87

Begeleidingsformulier aanvraag dierproef DEC- UM

Versie 2006

Herziene versie

DECNR: 2011-118

Ontvangen: 01-09-2011

DEC datum goedkeuring#	Type aanvraag [2]
05-09-2011	Nieuw / ~~Herz.versie~~ / ~~Pilot~~

VROM/GGONR[3]

LNV/CBDNR[4]

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

Financieel beheerder	

Budgetnummer	31961985N

Titel van het onderzoek:

Overexpressing the genes FASN, Glut1, beta-HAD, and PDP: Manipulating metabolic flexibility in skeletal muscle reveals the mechanism of insulin resistance

startdatum [redacted] einddatum [9] [redacted] *Duur van de proef [10]:* *6 weken*

	Naam	Tel (+ Tel privé enkel VO, VVO en VM)	E-mailadres	Bevoegdheid[5]	Cap. groep /afdeling
1.Verantwoordelijk onderzoeker (VO)				Art.9	
2. Vervanger VO (VVO)				Art.9	
3. overige uitvoerenden				Art. 9	
4. overige uitvoerenden				Art.9	
5. overige uitvoerenden				Art. 9	

Diergroep	Group A	1	2	3	4			
ctrl/exp/sham	Exp	Exp	Exp	Exp	Exp			
Diersoort	02	02	02	02	02			
Stam	Wistar	Wistar	Wistar	Wistar	Wistar			
Construct / mutatie ?	-	-	-	-	-			
Herkomst (leverancier) *	01	01	01	01	01			
Aantal	20	44	36	44	36			
Geslacht	male	male	male	male	male			
Dieren immuuncompetent ?	ja	ja	ja	ja	ja			
Leeftijd/gewicht	8 wk	8 wk	8 wk	8 wk	8 wk			
Doel van de proef *	37	37	37	37	37			
Belang van de proef *	01	01	01	01	01			
Toxicologisch onderzoek *	01	01	01	01	01			
Bijzondere technieken *	01	01	01	01	01			
Anesthesie *	04	04	04	04	04			
Pijnbestrijding *	04	04	04	04	04			
Mate ongerief *	04	04	04	04	04			
Toestand dier einde exp*	01	01	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: Overexpressing the genes FASN, Glut1, beta-HAD, and PDP: Manipulating metabolic flexibility in skeletal muscle reveals the mechanism of insulin resistance

1. Doel van de proef.

Metabolic flexibility is known as the capacity to switch from predominantly lipid oxidation and high rates of fatty acid uptake during fasted conditions to the suppression of lipid oxidation and increased glucose uptake, oxidation, and storage under insulin-stimulated (fed) conditions. In (pre-) diabetic conditions chronic elevation of circulating insulin levels can disrupt normal metabolic flexibility of insulin-responsive tissues such as skeletal muscle. As a consequence, diabetes patients are characterized by reduced capacity to oxidize fat during fasting, and impaired postprandial (after a meal) switch from lipid to glucose oxidation. This can lead to (increased) insulin resistance, fat accumulation in skeletal muscle, mitochondrial lipotoxicity and mitochondrial dysfunction. All these symptoms are causally linked to insulin resistance, indicating that metabolic inflexibility plays a role in the early development of type 2 diabetes mellitus (T2DM). Therefore, the aim of this study is to establish a causal link among impaired metabolic flexibility, muscle lipotoxicity, and insulin resistance.

The present study aims to manipulate the metabolic flexibility and study the effects on mitochondrial function and insulin resistance under conditions of low- and high lipid availability. Metabolic flexibility can be influenced *in vivo* through overexpression of key regulatory genes in the glucose and fatty acid oxidation pathways. By using gene-electroporation (as is described in DEC 2006-024 and 2010-036) we would like to overexpress βHAD (enzyme involved in β-oxidation), PDP (enzyme involved in glycolysis), Glut1 (transporter involved in glucose uptake), and FASN (enzyme involved in lypogenesis) in the skeletal muscle. Our hypothesis is that manipulation of the metabolic flexibility, via increasing fat oxidation or improving glucose uptake, could be a strategy to prevent the development of insulin resistance and T2DM.

2. Maatschappelijke relevantie en/of wetenschappelijk belang

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. 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 metabolic inflexibility are associated with insulin resistance. The exact causes of insulin resistance are unknown. Therefore, we want to use a systematic approach existing of gene-electroporation and diet interventions to manipulate metabolic flexibility. This way we can improve our understanding in the association between metabolic flexibility and insulin resistance. This study may provide novel targets for pharmaceutical interventions that can prevent the development of T2DM.

