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Extended Fasting Durations Delayed Gastric Emptying and Colonic Motility in Normal Male Rats

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Abstract

Background: Previous studies on fasting and gastrointestinal motility were reported with information lacking concerning prolonged continuous fasting and gastrointestinal motility. This study investigated the effect of prolonged fasting duration on gastrointestinal motility. **Methods:** Forty-five (45) male Wistar rats, with body weights between 180 - 200 g were used. They were randomly assigned into three (3) groups. Group1: control (rats fasted for 18 h—common duration of fasting for motility studies), groups 2 and 3 fasted for 48 and 72 h respectively. Five (5) rats per experiment and per group were considered. Blood glucose was determined by glucose oxidase method, gastric emptying was assessed by hydrated carbohydrate meal, intestinal motility by charcoal meal, and colonic motility was assessed using bead test. Data were reported in Mean ± SEM and analyzed with one-way ANOVA. Differences in results were considered significant at $p \leq 0.05$. **Results:** There was no significant change in the blood glucose level (mmol/L) of rats in the 48 h group (2.94 ± 0.35) and 72 h group (3.20 ± 0.32) as compared with the control (3.62 ± 0.19). There was a significant decrease in the rate of gastric emptying (g) in the 72 h group (0.20 ± 0.08) compared with the control (0.64 ± 0.16). The intestinal transit (cm) in the 48 h group (67.54 ± 6.15) and 72 h group (72.10 ± 7.60) increased significantly when compared with the control (42.14 ± 3.14). There was a significant decrease in the colonic motility time (Sec.) in the 48 h group (2707 ± 864.1) and 72 h group (6363 ± 968.1) when compared with the control (263.8 ± 64.26). **Conclusion:** Extended fasting durations decrease the rate of gastric emptying and colonic motility. It suggests that extended fasting durations could be beneficial in intestinal spasms or where the gut is required to relax.

Keywords

Fasting, Gastrointestinal Motility, Gastric Emptying, Intestinal Transit,

Colonic Motility, Rats

1. Introduction

The gastrointestinal system provides the body with essential water, electrolytes, and nutrients that it requires for day-to-day activities. This specialized system ingests food, thrusting it through the entire gut, processing it, and absorbing the important ingredients from the lumen of the gastrointestinal tract [1]. Gastrointestinal motility is an essential function of the digestive and absorptive processes of the gut, it is vital in thrusting intestinal substances, getting them mixed with digestive juices, and removing waste metabolites from the system [2]. Gastrointestinal motility encompasses myoelectrical activity, contractile movement, tone, compliance, and transit. These motility properties can be produced and modified by neurohormonal substances that are present either locally or within the circulatory system [3]. The motility of the gastrointestinal tract depends on many factors such as the good coordination of the tunica muscularis via the interaction of the intrinsic and extrinsic nerve plexuses [4], among others. Assessment of the motility of the stomach helps in the knowledge of the gastrointestinal function and its pathophysiology [5]. To further evaluate gastrointestinal (GI) motility, the therapeutic potential of new substances in motility disorders, the alteration in motility secondary to physiological or pharmacological stimuli, and the effect of pathological conditions on GI transit, [6] are crucial.

Fasting is the act of freely withholding food and fluids [7] [8]. Various research work have shown that fasting is beneficial to the well-being of man and it also improves health [9] [10] [11]. These benefits include reducing the risk of metabolic non-communicable diseases, and immune disorders, improving aging and potentiating a saver and longer life [11].

Studies surrounding gastrointestinal motility and fasting are little to none. In contrast to human studies, the *in vivo* gastrointestinal motility studies in rats are usually performed in the fasted state [12], rather than in the fed state for eliminating interference caused by the presence of food [13]. Obembe *et al.* [7] described the *in vitro* intestinal motility studies in the normal fasting duration period. Delayed gastric emptying was reported by Heruc *et al.* [14] after 4 h fasting in an anorexia nervosa patient. Other studies described the relationship between gastrointestinal motility and feeding disorders such as anorexia nervosa and this is because more complaints of gastrointestinal motility disorders are common in individuals grieving from eating disorders [15]. Prolonged intermittent and continuous modes of fasting are usually due to religious purposes, this study investigated the effect of different extended fasting durations on gastrointestinal motility.

2. Materials and Methods

2.1. Materials

Acacia gum/Arabian gum, Glass beads, Activated charcoal, Weighing scales, Rat feeds, Feeding troughs, Plastic cages, Drinkers, Distilled water, Glucometer and Glucometer strips, Filter paper, Syringes (1 ml and 5 ml), Catheter, Oral cannula, Measuring cylinder, Tween 80 oil (lubricant), Methylated spirit, Cotton wool, Hand gloves, Thread, Measuring ruler, Stopwatch, Dissecting set, Dissecting board.

