Video Article

An Experimental Model of Diet-Induced Metabolic Syndrome in Rabbit: Methodological Considerations, Development, and Assessment

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Abstract

In recent years, obesity and metabolic syndrome (MetS) have become a growing problem for public health and clinical practice, given their increased prevalence due to the rise of sedentary lifestyles and unhealthy eating habits. Thanks to animal models, basic research can investigate the mechanisms underlying pathological processes such as MetS. Here, we describe the methods used to develop an experimental rabbit model of diet-induced MetS and its assessment. After a period of acclimation, animals are fed a high-fat (10% hydrogenated coconut oil and 5% lard), high-sucrose (15% sucrose dissolved in water) diet for 28 weeks. During this period, several experimental procedures were performed to evaluate the different components of MetS: morphological and blood pressure measurements, glucose tolerance determination, and the analysis of several plasma markers. At the end of the experimental period, animals developed central obesity, mild hypertension, pre-diabetes, and dyslipidemia with low HDL, high LDL, and an increase of triglyceride (TG) levels, thus reproducing the main components of human MetS. This chronic model allows new perspectives for understanding the underlying mechanisms in the progression of the disease, the detection of preclinical and clinical markers that allow the identification of patients at risk, or even the testing of new therapeutic approaches for the treatment of this complex pathology.

Video Link

The video component of this article can be found at https://www.jove.com/video/57117/

Introduction

Obesity and metabolic syndrome (MetS) have become a growing problem for public health and clinical practice, given their increased prevalence due to the rise of sedentary lifestyles and unhealthy eating habits[1]. There are several definitions of MetS, but most of them describe it as a cluster of cardiovascular and metabolic alterations such as abdominal obesity, reduced HDL and elevated LDL cholesterol, elevated triglycerides, glucose intolerance, and hypertension[2,3,4]. Diagnosis requires that three out of these five criteria are present.

Owing to animal models, basic research has been able to investigate the mechanisms underlying pathological processes such as MetS. Several animal models have been used, but it is of crucial importance that the model of choice reproduces the main clinical manifestations of the human pathology (**Figure 1**). With this aim, animal models considered similar to humans, mainly canine and swine, have been developed (see Verkest[5] and Zhang & Lerman[6] for review). However, canine models do not show all the components of MetS, given that the development of atherosclerosis or hyperglycemia in dogs by means of the diet is questionable[5]. Swine models present the most anatomical and physiological similarity with humans, and thus offer significant predictive power for elucidating the mechanisms underlying MetS, but their maintenance and the complexity of the experimental procedures make the use of this model very labor intensive and costly[6].

On the other hand, rodent models (mouse and rat), diet-induced spontaneous and transgenic, have been extensively used in the literature for the study of obesity, hypertension, and MetS, and its pathological consequences in different organs and systems (see Wong *et al.*[7] for review). Although the use of these models is more affordable than canine or swine, they have important drawbacks. Indeed, depending on the strain, animals develop some components of MetS, whereas others such as hypertension, hyperglycemia, and hyperinsulinemia are absent[7]. Furthermore, one of the main components of MetS, obesity, in some genetically modified strains, does not only depend on factors associated with the diet, rather it has been shown that some animals become obese with normal or even reduced food intake[8]. Finally, mice and rats show a natural deficiency in cholesteryl ester transfer protein (CETP) and use HDL as the major means of cholesterol transport, which makes them

relatively resistant to the development of atherosclerosis. This is an important difference in lipid metabolism with humans, who express CETP and transport their cholesterol mainly in LDL[9].

Conversely, the laboratory rabbit represents an intermediate stage between the larger animal and rodent experimental models. Thus, the rabbit can be easily submitted to different types of protocols with minimal requirements of personnel and maintenance, being more easily handled in experimental procedures than larger animal models. Furthermore, it has been reported that rabbits fed with a high-fat diet have similar hemodynamic and neurohumoral changes as obese humans[8,10,11]. Of note, regarding lipid metabolism, the rabbit has abundant CETP in plasma and their lipoprotein profile is LDL-rich[12], which is also similar to humans. Additionally, rabbits develop hyperlipidemia quite rapidly given that, as herbivores, they are very sensitive to dietary fat[13].