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 this rat model we propose to evaluate the influence of genetic and environmental (diet) factors on the development of insulin resistance, which is one of the characteristics of T2DM. Since this study may identify mechanisms causing insulin resistance, which can contribute to prevention and treatment of T2DM, we think it justifies the use of animals. In addition, in our gene-electroporation model, the gene of interest will be incorporated into the skeletal muscle rat genome in one leg while the contralateral leg is used as a control. This way the rat serves as its own control, which reduces the amount of test animals needed.

5. Wetenschappelijke onderbouwing

Obesity, insulin resistance and T2DM are linked to reduced lipid oxidation during fasting and an impaired postprandial switch of lipid to glucose oxidation. Furthermore, a reduced postprandial switch from fat to glucose had been observed in people with an impaired glucose tolerance. This indicates that metabolic inflexibility plays a causal role in the development of T2DM (1). Although several studies have shown associations between metabolic inflexibility, muscle lipotoxicity, and insulin resistance, the exact mechanism of the development of T2DM remains unknown. Because skeletal muscles are responsible for ~80% of postprandial glucose uptake (2), the muscle is an important target to improve insulin sensitivity. Our hypothesis is that by manipulating metabolic flexibility at the level of fat and glucose oxidation in the skeletal muscle, the development of insulin resistance and T2DM can be influenced. This study may provide novel targets for pharmaceutical interventions that can prevent the development of T2DM.

Both glucose oxidation (glycolysis) and fatty acid oxidation (β-oxidation) result in the end product of a 2-carbon acetyl-CoA molecule. Acetyl-CoA can be used in the citric acid (TCA) cycle in order to produce ATP. Currently, it is believed that reduced entry of acetyl-CoA into the TCA cycle causes an energy mismatch underlying skeletal muscle insulin resistance (3). Supporting this theory, type II diabetic and insulin resistant models are characterized by an increased amount of incomplete fatty acid oxidation products (4). Furthermore, excess cytosolic acetyl-CoA is rapidly carboxylated to malonyl-CoA by ACC (acetyl-CoA carboxylase). In skeletal muscle, malonyl-CoA regulates the entry of long chain fatty acids into the mitochondria to undergo β-oxidation by allosterically inhibiting CPT1 activity. The present study aims prove this theory by manipulating acetyl-CoA content *in vivo* through the overexpressing key genes in the glucose-fatty acid cycle.

Fatty acid synthase
Fatty acid synthase (FASN) is the rate-controlling enzyme in *de novo* lipogenesis (new fat generation) by converting malonyl-CoA into long chain fatty acids. Malonyl-CoA levels are elevated in mouse models of obesity and insulin resistance (5) and reports in humans have shown that malonyl-CoA levels are elevated in obese and T2D subjects (6) while reduced malonyl-CoA content is associated with improved insulin sensitivity (7). FASN is mainly expressed in lipogenic tissue such as adipose tissue or liver, however FASN is mildly expressed in the skeletal muscle. Recent studies have showed that skeletal muscle FASN expression and activity are increased with training in both rats (8) and humans (9). Furthermore, it was proposed that elevated FASN in muscle is responsible for increased lipid stores in trained athletes (9). In the present study, we intend to overexpress FASN in rat muscle. We believe this strategy will increase fatty acid oxidation, by re-directing malonyl-CoA (and thus acetyl-CoA) towards lipid synthesis, so dietary lipids can efficiently be utilized for energy. Furthermore, FASN overexpression will improve insulin resistance since the generation of 'new fats' has been reported to stimulate the insulin sensitizing PPAR pathway (10).

Glucose transport 1
Glucose transport 1 (Glut1) imports skeletal muscle glucose under basal (non-insulin stimulated conditions). Transgenic mice overexpressing muscle Glut1, display reduced plasma glucose, increased basal skeletal muscle glucose transport and utilization. Furthermore, clamp studies have shown that these mice are insulin resistant, mainly due to increased glucose flux through the hexosamine pathway (11). In the present study we plan to transiently overexpress Glut1 to

investigate the impact of increased glucose flux on fatty acid oxidation and insulin sensitivity *in vivo*. We believe that short-term increased glucose utilization will inhibit fatty acid oxidation due to an increase in malonyl-CoA. This will result in an increase in incomplete fatty acid products and ultimately leading to insulin resistance.

