

Influence of Diabetes and Gestation in Blood Biochemistry Variables in Wistar Rats

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Abstract

Background: Several factors could affect physiologic and blood biochemical variables in laboratory animals. Bearing this in mind, it is recommended to characterize the animal's variables in each laboratory. The aim of this study was to evaluate the effect of diabetes and gestation on blood biochemical variables in Wistar rats.

Methodology: Female Wistar rats were randomly separated in four groups (healthy non-pregnant, diabetic, healthy mid gestation and final gestation period). Blood concentration of glucose, cholesterol, triglycerides, creatinine, uric acid, total proteins and albumin were determined. Statistic studies were performed using t-test and ANOVA with Bonferroni test for those with normal distribution or Mann-Withney U and Kruskal-Wallis with Dunn test for those without normal distribution; significant differences were considered with $p < 0.05$.

Results: Blood concentration of glucose, cholesterol, triglycerides, creatinine and uric acid were significantly higher in diabetic rats compared with healthy non-pregnant rats. Healthy pregnant rats presented significantly bigger concentrations of triacylglycerols and minors of total proteins and albumin than the not have gotten pregnant.

Conclusions: Diabetes and gestation induces modifications in blood biochemical variables in consonance the metabolic changes characteristics of this states and the renal damage caused by diabetes.

Introduction

Research in laboratory animals has contributed to the discovery of new mechanisms and therapeutics for diseases affecting human beings. Several species has been used as laboratory animals while the rat is one of the most used and well characterised [1].

Several factors are known to modify physiological and biochemical variables. These factors may include sex, age, genetic factors and environmental factors such as animal housing environment, nutrition, animal care and weather characteristic of the area of the planet, as well as the collection of the sample [1-5].

In this context, the characterization of laboratory animals in each laboratory or Bioteriumis usefully in the research. This knowledge is important during the selection of the animal room, analysis and validation of results as well as the experimental procedures and the interpretation of the modifications introduced by diseases¹.

Due to its high incidence and prevalence Diabetes Mellitus is much studied. Diabetes is one of the first 10 causes of incapacity worldwide. 4.6 millions of peoples die every year by this disease, one each 7 seconds [6].

In diabetic woman, aside of the common diabetic complications, it affects fertility and gestation. There is an increase in the frequency of miscarries, placental dysfunctions as well as maternal and offspring morbidity [8,9].

Even when the preconceptionals cares are the foundation of a successfully gestation, in the diabetic woman in hard to achieve a stable metabolic control [9]. Finding new methods to prevent part of the damage caused by the diabetes could ameliorate maternal and offspring health. Discoveries in experimental models could help the development of new methods to keep the intrauterus environment metabolically healthy and to prevent the damaging effects of maternal diabetes on the offspring [10].

Motivated by the above, our research group has developed a series of studies with an experimental system of diabetes and pregnancy in Wistar rats. The results obtained are sufficient for the characterization of this system, so the objective of this study was to analyze the influence of diabetes and gestation on blood biochemical variables of Wistar rats.

Materials and Methods

Ethical considerations

The data used for this study are results obtained in different research projects developed in the Department of Biochemistry of the Institute for Basic and Preclinical Sciences "Victoria de Girón", approved by the Scientific Council and the Center Ethics Committee.

General design

Data were obtained from healthy and diabetic female Wistar rats used as controls in three research projects developed by our group from 2009 to 2017, recorded in Excel sheets (Microsoft Office) and in the individual clinical records. Four groups were formed: healthy non-pregnant, diabetic, healthy mid-gestation and healthy at the end of pregnancy. The results of biochemical variables in plasma were analyzed: glycemia, cholesterol, triacylglycerols, creatinine, uric acid, total proteins and albumin.

General aspects of projects

The animals were obtained from the National Center for Laboratory Animal Production of Cuba (CENPALAB). The weight of the rats at the time of inclusion in the corresponding studies was 200 ± 20 grams.

Diabetic rat model was obtained by intraperitoneal administration of streptozotocin (Applichem) at a dose of 65 mg / kg. The pregnant rats were obtained by nocturnal matings with healthy males of the same strain, during the estrus phase of the estrous cycle [11,12].

At the end of the experiments, the animals were euthanized by exsanguinations (puncture of the inferior vena cava) under anaesthesia. The plasma was extracted by centrifugation of the blood (Eppendorf centrifuge) at 3000 rpm for 15 min at a temperature of 4° C [11,12].