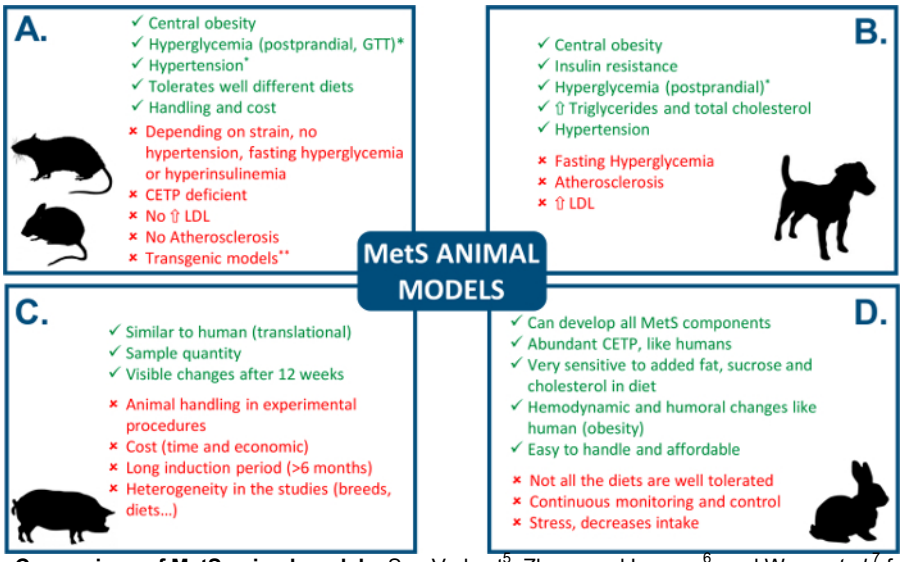

Figure 1: Comparison of MetS animal models. See Verkest[5], Zhang and Lerman[6], and Wong *et al.*[7] for review. "✓" indicates an advantage and "×" indicates a disadvantage. *controversial, depends on the study, **as pointed out by Carroll *et al.*[8], some genetically modified strains become obese independently of food intake. CEPT: cholesteryl ester transfer protein. GTT: glucose tolerance test. Please click here to view a larger version of this figure.

In order to elucidate the basic mechanisms underlying the pathological remodeling produced by MetS in the different organs and systems, and to gain understanding of this complex pathology, the choice of an experimental model that reproduces the main components of human MetS is essential. The rabbit can provide many advantages given its similarity with human physiology and the affordability of use in chronic protocols and measurements. In this line, few diet-induced rabbit models using high-fat and high-sucrose diet have been used[14,15,16,17,18,19] (**Table 1**), and a characterization of the different components of MetS is of great importance when relating a phenotype with organ remodeling. Thus, this article's main objective is to describe the methods to develop a model of diet-induced MetS in rabbits that allows the study of its pathophysiology and impact in organ remodeling.

Study	Diet	Duration	Breed	MetS components			
				Ob	HT	HG	Dl
Yin et al. (2002)[14]	· 10% fat · 37% sucrose	24 weeks	· Male NZW · 2 kg	✗	-	✓	✓
Zhao et al. (2007)[15]	· 10% fat · 30% sucrose	36 weeks	· Male JW · 16 weeks	✓	✗	✗	✗
Helfestein et al. (2011)[16]	· 10% fat · 40% sucrose · 0.5-0.1 cholesterol	24 weeks	· Male NZW · 12 weeks	✗	-	✓	✓
Ning et al. (2015)[17]	· 10% fat · 30% fructose*	8-16 weeks	· Male WHHL · 12 weeks	✗	-	✗	✓
Liu et al. (2016)[18]	· 10% fat · 30% sucrose	48 weeks	· Male NZW · 12 weeks	✗	-	✓	✓
Arias-Mutis et al. (2017)[19]	· 15% fat	28 weeks	· Male NZW	✓	✓	✓	✓

Table 1: Diet-induced MetS rabbit models using high-fat, high-sucrose diet. The symbol "✗" indicates absence, "✓" presence, and "-" not evaluated. *restricted. WHHL, Watanabe heritable hiperlipidemic rabbits. JW, Japanese white rabbits. Ob, obesity. HT, hypertension. HG, hyperglycemia. Dl, dyslipidemia.

Protocol

Animal care and the experimental protocols used in this study complied with EU directive 2010/63 on the protection of animals used for scientific purposes, and were approved by the Institutional Animal Care and Use Committee (2015/VSC/PEA/00049).

NOTE: The protocol consists of the chronic administration of a high-fat, high-sucrose diet for 28 weeks, and the assessment of the main components of MetS. We used 11 adult male New Zealand White (NZW) rabbits weighing 4.39 ± 0.14 (s.d.) kg, which were 20 - 22 weeks old at the beginning of the experimental protocol. They were housed in a room with humidity (50 ± 5%) and temperature (20 ± 1.5 °C) controlled conditions with a 12-h light cycle. The words "chow" and "diet" may be used interchangeably in the protocol steps.

