

## Effects of Hypothyroidism and Exogenous Thyroxine on Gastrointestinal Organs of Rat

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**Summary:** Thyroxine (T4) is important in gut development and maturation, and its use in treating hypothyroidism is becoming more popular. This study was conducted to evaluate the effect of thyroidectomy and thyroxine replacement on some gastrointestinal organs. Ten out of 20 thyroidectomised rats received 100pg/kgbw of T4 for five weeks to become euthyroid while the rest were left to become hypothyroid. Ten sham operated rats were made hyperthyroid by giving 100pg/kg.bw of T4 for five weeks, while the other ten sham operated rats served as control. 10mg/kg.bw intraperitoneal injection of ketamine was given as anesthesia for thyroidectomy and sham operation. At the end of the fifth week, the animals were sacrificed. Liver, stomach and small intestine were harvested and their morphological dimensions measured. Everted sacs were made from the small intestine for glucose transfer studies and slides for histomorphometry. There was no significant difference in the weights of the liver and stomach of the groups when compared with the control group. There was significant ( $p<0.05$ ) increase in length and diameter but reduced wall thickness in the hyperthyroid small intestine; unlike that of hypothyroid which had significant ( $p<0.05$ ) shorter length, decreased diameter but increased wall thickness. Villi length and crypt depth was higher in hyperthyroid ( $p<0.01$ ) but smallest in the hypothyroid ( $p<0.05$ ). Glucose transfer was lesser in the hypothyroid but greater in the hyperthyroid intestine. These findings show that hypothyroidism diminishes the morphological variables of absorption in the small intestine as a mechanism to reducing its transfer capacity, while thyroxine replacement increases these variables as mechanism to increasing intestinal transfer capacity.

**Keywords:** Thyroidectomy, Thyroxine, Gastrointestinal organs, Everted sac, Glucose transfer.

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### INTRODUCTION

Thyroxine (T4) is important in the development of the gut (Hodin, et al., 1992) and postnatal intestinal maturation (Hodin, et al., 1996). The potential role of the intestine, in the interactions of the gut with thyroid hormone, is to serve both as a reservoir for thyroid hormones and as a regulator of the hormone activity. Excess of or deficiency in thyroid hormone alters the function and metabolism of the gastrointestinal tract with hypothyroidism appearing to affect the gastrointestinal (GI) tract more profoundly than hyperthyroidism. Thyroxine affects the transportation of substances, including glucose, across the small intestine. A good number of researchers have reported an increased glucose transport after thyroxine treatment (Adeniyi and Oloowokurun 1987; Olaleye and Elegbe 2005), while some have reported an inhibition of glucose transport in the small intestine upon thyroxine ingestion (Halliday, et al., 1962; Matty and Seshadri 1965). Khoja and Kellett (1993), for instance, have reported normal glucose absorption and an increased net transmural transport of glucose. Thus,

animal studies have demonstrated complex and conflicting effects of thyroxine on active, electrogenic transfer of glucose in the small intestine, even though it was shown, in a more recent study that glucose transport across the small intestine was increased (Fabiyi, et al., 2014). GI tract disorders common to hypothyroidism include: intestinal pseudo-obstruction, intestinal myxedema, dilatation of the GI tract and decreased intestinal motility among others. Thyroxine (T4) is the first hormone replacement therapy, first initiated over a century ago. Shafer, et al., (1994) has been able to show that gastrointestinal motility was corrected in hypothyroid patients upon T4 replacement. It is, however, observed that there is paucity of comprehensive research on how chronic administration of oral T4 alters the morphology of the gastrointestinal tract and the influence of such alteration on absorption of substances in the small intestine. Again, considering the fact that exogenous Thyroxine (T4) orally administered is absorbed from the lumen of the jejunum and ileum within hours of ingestion (Hays 1991); this study seeks to investigate

how exogenous thyroid hormone, upon chronic administration, affects gastrointestinal function and morphology, especially those concerned with absorption. And the extent to which the structural gastrointestinal alterations seen in hypothyroidism can be reversed by T4 replacement.