Methods used to determine the biochemical variables

1. Glycaemia: using SUMA glucometer
2. Cholesterol, triacylglycerol, total proteins, albumin, creatinine, uric acid: through commercial games HELFA Diagnostics, QUIMEFA Epb Carlos J. Finlay.

Table 1: Biochemical variables in plasma of healthy and diabetic non-pregnant female Wistar rats.

Variables	Healthy	Diabetic
Glycaemia (mM)	6,7 ± 1,3 (38)	22,3 ± 5,9* (47)
Cholesterol (mM)	1,9 ± 0,8 (38)	2,7 ± 1,8† (26)
Triacylglycerol (mM)	1,0 ± 0,5 (38)	1,7 ± 1,3† (26)
Creatinine (mM)	40,3 ± 16,6 (38)	91,7 ± 69,4† (26)
Uricacid (mM)	125,0 ± 73,5 (38)	218,5 ± 135,3† (24)
Total proteins (g/L)	64,6 ± 15,0 (38)	58,8 ± 10,6 (26)
Albumin (g/L)	37,6 ± 6,5 (38)	34,8 ± 6,4 (22)

The values represent the mean ± standard deviation and the number of rats (n)

*p <0.05 (t-test) † p <0.05 (U Mann Whitney).

Table 2: Plasma biochemical variables during the pregnancy of healthy Wistar rats.

Variables	Not pregnant	Pregnant in the middle of pregnancy (11-12 days)	Pregnant at term (20 days)
Glycaemia (mM)	6,7 ± 1,3 (38)	6,9 ± 2,2 (37)	5,8 ± 1,7 * (29)
Cholesterol (mM)	1,9 ± 0,8 (38)	1,4 ± 0,7 (37)	1,5 ± 0,5 (29)
Triacylglycerol (mM)	1,0 ± 0,5 (38)	1,6 ± 1,4 (37)	2,9 ± 2,8 # (29)
Creatinine (mM)	40,3 ± 16,6 (38)	49,1 ± 17,9 (37)	47,5 ± 14,1 (29)
Uricacid (mM)	125,0 ± 73,5 (38)	136,5 ± 83,3 (35)	110,3 ± 78,2 (29)
Total proteins (g/L)	64,6 ± 15,0 (38)	55,8 ± 11,7 # (37)	58,3 ± 8,5 (29)
Albumin (g/L)	37,6 ± 6,5 (38)	27,8 ± 7,5 † (34)	37,7 ± 11,7 (29)

The values represent the mean ± standard deviation and the number of rats (n). Glycaemia and total proteins: One-Way ANOVA with Bonferroni test; the rest of the variables: Kruskal Wallis and Dunn.

*p <0.05 against pregnant women in the first half of gestation days.

p <0.05 against non-pregnant.

† p <0.05 against the rest of the groups.

For the control of the quality of the results, the determination of the concentrations of all the variables was carried out in duplicate.

Statistical analysis

The data was processed using the Graph Pad Prism 5.01 software. The distribution of the variables was analyzed with the Kolmogorov Smirnov test. For the variables with normal distribution, the comparisons between the groups were made by means of the t-test (two groups) and one-way ANOVA with Bonferroni Multiple Comparisons test (three groups). For the variables without normal distribution, the groups were compared using the U Mann Whitney tests (2 groups) and Kruskal Wallis with Dunn Multiple Comparisons test (three groups). The results were expressed as the mean ± the standard deviation. The differences were considered significant with values of p <0.05.

Results

Tables 1 and 2 show the results obtained in the healthy and diabetic female Wistar rats used as controls in the studies carried out from 2009 to 2017 in the ICBP Biochemical laboratories "Victoria de Girón".

At the time of taking the blood sample, healthy non pregnant rats had a body weight of 200 g - 300 g and diabetics of 180 g - 300 g. The time of evolution of diabetes was 22 days - 46 days.

The animals of the group of pregnant rats in the middle of gestation presented 11-12 days of pregnancy and those of the group of pregnant rats at term 20 days of gestation.

Discussion

Diabetes mellitus is a complex metabolic syndrome, characterized by alterations in the metabolism of carbohydrates, lipids and proteins, as a consequence of a deficiency of insulin secretion, its action or both [13]. The manifestations of these metabolic changes include changes in the values of biochemical variables, such as those shown in this research.