1. Diet Administration

1. **Obtain or prepare diets**
   1. Obtain a commercially available **high fat diet** with added hydrogenated coconut oil (10%) and lard (5%)[19]. This diet will provide 3.7 kcal·g^{-1}.
   2. Prepare 5 to 15% **sucrose solutions** by dissolving the appropriate amounts of sucrose in sterilized water (*e.g.*, use 300 g sucrose in 2 L stock for a 15% sucrose solution). A 15% solution will provide 0.6 kcal·mL^{-1}.
   3. Obtain a commercially available **control diet**[19], which provides 2.7 kcal·g^{-1}.

2. **Acclimate the animals for 4 weeks**
   1. Feed each animal in the control group 120 g of control diet daily. Provide water *ad libitum*.
   2. Feed animals in MetS group 250 g chow starting with a 50% control and 50% high-fat chow, increasing progressively to 100% high-fat chow by the end of week 4.
      NOTE: The aim would be to achieve: (i) 35% control and 65% high-fat chow by the end of week 1; (ii) 25% control and 75% high-fat chow by the end of week 2; (iii) 15% control and 85% high-fat chow by the end of week 3. (iv) 100% high-fat chow by the end of week 4.
   3. Give animals in the MetS group water with 5% sucrose at the start, and increase sucrose concentration to 15% by the end of the 4th week.
   4. Register the daily intake of chow and sucrose solution to calculate caloric intake as per values provided in 1.1.1. and 1.1.2.

3. **Induce MetS (28 weeks)**
   1. Feed each animal in control group 120 g of control chow and water *ad libitum* daily.
   2. Feed the animals in the MetS group 250 g of high-fat chow and 15% sucrose in water. Replace chow daily and the sucrose solution every third day.
   3. Weigh the remaining chow and water daily to estimate daily intake.

2. Morphological Assessment

1. Measure animal **bodyweight** on a weekly basis.
2. Measure **height**, **length**, **abdominal contour**, and **tibial length**, and estimate **BMI** before administration of the experimental diet and at weeks 14 and 28 in anesthetized animals.
    1. Cannulate right ear marginal vein with a sterile disposable catheter (18 - 22 G) and inject propofol (8 $mgkg^{-1}$) followed by 1.5 mL of 0.9% NaCl solution. In the anesthetized rabbit, perform the measurements listed in the subsequent steps.
    2. Measure height and length. Using measuring tape, measure and record the distance from the nose to the heel in lateral decubitus position (length). In the same position, take the distance from the acromion in the shoulder to the tip of the paw (height).
    3. Calculate Body mass index (BMI)[20] as bodyweight (kg) · [body length (m) × height (m)]$^{-1}$.
    4. Place the measuring tape gently around the abdominal contour and take a measurement with the animal in supine position.
    5. Measure tibial length from the lower part of the knee joint to the insertion of Achilles tendon.

3. Fasting Glycemia and Intravenous Glucose Tolerance Test (IVGTT)

NOTE: It is advisable to start the procedures the same time of day (*i.e.*, 2 - 3 PM).

1. Prepare a glucose stock solution (60%) with 60 g glucose in 100 mL of 0.9% NaCl solution.
2. Fast the animal for 7 h (removing food and maintaining water), then place the conscious rabbit in a restrainer in the prone position. Prepare the glucose meter (insert a new strip into the meter), and take the first sample from the left ear marginal vein using a lancet to get a drop of blood. Then touch the blood drop with the test strip and measure blood glucose levels using the glucose meter to determine fasting glycemia.
3. Cannulate right ear marginal vein with a disposable catheter (18 - 22 G) and inject a bolus of a 60% glucose solution (0.6 $g{\cdot}kg^{-1}$).
   NOTE: To prepare the bolus, add 1 mL/kg of the glucose stock.
4. Take blood samples using the lancet (one drop of blood) at 15, 30, 60, 90, 120, and 180 min after glucose injection and analyze them with the glucose meter as in 3.2.
5. Remove the disposable catheter and pinch the site of catheter insertion with a gauze. Once blood has coagulated, remove the gauze and check the status of the animal.

