Evaluation of metabolic, endocrine and growth features in the Mexican hairless pig to determine its potential as model for obesity in comparison with commercial pigs

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Abstract

Biochemical, endocrine and growth performance variables were evaluated and compared in the Mexican hairless pig (MHP) and in Landrace-Yorkshire pigs (LYP) from first to ninth month of age in order to establish if the MHP could be a better model for the study of obesity than the LYP. Serum concentrations of total cholesterol (TC), triglycerides (TG), high-density lipoproteins (HDL), low-density lipoproteins (LDL), glucose, free fatty acids (FFA), and insulin were evaluated and compared in these variables. The results showed that the MHP partitioned more energy to backfat than the LYP (P<0.01), and had higher serum concentrations of insulin, TC, TG, LDL, and FFA than the LYP (P<0.05). Based on the high capacity of the MHP to deposit body fat, and its higher serum concentrations of insulin, TG, CT, LDL, and FFA, we conclude that the MHP is a better model for the study of obesity than the Landrace-Yorkshire pigs and could be used under experimental conditions as a model for obesity, particularly the observed in metabolically healthy but obese humans.

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Introduction

Obesity has been identified as a risk factor that predisposes to the development of different metabolic alterations (Kahn and Flier, 2000). Usually, obesity is characterized by high insulin concentrations and a range of abnormalities in lipid metabolism, such as increased triglycerides, low-density lipoproteins (LDL), very low-density lipoproteins (VLDL), and reduced high-density lipoproteins (HDL). However, there is a unique subset of metabolically healthy obese individuals (MHO), which appear to be protected against obesity related metabolic disturbances (Karelis et al., 2004a). These individuals despite having excessive body fatness display a favorable metabolic profile, characterized by high levels of insulin sensitivity and a favorable lipid profile (Dvorak et al., 1999). Evidence suggests that these obese individuals may account for as much as 20%-30% of the obese population (Karelis et al., 2004b). The research to develop a better animals model in biomedicine, has conducted to the use of swine in different areas of scientific research because of their similarity to humans with respect to digestive physiology, the endocrine system, intermediary metabolism (Panepinto and Phillips, 1986; Bhatthena et al., 1996), and like humans, pig carry a large fraction of total cholesterol in LDL (Swinkels and Demacker, 1988; Dixon et al., 1999).

The Mexican hairless pig is an unselected genotype originated from the Iberic, Celtic, Napolitano, and Asiatic pigs that were introduced to Mexico during the Spanish colonization (Flores, 1970). This kind of pig belongs phylogenetically to a genetic lineage not related to the commercial modern breeds (Lemus-Flores et al., 2001). With a good food supply, the Mexican hairless pig can accumulate up to 45% of its body mass as adipose tissue (Cárdenas, 1966). Very little is known about the biochemical and endocrine features associated to the high adiposity of this kind of pig and about the similarities that it could have with human obesity.

Therefore, the objective of this study was to compare some biochemical, endocrine and growth performance features including: backfat deposition, body weight, feed intake, serum lipids profile and serum glucose, insulin, growth hormone, free fatty acids concentrations between Landrace-Yorkshire pigs and Mexican hairless pigs, as an initial step in the characterization of the Mexican hairless pig, in order to determine if it can be used as a model for the study of obesity and its associated complications.

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Materials and methods

Animals and diet

All experimental procedures were conducted in accordance with the animal care committee of the National Autonomous University of Mexico. Twelve Mexican hairless female pigs and 14 crossbred Landrace-Yorkshire female pigs of 28 days of age with an average weight of 5.2±1.03 and 7.6±0.95 kg (mean±S.D) were used in this study. The Mexican hairless pigs were housed in two elevated pens with six animals each, and the Landrace-Yorkshire pigs were housed in two elevated pens with seven animals each. All pigs were kept in the weaning room until 12 weeks of age, after this period the animals were moved to a growth area with cement floor pens. In both installations, the pigs were provided with hopper feeders, nipple drinkers, natural ventilation and gas heater only in the weaning room. In order to evaluate the biochemical, endocrine and growth performance variables included in this study, the pigs were fed ad libitum a diet sorghum-soybean with 5% higher energy content than the one recommended by the National Research Council (1998) (Table 1) rather than a western type high fat diet.
Serum collection

Every fifteen days and following an overnight fast, blood samples from each pig were collected via venipuncture into vacutainer tubes (Becton, Dickinson and Co., Oakville, Ontario, Canada). The blood was incubated for two hours at room temperature to permit clotting and then centrifuged at 3000g for 10 min. Serum samples were used immediately after centrifugation to assay the lipid profile and glucose concentrations, the remaining serum was stored at -20°C for hormone analysis, and free fatty acids assay.

