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Full Length Research Paper

Effects of Thyroidectomy and Thyroxine on Glucose Transport Capacity in the Jejunum and Ileum of Rat

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ABSTRACT

Thyroid hormone has been known to alter glucose metabolism. This study was conducted to investigate the effect of thyroidectomy and thyroxine on glucose transport in the small intestine. Forty rats were randomly selected into four groups of ten rats. Groups one and two rats were thyroidectomised to make them hypothyroid after which group two rats received T4 replacement of 10ug/100g b/w for thirty-five days to make them euthyroid. Rats in groups three and four were sham operated thereafter group three rats received 10ug/100g b/w thyroxine for thirty-five days to make them hyperthyroid. 10mg/kg b/w Ketamine was administered intraperitoneally as anesthesia before the surgeries. On the thirty-fifth day post-surgery all the animals were sacrificed and their small intestines were harvested. 10cm length of jejunum and ileum respectively were used to make everted sacs for the *in vitro* study. Mucosa glucose transfer (MGT), Final glucose concentration gradient (FCG) and Gut glucose uptake (GGU) were significantly higher ($P< 0.05$) in the hyperthyroid group and lower ($P< 0.05$) in the hypothyroid group compared with the control with transport in the jejunum greater ($P< 0.05$) than the ileum in all groups. Serosal glucose transfer (SGT) was Negative in the hyperthyroid group. These findings suggest that thyroidectomy reduced glucose transport while thyroxine increased glucose transport in different segments of the small intestine with the transport in the jejunum greater than that of the ileum. But excess thyroxine may cause reverse glucose transport in the small intestine.

Key words: Glucose, transport, thyroxine, thyroidectomy, Small intestine

INTRODUCTION

Thyroid dysfunction is one of the most common endocrine disorders and it appears to be closely linked with Diabetes Mellitus (DM) (Perros *et al* 1995). A recent meta-analysis, by Kadiyala, of all available data in 10 920 patients with DM revealed a mean frequency of thyroid disease of 11% (Kadiyala*et al* 2010).Thyroid hormones (TH) have well-described effects on glucose metabolism (Roubsanthisuk *et al* 2006) both by short-term and long-term interaction with the regulatory network for glucose homeostasis. However glucose regulation begins with intestinal absorption or transport. In the past there have been reported studies on intestinal glucose transport under altered thyroid states. Abdel-Fattah and Al-Balool (1977) studied the effect of thyroxine and adrenaline on the rates of glucose absorption by the small intestine of the Lizard (Mromastyx microlepis). They made the lizards hypothyroid with thiouracil and reported that thyroxine treatment increased glucose absorption with a rate proportional to the rate of thyroxine treatment. However

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this study did not account for the effect of long term administration of thyroxine on intestinal glucose absorption, in addition the effect may vary in mammalian intestine considering that they used reptiles. Cramer and M'Call (1918) reported that in rats, removal of the thyroid and the parathyroid glands does not produce any severe disturbance of carbohydrate metabolism. There is at first a diminution of the total metabolism, which is followed later by a compensatory increase. Halliday *et al* (1962) reported that glucose transfer across the isolated everted intestine of mice was inhibited when the animals were fed with 0.5 % desiccated thyroid for 14 days; Levin and Smyth (1963) observed that hyperthyroid rats show little change in their hexose transfer mechanism across the isolated intestine while Matty & Seshadri 1965 presented that thyroxine can produce inhibitory effects on the metabolic and transport processes of the intestine of the rat. On the contrary, Adeniyi and Oloowookurun (1987) reported that intestinal glucose transport is decreased in the hypothyroid state and increased in hyperthyroid state. Olaleye and Elegbe (2005) further studied the effect of thyroxine and catecholamine in altered thyroid states and reported that Catecholamines potentiated the effect of thyroid hormone on intestinal absorption of glucose in the rat. There seems to be more report in favor of exogenous thyroxine inhibiting glucose transport in the small intestine and studies did not report if thyroxine may influence glucose transport capacity in different segments of the small intestine differently as variational differences exist in the transfer capacity of different parts of the small intestine. Hence the reason for the present study

MATERIALS AND METHODS

Experimental Animals: Matured Sprague Dawley albino rats (100-180g) were obtained from the Central Animal house of the College of Medicine, University of Ibadan, Nigeria. Animals were maintained under standard nutritional and environmental conditions of normal relative humidity, room temperature, 12 hour light and 12 hour dark cycle. The animals were provided with standard food pellets and clean water *ad libitum.* Experimental protocols complied with the 'Principle of Laboratory Animal Care' (NIH publication No. 85-23) guidelines (PHS, 1996).