## **MATERIALS AND METHODS**

### **Experimental animals**

Forty albino Sprague Dawley rats weighing 150-200g were obtained from the animal house in the Department of Anatomy, University of Ibadan. They were kept in a 12-hour light and dark cycle animal house with standard animal housing conditions and were fed with standard rat pellets and clean water ad libitum until the experiment was carried out (Guide for the Care and Use of Laboratory Animals, NIH publication 86-23 revised 1985). Experimental protocols complied with the 'Principle of Laboratory Animal Care' (NIH publication No. 85-23) guidelines (PHS, 1996).

### **Animal grouping**

Forty (40) animals were used for this study. Twenty (20) of them were sham operated (SO), while the remaining twenty (20) were thyroidectomised (TX), after anesthesia with an intraperitoneal injection of 10mg/kgbw ketamine hydrochloride. Ten SO rats were given 100pg/kgbw of levothyroxine (Forley Generics Ltd, UK), which was administered orally for 35 days to make them hyperthyroid; while the other ten SO rats served as control. Again, ten TX rats orally received 100pg/kgbw levothyroxine orally for 35 days to serve as the Euthyroid group, while the other ten TX rats served as the hypothyroid group.

### **Thyroxine (T4) Assay**

On the 35th day, blood was collected from the animals through cardiac puncture. The blood was centrifuged at 3,000rpm for 5 minutes, and the serum was separated. Serum T4 was quantified using immunochemiluminiscence.

### **Animal sacrifice**

The rats were sacrificed on the 35th day post-surgery via cervical dislocation. Their stomachs, small intestines and liver were harvested and weighed.

### **Morphological measurements**

Length of the small intestine of the rats was measured using thread and a standard meter ruler. Intestinal thickness and diameter were measured by means of a digital vernier caliper.

### **Glucose transport studies**

Everted sacs were made from jejunum and ileum (- as described in an earlier study- Fabiyi, et al., 2014). 2mls of Krebs solution containing 522mg/dl concentration

of glucose was injected into the sacs (serosal fluid) before tying its ends. The sacs were incubated in 15mls of Krebs bicarbonate solution containing 522mg/dl (mucosal fluid) for 30minutes. Glucose concentration in the sac and in the suspending fluid was measured using a glucometer. Disappearance of glucose from the outer mucosal fluid was termed mucosal glucose transfer (MGT) while increase in glucose concentration in the inner serosal fluid was termed serosal glucose transfer (SGT).

### **Preparation of Tissues for Microscopic examination**

Tissue preparation for microscopic examination was done in line with the method of Drury and Wallington (1994). The small intestine was cut open and its contents emptied and the intestine rinsed in normal saline. Tissue blocks from the small intestine was fixed in 10% neutral formalin after which they were dehydrated using alcohol and then cleared in xylene. They were embedded in paraffin wax and thin sections cut at 5 microns. The sections were stained with hematoxylin for 15 minutes, differentiated with 1% acid alcohol counter-stained in eosin for 2 minutes and mounted with dextrene polystyrene xylene (DPX). The sections were viewed under a microscope, after which photomicrographs were taken.

### **Determination of Villus Dimension (Histomorphometry)**

The dimension of villi was measured in sections of the intestine examined under a dissecting microscope. Images of jejunum and ileum were captured using a digital camera, and were displayed on a computer connected to the microscope. Five villi each were selected for macroscopic analysis using x40 magnifications. The villus height was taken as the distance from the crypt opening to the tip of the villus and the crypt depth measured from the base of the crypt to the level of the crypt opening (Obembe et al., 2011). Motic image plus 2.0ML software was used to measure the villus height and crypt depth.