2.2. Experimental Animals and Grouping

Forty-five (45) male Wistar rats, weighing between 180 - 200 g were used. The rats were purchased from the Central Animal House, University of Ibadan. The rats were kept in plastic cages bedded with wood shavings and with a wire mesh cover, which was maintained at room temperature. They were acclimatized for two weeks and were allowed free access to water and adequate food before the start of the different experimental protocols. The rats were randomly assigned to three (3) groups: Control, rats fasted for 18 (18 H), 48 hours fasted group (48 H) and the 72 hours fasted group (72 H). Each group comprised fifteen (15) rats and five (5) rats per group were used for gastric emptying, intestinal transit time, and colonic motility experiments.

2.3. Experimental Design

2.3.1. Preparation and Administration of Hydrated Feed

The rats used for the gastric emptying study were introduced to a hydrated diet three (3) days into the experiment. This was to ensure that they adapted to the new food before the day of the experiment [16]. The hydrated meal was prepared by adding 45 g of grind pellet to distilled water to make 100 ml of the paste (meal) after which it was stored in the refrigerator at 4˚C for 16 hours.

2.3.2. Determination of Blood Glucose Level

The blood glucose level measurements were taken twice, before fasting and after fasting using the glucose oxidase method with an Easymax® glucometer. A drop of blood was obtained and used after a mild prick on the tail vein of a restrained rat.

2.3.3. Determination of Gastric Emptying

Five (5) rats fasted at certain period intervals from each group: 18 hours (Control), 48 hours (48 H), and 72 hours (72 H). The rats were then given 2 mL of hydrated feed orally through oral gavage administered with an oral cannula. The rats were sacrificed 2 hours after by fast cervical dislocation and laparotomy was done subsequently to allow assessment of the experiment. The pyloric and cardiac ends of the stomach were ligated to prevent the escape of stomach contents. The stomach was carefully excised, removed, and weighed with the food content after which it was opened along its greater curvature and its contents rinsed off with normal saline and dried with filter paper. The emptied stomach was then weighed. Measurements of the weight of the full stomach, empty stomach, and body weight were obtained. The weight of food remaining in the stomach was expressed per gram of body weight. The gastric emptying was calculated thus;

$$\text{Gastric Emptying} = \frac{\text{Full stomach weight} - \text{Weight of Empty Stomach}}{\text{Body weight}} \quad [17].$$

2.3.4. Determination of Intestinal Transit

Five (5) rats per group fasted for the assigned duration of 18 hours (Control), 48 hours (48 H), and 72 hours (72 H). After fasting, 1 mL of the meal containing 10% (10 g) of charcoal in 5% (5 g) of acacia gum was constantly stirred and the aqueous suspension was fed orally to rats by gavage. After 30 minutes of administration of the meal, rats were sacrificed through quick cervical dislocation, and then laparotomy was conducted to expose the intestine for excision. The entire small intestine starting from the pyloric end of the stomach to the ileocaecal junction was rapidly removed and measured. The total length of the intestine and the distance moved by the charcoal meal within the intestine were measured and expressed as percentage inhibition, using the formula:

$$\%\ \text{Intestinal transit} = \frac{\text{Distance traveled by charcoal} \times 100}{\text{The total length of the small intestine}} \quad [18].$$

2.3.5. Determination of Colonic Motility

This experiment was executed according to the method described by Coates *et al.* [19] as modified by Odukanmi *et al.* [20]. Briefly, thereafter the duration of fasting by the groups, two 3 mm gold colored plastic beads were introduced to the distal 2 cm end of the colon from the anal opening with the aid of a lubricated glass rod. Each rat was timed in the plastic cage. The time taken for the expulsion of the beads was obtained in seconds. The colonic motility was taken as the amount of time between the time of insertion of beads and the time of the expulsion of beads.

2.4. Statistical Analysis

Data was collated, computed and the results were expressed as Mean ± Standard Error of Mean (SEM) and analyzed using one-way analysis of variance (ANOVA), Newman-Keuls *Post hoc* test was adopted using GraphPad Prism version 5.0 for Windows (GraphPad Software Inc., San Diego, CA), $p \leq 0.05$ was considered significant.

3. Results

3.1. Effect of Extended Fasting Duration on Blood Glucose Level

Post fasting durations show that 48-hour (2.94 ± 0.35) and 72-hour (3.20 ± 0.32) fast has no significant effect on the blood glucose level of rats compared to control (3.62 ± 0.19) which was fasted for 18 hours, **Figure 1**.

3.2. Effect of Extended Fasting Duration on Gastric Emptying

The effect of 72 H fasting (0.20 ± 0.08) on the gastric emptying of rats decreased significantly, $p \leq 0.05$ compared to the control (0.64 ± 0.16) which was fasted for 18 hours. The 48 hours (0.60 ± 0.07) fast had no significant effect on gastric emptying compared to the control, Figure 2.

