Physiological and biochemical measurements before, during and after pregnancy of healthy rats[1]

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ABSTRACT

PURPOSE: To analyze the physiological and biochemical measurements before, during and after pregnancy of healthy rats.

METHODS: Wistar adult females rats (n=8) were weighed and blood samples were obtained before, during and after pregnancy for biochemical determinations, chow intake, water consumption and milk production were evaluated. At day 10 postpartum, the rats were killed for weighing of organs and adipose tissues.

RESULTS: The results showed increase in body weight, serum insulin and ingestion of water and chow. At day 17 pregnancy, presented normal values in the OGTT. At days 7, 14 and 20 of pregnancy, there was increase in triglyceride levels. At term pregnancy, there was weight gain due to fetal growth. In the postpartum period presented reduced blood glucose levels. The glycemic means were reduced during and after pregnancy compared to after pregnancy. The triglyceride concentrations were increased before and during pregnancy in relation to after pregnancy. The total cholesterol levels presented no changes.

CONCLUSION: The use of experimental animals is suitable for evaluation of metabolic changes because the profile of answers found in this study was similar to human profile, showing the relevance of translational research to better understand the pathophysiological mechanisms and possible treatment for diseases.

Key words: Pregnancy. Physiology. Rats.

Introduction

The studies conducted by animal experimentation are relevant related to its contribution to human health, the knowledge development and the society improvement[1]. During researches related to reference values of healthy animals in several database, our research group found difficulties involving reference values in pregnancy and postpartum-related healthy laboratory rats. To indicate reference values for biochemical parameters it is necessary to develop manual techniques characterized by a low risk of laboratory error, leading to precision and repeatability of measurements[2,3].

The methods of blood analysis in experimental animals may be applied to evaluate biochemical markers, which are based on various laboratory techniques[4]. Therefore, it is necessary to develop adequate experimental models using laboratory animals. These models are relevant to expand and improve the understanding of the pathophysiological mechanisms[5] and to contribute for scientific knowledge in various research areas[6]. We hypothesized that the physiological and biochemical parameters are changed in different moments (pre, pregnancy and post pregnancy). Thus, the objective of this study was to analyze physiological and biochemical measurements in healthy Wistar rats and to compare them before, during and after pregnancy of these animals.

Methods

This study was approved by Ethical Committee for Animal Research of the UNESP, Brazil (protocol number: 938/2012).

Female and male Wistar rats were obtained of CEMIB (Universidade Estadual de Campinas - UNICAMP, Sao Paulo) weighing approximately 200 grams (g), and the animals were housed in a certified animal care. The rats were maintained on Laboratory of Experimental Research on Gynecology and Obstetrics under controlled conditions (temperature 22±2°C, humidity 55±5% and 12h light/dark cycle), and chow (Purina® rodent chow, Brazil) and water were provided *ad libitum* and cared for in accordance with the principles of the Guide for Care and Use of Experimental Animals. According the statistical design, the same eight (n=8) rats were used throughout the experiment.

Evaluation of physiological and biochemical parameters before pregnancy

Oral glucose tolerance test (OGTT)

For OGTT, at mornings of days 90 (adult life) and 120 (adult life and early period of mating), after fasting for six hours, a glucose solution (200 g/L) was administered by *gavage* at a final dose of 2 g/kg body weight. Following, the blood samples were obtained by a small cut tip of the tail for blood glucose levels determination using a specific glucometer (OneTouch Ultra—Johnson & Johnson®) in the first timepoint (zero). For obtaining of the blood samples at 30, 60, and 120 minutes (min), a massage on the tail was performed toward the same small cut tip. The glycemic values were expressed in mg/dL[7] (Figure 1).

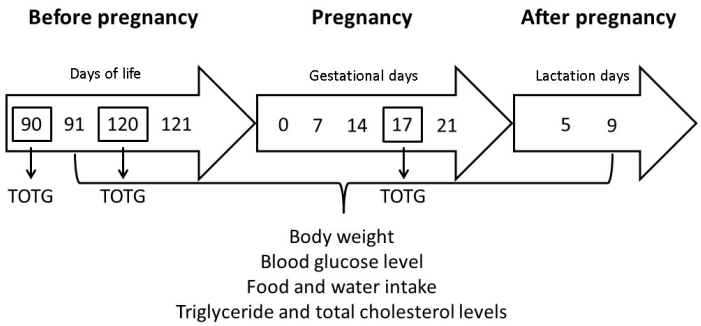

FIGURE 1 - Experimental design.