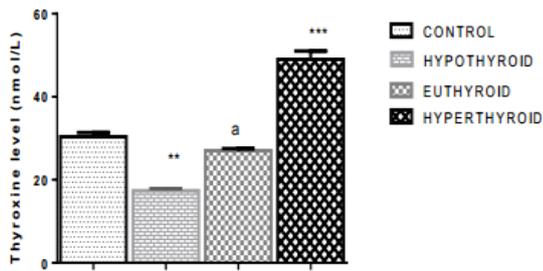
### **Statistical Analysis**

Data was expressed as mean  $\pm$  SEM and analysed using GraphPad Prism version 4.00 (GraphPad Software, San Diego California USA). Statistical significance was tested using One-way ANOVA with Newman Kaul's post-test. P value of 0.05 was considered significant.

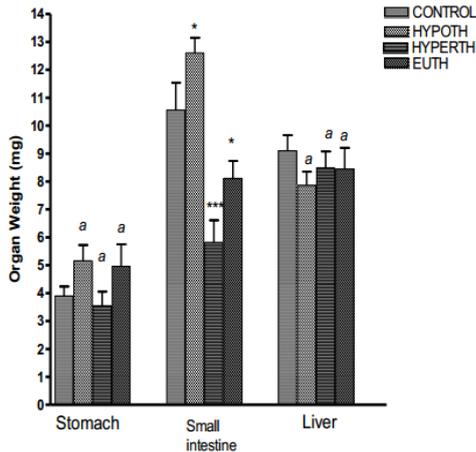
## **RESULTS**

Hyperthyroid group had significantly ( $p < 0.001$ ) high thyroxine level, while hypothyroid T4 level was markedly ( $p < 0.05$ ) lower than that of the control (Figure 1).

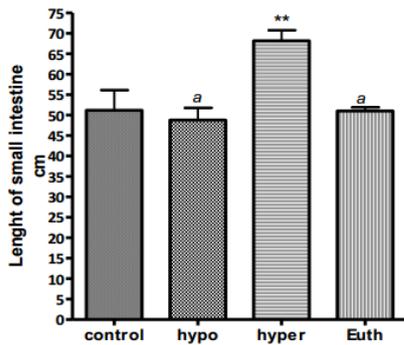
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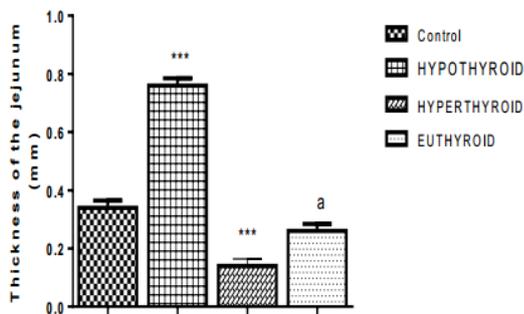
**Figure 1:** T4 level in the rats after the treatment period. The Values are expressed as Mean  $\pm$  S.E.M. (n=10 rats). \*\* $p$ <0.01, \*\*\* $p$ <0.001,  $a$  $P$ >0.05



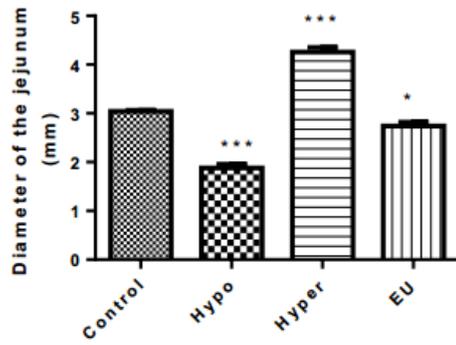
**Figure 2:** Gastrointestinal organ weights of the rats after the treatment period. Values are expressed as Mean  $\pm$  S.E.M. (n=10 rats). \*\* $p$ <0.01, \*\*\* $p$ <0.001,  $a$  $P$ >0.05



