwaarschijnlijk dat de sigma hier in elk geval 25% bedraagt en verzoekt de groepsgroottes per parameter (dus experiment) te berekenen op basis van de bijbehorende sigma.
- Punt 10a- De DEC schat het totale ongerief in op code 05 en verzoekt dit aan te passen (ook op het voorblad).

**Conclusie:**
**Het project wordt aangehouden.**

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 **2011-131**, gelieve dit nummer in verdere correspondentie te vermelden.

Hoogachtend,

Voorzitter DEC-UM

Geachte leden van de DEC,

Jullie hadden een aantal opmerkingen betreffende mijn projectaanvraag "*The role of miR-216 in type 2 diabetes*" (nummer **2011-131**):
:

- De DEC merkt op dat de ethische afweging (punt 4), geen afweging is en verzoekt dit aan te vullen.

De DEC heeft gelijk en daarom heb ik het nu aangepast. Volgende tekst is ingevoegd:
Bij de ethische afweging wordt rekening gehouden met de intrinsieke en instrumentele waarde van de proefdieren. De mate van ongerief en het aantal proefdieren wordt beperkt tot het minimale. Ervaring uit eerdere experimenten speelt hierbij een belangrijke rol. Via de juiste omgang met de muizen en anesthesie wordt de mate van het ongerief beperkt. Gebruik van een combinatie van technieken en van de juiste statistische analyse reduceert het aantal proefdieren.
Kijkend naar de instrumentele waarde worden de experimenten gedaan met een duidelijk doel. Diabete patiënten hebben als complicatie vaak hartfalen, dit is tevens de belangrijkste doodsoorzaak bij deze patiëntengroep. Onderzoek naar de moleculaire mechanismen betrokken bij de ontwikkeling van insuline resistentie, diabetes type 2 en hartfalen zal zorgen voor de juiste aangrijpingspunten voor de behandeling van patiënten. Deze behandeling is zowel gericht op preventie als het voorkomen van hartfalen bij diabete patiënten.
Geconcludeerd kan worden dat we verwachten dat het belang van de beoogde resultaten opwegen tegen het ongerief van het proefdier.

- De DEC verzoekt bij punt 7 de namen te verwijderen. Het is niet de bedoeling dat er verwezen wordt naar personen in de aanvraag in verband met de Wet Openbaarheid van Bestuur.

Dit is nu aangepast als volgt: The relative effect (difference in experimental parameters) that we can expect in our study is known. A couple of parameters will be measured in this study. For the sample size calculation we adopted the equation proposed by Sachs (N=2[(Zα/2-ZΠ)2/(δ/σ)2]). We will start from a minimal relative effect (δ) of 30%. The alpha for each experiment is based o previous experimentation and literature.[1]

- Punt 7c- De powerberekening ten behoeve van bepaling van de groepsgroottes, berust op de variatie coëfficiënt van 25%.
  Echter in de 8 genoemde "experimenten" worden tenminste 6 verschillende primaire parameters bepaald. De DEC acht het onwaarschijnlijk dat de sigma hier in elk geval 25% bedraagt en verzoekt de groepsgroottes per parameter (dus experiment) te berekenen op basis van de bijbehorende sigma.

Ik heb nu voor ieder experiment de groepsgrootte apart berekend. En als volgt aangepast in het portocol:
Experiment 1: the variation coefficient (σ) is 25% (variability between the animals that undergo the same experimental procedure and are genetically related). It was also adopted a power value of 0.80 (Π) and a 0.05 for alpha (α). The minimal number of animals is **8,18** (or **9** animals per group).

Experiment 2: the variation coefficient (σ) is 30% (variability between the animals that undergo the same experimental procedure and are genetically related). It was also adopted a

power value of 0.80 (Π) and a 0.05 for alpha (α). The minimal number of animals is **11,78** (or **12** animals per group).

Experiment 3: the variation coefficient (σ) is 25% (variability between the animals that undergo the same experimental procedure and are genetically related). It was also adopted a power value of 0.80 (Π) and a 0.05 for alpha (α). The minimal number of animals is **8,18** (or **9** animals per group).

Experiment 4+5: the variation coefficient (σ) is 20% (variability between the animals that undergo the same experimental procedure and are genetically related). It was also adopted a power value of 0.80 (Π) and a 0.05 for alpha (α). The minimal number of animals is **5,23** (or **6** animals per group).

Experiment 6+8: the variation coefficient (σ) is 25% (variability between the animals that undergo the same experimental procedure and are genetically related). It was also adopted a power value of 0.80 (Π) and a 0.05 for alpha (α). The minimal number of animals is **8,18** (or **9** animals per group).

Experiment 7: the variation coefficient (σ) is 25% (variability between the animals that undergo the same experimental procedure and are genetically related). It was also adopted a power value of 0.80 (Π) and a 0.05 for alpha (α). The minimal number of animals is **8,18** (or **9** animals per group).

Experiment 1 and 2 can be combined and experiment 3 and 4 too. For Experiment 5 and 6 we don't need extra mice.
Thus, in total we will do 4 separated experiments Thus, we need 12+9+9+9= 39 mice per group. We have 6 WT groups, so we will need 234 WT mice. We also have 4 miR-216 KO groups, so we need 156 KO mice in total.

- Punt 10a- De DEC schat het totale ongerief in op code 05 en verzoekt dit aan te passen (ook op het voorblad).

Di is nu aangepast in het portocol als volgt:
Intraperitoneale injection en verwijdering van weefsel na anesthesie: gering/matig
Alle dieren ontvangen een dieet gedurende 20 weken (leeftijd: week 10-30). Dieren krijgen of een hoog vet-dieet, of het controle laag vet-dieet.

- Measuring body weight every week. Estimated degree of discomfort: 1
- Euthanasia. Estimated degree of discomfort: 2
- Glucose tolerance test:1 time in week19. Estimated degree of discomfort: 5
- Insulin tolerance test: 1 time in week 19. Estimated degree of discomfort: 5
- Antagomir/mimic/vehicle injection:i.p. Week 1, 6, 12 and 18: 3 times each week. Estimated degree of discomfort: 4
- s.c. Temgesic: Estimated degree of discomfort: 1
- Collecting blood from tail vein: In week 8, 12, and 16. Estimated discomfort: 3

Uiteraard is de codering op het voorblad ook aangepast.

Al deze aanpassingen zijn ook grijs gemarkeerd in bijgevoegd DEC protocol.
Mvg,

Maastricht University

Faculty of Health, Medicine and Life Sciences

Aan:

| *Ons kenmerk* | *Doorkiesnummer* | *Maastricht* |
|---|---|---|
| | | 22-11-2011 |

**Project:** *The role of miR-216 in type 2 diabetes.*

**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:** | **2011-131** |
| **Diersoort:** | **muis** |
| **Aantal dieren:** | **390** |
| **Einddatum:** | **18-11-2015** |

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*

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