Physiological and biochemical parameters analyzed before pregnancy (pre pregnancy period)

At mornings of days 91 and 121 of life, maternal body weights, post prandial glycemia, triglyceride and total cholesterol levels were determined. All blood samples were obtained from a small cut tip tail. Blood glucose concentrations were measured by conventional glucometer and these values were expressed in mg/dL. For evaluation of triglycerides and total cholesterol levels, Accutrend® Plus (Roche Diagnostics GmbH, Mannheim, Germany) was used and the values were also expressed as mg/dL. The 24h-chow (g) and water (mL) intakes were calculated.

Mating and pregnancy

At day 120 of life, the same female Wistar rats were mated overnight with male rats. The day when sperms were found in the vaginal smear was designated as gestational day 0[8]. The mating period consisted of 15 consecutive days, a period comprising approximately three estral cycles, until a replicate number was obtained. However, during this period, nonmated female rats were considered to be infertile and were discarded from the study[9].

Evaluation of physiological and biochemical parameters during pregnancy

During pregnancy, post prandial blood samples were collected at days 0 (early pregnancy), 7 (embryonic period), 14

(fetal period) and 20 of pregnancy (end of pregnancy – at term pregnancy), with respect to the same parameters following the methodology described in item 2.2. For this, at day 14 of pregnancy, post prandial blood samples were collected to determine insulin concentration (Crystal Chemical - ELISA Kit 90010 - Rat Insulin Elisa kit; Crystal Chemical Company, Harris County, Texas). At day 17 of pregnancy, OGTT was performed in all these rats to evaluate the presence or absence of glucose intolerance following the methodology described in item 2.1.

Evaluation of physiological and biochemical parameters after pregnancy

The litter size was adjusted at birth eight newborns (four male and four female), which were maintained with their mother during 10 days of lactation. At days 5 (half of lactation period) and 9 (end of experiment), maternal body weights, post prandial glycemia, triglyceride and total cholesterol levels were determined. All blood samples were obtained by venous puncture of the tail. Blood glucose concentrations were measured by conventional glucometer. For evaluation of triglycerides and total cholesterol levels, Accutrend® Plus was also used. In the same days, the pups were removed from their mothers for 4h during which mothers ate *ad libitum* and were weighed at the beginning and end of the 4h period for obtaining indirect milk production in the absence of breastfeeding to offspring[10]. The water consumption (mL) and chow intake (g) consumed were measured after 24 hours in analytical balance and beakers, respectively.

At day 10 after delivery, the dams were weighed and anesthetized with sodium pentobarbital (Hypnol® - 50 mg/kg body weight) for laparoscopy procedure. This procedure was carried out by an incision in the medium line beginning in the xiphoid cartilage and ending in the pubis. The intestinal loops were moved cranially for uterus exposure. The hysterectomy was accomplished with the ligament, artery and ovarian vein section and incision of the body uterine above the cervix. Afterward, the uterus and his content were weighed using analytical balance. Rapidly, the stomach was moved to the right to expose the liver, heart, lung pancreas, adipose tissues (peritoritoneal, periovarian, periuterine, pancreatic and sternal) and mammary gland. These organs were dissected and weighed to obtain the maternal relative weight (absolute weight / body weight x 100). Regarding adipose tissues, the calculation of total fat (sum of all adipose tissues) and relative weight (total body fat / body weight x100) were performed.

Statistical analysis

The number of rats used was eight, which were not sisters because were obtained of different mothers to minimize the maternal influence. The data were presented as mean ± standard deviation. The comparison were analyzed by t test, Gamma distribution followed by repeated measures, and two-way ANOVA followed Tukey´s multiple comparison test. $P< 0.05$ was considered as statistical limit of significance.

Results

Data obtained before pregnancy

It was verified increase in body weight, serum insulin, water intake and chow consumption of Wistar rats at day 121 of life compared to these same parameters at day 91 of life (Table 1).

