

The Effect of Fasting on Haematology Serum Biochemistry Parameters on STZ Induced CD1 Mice and Diabetic db/db Mice

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Abstract

Modest information is available on how fasting affects clinical pathology variables in (Streptozocin) STZ induced diabetes mice and db/db mice. This study was carried out to evaluate the influence of fasting on clinical pathology variables in diabetic mouse models. Seven male STZ induced diabetic CD1 mice and db/db mice were fasted for 18 hours and change in body weight (BW), hematologic and biochemical variables were evaluated. Fasting provoked significant variation in body weight, haematology and biochemical variables in diabetic animal models.

The results suggested that clinical pathology variables will vary after fasting. The decision to feed or fast before blood collection for analyzing the results should be made based on fasting in animal models for diabetic research.

Keywords: Fasting; Animal models; Hematology; Biochemistry

Abbreviation: CREA: Creatinine; T-BIL: Total Bilirubin; BUN: Blood Urea Nitrogen; ALT: Alanine Transaminase; AST: Aspartate Transaminase; ALP: Alkaline Phosphatase; GLU: Glucose; T-CHO: Total Cholesterol; TP: Total Protein; TG: Triglyceride; Ca: Calcium; Na: Sodium; K: Potassium; RBC: Red Blood Cells; Hb: Hemoglobin; HCT: Hematocrit; MCV: Mean Corpuscular Volume; RDW: Red Cell Distribution Width; MCH: Mean Corpuscular Haemoglobin; MCHC: Mean Corpuscular Haemoglobin Concentration; WBC: White Blood Cells; G: Granulocytes; L: Lymphocytes; M: Monocytes; PDW: Platelet Volume Distribution; MPV: Mean Platelet Volume; PCT: Platelet Crit; PLT: Platelet

Introduction

Clinical pathology data of animal models used in diabetic research play an important part in non-clinical toxicity studies and safety evaluations of new drugs, food additives, and chemicals [1]. Many regulatory guidelines and the recommendations of American Association for Clinical Chemistry's Division of Animal Clinical Chemistry (AACC-DACC) and the American Society for Veterinary Clinical Pathology (ASVCP) joint committee on clinical pathology testing of laboratory species requires overnight fasting of animals before blood sampling [2-4]. An important reason for fasting in laboratory animals before blood collection is to reduce variability of some clinical chemistry parameters between feeding and fasting conditions. However, intestinal physiologic functions and drug-metabolizing enzymes may have some difference under feeding and fasting conditions [5-7]. Thus, the fasting in animals should be decided case by case rather than made uniform for every study. There are limited reports on the effects of fasting on certain clinical pathology variables; most of them focus on rats [8,9] but none defined in diabetic animal models.

This study was undertaken to recognize variables after fasting and this study will provide a more complete picture of fasting effects on the on haematology and biochemical variables in diabetic animal models. In addition this result will help for designing toxicity or pharmacodynamics studies on diabetic animal models.

Materials and Methods

Experimental protocols were approved by the Institutional animal ethics Committee and experiments were conducted in accordance

with the guidelines of Institutional Animal Use and Care of National Institute of Immunology New Delhi. Diabetic mice (*db/db*) mice on a C57BLKS/J background were obtained from the Jackson Laboratory (Bar Harbor, ME). The animals were maintained in individual ventilated cages at standard environmental conditions (temperature 22–25°C, humidity 40–70%) with 14:10 dark/light photoperiods. Two diabetic models were used for this study, STZ induced CD1 mice and db/db mice. These strains are preferred as CD1 has greater induction of diabetes as compared to other strains and out bred CD-1 mice carry a spectrum of genetic susceptibilities for obesity and type 2 diabetes. db/db on the other hand spontaneously develop hyperphagic, obese, on the other hand spontaneously develop hyperphagic, obese, hyperglycaemic, hyperinsulinaemic and insulin resistant within first month of age and develop hypoinsulinaemia, hyperglycaemia later with a peak between 3-4 months of age.

STZ induced diabetes in CD1 male mice

Diabetes was induced in CD1 mice by Streptozotocin (STZ), as described previously [10]. STZ (Sigma-Aldrich) was dissolved in 50 mM sodium citrate buffer (pH 4.5) and a final concentration of 6 mg/ml was made. The animals were injected intraperitoneally at a dose of 45 mg/kg/day. Treatment was repeated for 5 consecutive days. Blood glucose level was measured, in non fasted animals on alternate days and mice were considered diabetic when blood glucose levels exceeded 250 mg/dL. The animals achieved diabetic 7 to 9 days after the last STZ injection.

Fasting experiment: Seven male six to eight weeks db/db and STZ

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induced CD1 mice were fasted for 18 h. The animals were housed in individual ventilated cages with free access to water. For pre fasting variables seven db/db and STZ induced diabetic male mice were used.