Lipid profile

The lipid profile was determinate by enzymatic colorimetric methods. Total cholesterol (TC) was measured using the commercial kit Cholesterol esterase/Peroxidase CHOP-POD (Spinreact, S.A. Sant Esteve de Bas, Spain). The triglycerides concentration was measured using the kit Glycerol Phosphate Oxidase (GPO-PAD) (GPO-PA, Spinreact). The high-density lipoproteins (HDL), and low-density lipoproteins (LDL), were measured using the commercial kits (HDLColesterol-D, and LDL-cholesterol-D (Spinreact)); all tests were realized using an automatic clinical chemistry analyser (Spinlab 180, Spinreact).

Glucose concentrations

Glucose concentrations were measured in duplicate for each animal by the method Glucose Oxidase/Peroxidase (GOP-POD) using the commercial kit GOP-POD-Spinreact (Spinreact) and the automatic clinical chemistry analyser Spinlab 180, previously mentioned.

Free fatty acids

Free fatty acids concentrations were determinate in duplicate for each animal at 1, 3, 6, and 9 months of age by the ACS-ACOD method, using the commercial kit (NEFA-C, Wako Chemicals, Richmond, VA, USA).

Hormone assays

Insulin concentrations were determined each month from 1 to 9 months of age with the same method using the commercial kit (Porcine insulin RIA kit, Linco Research, Inc., St. Charles, MO, USA). The limit of sensitivity of the assay was 1 ng/mL, the intra-assay and inter-assay coefficients of variation were 7.9% and 9.4%, respectively.

Growth performance

The pigs were weighted every two weeks before feeding. Since the two genotypes are different in body size, the feed intake was measured as a percent of the body weight of the animals. The feeders were checked daily for proper feed flow to minimize wastage, and the drinkers were checked for adequate water flow. Feed refusals were collected and weighed weekly. Backfat thickness measurements were obtained every two weeks at the 10th rib using real time ultrasound fitted with a 3.5 MHz transducer (Aloka 500V, Corometrics Medical Systems, Wallingford, CT, USA).

Data analyses

To identify differences between genotypes in blood metabolites and hormone concentrations, growth performance, and food intake, the data were analyzed using an analysis of variance under a repeated measurements design that include the effects of the genotype, the age and the genotype by age interaction. The pen was used as the experimental unit for the analysis of feed intake, and the individual pig was considered as the experimental unit for the analyses of hormones, metabolites, body weight and backfat thickness.

A Shapiro-Wilk test was performed to determine if the data of the variables included in the study were normally distributed. Those variables that significantly deviate (P<0.05) from a normal distribution were transformed to fulfill the conditions of normality, logarithmic transformations were performed on insulin, backfat thickness and triglycerides data. Serum glucose was transformed as the reciprocal of the square root, and free fatty acid and high density lipoproteins concentrations were

Table 1. Dietary ingredients and chemical composition. The diets were formulated according to the NRC (National Research Council) requirements for commercial pigs at different growth phases, with the exception of the energy level.

| Ingredients | 1-2 | 2-3 | 3-4 | 4-9 |
|-------------|-----|-----|-----|-----|
| Sorghum 8% CP, kg | 764.0 | 783.0 | 738.0 | 802.0 |
| Soya protein concentrate, kg | 88.66 | ------ | 20.86 | ------ |
| Fish protein concentrate, kg | 50.00 | 49.45 | ------ | ------ |
| Soybean meal 44% CP, kg | 49.67 | 123.09 | 184.43 | 143.52 |
| Powder fat*, kg | 24.00 | 25.00 | 35.00 | 35.00 |
| Dicalcium phosphate 18/20, kg | 8.59 | 4.14 | 5.49 | 4.12 |
| Calcium carbonate 30%, kg | 7.81 | 7.76 | 8.35 | 8.24 |
| Sodium, kg | 2.50 | 2.50 | 2.50 | 2.50 |
| Vitamins premix², kg | 2.50 | 2.50 | 2.50 | 2.50 |
| Minerals premix³, kg | 1.50 | 1.50 | 1.50 | 1.50 |
| Choline 60%, kg | 0.40 | 0.40 | 0.40 | ------ |
| Lysine, kg | ------ | ------ | ------ | 0.469 |
| Metabolizable energy, Kcal/kg | 3400 | 3400 | 3400 | 3400 |
| Total lysine, % | 1.00 | 0.830 | 0.750 | 0.690 |
| Total threonine, % | 0.719 | 0.600 | 0.575 | 0.463 |
| Total valine, % | 0.987 | 0.840 | 0.793 | 0.661 |
| Calcium, % | 0.700 | 0.600 | 0.500 | 0.450 |
| Crude fibre, % | 2.20 | 2.50 | 2.77 | 2.62 |
| Dry matter, % | 88.71 | 88.64 | 88.73 | 88.59 |
| Crude protein, % | 16.00 | 15.29 | 15.36 | 12.79 |
| Total methionine, % | 0.340 | 0.323 | 0.248 | 0.214 |
| Total tryptophan, % | 0.190 | 0.189 | 0.194 | 0.157 |
| Total phosphorus, % | 0.600 | 0.500 | 0.450 | 0.400 |
| Ether extract, % | 5.31 | 5.30 | 5.63 | 5.69 |