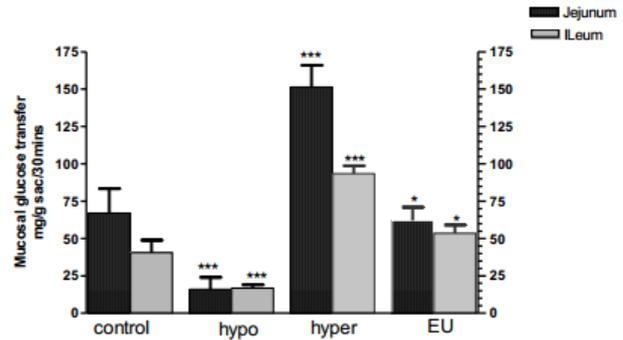
**Figure 3:** The length of the small intestine after the treatment period. Values are expressed as mean  $\pm$  S.E.M. (n = 10 rats). \*\* $p$ <0.01;  $a$  $P$ > 0.05



**Figure 4:** Thickness of the small intestine of thyroxine treated and thyroidectomised rats. Values are expressed as mean  $\pm$  S.E.M. (n = 10 rats). \*\* $p$ <0.01 significantly different from control;  $a$  $P$ >0.05 no significant difference from control.



**Figure 5:** Luminal diameter of the small intestine of thyroidectomised and thyroxine treated rats. Values are expressed as mean  $\pm$  S.E.M. (n = 10 rats). \*\*\* $p$ <0.001; \* $p$ <0.05



**Figure 6:** Mucosal glucose transfer capacity in the small intestine of TX and T4 treated rats. Values are expressed as mean  $\pm$  S.E.M. (n = 6). \* $p$ <0.05, \*\*\* $p$ <0.001 significantly different

The weights of the stomach and liver were significantly unaffected by either TX or T4. However, the hypothyroid small intestine showed a significant ( $p$ <0.05) decrease in weight, while the hyperthyroid and euthyroid small intestines showed significant ( $p$ <0.5) increase in weight (Figure 2). In addition, the hyperthyroid small intestine was significantly ( $p$ <0.01) longer than the control (Figure 3). Hypothyroid small intestine showed significant ( $p$ <0.001) increase in thickness and decrease in luminal diameter, while hyperthyroid small intestine was greatly ( $p$ <0.001) reduced in thickness but increased in luminal diameter (figures 4 and 5). Mucosal glucose transfer (MGT) is an index of Glucose transfer capacity in the small intestine. Hyperthyroid small intestine had a greater ( $p$ <0.001) MGT while hypothyroid small intestine had the least MGT (figure 6).

### Histomorphometry

Villi length was highest ( $p$ <0.05) in hyperthyroid jejunum and ileum, but was smallest ( $p$ <0.05) in the hypothyroid jejunum and ileum. Euthyroid jejunum villi length was greater ( $p$ <0.05) than the control. Crypt dept was lower ( $p$ <0.01) in hypothyroid but higher ( $p$ <0.05) in hyperthyroid than control (table 1). The villi of the hypothyroid jejunum appeared shorter

Table 1: Villus length and crypt depth in the small intestine of hypothyroid and thyroxine treated rats

	Villus Length		Crypt depth	
	Jejunum (µm)	Ileum (µm)	Jejunum (µm)	Ileum (µm)
<b>CONTROL</b>	911.16 ±33	636.53±52.6	257±15	126.77±17.6
<b>HYPO</b>	608.29±29.5*	491.3±60.5+	122.6±19.4*	148.6±23.4 <sup>a</sup>
<b>HYPER</b>	2548.63±115**	945.6±54.7*	316±11.7+	224.73±7.4*
<b>EUTH</b>	1052.97±130+	501.8±35.8 <sup>a</sup>	237±11.4 <sup>a</sup>	127.17±12.6 <sup>a</sup>

Values are mean ± SEM with n=6. \*\*p< 0.001, \*p<0.01, +p<0.05 vs control, aP>0.05 not significant