Blood collection: At the end of the respective fasting period, blood was collected from each mouse by retro-orbital venous puncture under ketamine anaesthesia. 200 µl of blood sample were collected into micro centrifuge tubes containing 2% EDTA for hematologic analyses using MS 4e Vet Haematology Analyzer (Melet Schloesing) and analyzed as per manufactures instruction. 500 µl blood without EDTA were collected through cardiac puncture before euthanasia kept in refrigerator, allowed to clot for 60 min and centrifuged at 3000 g×15 min at 4°C for biochemical analysis using Tulip 3000 Evolution biochemical Analyzer, India. The above procedures were followed in the non fasted control db/db, STZ induced CD1 mice. Following blood collection the animals were euthanized.

Statistics: All data's were analyzed with GraphPad Prism version 6 for Windows (GraphPad Software, San Diego, CA, USA). The data are expressed as the mean ± s.d. with median and range. Values for blood parameters obtained from pre fasted blood samples and post fasted were compared by using unpaired *t* tests. A p value of 0.05 was used as the threshold for statistical significance significant.

Results

The results of are summarized in table 1 and table 2. As shown in the table 1 there were significant differences in haematology after fasting in both db/db and STZ induced CD1 diabetic mice. There was significant increase in M, G, RBC, HCT, RDW, Hb and MPV in STZ induced CD1 mice and significant increase in G, PLA and Pct in db/db mice. There was significant decrease in L, RBC, MCV and HCT on both models.

As shown in the table 2 there was significant increase in T-BIL, Total P, Na, CREA in STZ induced CD1 mice and decrease in ALP, AST, ALT, CHO ,TGY, GLU Ca in STZ induced CD1 mice. In db/db mice there was significant increase in T- BIL, TGY, CHO, Uric acid and Total P and significant decrease in ALP and GLU.

Discussion

In diabetic research, the fasting is generally primed to study the various effects. As stated in the introductory section, it is evident that variables many variables are noticed after fasting and has large impact on the research. Given the almost mandatory use of the fasting we are surprised that an attempt has not previously been made to validate the various variables of this test in diabetic mice. The animals are in normal physiological state when given enough food and when food is withdrawn or fasted the physiological and pathological variation may take place. Fasting will affect the animal health, behaviour, absorption rate of test substances, carbohydrate and lipid metabolism [11]. Researchers using diabetic animals for research employ 16-24 hours fasting, but this fasting brings about important changes. These changes will affect internal cellular biochemistry and one should therefore expect differences in the effects of preparations on isolated cells, tissue or organs removed from animals that have, or have not, been fasted. Claassen [12] studies explain that many plasma values fall, including glucose, urea, lactate and amino acids, while glycerol and free fatty acids increase, after only 3 hours of fasting

After different durations of fasting, a significant loss of 1.8 to 11.77% of BW was observed in rats and an average of 14% in pigs [13]. This similar effect on BW was observed in our study at 18 hours of fasting in STZ induced CD1 mice but not in db/db mice. This changes might be due to lack of long-form leptin receptor. Most published articles stated that RBC, Hb, HCT increases after fasting laboratory