Animal grouping: The rats were grouped into four of ten rats each: Rats in the first group were thyroidectomised and kept for 35 days and served as the hypothyroid group, the second group was thyroidectomised and given 10ug/100g body weight levothyroxine (Forley Generics Ltd, UK) replacement, orally, for 35 days to serve as the Euthyroid group. The third group was sham operated and given 10ug/100g body weight of levothyroxine orally for 35 days to serve as the hyperthyroid group. While the fourth group was sham operated only and served as the control.

Thyroidectomy Procedure: The thyroid gland was completely removed surgically via the following procedure. The rats were anaesthesied with 10mg/Kg body wt Ketamine Hydrochloride injection USP (Rotex medica Germany) injected intraperitoneally. Each rat was pinned on a dissecting board with the ventral side up. A vertical or horizontal incision was made in the neck of the rat and blunt dissection made in the layers underneath the skin until the larynx is exposed. Essential structures such as the superior laryngeal nerve and parathyroid gland were identified as landmarks to the thyroid glands. The left and right lobes of the thyroid gland were devascularised from the thyroid arteries by tying a suture at the base of each after which the glands were cut and removed. The cut was closed by suturing and Peniciline powder was applied to the wound. The rats were allowed to recover and returned to standard diet and tap water.

Sham operation: For the sham operation the same surgical procedure as that of the thyroidectomy was followed except that the thyroid glands were neither devascularised nor extirpated.

Thyroxine (T4) Assay: On the thirty fifth day post surgery blood was collected by cardiac puncture from the rats in each group to determine serum T4 levels. The blood was put in clean sample bottles, allowed to clot at room temperature and centrifuged at 4000rpm for 30 minutes to separate the serum which was carefully pipetted into labeled serum bottles and frozen until analysis. T4 level was determined using chemi-immunoluminiscence, which was done in the immunology laboratory of the University college hospital, University of Ibadan, Nigeria.

The everted sac study: The rats were killed on the 35th day post- surgery, by cervical dislocation. The abdomen was opened by a midline incision, and the entire small intestine washed out with a solution of 0.9% (w/v) NaCl. The whole of the small intestine was removed by cutting across the upper end of the duodenum and the lower end of the ileum at the ileoceacal junction, and manually stripping the mesentery from the intestine. The intestine was everted using a stainless steel rod to push the ileal end of the gut into the gut lumen until it appeared at the

duodenal opening of the intestine, and rolling the proximal half of the intestine on the rod. The eversion exposed the highly active mucosa to the oxygenated suspending medium with the mucosa on the outside and the serosal now on the inside (Fisher and Parsons, 1949b; Wilson and Wiseman 1954). A thread ligature was tied around one end of the everted intestine to facilitate subsequent identification and sections of the jejunum and ileum was cut. 10cm length of everted jejunum or ileum was tied off at one end by a thread ligature and a second ligature was placed loosely around the other end. A blunt needle, attached to a syringe was introduced into the intestinal lumen and the loose ligature pulled tight over the needle. 2-3 mls of Krebs bicarbonate solution (Krebs & Henseleit, 1932) was injected into the sac, the needle was withdrawn and the ligature tied tight thus distending the sac. The distension increased the surface area of the sac and reduced the thickness of the sac wall while the fluid in the sac served as the serosal fluid. The distended sac was suspended in a mucosal fluid of 15ml of krebs bicarbonate solution according to Beryl (1961). The relatively small volume of fluid contained in the sac (serosal side) allows a rapid change in concentration of transferred substances across the intestinal wall. At the end of the experiment, the sac was removed, opened and the fluid drained out using a needle attached to a syringe. Samples of the final serosal and final mucosal fluids were taken for estimation of glucose concentration.