In experimental studies, the method used to induce diabetes can be a source of variation in the levels reached by different analytes. There are several methods to obtain experimental models of diabetes, such as chemical induction, partial pancreatectomy and genetic modifications [10]. Each one has different characteristics regarding the origin of diabetes, glycaemia levels and the severity of complications, which influence the values of the variables analysed in each case [10].

The results obtained show that the glycaemia of healthy rats was below 7.8 mM (minimum reference value for glucose intolerance) and 11.1 mM (minimum reference value for diabetes mellitus). However, administration of 65 mg/kg of Streptozotocin intraperitoneally to adult rats led to severe diabetes, with blood glucose levels above 16.7 mM (300 mg/dL).

The glycaemia values observed in the diabetic rats of this study are higher than those produced by administering 100 mg/kg of streptozotocin subcutaneously to rats during the neonatal period (120-200 mg/dL in adulthood) [15]. This shows that the dose of streptozotocin and the age of the animal contribute to the differences between the models of diabetes that are obtained by chemical induction [10,15,16].

Diabetic rats showed a significant increase in blood triglyceride and cholesterol levels, which correspond to the decompensated diabetes that characterizes this experimental model [9,15-17]. Blood creatinine and uric acid concentrations were also increased, which are signs of impaired renal function [18,19].

Uric acid has beneficial effects on the body, acting as an antioxidant, but its increase can lead to the accumulation of urate crystals that affect tissues, especially kidney [19]. Recent research has shown a link between hyperuricemia and chronic kidney disease in diabetic patients [20], but studies continue to elucidate its causal role or as a marker of renal dysfunction [21].

A balance between its generation, reabsorption and renal excretion determines the levels of uric acid in the blood [19]. One of the endogenous mechanisms of its production is fructose, which in diabetic patients is increased from the polyol pathway [22]. In addition, the reabsorption and secretion of urate in the kidney are regulated by several transporters, one of which (GLUT 9) is a glucose and fructose exchanger for uric acid [23]. On the other hand, hyperuricemia can induce renal damage due to vasoconstriction mediated by endothelial dysfunction, inflammation and activation of the renin-angiotensin system, as well as tubulointerstitial fibrosis [19]. The above shows possible mechanisms that relate hyperuricemia and kidney damage caused by diabetes.

Insulin deficiency causes a decrease in protein synthesis and an increase in its catabolism [16]. However, the diabetic rats did not show changes in the concentrations of total proteins and albumin in

the plasma, which could be due to the fact that the time of evolution of diabetes was not sufficient for these metabolic changes to manifest.

These results show that diabetic rats present biochemical manifestations that are common to those that occur in diabetic patients. The above shows the usefulness of the experimental model used for diabetes studies.

Pregnant healthy rats were used for the analysis of the influence of pregnancy on blood biochemical variables. Ind diabetic rats pregnancy was not consider, taking into account that animals differ greatly in the time of evolution of diabetes, so that their grouping would not offer results attributable only to the influence of pregnancy.

The significant decrease in the levels of total proteins and albumin in the first half of pregnancy, as well as the glycaemia at the end of pregnancy (day 20), reflects the contribution of nutrients from the mother to the fetus, which guarantees its growth and development [24]. In addition, a decrease in hepatic gluconeogenesis at the end of pregnancy has been reported, which contributes to the decrease in blood sugar levels in this stage [25].

Healthy pregnant rats also had a significant increase in triacylglycerol in their blood at the end of pregnancy (20 days). It is known that the anabolic phase at the beginning of pregnancy stimulates lipogenesis and fat storage, which contributes to rapid fetal growth in later stages. Because of the development of insulin resistance, lipolysis is favored and the activity of lipoprotein lipase decreases, which increases circulating lipids, more evident at the end of pregnancy [24-26]. The progressive increase in circulating lipids may also be related to the preparation for lactogenesis [24].

The previous results show that gestation is another factor that influences the variables analyzed in laboratory animals.

Due to the large number of factors that can modify the homeostasis in experimental animals, the characterization of the models used in each laboratory is useful and necessary to evaluate the state of the animals at any stage of the studies. However, differences may occur between animals of the same strain and kept under the same conditions, attributable to environmental changes in the course of the experiments [6]. For this reason, the need for new determinations every time the animals are subjected to different experimental conditions is not excluded.

Conclusions

Diabetes and gestation in rats modify the values of biochemical variables in blood, which corresponds to metabolic changes characteristic of these states and to kidney damage caused by diabetes.

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