4. Blood Pressure

1. Prepare the acquisition system including a pressure transducer, a 10-mL syringe with 0.9% NaCl, a three-way stopcock, an amplifier, and a PC/laptop with the acquisition software (for blood pressure recording).
2. Set up the equipment. First, place the three-way stopcock and the syringe in the pressure transducer, between the transducer and the catheter, and connect the pressure transducer to the amplifier. Then connect the amplifier to the PC/laptop.
3. Perform the pressure transducer calibration according to the manufacturer's recommendations.
4. Place the conscious animal in a rabbit restrainer in the prone position. Warm up the ear before cannulation, then topically apply a local anesthetic (2.5% lidocaine/prilocaine) in the ear around the site of insertion. Gently tap the area where the vascular package runs to easily identify the artery. Then insert a sterile catheter (18 - 22 G) in the left ear central artery. Loosen the restraints and allow the animal to stay quiet for 30 min.
5. Record blood pressure continuously for 20 min directly from the arterial catheter, placing the pressure transducer positioned next to the animal at the heart level (sampling frequency: 1 KHz, see **Figure 5B**).
   NOTE: To keep the blood pressure (BP) recording free from blood coagulation interference (BP signal loses amplitude or disappears), an NaCl (0.9%) injection should be made. Using the three-way stopcock, close the circuit that goes from the transducer to the catheter, open the circuit that goes from the syringe to the catheter, and inject 1 - 2 mL. This will remove blood clots that may form in the catheter. Then, open the circuit between the transducer and the catheter, and continue the recording once the signal has been recovered.
6. Once the recording is finished, remove the catheter and pinch with a gauze in the site of catheter insertion to stop blood loss. Once the blood has coagulated, remove the gauze and check the status of the animal.

5. Plasma Measurements

NOTE: It is advisable to start the procedures the same time of day (*i.e.*, 2 - 3 PM).

1. Fast the animal for 7 h (removing food and maintaining water), then place the conscious animal in a restrainer in the prone position and insert a sterile 21 G needle in the left ear marginal vein. Once blood begins to drip, discard the first drop and collect the blood samples in EDTA tubes up to the level indicated in the tube. Store the samples on ice.
2. Centrifuge blood samples at 1,500 x g, 15 min, 4 °C. After centrifugation, suction the plasma using a pipette and prepare aliquots of 250 µL.
3. Analyze the fresh samples immediately. Basic control parameters are as follows: triglycerides, total cholesterol, HDL, and LDL cholesterol.
   NOTE: Samples not freshly analyzed should be stored immediately in a -80 °C freezer. If interested in analyzing blood glucose from plasma samples, the blood glucose test should use tubes with Fluoride Oxalate instead of EDTA.

Representative Results

MetS represents a cluster of metabolic and cardiovascular abnormalities whose study can be facilitated by the use of experimental models. Indeed, to elucidate the mechanisms underlying the pathological remodeling produced by MetS, the choice of an experimental model that appropriately resembles the human condition and is suitable for research is of crucial importance. Here, we present the methods to induce MetS in rabbit using a diet high in saturated fat and sucrose, and a detailed characterization for its evaluation. The use of diet instead of a genetically modified animal model is of great importance since diet affects whole-body metabolism[19], thus resembling closely what happens in human MetS. We used a factorial (mixed model) ANOVA with two factors, one repeated measures or "within" factor (time: pre, week 14 and week 28, depending of the analysis) and one "between" factor (group: control and MetS) for statistical analysis. Significance was accepted when p <0.05.

The high-fat, high-sucrose diet is well tolerated by the animals. An acclimation period of 4 weeks is necessary for the correct transition from the previous feeding regime to the high-fat, high-sucrose diet. Animals in control group are fed 120 g chow, which has been shown to be appropriate for the maintenance of the adult rabbit[8]. Rabbits in the MetS group increased progressively in weight until the end of the experimental protocol (**Table 2**). Animals should gain 50 - 100 g per week. It is important that rabbits are housed individually in cages with enough space, light, and environmental enrichment (**Figure 2C**), and a daily check of the animals is performed. Also on a daily basis, chow and drink intake must be supervised and registered, in order to achieve weight gain and detect possible health problems, since rabbits are easily stressed and the response may be to stop nourishment consumption. In addition, since high-fat pellets tend to be very unstable and lose consistency very easily, turning into powder which rabbits do not eat, it is of critical importance to prepare the daily portions of chow very carefully (**Figure 2A**). In **Figure 3A**, we can observe the behavior of energy intake and its fluctuations, ranging from 250 to 815 kCal in the MetS group. In **Figure 3B**, the relative contribution of the different sources of energy (chow and drink) is depicted. There are critical periods in weeks 14 and 28 because, given the stress produced by experimental procedures, rabbits might decrease chow and water intake. The daily quantification allows the rapid identification of this problem, which can be avoided by introducing control chow (80% high-fat, 20% control) and decreasing the sucrose solution from 15% to 10%, or even 5%, during 2-3 days until animals recover their normal intake values. Animals also developed central obesity as shown by the increase in weight, abdominal perimeter and BMI (**Table 2**), which is closely related with the risk factors that define MetS[3].