Measurement of glucose concentration: The initial and final concentrations of glucose in the Krebs Bicarbonate solution in the intestinal sac (serosal fluid) and the suspending medium (mucosal fluid) before and after incubation respectively were determined using a glucometer.

The methods of calculating and expressing the results: The methods of calculating the results are as follows: The fluid in which the sacs were suspended is called the mucosal fluid, and the fluid inside the sac the serosal fluid. The initial mucosal and serosal glucose concentrations are the concentrations in the mucosal and serosal fluid at the beginning of the experiment; the final mucosal glucose concentration is the glucose concentration in the mucosal fluid at the end of the experiment; the final serosal concentration is the concentration of glucose in the serosal fluid at the end of the experiment; the final concentration gradient is the difference between the final serosal glucose concentration and the final mucosal glucose concentration; the mucosal glucose transfer is the amount of glucose which disappears from the mucosal fluid; the serosal glucose transfer is the increase in glucose concentration in the serosal fluid; the gut glucose uptake is the difference between the mucosal glucose transfer and the serosal glucose transfer and includes glucose metabolized and also glucose present in the gut wall at the end of the experiment (Jervis & Smyth 1960).

Statistical Analysis: The results of the experiments were expressed as mean ± *S.E.M*. The statistical significance of differences was estimated with GraphPad Prism version 4.00 for Windows using "Newman-Keuls Multiple Comparison Test ANOVA". The value with $P < 0.05$ is considered to be significant..

RESULTS

Thyroxine assay: Results in Table 1 showed that T4 level was significantly ($P<0.01$) reduced in the hypothyroid group and increased ($P<0.001$) in the hyperthyroid group compare with control and euthyroid group.

Table 1:
Thyroid hormone concentration in the rats on the 35th day post-surgery

Group	Thyroxine level (nmol/L)
Control	30.4
Hypothyroid	17.4**
Hyperthyroid	49***
Euthyroid	27.1[a]

*The Values are expressed as mean ± S.E.M. (n = 10 rats). **P<0.01,*
****P<0.001 significantly different from the control value;[a]P> 0.05 no significant difference*

Glucose transport studies: Table 2 shows the initial and final glucose concentration in the mucosal and serosal fluids at the beginning and end of the incubation periods respectively. There was disappearance of glucose from the mucosal fluid as glucose is being transported across the sacs. Glucose transfer capacity is presented as percentage mucosa and serosal glucose transfer (Fig 1& 2). Percentage mucosal glucose transfer (MGT) was significantly ($P<0.01$) higher in the hyperthyroid and euthyroid groups but lower ($P<0.05$) in the hypothyroid group compared with control. In addition transfer was highest in the hyperthyroid group. Also percentage MGT was greater in the jejunum than in the ileum across the groups (Fig 1).

Table 2:
Glucose concentrations in the mucosal and serosal fluids at the beginning and end of the 30 minutes incubation period of the everted sac experiment.

Group	Control		Hypothyroid		Hyperthyroid		Euthyroid	
Glucose concentration (mg/dl)	J	I	J	I	J	I	J	I
Initial mucosal & serosal concn.	522	522	522	522	522	522	522	522
Final mucosal concn.	468.4 ±13	489.0 ± 6.7	494.4 ±6.8	501 ±2.9	400.8 ± 11.6	447.2 ± 4.2	463 ±8.4	475.4 ± 2.8
Final serosal concn.	544.2 ±4.3	534.8 ± 1.7	543 ±6.8	541.8 ±3.5	497.4 ±2.4	501.8 ± 2.5	529.8 ± 5.8	518.6 ±5.5