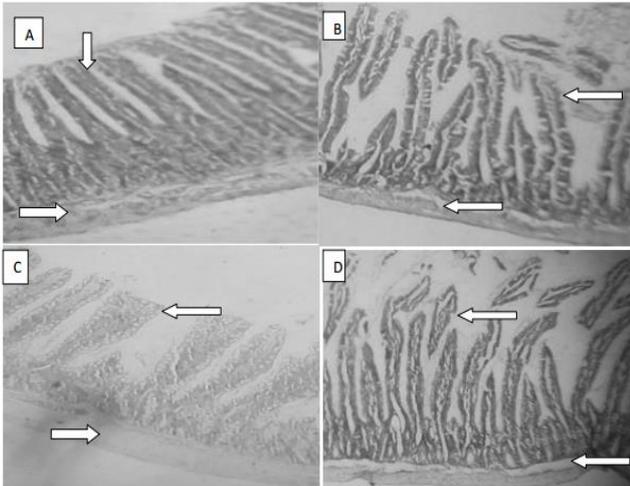


Figure 7: Histology of the small intestine (jejunum) in different thyroid states. A-Control; B- Euthyroid; C- Hypothyroid; D-Hyperthyroid

and wider (thicker), and the wall was thicker than that of other groups, as well as control. However, thinning of the intestinal wall was observed in the hyperthyroid jejunum (Figure 7).

**DISCUSSION**

It has previously been shown by Tata et al (1962) that the basal metabolic rate of rats is raised by the T4 administration of 10-25ug/100g body weight every fourth day. As a result of this, thyroxine dose of 100mg/kgbw/day was used in this study to induce hyperthyroidism and euthyroidism. Treatment efficacy was confirmed by determination of serum total T4 level to ascertain the establishment of the altered thyroid states in the rats. The hypothyroid group has a significantly lower serum thyroxine level than the control after the removal of the thyroid gland, thus showing a progressive fall in extrathyroidal store of thyroxine overtime.

Thyroid hormones act on almost all organs throughout the body and regulate basal metabolism. The gut and viscera are not excluded, and disturbances in thyroid function have numerous gastrointestinal manifestations (Maser et al., 2006). The seemingly reduced stomach size in the hyperthyroid and increased stomach weight in the hypothyroid may be due to increased rate of gastric emptying and muscle

edema respectively. The stomach empties rapidly in hyperthyroidism, whilst in hypothyroidism gastric emptying is prolonged (Holdsworth and Besser 1968; Gunsar et al., 2003). Gastric dysmotility seen in hypothyroidism, may be as a result of altered myoelectrical activity and muscle edema (Greenspan and Rapaport 1992). The hypothyroid group had a slightly reduced liver size which may be due to decreased hepatic glucose output and activities (Liverini 1992).

The Significant increase in weight gain and intestinal wall thickness observed in the hypothyroid small intestine may be due to myxoedematous infiltration or fluid retention in the intestinal wall. According to Shafer et al., (1984), the effect of hypothyroidism on the gastrointestinal tract seems to be multifactorial and caused by infiltration of the intestinal wall with a reduction in peristalsis, being the main pathophysiologic process. The hyperthyroid group had significant decrease in the weight of their small intestines. This may be due the thinning or reduction in the thickness of the intestinal wall observed in this study, such thinning of the intestine had also been observed in dietary restricted (semi starved) rats by Neale and Wiseman (1969). The decrease in the weight of the hyperthyroid intestine observed in this study is at variance with the findings reported by Liberman et al., (1979), who observed that there was no significant change in intestinal weight of euthyroid rats made hyperthyroid, and Levin and Smyth (1963) who observed an increase in intestinal weight in hyperthyroid rats. The disparity in these studies may be due to differences in dose, duration and methods of thyroxine administration. This study, in particular, has shown that chronic administration of thyroxine to euthyroid rats results in a decrease in intestinal weight and wall thickness. Hypersecretory state within the hyperthyroid intestinal mucosa has been reported (Tenore et al., 1996, Kim and Ryan 2002), and this could be a reason for the thinning or decrease in the thickness of the intestinal wall. This may also be due to tissue wasting associated with hyperthyroidism resulting from increased metabolism. However, it was also observed, though not reported, that some of the hyperthyroid intestine had very thin

wall which bleed, resulting, suggestively, in excessive erosion of intestinal wall.