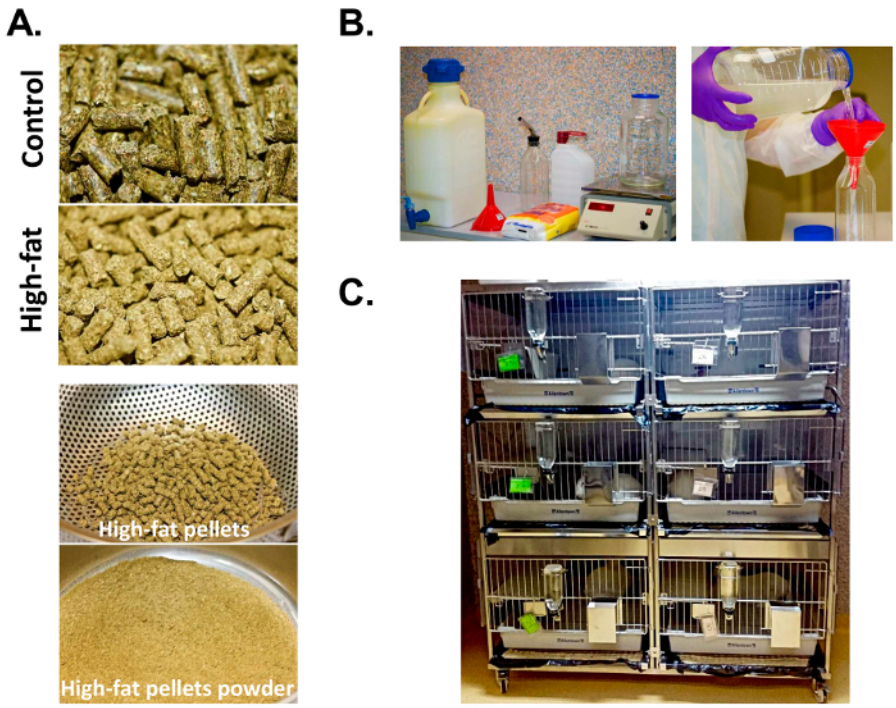

Figure 2: Diet administration. In panel **A**, control chow (top) and high-fat chow (below) are depicted, showing the differences between the two due to the added fat. In order to avoid the powder that makes high-fat pellets less palatable, it is necessary to use a strainer to separate high-fat pellet powder (Panel A, bottom). In panel **B**, we can observe the materials needed to make the drinking solution (left), and how it is advisable to make a stock solution to distribute in the water dispenser. Lastly, the welfare of the animals is very important, and they must be housed individually in cages (C) with enough space and light and, if possible, environmental enrichment (*i.e.*, platforms, toys, *etc.*). Please click here to view a larger version of this figure.

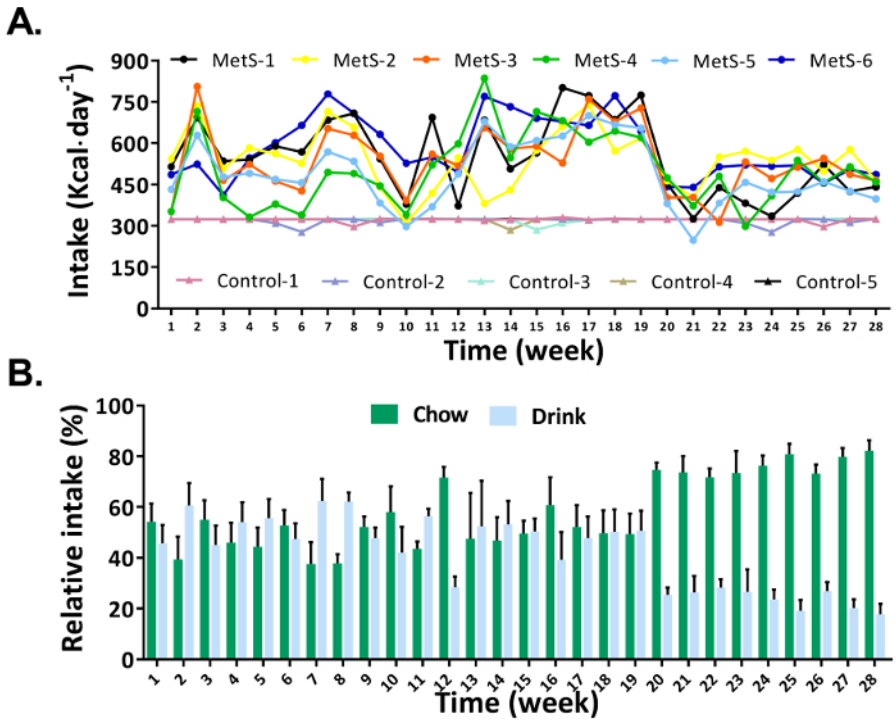

Figure 3: Energy intake. The evolution of weekly intake during the 28 weeks of the experimental period is depicted in panel A for control and MetS. The relative intake (in percentage) of kCal from high-fat chow and the drinking solution of MetS animals is shown in panel B. Control (n = 5), MetS (n = 6). Error bars: SD. Modified from Arias-Mutis *et al.*[19] Please click here to view a larger version of this figure.