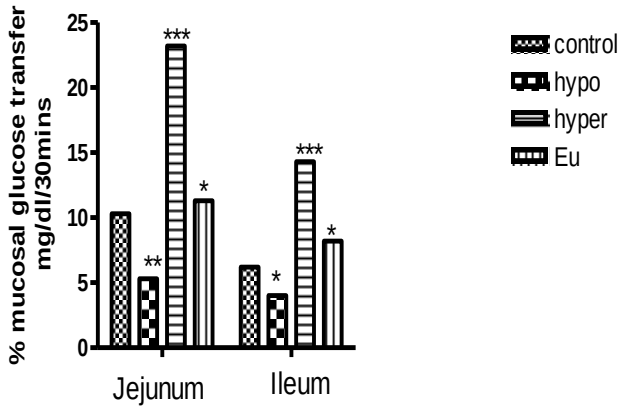

Figure 1:
Percentage Mucosa glucose transfer across the jejunum and ileum. *Values are expressed as percentage mean ± S.E.M. (n = 6 rats). *P<0.05, **P<0.01, ***P<0.001 significantly different from control*

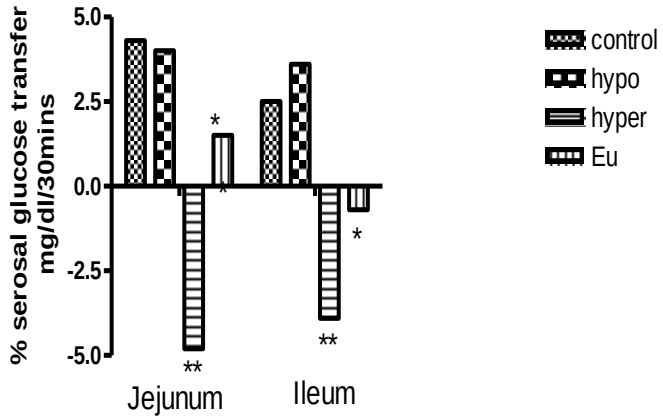

Figure 2:
Percentage serosal glucose transfer
*Values are expressed as mean ± S.E.M. (n = 6 rats). *P<0.05,**P<0.01 significantly different from control*

Positive serosal glucose transfer was seen in the groups except in the hyperthyroid intestine and euthyroid ileum where significant ((P<0.05) negative transfer was observed (Fig 2).

Gut glucose uptake (GGU) was significantly (P<0.01) lower in the hypothyroid jejunum and ileum and higher in the hyperthyroid jejunum and ileum. However GGU was not significant in the euthyroid jejunum compared with control (fig 3). Final concentration gradient (FCG) was significantly (P<0.05) greater in the hyperthyroid jejunum. However, FCG appeared to be lower in the hypothyroid compared with control and in the ileum compared to the jejunum though not significantly so (fig 4).

Mucosal glucose transfer (mg/g.sac/30mins) indicates the amount of glucose leaving the mucosal fluid per gram of sac in the course of the thirty minutes experiment. Least transfer was seen in the hypothyroid group (P>0.001) while the greastest mucosal transfer activity was observed in the hyperthyroid group (P>0.001), with lesser transfer in the ileum (fig 5).

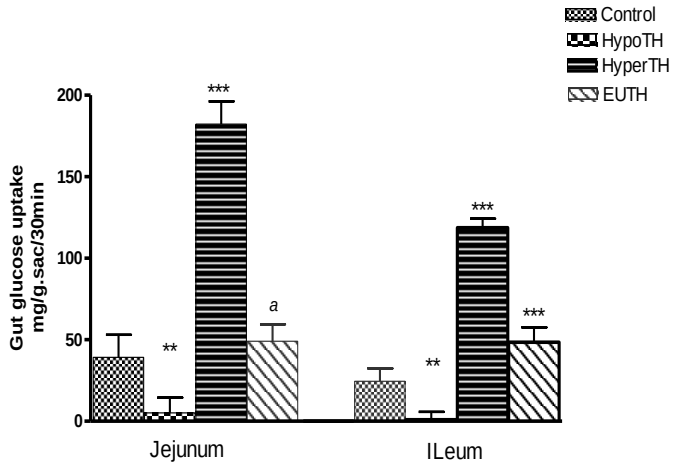

Figure 3:
Gut glucose uptake in different parts of the small intestine of altered thyroid rats