Again, unlike the reduction in the intestinal weight, there was a marked increase in the length of the hyperthyroid small intestine, contrary to the shortening of the small intestinal length of rats reported by Hindmarsh et al., (1967). Hindmarsh actually reported thinning of the intestinal wall in rats subjected to semistarvation. The semistarved rats had a weight loss of 18 -28% of their initial body weight and about 24 -29% loss in intestinal dry weight. This was accompanied by a slight shortening in the length of the intestine. Hyperthyroidism in this study mimicked this weight loss and thinning of intestine, but not the shortening of intestinal length. Our current study observed that thyrotoxic rats eat more, have increased lipogenesis and predominantly burn fat, just as Oppenheimer et al., (1991) observed in his study. This implies that although the rats' food intake was voracious, it was almost immediately burned out, and never enough to compensate for the increased metabolism induced by excess thyroxine. Thus, some conditions seen in starvation may appear. Intestinal hypermotility and increased myoelectrical activity may have led to the stretching or lengthening of the intestine in hyperthyroid rats (Wegener et al., 1992). Glucose may leave the intestine at a faster rate in the hyperthyroid state, and in the presence of intestinal hurry, may be distributed to a greater length and mucosal surface area. This could be a mechanism to make up for the short transit time and highly increased absorption seen in hyperthyroidism.

Diameter of the small intestine was significantly reduced in hypothyroid rats but increased in hyperthyroid rats. This is in agreement with the report of Fraichard et al., (1997) that the diameter of the jejunum and ileum were reduced in thyroxine receptor knockout mice.

The Initial glucose concentration was set at 522mg/dl which was close to 500mg/dl used by Beryl et al (1961). The changes in concentration in mucosal and serosal solutions depended on the relative movements of fluid and glucose. Across the groups, the concentration of glucose in the mucosal fluid at the end of the everted sac experiment was lower than the initial glucose concentration. The lower mucosal glucose concentration at the end of the experiment shows a positive glucose transfer from the outer mucosal fluid to the inner serosal fluid in the sacs. The greatest mucosal transfer was observed in the hyperthyroid and the least transfer was seen in hypothyroid intestine. There was also a significant decrease in glucose concentration in the serosal fluid of hyperthyroid intestine. This means that glucose transport was impaired in hypothyroid intestine but increased in euthyroid and hyperthyroid intestines. In this study, histomorphometry showed an increase in Hypothyroidism and gastrointestinal organs of rats

height of the villi of hyperthyroid rat indicating hypertrophy of the microvillus border (Wall, et al., 1970). The decreased villus height and crypt depth in the hypothyroid intestine and generally in the ileum suggest reduced absorptive and secretive activity (Kelly, et al., 1998). In addition, the infiltration of the hypothyroid intestine and thickening of the wall may slow down the rate of glucose transport, thus, contributing to impaired glucose absorption. Increased villi length and crypt depth in the hyperthyroid jejunum and ileum, compared to the other groups, evidently support, the increased glucose absorption observed in hyperthyroidism, while increase in Euthyroid jejunum suggests that thyroxine replacement ameliorates the effect of thyroidectomy on the villi size.

In conclusion, thyroxine caused the thinning of the intestinal wall to reduce transfer barrier; caused the lengthening of the small intestine; increased luminal diameter of the small intestine; increased villi length and crypt depth to increase absorption; and increased glucose transport in the small intestine. But the reverse of these is obtained by thyroidectomy. These findings show that thyroxine increases morphological absorption variables in the small intestine to match the increase in absorption of nutrient such as glucose induced by thyroxine, while these variables, as well as glucose transport activities, are reduced by thyroidectomy.

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