	Pre-diet		Week 14		Week 28	
	Control	**MetS**	**Control**	**MetS**	**Control**	**MetS**
Weight (Kg)	4.35(0.15)	4.43(0.14)	4.49(0.12)	5.42(0.17)	4.51(0.13)	5.75(0.6)
Length (cm)	52.4(1.6)	53.6(1.7)	52.5(0.8)	54.4(1.7)	53.7(0.7)	54.6(0.8)
Height (cm)	25.9(0.7)	25.5(1.1)	25.9(2.2)	26.1(5.3)	26.0(1.0)	26.1(1.5)
Abdom. perimeter (cm)	39.8(1.7)	40.5(1.4)	38.5(1.5)	47.5(2.2)	38.1(1.0)	49.7(3.5)
Tibial length (cm)	16.4(0.8)	16.3(0.7)	16.7(0.3)	16.7(0.4)	17.4(0.4)	16.8(0.6)
BMI (Kg/m^2)	32.8(1.9)	32.9(2.6)	32.8(1.2)	36.8(1.9)	32.6(2.1)	39.3(6.0)

Table 2: Morphological characteristics. We found differences when comparing control vs. MetS at weeks 14 and 28 in weight (main effect p = 0.003, η^2 = 0.6; pairwise comparisons at week 14 p <0.001 and week 28 p <0.001), abdominal perimeter (main effect p<0.001, η^2 = 0.9 pairwise comparisons at week 14 p <0.001 and week 28 p <0.001), and BMI (main effect p = 0.016, η^2 = 0.5; pairwise comparisons at week 14 p <0.001 and week 28 p <0.001). Control (n = 5) and MetS (n = 6). Values are expressed as Mean (SD). Modified from Arias-Mutis *et al.*[19].

Regarding fasting blood glucose, the response to the IVGTT plays a key role in the characterization of glucose homeostasis[21]. We observe mild hyperglycemia at week 14, which reaches a plateau and maintains similar values at week 28 (**Figure 4A**). The area under the curve (AUC) also increases in the MetS group (**Figure 4B**). Even though cut-off values to identify type II diabetes in rabbits based on fasting blood glucose have not yet been recognized[19], with this experimental protocol, rabbits submitted to 28 weeks of high-fat, high-sucrose feeding developed pre-diabetes with impaired fasting glucose and glucose intolerance.

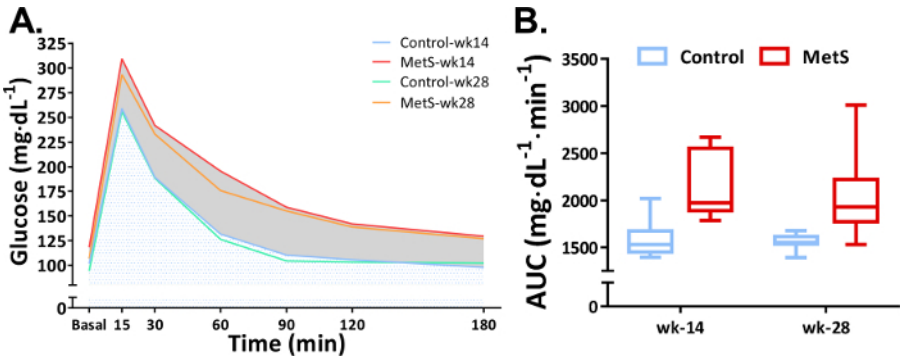

Figure 4: Blood glucose regulation. The results of the IVGTT in control and MetS animals at weeks 14 and 28 are shown in panel A. The quantification of the area under the curve (AUC) from 0 to 180 min is depicted in panel B with a box and whiskers plot. This parameter increased in MetS animals in weeks 14 and 28 versus controls (main effect p = 0.001, η^2 = 0.5; pairwise comparisons at week 14 p = 0.001 and week 28 p = 0.002). Control (n = 5), MetS (n = 6). Modified from Arias-Mutis *et al.*[19] Please click here to view a larger version of this figure.

Hypertension is closely and directly related to the severity of obesity. Rabbits fed a high-fat, high-sucrose diet for 28 weeks showed an increase in systolic, diastolic, and mean blood pressure already at week 14, and this increase in blood pressure is maintained at week 28 (**Figure 5C - E**). Given the close relationship between blood pressure and BMI[22], it is of great importance to ensure that animals gain weight progressively to obtain a significant increase in blood pressure.