The composition of the diet was estimated using tables of ingredients from the Department of Nutrition and Biochemistry, School of Veterinary Medicine and Animal Sciences, National Autonomous University of Mexico. °VANAGRASA 80CTM; fat powder with a base of lard/suneh, glucose syrup and milk derivatives; fatty composition: Lauric, 0.1%; Myristic, 1.0%; Palmitic, 45.0%; Palmitoleic, 0.2%; Margaric, 0.1%; Stearic, 4.5%; Oleic, 30.0%; Linoleic, 10.0%; Linolenic, 0.5%; Arachidic, 0%. #Provided the following per ton of diet: manganese, 30 g; zinc, 90 g; Fe, 90 g; Cu, 90 g; iodine, 0.15 g; selenium, 0.2 g; calcium, 24.3 g. §Provided the following per ton of diet: 8000 U of vitamin A, 6000 U of vitamin D₃, vitamin E, 15 g; vitamin K₂, 2 g; vitamin B₁, 4g; vitamin B₂, 4 g; vitamin B₆, 1.5 g; vitamin B₉, 18 mg; biotin, 62.5 mg; nicotinic acid, 20 mg; pantothenic acid, 8 g; of calcium, 62 g §Provided the following per ton of diet: 8000 U of vitamin A, 6000 U of vitamin D₃, vitamin E, 15 g; vitamin K₂, 2 g; vitamin B₁, 4g; vitamin B₂, 4 g; vitamin B₆, 1.5 g; vitamin B₉, 18 mg; biotin, 62.5 mg; nicotinic acid, 20 mg; pantothenic acid, 8 g; of calcium, 62 g.
transformed by squaring the data (Neter et al., 1996). Statistical analyses were performed with JMP 4.04 software (SAS, 2001)

**Results and discussion**

Swine have been considered a good animal model for the study of cardiovascular disease, diabetes (Ratcliffe and Lugninhb, 1971) and pathologies related with obesity due to similarities with the human in anatomy, organ development, digestive physiology, endocrine system, and intermediary metabolism (Bhathena et al., 1996; Lunney, 2007).

In this study, the Mexican hairless pigs have a higher level of fat deposition than de Landrace-Yorkshire pigs. At the beginning of the study, both genotypes had similar backfat thickness, but, at 12 weeks of age, the Mexican hairless pigs started to partition more backfat and this difference increased with age. At the end of the experimental period the backfat thickness was two fold higher in the Mexican hairless pigs than in the Landrace-Yorkshire pigs (F=64.67; df=1, 24; P<0.01) (Figure 1). This result indicates that the Mexican hairless pigs have a higher predisposition to convert food energy more readily into fat stores, and thereby higher propensity toward obesity, which can be an advantage for this kind of pig to be a model to study obesity.

There were no differences in the feed intake between the two genotypes (F=15.89; df=1, 2; P>0.05). The feed intake peaked at eight weeks of age and decreased gradually over the study period (Figure 2). These results suggest that the adiposity of the Mexican hairless pig did not result from a higher feed intake. This observation agrees with other studies in which there were no differences in feed intake between high fat pigs and low fat pigs of contemporary lines.

As it was expected, throughout the study period the mean body weight of the Landrace-Yorkshire pigs was higher than that of the Mexican hairless pigs (F=205.16; df=1, 23; P<0.01) (Figure 3). This result is due to differences in body composition, size and growth rate between the two types of pigs. The differences observed in our study are similar to those reported between Yorkshire pigs and Ossabaw pigs (Wangsness et al., 1977) an obese pig with a similar phenotype as the Mexican hairless pigs.

Obesity is associated with high serum insulin concentrations as result of insulin resistance (Klein et al., 2004; Utzschneider et al., 2006). There were significant differences

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**Figure 1.** Backfat thickness in the Mexican hairless pigs (n=12) (open square □) and Landrace-Yorkshire pigs (n=14) (solid square ■). Data points represent least squares means ± SE.

**Figure 2.** Feed intake in the Mexican hairless pigs (n=12) (open square □) and Landrace Yorkshire-pigs (n=14) (solid square ■). Data points represent least squares means ± SE.

**Figure 3.** Body weight in the Mexican hairless pigs (n=12) (open square □) and Landrace Yorkshire-pigs (n=14) (solid square ■). Data points represent least squares means ± SE.