TABLE 1 - Body weight and biochemical determinations before pregnancy of healthy Wistar rats.

Variables	(n = 8)
Body weight (g)	
Day 91	244.8±**19.**3
Day 121	268.3±**16.**5*
Blood glucose level (mg/dL)	
Day 91	110.5±**5.**5
Day 121	104.5±**9.**2
Chow intake (g)	
Day 91	14.6±1.0
Day 121	16.6±**1.**1*
Water consumption (mL)	
Day 91	39.6±**2.**5
Day 121	57.3±**2.7***
Triglyceride level(mg/dL)	
Day 91	130.5±**14.**6
Day 121	114.5±**27.**1
Total cholesterol level (mg/dL)	
Day 91	158.7±**1.**3
Day 121	162.2±**8.**3

Values are expressed as mean ± standard deviation (SD)
*p<0.05 - between days 91 and 121 of life (t test).

During OGTT, there was increase in blood glucose levels at timepoint 30 min (after glucose overload) compared to timepoint 0 (test start), and reduced blood glucose levels at timepoint 120 min (test final) compared to timepoint 60 min at days 91 and 121 of life (Figure 2).

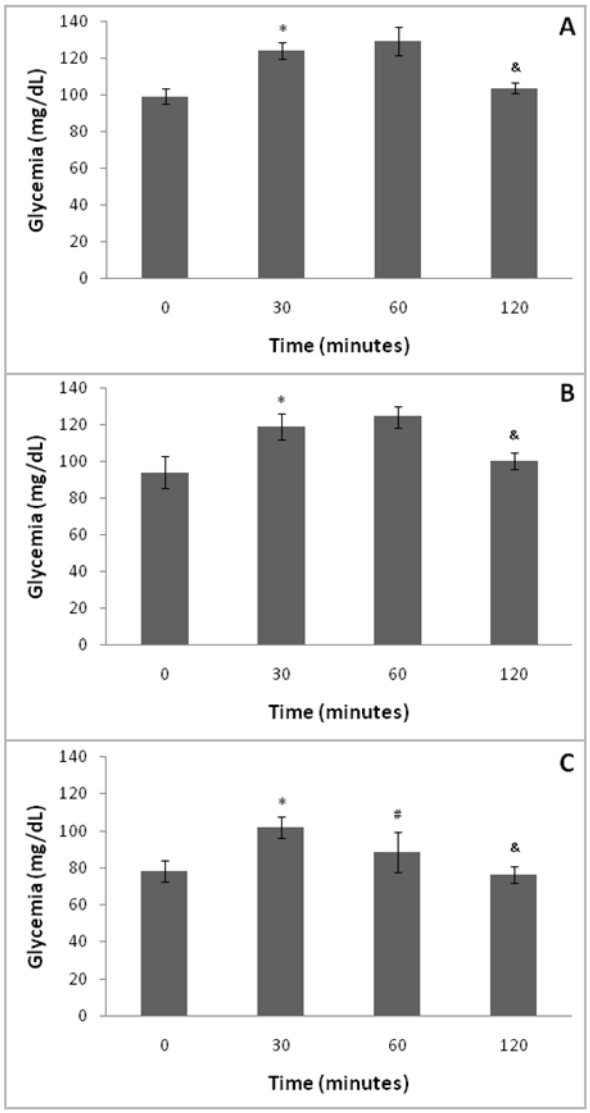

FIGURE 2 - Oral glucose tolerance test (OGTT) at day 90 of life (**A**), at day 120 of life (**B**) and at day 17 of pregnancy (**C**) of healthy Wistar rats (n=8).
Values are expressed as mean ± standard deviation (SD). Gamma distribution followed by repeated measures.
*p<0.05 - between timepoint zero and timepoint 30 minutes (t test).
#p<0.05 - between timepoint 30 minutes and timepoint 60 min (t test).
&p<0.05 - between timepoint 60 min and timepoint 120 min (t test).