Pyruvate dehydrogenase phosphatase

The pyruvate dehydrogenase complex (PDH) catalyzes the irreversible oxidative decarboxylation of pyruvate to acetyl-CoA, a reaction that links the utilization of glycogen, glucose, and lactate with the TCA cycle to meet the energy needs of cells. The activity of PDH is tightly controlled by a reversible phosphorylation/dephosphorylation cycle, with the extend of phosphorylation determined by the activities of pyruvate dehydrogenase kinase (PDK) and pyruvate dehydrogenase phosphatase (PDP) (12). PDH activity is decreased in skeletal muscle of T2DM patients compared to healthy subjects (13). This inhibition of PDH is due to an increase in PDK activity and a decrease in PDP expression (14). PDK is activated by increased levels of acetyl-CoA (4). Decreased PDH activity leads to decreased glucose uptake this could lead to insulin resistance. By overexpressing PDP we expect to counteract the PDK activation and thereby increasing the PDH activity. As a result glucose uptake will be improved and this can lead to enhanced insulin sensitivity.

β-Hydroxyacyl-CoA dehydrogenase (β-HAD)

β-HAD is the rate limiting enzyme of the β-oxidation. β-HAD catalyzes the third step reaction of the β-oxidation, which involves conversion of 3-hydroxyacyl-CoA to 3-ketoacyl-CoA. Various inherited disorders of β-HAD deficiencies have been described. Most often, these deficiencies are manifested as hypertrophic cardiomyopathy, skeletal myopathy, hypoketotic hypoglycemia, and liver dysfunction (15). This indicates that β-HAD is an important enzyme. Although there are studies described upon β-HAD deficiency, not so much is known about β-HAD overexpression. By overexpressing β-HAD and herewith increasing the β-oxidation more acetyl-CoA is expected to be used in the TCA cycle. By this method we expect to decrease the acetyl-CoA accumulation, and additionally, increase PDH activity as well.

References:

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6. Wetenschappelijke beoordeling

The proposed research plan has been reviewed and approved by

6 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 5 groups; Group A- pilot phase, Groups 1 and 3- insulin sensitivity measurements and Groups 2 and 4- 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 determine the optimal electroporation conditions in order to reach the best possible transfection efficiency. Parameters that have to be optimized are variations in electric pulse, the optimal concentration of vector injected, and the duration of overexpression of the gene (dependent on the half-life of the protein). Based on our experience from previous studies (DEC 2010-036 and DEC 2006-024), a total of 5 pilot rats per gene should be sufficient to carry out the abovementioned optimization. If required, the amount of pilot rats necessary for determination of the optimal electroporation conditions can be responded to the DEC.

Group 1 and 3 (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 1 and 3 together.

Group 2 and 4 (mitochondrial function): Besides insulin sensitivity we are also interested in the mitochondrial function. Therefore, we will need tissue that is not treated with insulin. For this reason we need parallel groups that receive the same treatment but do not get a hyperinsulinemic/euglycemic clamp. 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 2 and 4 jointly.

The total number of test animals is 180 based on the following calculations.
Pilot = 5 (group A: pilot) x 4 genes = 20.
Experiment group 1: 11 (insulin sensitivity) x 4 genes = 44 rats.
Experiment group 2: 9 (mitochondrial function) x 4 genes = 36 animals.
Experiment group 3: 11 (insulin sensitivity) x 4 genes = 44 rats.

Experiment group 4: 9 (mitochondrial function) x 4 genes = 36 animals.

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.

Per gene the rats will be divided over a pilot group (group A) and 4 experimental groups (1, 2, 3, 4). Two of the 4 groups (1 and 2) will get a high fat (HF) diet. The other two groups (3 and 4) will receive the normal chow diet. Groups 1 and 3 will receive a hyperinsulinemic/euglycemic clamp in order to determine the insulin sensitivity. Groups 2 and 4 will not get this clamp so that mitochondrial function can be determined.

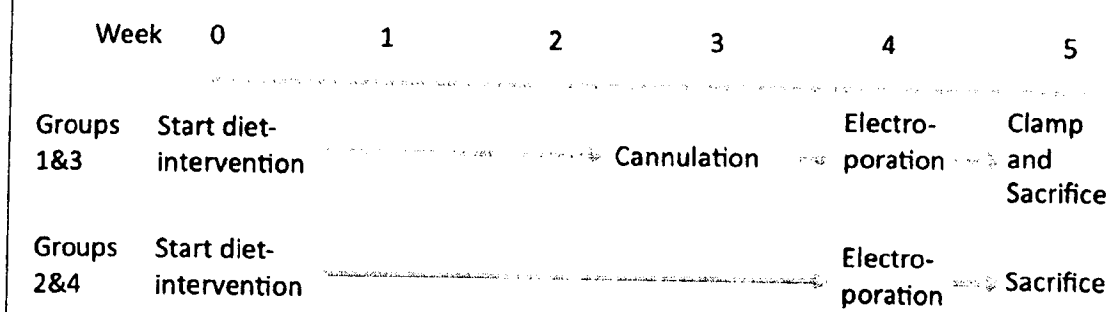
Pilot (Group A) – Optimization phase

12-week old rats will animals will undergo an electroporation procedure (SOP 2) to induce overexpression of the specific gene. The electroporation procedure introduces exogenous DNA into the skeletal muscle genome by electro-stimulation (200V/cm). According to the scheme below, the electroporation procedure will be combined with the cannulation surgery in week 4 in order to reduce the harm for the animals. Vector concentrations are varied between left and right legs. This approach will generate the necessary information on transfection efficiency, duration of gene overexpression and optimal vector concentration needed for the main experiment. All rats in this group will be sacrificed at week 5.