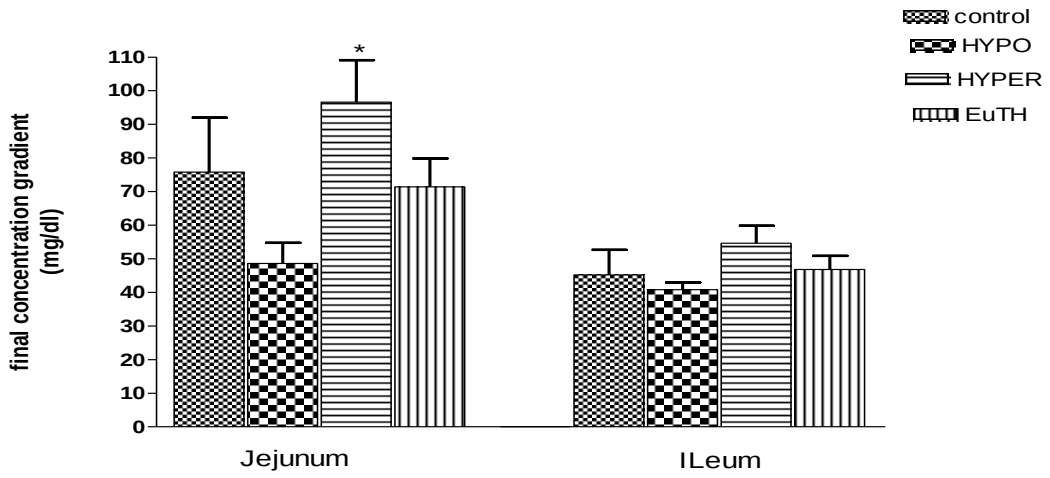

Figure 4:
Final concentration gradient across the jejunum and ileum.
*Values are expressed as mean ± S.E.M. (n = 6 rats). *P<0.05 significantly different from the control value*

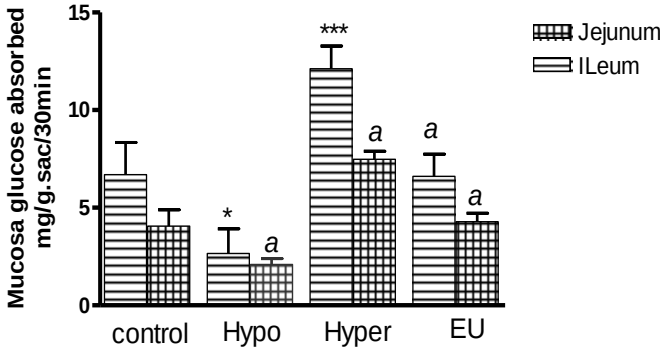

Figure 5:
Mucosal glucose absorption in the small intestine of different thyroid states. *Values are expressed as mean ± S.E.M. (n = 6rats). *P<0.05, ***P<0.001; [a]P > 0.05 no significant difference*

DISCUSSION

This study has demonstrated that thyroidectomy reduces intestinal glucose transport significantly while thyroxine increases glucose transport in both the jejunum and ileum. Glucose transport was increased in the euthyroid group in a manner similar to that of the control.

Tata (1962), have shown that the basal metabolic rate of rats is raised by the administration of only 10-25ug thyroxine/100g body weight every fourth day thus thyroxine dose of 10ug/100g b/w was used in this study to induce hyperthyroidism and euthyroidism. Treatment efficacy was confirmed by determination of serum thyroxine 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). Hypothyroidism is characterized by impaired glucose absorption from the gastrointestinal tract and delayed peripheral glucose assimilation and gluconeogenesis, decreased or normal hepatic glucose output and decreased peripheral tissue glucose disposal (Althausen, 1949).

The results provide detailed information of the activity of different part of the intestine for glucose transfer using the everted sac model. They include nine of the twelve different variables of absorption recommended by Beryl *et al* (1961). Although all these variables are dependent, there is a strong case for taking all of them into account in investigating any problem of intestinal transfer in vitro, and it is suggested that data from these variables should form an indispensable baseline for studying any effects on intestinal transport in vitro. The everted sac technique is an *in vitro* technique with the advantage that all regulatory factors whose influence may be unwanted or difficult to account for in an *in vivo* study have been eliminated by the isolation of the intestine (Ingmar *et al*, 2010), it also enables determination of regional variation in absorption in the different part of the intestine (Stephens *et al*, 2002).