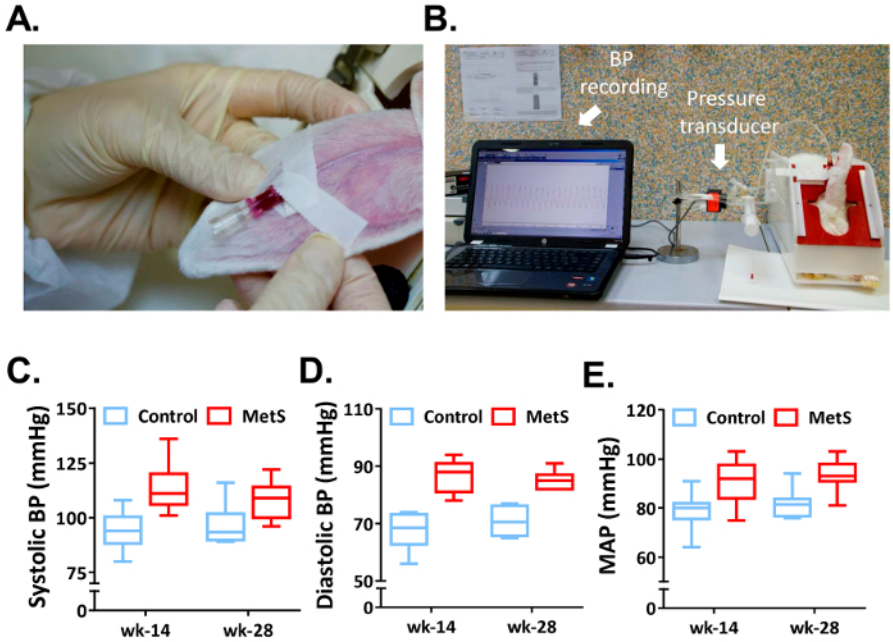

Figure 5: Modifications in blood pressure. Panel A depicts the catheter inserted in the auricular artery. Of note, given that the vein and the auricular artery run through the dentition of the ear very closely, it is of crucial importance to differentiate them. Before cannulation, it is advisable to warm up the ear and, after topical anesthesia, to tap gently the area where the vascular package runs. The artery has a thicker vascular wall and a lighter color than the vein, and blood pulses can be observed. Panel B shows the experimental setup with the pressure transducer, which is connected to an amplifier and records continuously the signal (BP recording). Panels C and D show box and whiskers plots of systolic and diastolic blood pressure at week 14 and 28 in both experimental groups. Mean arterial pressure (MAP) is presented in panel E. We found differences when comparing control vs. MetS at weeks 14 and 28 in systolic (main effect p = 0.003, η^2 = 0.4; pairwise comparisons at week 14 p = 0.029 and week 28 p = 0.013), diastolic (main effect p = 0.027, η^2 = 0.3; pairwise comparisons at week 14 p = 0.036 and week 28 p = 0.001) and MAP (main effect p = 0.006, η^2 = 0.4; pairwise comparisons at week 14 p = 0.027 and week 28 p = 0.001). Control (n = 5), MetS (n = 6). Modified from Arias-Mutis *et al.*[19] Please click here to view a larger version of this figure.

Finally, to assess the development of MetS, an evaluation of changes in plasma biochemical markers is needed. In this chronic model, we observed an alteration in the lipid profile as early as week 14, and this alteration remained stable until week 28, without further increases in the differences. Modifications in plasma lipid profile are characterized by an increase in triglycerides and LDL, a decrease in HDL, and no changes in total cholesterol in MetS animals versus controls at both time points (weeks 14 and 28) (**Table 3**).

	Week 14		Week 28	
	Control	MetS	Control	MetS
Total cholesterol ($mg \cdot dL^{-1}$)	20.4(2.3)	24.0(9.1)	27.4(15.7)	21.2(4.4)
HDL ($mg \cdot dL^{-1}$)	9.1(4.2)	4.3(1.7)	11.2(4.2)	5.1(2.9)
LDL ($mg \cdot dL^{-1}$)	3.8(1.1)	8.7(4.5)	4.0(1.2)	13.8(9.3)
Triglycerides ($mg \cdot dL^{-1}$)	71.2(58.8)	118.0(40.7)	30.2(11.4)	76.8(28.2)