3.3. Effect of Extended Fasting Duration on Intestinal Transit

Figure 3 describes that both 48 hours (67.54 ± 6.15) and 72 hours (72.10 ± 7.60) fasting increased significantly the intestinal transit of charcoal meal compared to control (42.14 ± 3.14).

3.4. Effect of Extended Fasting Duration on Colonic Motility

Figure 4 shows that the effect of 48 (2707 ± 864.10) and 72 (6363 ± 968.10) hours

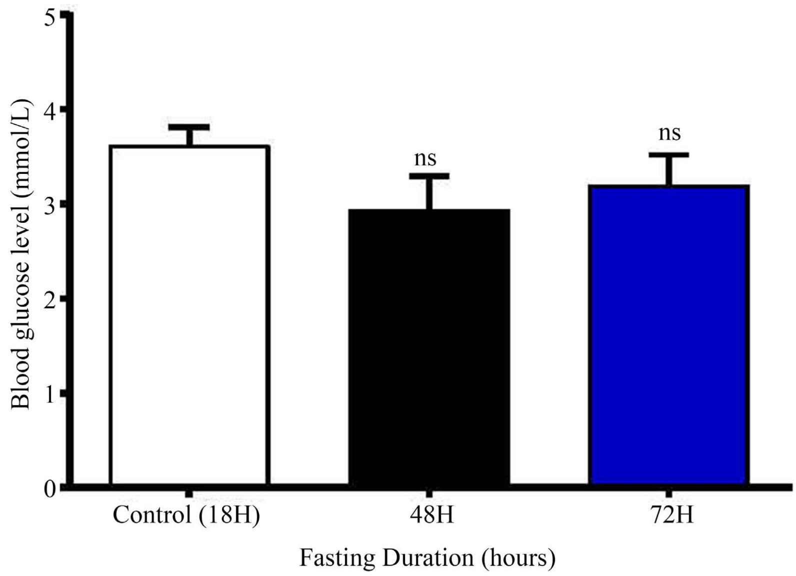

Figure 1. Effect of different fasting durations on fasting blood glucose levels of fasted rats. [ns]Not significant compared to control, $p > 0.05$.

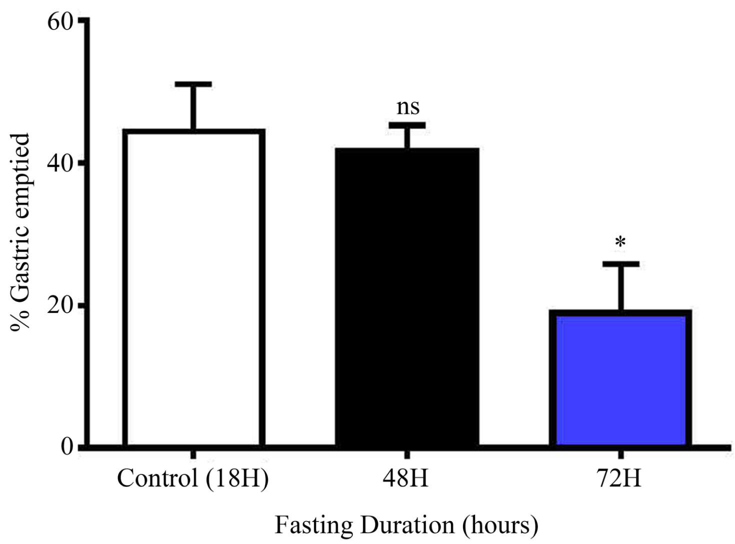

Figure 2. Effect of different fasting durations on gastric emptying of fasted rats. [ns]Not significant compared to control, $p > 0.05$; *Significant decrease compared to control at $p \leq 0.05$.

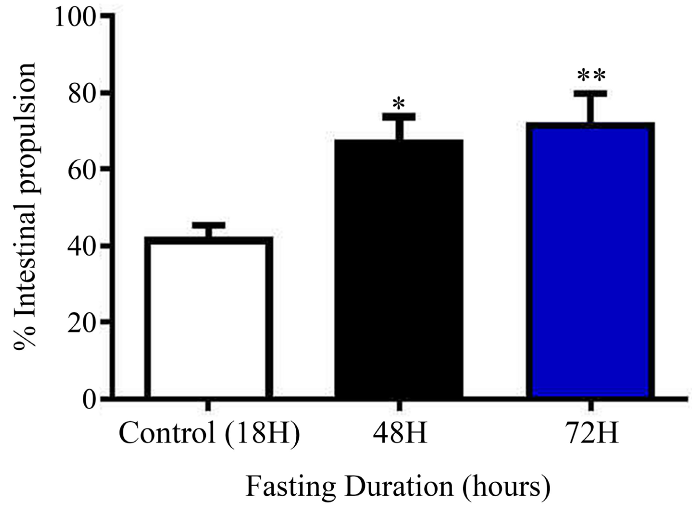

Figure 3. Effect of different fasting durations on intestinal transit of fasted rats. *A significant increase compared to control at $p \le 0.05$; **Significant increase compared to control at $p < 0.01$.