Analyte Mean ±SD Median (Range-Max – Min)	Diabetic CD1mice- Pre fasting	Diabetic CD1 mice- Post fasting	Diabetic db/db mice Pre fasting	Diabetic db/db mice Post fasting
Body weight-BW	24.02 ± 3.10 25.75 (25.30-24.20)	22.60 ± 2.60* 22.5 (24.50-21.90)	36.68 ± 4.10 36.90 (37.50-33.10)	35.43 ± 2.40 35.10 (36.50-34.30)
Total WBC (× 103/µL)	6.71 ± 1.31 (6.81) (8.21-5.15)	4.65 ± 1.03* 4.57 (5.97-3.46)	8.47 ± 2.11 8.18 11.88-6.04	6.87 ± 2.17 6.48 10.89-4.69
Lymphocytes-L (%)	71.32 ± 3.07 71.95 (74.50-66.40)	41.15 ± 10.37* 40.90 (52.70-32.20)	79.98 ± 3.66 81.10 83.70-73.10	72.59 ± 3.51* 72.00 77.00-68.20
Monocytes -M(%)	7.53 ± 1.38 (8.05) (8.80-5.70)	14.08 ± 3.78* (14.05) (17.40-10.80)	6.43 ± 1.42 6.25 8.90-5.00	6.41 ± 1.60 5.95 9.00-4.70
Granulocytes-G (%)	21.15 ± 2.30 21.20 (24.90-18.10)	44.78 ± 6.89* 45.00 (52.60-32.60)	13.60 ± 2.41 13.15 18.00-11.20)	21.00 ± 2.58* 21.55 23.60-16.60
RBC (× 106/µL)	6.97 ± 0.49 7.02 (7.52-6.23)	7.78 ± 0.28* 7.90 (7.94-7.35)	9.18 ± 0.50 9.11 (9.93-8.35)	8.67 ± 0.40* 8.79 (9.26-7.91)
MCV (fL)	59.33 ± 0.56 59.20 (60.10-58.70)	59.63 ± 0.56 59.60 (60.20-59.10)	58.20 ± 0.68 58.40 (59.00-57.10)	57.34 ± 0.53* 57.30 (58.10-56.40)
HCT (%)	41.33 ± 3.09 41.70 (45.00-36.50)	46.33 ± 1.91* 47.10 (47.60-43.50)	53.31 ± 3.33 52.65 (58.50-47.60)	49.66 ± 2.71* 50.30 (53.80-44.60)
MCH (pg)	17.43 ± 0.22 17.45 (17.70-17.10)	17.95 ± 0.24 17.85 (18.30-17.80)	17.50 ± 0.34 17.40 (18.10-17.10)	17.46 ± 0.38 17.40 (18.00-17.00)
MCHC (g/dL)	29.43 ± 0.39 29.35 (30.10-29.10)	30.20 ± 0.6229.95 (31.10-29.80)	30.13 ± 0.36 30.10 (30.80-29.70)	30.50 ± 0.63 30.40 (31.50-29.80)
RDW (%)	9.52 ± 0.10 9.50 (9.70-9.40)	10.28 ± 0.25* 10.40 (10.40-9.90)	9.78 ± 0.56 9.60 (10.80-9.30)	9.66 ± 0.45 9.50 (10.40-8.90)
Hemoglobin-Hb(%)	12.18 ± 0.76 12.25 (13.1-11)	14.00 ± 0.64* 14.15 (14.6-13.1)	16.10 ± 0.98 16.1 (17.4-14.3)	15.18 ± 0.90 15.2 (16.5-13.5)
Platelets -PLT(× 103/µL)	1,097.00 ± 28.74 (1052) (1489-842)	1,183.00 ± 218.42 (1134) (1488-976)	646.75 ± 63.49 625 (779-577)	909.75 ± 69.03* 904 (995-833)
MPV (fL)	5.17 ± 0.23 5.15 (5.50-4.90)	5.53 ± 0.22* 5.50 (5.80-5.30)	5.48 ± 0.22 5.45 (5.80-5.10)	5.25 ± 0.20 5.30 (5.50-4.90)
Pct %	0.57 ± 0.12 0.55 (0.79-0.45)	0.65 ± 0.10 0.62 (0.80-0.57)	0.35 ± 0.02 0.35 (0.40-0.33)	0.47 ± 0.04* 0.48 (0.54-0.41)
PDW	8.58 ± 0.37 8.50 (9.10-8.20)	9.00 ± 0.32 9.05 (9.30-8.60)	8.15 ± 0.49 8.15 (8.90-7.40)	8.51 ± 0.53 8.55 (9.10-7.60)

Table 1: Haematology values in Pre and post fasting variables of STZ induced CD1 mice and db/db Presented as mean ± standard deviation (n=7) with median and range *p<0.05. * p<0.05 compared with pre and post fasted mice using unpaired student t test