**Figure 4.** Serum insulin concentrations in the Mexican hairless pigs (n=12) (open square □) and Landrace Yorkshire pigs (n=14) (solid square ■). Data points represent least squares means ± SE.
in serum insulin concentrations between genotypes (F=39.78; df=1, 24; P<0.05). At the beginning of the study both genotypes showed similar serum insulin concentrations, in the second month of age the Mexican hairless pigs started to have higher levels of this hormone than the Landrace-Yorkshire pigs and the difference was more notorious by the fourth month of age, which suggested an hyperinsulinemic state in the Mexican hairless pig at this age. However, from the 5th to the 9th month of age the insulin concentrations in the Mexican hairless pig started to decrease to levels similar to those observed at the beginning of the study (Figure 4). This finding are different to those reported by Sébert et al., (2005), who induced obesity and insulin resistance in overfeed Yucatan pigs. It is possible that the Mexican hairless pigs regulate its levels of insulin as they age regardless of its adiposity, and this could be a mechanism to prevent the development of insulin resistance. Future investigations are necessary to confirm what we observed in this study and to elucidate the mechanisms that regulate the insulin production in the Mexican hairless pigs, which could be useful to understand the mechanism underlying insulin resistance.

No differences in growth hormone concentrations between genotypes were found during the course of the study (F=0.61; df=1, 24; P>0.05). Fasting serum glucose concentrations did not differ between genotypes throughout the study period (F=0.05; df=1, 13; P>0.05) (Figure 5). This result suggests that Mexican hairless pigs despite of an excessive adiposity, apparently does not develop impaired fasting glucose levels. Something similar has been observed in some obese humans, who maintain normal glucose tolerance levels regardless of their high adiposity due to a compensatory hyperinsulinemia that is the result of insulin resistance (Utzschneider et al., 2006). In this study there was however no evidence of insulin resistance in the Mexican hairless pig.

In obesity state is common to observe cholesterol synthesis enhanced and cholesterol absorption efficiency decreased (Miettinen and Gylling, 2000). The total cholesterol concentrations in the Mexican hairless pigs were higher than in the Landrace-Yorkshire pigs (F=11.60; df=1,13; P<0.05). The cholesterol concentrations started to be higher in the Mexican hairless pigs at the two months of age, time in which this kind of pigs started to partition more backfat than the Landrace-Yorkshire pigs, and this tendency was maintained throughout the study (Figure 6). These results suggest that the Mexican hairless pig has a major de novo cholesterol synthesis and like obese humans it could be susceptible to develop disturbances in the lipid metabolism. However, it is necessary other studies to determine whether the cholesterol levels in this genotype are due to low intestinal absorption which triggers an increase in hepatic endogenous cholesterol synthesis, which is commonly observed in obese humans (Miettinen and Kesäniemi, 1986).

In addition, the Mexican hairless pigs have higher serum concentrations of low-density lipoproteins compared with the Landrace-Yorkshire pigs (F=9.38; df=1, 12; P<0.05).
(Figure 7), similar results have been reported in obese humans (Pi-Sunyer, 1993). This result led us to speculate that this genotype could have higher catabolism of the very low density lipoproteins, which seems to be reasonable because in pigs like humans a greater fraction of the very low density lipoprotein remnants are converted to low density lipoproteins (Gotto et al., 1986).

There were no differences between genotypes in serum high-density lipoproteins (F=2.78; df=1, 13; P>0.05).

The Mexican hairless pigs showed higher concentrations of free fatty acids than the Landrace-Yorkshire pigs (F=10.19; df=1, 24; P<0.05) (Figure 8). The higher free fatty acids concentrations found in the Mexican hairless pig could have contributed to the high levels of low density lipoprotein concentrations observed in this genotype.

There were differences between genotypes in serum triglycerides concentrations (F=11.46; df=1, 13; P<0.05). The higher concentrations of triglycerides observed in the Mexican hairless pig were more apparent during the last three months of the study (Figure 9). This could be related with the free fatty acids levels since free fatty acids can be converted into triglycerides by reesterification. These results suggest that it is possible that the Mexican hairless pigs may have a higher rate of de novo triglycerides synthesis compared to the Landrace-Yorkshire pigs.

Conclusions

The pig is emerging as an attractive biomedical model for the study of energy metabolism and obesity; however, contemporary pigs are a result of intense selection for growth rate and leanness, which is a disadvantage for a model for obesity. The Mexican hairless pigs have been under natural selection for the thrifty genotype, a genetic predisposition to obesity. The results of this study indicates that under a conventional diet the Mexican hairless pigs display higher adiposity and higher but normal levels of serum lipids, fasting glucose and insulin concentrations than the Landrace-Yorkshire pigs. Based on these results we conclude that the Mexican hairless pigs is a better animal model for the study of obesity than the Landrace-Yorkshire pigs and could be used under experimental conditions as a model for obesity, particularly the observed in metabolically obese but normal-weight young women. Diabetes 48:2210-2214.

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