Table 3: Assessment of plasma biochemistry. We found differences when comparing control vs. MetS at weeks 14 and 28 in HDL (main effect p = 0.008, η^2 = 0.3; pairwise comparisons at week 14 p = 0.006 and week 28 p = 0.037), LDL (main effect p = 0.040, η^2 = 0.2; pairwise comparisons at week 14 p = 0.028 and week 28 p = 0.034), and triglycerides (main effect p = 0.002, η^2 = 0.4; pairwise comparisons at week 14 p = 0.004 and week 28 p = 0.001). Control (n = 5) and MetS (n = 6). Values are expressed as Mean (SD). Modified from Arias-Mutis *et al.*[19]

Discussion

The establishment of an appropriate experimental model can provide a more consistent and reliable method to study the development of MetS, and it is also necessary to understand the basic mechanisms that underlie the organs and systems remodeling. Here, we describe the methods used to develop a relevant experimental model of diet-induced MetS and how to assess the main components of this cluster of metabolic and cardiovascular abnormalities that characterize this model: central obesity, hypertension, glucose intolerance, and dyslipidemia with low HDL, high LDL, and an increase of TG levels.

A major strength of the model is the ability to study the condition that precedes the clinical manifestation of the pathology. Indeed, regarding metabolic changes, in 28 weeks animals did not develop type II diabetes and were in a state of prediabetes (**Figure 4**). Similarly, plasma biochemical markers showed an evident alteration in the lipid profile with an increase in LDL and TG, a decrease in HDL, but no changes in total cholesterol (**Table 3**), which is a key factor in the development of atherosclerosis. Even though we can observe an increase in systolic, diastolic, and MAP at week 28 (**Figure 5**), this can be considered mild hypertension. Overall, the effects in those metabolic and cardiovascular markers are modest, but this model can enable the research of the state before the manifested (and in most cases irreversible) pathology, thus allowing the identification of preclinical and clinical markers that might allow the detection of patients at risk.

Furthermore, unlike other MetS animal models (mouse, rat, and dog), spontaneous or transgenic rabbits models can develop all the components of the MetS. Interestingly, it has been reported that the combination of the different components of MetS can amplify cardiovascular risk. Indeed, the pathological remodeling produced by hypertension is aggravated when more components of MetS appear[23]. This experimental model could allow the study of the underlying mechanisms, and the effect of the different components combined. Furthermore, given that diet affects whole-body metabolism, the use of a diet-induced model has important significance, closely emulating what happens in human MetS[19].

The last, but not least important strength, is the balance between the relevance and impact on translational research and the economic cost. On one side we find the swine models, very similar to humans, but very expensive in terms of time, resources, and economic cost. On the other side, we have rodent models, which are easy to implement with very little cost, but have a lower generalization power. The rabbit model represents the middle point, as it is flexible enough for many different types of studies while avoiding some of the drawbacks of large animal models, and shows similar hemodynamic and neurohumoral changes observed in human MetS[8,10,19].

The following limitations of the methods described should be considered. With respect to central obesity and body fat distribution, the use of magnetic resonance imaging would be the gold standard, if available, otherwise use the quantification of visceral fat at the end of the 28 weeks. Other non-invasive methods for longitudinal studies, such as X-ray computed tomography, would be more adequate[24]. We measured abdominal circumference and BMI instead (**Table 2**), which have also been used in several studies in rabbits as a measure of central obesity[25,11,26]. Tibial length measurement could also be more precise using echography or a leg radiography. In order to establish if the cause of glucose intolerance in this chronic model is insulin resistance or decreased insulin production, insulin resistance should be determined using an insulin tolerance test or determining fasting insulin levels.

Finally, in order to improve the model, several measures could be taken. We could probably have obtained a faster increase in glycaemia with the combination of brief periods of alloxan injection and the high fat, high sucrose diet, but then the phenotype could not be attributed only to the diet. Age could play also an important role, since we worked with young adult rabbits (4.5 months old when animals arrived in the animal facilities, 12.5 - 13 months old by the end of the experimental protocol) and MetS often occurs at older age[27]. Unfortunately, older rabbits were not commercially available. It would be interesting to test this model in older animals and observe if the phenotype is aggravated.

The methods presented here for the development of this experimental model of MetS in laboratory rabbits should provide a valuable tool for studies aiming to elucidate the basic mechanisms underlying the pathological remodeling produced by MetS in the different organs and systems, and to gain understanding of this complex pathology. Finally, since NZW rabbits are sedentary animals, this diet-induced model can be useful to study how the different components of the pathology evolve in a similar way than occurs in human MetS, and could allow new perspectives for understanding the pathophysiological mechanisms involved in the progression of the disease, the identification of preclinical and clinical markers to identify patients at risk, or even the testing of new therapeutic approaches for the treatment of this complex pathology.

Disclosures

The authors declare that they have no competing financial interests.

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