Analyte Mean \pm SD Median (Range-Max – Min)	Diabetic CD1mice- Pre fasting	Diabetic CD1 mice- Post fasting	Diabetic db/db mice Pre fasting	Diabetic db/db mice Post fasting
Alkaline phosphatase (U/L) (ALP)	422.66 \pm 45.40 411 (494-366)	104.33 \pm 15.8* 101 (131-85)	265.11 \pm 38.79 268 (342-228)	89.85 \pm 35.22* 100 (134-40)
Bilirubin (μ mol/L) (T-BIL)	3.70 \pm 1.99 3.42 (6.84-1.70)	13.39 \pm 2.73* 13.68 (17.10-10.26)	6.15 \pm 1.94 6.8 (8.55-3.42)	12.14 \pm 1.41* 11.97 (12.65-11.62)
Aspartate aminotransferase (U/L)(AST)	191 \pm 12.31 191 (220-158)	115 \pm 35.82* 114 (180-81)	155 \pm 41.45 146 (226-113)	130.8571 \pm 8.83 132 (142-121)
Alanine aminotransferase (U/L)(ALT)	108 \pm 19.30 103 (142-92)	56 \pm 21.91* 51 (96-32)	87.66 \pm 32.48 90.00 (140-48)	86.1429 \pm 9.65 84.00 (100-71)
Triglyceride mmol/L (TGY)	4.21 \pm 1.17 4.23 (5.62-2.50)	3.48 \pm 0.47* 3.42 (4.27-2.97)	1.26 \pm 0.59 1.03 (2.32-0.76)	1.89 \pm 0.10* 1.94 (2.05-1.76)
Cholesterol mmol/L (CHO)	4.83 \pm 0.21 4.75 (5.23-4.63)	3.54 \pm 0.48* 3.39 (4.24-2.92)	3.01 \pm 0.66 3.36 (3.72-2.07)	3.57 \pm 1.52* 4.12 (4.71-3.21)
Serum Creatinine mmol/L	0.01 \pm 0.03 0.17 (0.02-0.01)	0.02 \pm 0.04* 0.02 (0.02-0.01)	0.02 \pm 0.007 0.01 (0.03-0.01)	0.08 \pm 0.02* 0.08 (0.10-0.03)
Urea nitrogen mmol/L(BUN)	19.63 \pm 6.03 17.13 (28.56-13.56)	17.37 \pm 3.53 18.74 (21.06-11.42)	17.25 \pm 1.84 17.49 (19.90-14.90)	14.58 \pm 7.32 13.2 (27.84-6.42)
Uric Acid mmol/L(U-ACID)	0.46 \pm 0.14 0.44 (0.74-0.30)	0.39 \pm 0.09 0.36 (0.55-0.32)	0.21 \pm 0.02 0.21 (0.24-0.17)	0.35 \pm 0.13* 0.35 (0.65-0.23)
Total Protein g/L (P)	58.32 \pm 10.08 59.50 (64-50)	178.88 \pm 36.59* 189.00 (214-121)	55.14 \pm 5.21 54 (63-48)	123.33 \pm 12.00* 122 (150-108)
Glucose mmol/L	38.09 \pm 5.41 39.9 (43.92-30.80)	16.62 \pm 3.65* 15.73 (23.57-13.06)	25.82 \pm 4.76 25.11 (33.30-18.98)	13.41 \pm 3.42* 13.07 (17.64-10.65)
Sodium mmol/L(Na)	146.50 \pm 6.34 145.50 (155-138)	304.5 \pm 43.58* 321 (330-216)	155 \pm 4.80 165 (172-141)	153.55 \pm 3.04 147 (165-147)
Potassium mmol/L(K)	7.16 \pm 1.72 7.00 (9-5)	9 \pm 3.00 10.00 (12-4)	7.65 \pm 2.10 8.20 (11.20-6.50)	8.0 \pm 7.52 9.12 (15.20-6.50)
Calcium mmol/L(Ca)	2.73 \pm 0.37 2.81 (3.1-2.2)	1.38 \pm 0.80* 1.25 (2.65-0.65)	2.71 \pm 0.46 2.75 (3.30-2.25)	2.55 \pm 0.49 2.38 (3.30-2.10)

Table 2: Serum biochemistry values of STZ induced CD1 mice, and db/db mice (Pre and post fasting variables) Presented as mean \pm standard deviation (n=7) with median and range. * p<0.05 compared with pre and post fasted mice using unpaired student t test.

animals [14,15]. But in this study there was significant increase in RBC and Hb but not HCT in STZ induced CD1 mice. The changes observed in WBC parameters were similar in both strains to those seen in SD rats and humans [14-16]. Studies on rats showed [8] PLT increased with percentage changes of 16.5% in male rats. In our study there was increase in PLT in db/db mice though not significantly in STZ induced CD1 mice. No noteworthy changes in RBC parameters were observed db/db mice but significant increase in RBC count in STZ induced CD1 mice as reported in Wistar rats [17].

Serum biochemical variables like CHO and TGY had significant effect with increase in db/db mice on the other hand there were significant decrease in CHO and TGY in STZ induced CD1 mice. The liver enzymes AST and ALT showed significant decrease in STZ induced CD1 mice as reported previously on other species after overnight fasting [18] but there were no significant changes in db/db mice. ALP and TOT-BIL were significantly increased in both the models. This change in ALP activity could be attributable to decreased production of the intestinal isoenzyme during fasting; as intestine is the major source of serum ALP [19]. There was significant increase in CREA level in db/db mice though not significant in STZ induced CD1 mice. Serum calcium obviously decreased after fasting, and it was similar to observations reported by Matsuzawa and Sakazume [11]. Calcium levels were decreased in STZ induced CD1 mice but not in db/db mice after fasting. This change in calcium in serum may be a consequence of a decrease in serum parathyroid hormone (PTH) after fasting, which might affect the reabsorption and secretion of Ca and P in kidney [20].

Clearly, the fasting has pronounced effects on clinical chemistry analytes and hematology in diabetic animal models. To conclude researchers using diabetic animal models for their research should consider effect of fasting for interpretation of their results.

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