Data obtained during pregnancy

The body weight and biochemical parameters were evaluated at days 0, 7, 14 and 20 of pregnancy. The rats presented increased body weight at day 20 of pregnancy (end of pregnancy) compared to at day 14 of pregnancy (early fetal growth). The pregnant rats gained 94,0 grams of weight at the end of pregnancy (day 20 of pregnancy) in relation to early pregnancy (day zero). It was verified a progressive increase of triglyceride level throughout pregnancy (from day 0 to day 7 of pregnancy, from day 7 to day 14, and from day 14 to day 20 of pregnancy) (Table 2). At day 17 of pregnancy the dams were submitted to OGTT, and it was observed increased blood glucose level at timepoint 30 min compared to timepoint 0, and decreased blood glucose level at timepoint 60 min in relation to timepoint 30 min, and at 120 min compared to at timepoint 60 min (Figure 2).

TABLE 2 - Body weight and biochemical determinations during pregnancy of healthy Wistar rats.

Variables	(n = 8)
Body weight (g)	
Day zero	270.8±18.0
Day 7	288.5±21.2
Day 14	313.7±21.3
Day 20	364.8±27.0&
Glycemia (mg/dL)	
Day zero	102.8±8.0
Day 7	98.0±4.2
Day 14	88.3±12.3
Day 20	90.0±9.3
Serum insulin (ng/mL)	
Day 14	1.2±**0.7**
Chow intake (g)	
Day zero	18.0±2.6
Day 7	21.4±4.0
Day 14	23.8±2.5
Day 20	24.3±3.1
Water consumption (mL)	
Day zero	54.0±**12.1**
Day 7	52.5±20.4
Day 14	74.0±19.7
Day 20	62.1±10.7
Triglycerides (mg/dL)	
Day zero	92.3±17.8
Day 7	115.1±10.6*
Day 14	169.8±25.0#
Day 20	255.7±37.9&
Total Cholesterol (mg/dL)	
Day zero	160.7±2.9
Day 7	116.0±2.9*
Day 14	161.8±3.8
Day 20	161.7±4.6

Values are expressed as mean ± standard deviation (SD). Gamma distribution followed by repeated measures.
*p<0.05 - between day zero and day 7 of pregnancy.
#p<0.05 - between day 7 and day 14 of pregnancy.
&p<0.05 - between day 14 and day 20 of pregnancy.

Data obtained after pregnancy

During lactation period (days 5 and 9), the body weight, blood glucose level, water intake, chow consumption, triglyceride and total cholesterol levels, and milk production presented no significant statistically difference throughout lactation period. The mean weights of organs (in grams) obtained at day 10 of lactation were: heart (0.35±0.04), pancreas (0.24 ±0.03), lung (0.50±0.06), liver (3.45±0.121), and mammary gland (6.32±0.89). In relation to mean weight (in grams) of adipose tissues: peritoneal (1.34±0.59), periovarian (0.28±0.12), periuterine (0.70±0.22), pancreatic (0.19±0.10), sternal (0.07±0.03), total fat (8.41±2.52), and relative fat (0.85±0.29) (Table 3).

TABLE 3 - Body weight, biochemical determinations and milk production after pregnancy (day 10 of lactation period) of healthy Wistar rats.

Variables	(n = 8)
Body weight(g)	
Day 5	300.3±25.9
Day 9	314.5±21.9
Glycemia (mg/dL)	
Day 5	97.6±11.0
Day 9	87.0±3.6
Chow intake(g)	
Day 5	34.4±7.1
Day 9	38.6±8.7
Water consumption (mL)	
Day 5	124.7±25.5
Day 9	139.8±41.9
Triglycerides (mg/dL)	
Day 5	86.1±7.2
Day 9	94.3±15.9
Total Cholesterol (mg/dL)	
Day 5	161.8±7.5
Day 9	160.2±7.4
Milk production (g)	
Day 5	3.3±1.1
Day 9	3.8±3.2

Values are expressed as mean ± standard deviation (SD)
p>0.05 - no statistically significant difference

Comparison of the biochemical data obtained before, during and after pregnancy

The glycemic means were reduced during and after pregnancy compared to after pregnancy. The triglyceride concentrations were increased before and during pregnancy in relation to after pregnancy. The total cholesterol levels presented no changes regardless of period (Table 4).

TABLE 4 - Biochemical determinations before, during and after pregnancy of healthy Wistar rats.