Experiments (Groups 1, 2, 3, and 4)

8-week old male Wistar rats purchased from Charles River Laboratories will be placed on either low fat (LF) chow or a high fat (HF) diet for 5 weeks in order to induce insulin resistance. Food intake and rat body weight will be measured weekly. After 3 weeks of diet intervention rats from group 1 and 3 will undergo a cannulation operation (SOP 1) for insulin sensitivity measurements via a hyperinsulinemic-euglycemic clamp, which will be performed after the dietary intervention (at week 5). The jugular vein and carotid artery are cannulated to sample blood and infuse glucose and insulin during the clamp. In week 4 the rats will be electroporated (SOP2). One week after the electroporation, groups 1 and 3 will undergo the hyperinsulinemic-euglycemic clamp (SOP 3) with the simultaneous infusion of labeled deoxyglucose to determine muscle-specific glucose uptake. After the clamp the rats will be sacrificed. Groups 2 and 4 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.

Study outline:



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, rats from groups 2 and 4 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 rats from group A, 1, and 3 will be sacrificed by an overdose injection of pentobarbital (200mg/kg IV or 1/10 diluted IP). Rats from group 2 and 4 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 these rat groups (2 and 4).

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).

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 electroporation protocol is estimated to cause a moderate discomfort (category 03).

Group 1 and 3 rats: 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 electroporation procedure (SOP2) (intramuscular injection with simultaneous transcutane electro-stimulation) will be performed after 4 weeks of diet intervention. The degree of discomfort experienced by the rats for the electroporation is estimated to be moderate (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 cannulation 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 2 and 4 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 1, 2, 3, and 4 rats: During the diet intervention period the rats will be housed individually to obtain food intake information. It is important that the rats are housed in clean rooms to prevent infections via the cannula. For groups 1 and 3, the cannulation operation is performed in room 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	Certified
	Certified and experienced	Certified and experienced
	Certified and experienced	Certified and experienced
	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

Aan:

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

Uw referentie:

Onze referentie : I

Maastricht, 31-08-2011

Geachte Onderzoeker,

Uw projectaanvraag: "*Overexpressing the genes FASN, Glut1, beta-HAD, and PDP: Manipulating metabolic flexibility in skeletal muscle reveals the mechanism of insulin resistance*", is op de DEC vergadering van 26 augustus 2011 besproken.

De DEC heeft een aantal vragen en opmerkingen:

- De DEC verzoekt het privételefoonnummer van de verantwoordelijke onderzoeker en de vervangend verantwoordelijke onderzoeker op het voorblad te vermelden.
- De aantallen op het voorblad en bij punt 7c stemmen niet overeen. De DEC verzoekt dit in overeenstemming te brengen.

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 **2011-118**, gelieve dit nummer in verdere correspondentie te vermelden.

Hoogachtend,

Voorzitter DEC-UM

Dear DEC committee members,

We have revised our protocol DEC 2011-118: "Overexpressing the genes FASN, Glut1, beta-HAD, and PDP: Manipulating metabolic flexibility in skeletal muscle reveals the mechanism of insulin resistance", to address the concerns listed below.

De DEC verzoekt het privételefoonnummer van de verantwoordelijke onderzoeker en de vervangend verantwoordelijke onderzoeker op het voorblad te vermelden.

The private gsm telephone numbers of both (VO) and VVO) have been added in the voorblad sheet.

De aantallen op het voorblad en bij punt 7c stemmen niet overeen. De DEC verzoekt dit in overeenstemming te brengen.

The animal numbers for each experimental group are now clearly marked in section 7c and changes have been made to the voorblad sheet.

Thank you.

Kind regards,

Maastricht University
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Maastricht 6200 MD
The Netherlands
Phone:
Fax:
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Maastricht University

Faculty of Health, Medicine and Life Sciences

Aan:

Ons kenmerk	*Doorkiesnummer*	*Maastricht*
		05-09-2011

Project: *Overexpressing the genes FASN, Glut1, beta-HAD, and PDP: Manipulating metabolic flexibility in skeletal muscle reveals the mechanism of insulin resistance.*

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:	**2011-118**
Diersoort:	**rat**
Aantal dieren:	**180**
Einddatum:	**05-09-2015**

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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