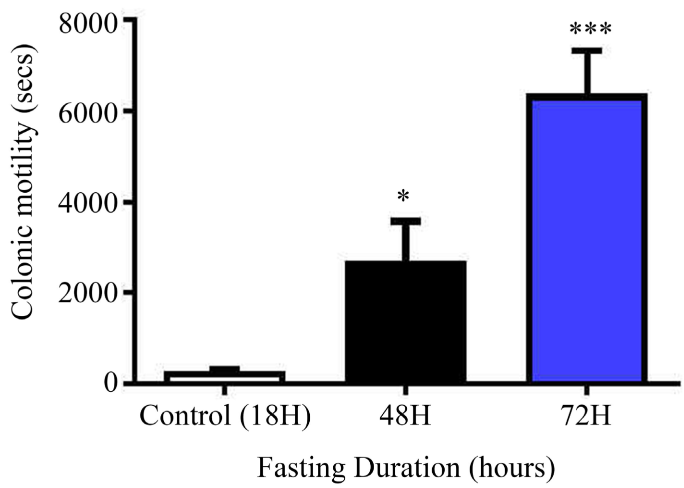

Figure 4. Effect of different fasting duration on colonic motility time. *Significant decrease in colonic motility compared to control at $p \le 0.05$; **Significant decrease in colonic motility compared to control at $p \le 0.01$.

fast on colonic motility time decreased significantly, $p \le 0.05$ compared to control (263.8 ± 64.26).

4. Discussion

Fasting as a topic have been meticulously studied in the general population of individuals both healthy and ill. The effect of fasting usually are profound and could affect cellular physiology and metabolism [8]. During fasting periods depending on the duration, different systems and mechanisms are called upon for the maintenance of glucose homeostasis. The result of this current study shows no significant change in the blood glucose level following a long-term fast compared to that of the control group that fasted for 18 h. This could be a result of the meshwork of mechanisms put in place by the regulatory systems. Within the initial 24 h fast, it has been described that blood glucose sustainability lies within the glycogen stored within the liver and the skeletal muscle [8] and that this is depleted after the initial 24 h. Subsequently, the blood glucose is sustained with the effort of adipose tissue catabolism, and protein catabolism, through the process of gluconeogenesis that concurrently occur during prolonged fasting [21] [22].

The decrease in the percentage of gastric emptied in the rats who fasted for 72 hours in this study is in agreement with the work of Corvilain *et al.* [23] where humans fasted and their gastric motility was monitored after short-term starvation of 4 days with intermittent 12 - 14 h fast each day [24]. Although the duration of fast was shorter and the mode is slightly different from what was obtainable in this study. The delay in gastric emptying may be because of the likely cause of the slower systemic appearance of glucose during refeeding following a fast [23]. The observed effects of fasting on gastric emptying are comparable in size to that observed in patients with restrictive anorexic behavior [15]. This delay appears to occur with the duration of fasting. It may not be unconnected with the reduced vagal tone during fasting [25] [26].

The increase in the intestinal transit in the long-duration fasted groups in this study agrees with the work of Kotal *et al.* [27] and Mittelstadt *et al.* [28]. Although, the duration of the fast was 48 hours for Kotal *et al.* [27] and 6 h for Mittelstadt *et al.* [28] while determining intestinal transit. Obembe *et al.* [7] used a lesser fast duration as well and reported that fasting at 4 h duration does not affect intestinal transit. This current study has buttressed the fact that it is even further reduced with an extended duration of fasting. The possible increase in intestinal transit may be due to an increase in enteric neuron signaling increasing the phase of the migrating motor complex during fasting [29].

The decrease in the colonic motility in the extended fasting duration groups in this study is in agreement with the study by Kotal *et al.* [27], where it was reported that fasting decreased intestinal motility in rats. Note that the rats in the study carried out by Kotal *et al.* [27] fasted for 48 h and this is the first work that shows the effect of a 72 h fast on colonic motility. The decrease observed in colonic motility could be due to a decreased fluid and fiber intake.

In conclusion, it can be inferred that a 48 - 72 hour fast decreases gastric emptying and colonic motility compared to 18 h fast. Thus suggesting that extended fasting durations might be beneficial in conditions presenting with spasms requiring relief or situations where the gut smooth muscles are required to relax. The mechanisms by which extended durations modulate gastrointestinal motility and the overall gain of its effect on the duration of fasting can be elucidated in further studies.

Conflicts of Interest

Authors declare no conflict of interest.

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