Variables	Pre-pregnancy	Pregnancy	Post-pregnancy
Glycemia (mg/dL)	110.9±**13.7**	93.1±**9.7***	92.3±**9.6***
Triglycerides (mg/dL)	122.5±**23.4**	180.2±**64.*7**	90.3±**12.5***#
Total Cholesterol (mg/dL)	160.5±**6.3**	163.2±**4.2**	161.0±**7.1**

Values are expressed as mean ± standard deviation (SD). Two-way ANOVA followed Tukey´s multiple comparison test
*p<0.05 - compared to Pre-pregnancy period.
#p<0.05 - compared to Pregnancy period.

Discussion

The increased consumption of water and chow (days 91 and 121 of life) as there was the body growth contributed to weight gain throughout life. This weight gain is compatible with the findings of Tomanari *et al*.[11], who found an increased weight in rats had free access to water and food due to the rapid mass weight gain in the first month of life and became slow in other periods of life.

Regarding the triglyceride concentration, our findings corroborate the findings of Całkosiński *et al*.[12], who found that triglyceride levels of laboratory animals did not differ throughout life during the period studied (from day 91 of life to day 10 of lactation). However, another study perfomed by Vega *et al*.[13] demonstrated that control rats with 120 days of life presented lower triglyceride levels in relation to those found in our investigation. In the present study, the cholesterol levels showed no difference in the two ages analyzed, however the values are not compatible with the literature[12], who verified low values, suggesting that the methods used for cholesterol concentration measurement could have interfered in controversial results.

It is already demonstrated that insulin secretion is biphasic. The first phase occurs in the first ten minutes after a stimulus, and it is acute and has a short duration. It consists of the preformed insulin. It is of fundamental importance for the control of postprandial glucose levels. A continuous glycemic stimulus induces the second phase of insulin secretion, which is less intense and more prolonged[14,15]. In our study, it was found before pregnancy that the pancreas was tolerant to glucose stimulus during oral

glucose tolerance test, confirmed by adequate insulin secretion due to the blood glucose levels maintenance below 140 mg/dL (minimum reference value for intolerance glucose) and below 200 mg/dL (minimum reference value for *Diabetes mellitus*)[16].

The weight gain at the end of pregnancy in rats shows fetal growth period starting from day 18 of pregnancy[17]. Then, the increased fetal weight consequently led to maternal weight gain on day 20 pregnancy[18-22]. The progressive increase in triglyceride levels during pregnancy may be related to preparation for lactogenesis and also for the milk production or feeding of offspring. Increments in circulating VLDL-triglycerides are seen in pregnant women during the third trimester of gestation play an important role in the fate of circulating triglycerides, which are diverted from uptake by adipose tissue to uptake by the mammary gland for milk synthesis[23]. Our findings showed no alteration in chow consumption and water intake during pregnancy. However, Bautista *et al.*[24] observed increased food intake from day 10 to day 16 of pregnancy, a period that corresponds to the initiation of major fetal organ growth and augmented cell proliferation.

The oral glucose tolerance test (OGTT) applied during pregnancy showed reduced glucose values compared to the other life periods. This finding may be due to test application period (day 17 of pregnancy), which is the peak of development and fetal growth. However, these rats showed adequate response to OGTT, i.e., showed an increase in blood glucose after glucose overload and adequate glycemic return at end of test, confirming the absence of glucose intolerance or *Diabetes mellitus.* The fact that there have been no change in parameters studied during lactation may be related to the period of analysis, suggesting that after day 14 of lactation there are variations noticeable in the physiological and biochemical parameters.

In relation the physiological and biochemical changes in the different periods analyzed, it was observed that the rats gained body weight during pregnancy related to fetal and extraembryonic membranes development, according to others studies of our laboratory[18-22]. The blood glucose level in pregnancy and lactation periods was reduced, showing nutrient exchange from mother to offspring as energy source. It was verified a reduction of triglyceride level in lactation period. Desoye *et al*.[25] described lipid and lipoprotein concentrations decreases in postpartum period, and the rhythm of this reduction increases in lactation period, corroborating with our results.

Conclusion

The use of experimental animals is suitable for evaluation of metabolic changes because the profile of answers found in this study was similar to human profile, showing the relevance of translational research (bench-to-bedside and bedside-to-bench) to better understand the pathophysiological mechanisms and possible treatment for diseases.

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