The Initial glucose concentration was set at 522md/dl which was close to 500mg/dl used by Parsons *et al* (1958) and Beryl *et al* (1961). The changes in

concentration in mucosal and serosal solutions depended on the relative movements of fluid and glucose. In all the sacs the concentration of glucose in the fluid leaving the mucosal side (mucosal concentration transferred) is much higher than the initial glucose concentration, and hence the final mucosal glucose concentration (FMC) is lower than the initial concentration. The lower mucosal glucose concentration at the end of the experiment shows a positive glucose transfer from the outer mucosal fluid. However, not all these glucose appear in the serosal fluid. Much more glucose disappears from the mucosal side than appears on the serosal side, and the reason for this, according to Parsons *et al* (1958), is presumably the metabolism by the tissues of part of the glucose disappearing from the mucosal side. This is because mucosal transfer depends to a large extent on glucose metabolism as well as on glucose transfer, and hence metabolism would also have a considerable effect on the final concentration gradient and final concentration ratio (Beryl *et al* 1961). The greatest mucosal transfer was observed in the hyperthyroid group.

There was also a significant reduction in the serosal glucose concentration, even though the final serosal glucose concentration was greater than the final mucosal glucose concentration. It has been reported by Levin (1969, 1974) that excess or deficiency of thyroid hormone alter the function and metabolism of the enterocytes of the small intestine and affect the alimentary tract blood flow, motility, cell population and luminal nutrition thus influencing intestinal function, therefore the markedly high glucose loss (253mg/dl jejunum, 96mg/dl ileum) observed in the hyperthyroid intestine was due to the increased metabolism of the enterocytes caused by hyperthyroidism while the slight glucose loss (12.6mg/dl jejunum, 1.2 mg/dl ileum) seen in the hypothyroid intestine was due to reduced metabolism. In addition transport and metabolic activity was greater in the jejunum than in the ileum in all the groups both with and without the influence of thyroxine; that is to say thyroxine elevated intestinal metabolism in a manner that maintained the variational differences in the intestine contrary to the suggestion of Spencer *et al* (2003) that regional variation in absorption in the small intestine is masked by different p-glycoproteins expression.

The positive gradient in all the sacs showed that the serosal glucose was greater than the mucosal glucose concentration at the end of the experiments. Final concentration gradient in the hypothyroid was reduced compared to the control in a manner similar to that reported for phlorizin inhibition of glucose transport (Parsons *et al* 1958). The reduced gradient may be responsible for the decrease in glucose transport across the intestine.

However, FCG was raised when thyroxine replacement was given (Euthyroid group) showing that thyroxine increases FCG and consequently glucose transport across the intestinal wall. Final concentration gradient was highest in the hyperthyroid intestine thus enabling the increased transport of glucose because the greater the FCG the greater the glucose transport since a steep concentration gradient is necessary for glucose to move appreciably across the submucosal tissues (Fisher and Parsons 1953). Transport was greater in the jejunum than ileum since FCG was greater in the jejunum than in the ileum. The thinning of the intestine in the hyperthyroid group may also be responsible for the increased FCG and glucose transport.

More glucose leaves the mucosal fluid in the hyperthyroid than the other groups however serosal glucose transfer was negative in the hyperthyroid jejunum and ileum and euthyroid ileum. This may mean that most of the glucose leaving the mucosal fluid did not get to the serosal fluid while marked amount of glucose was lost from the serosal fluid. This loss may be due to increased intestinal metabolism caused by excess thyoxine as gut glucose uptake was very high in the hyperthyroid intestine and euthyroid ileum. Thus exogenous thyroxine may greatly increase gut glucose uptake and metabolism in the ileum. Futhermore, gut glucose uptake was very small in the hypothyroid group and so was glucose gradient and absorption. This may be due to reduced intestinal metabolism and activity, and the thickening of the intestinal wall observed in this study (though not reported here) which may pose a barrier to transport. Hyperthyroidism caused a rise of mucosal glucose transfer and gut glucose uptake in this study as was previously shown by Adeniyi and Olowookorun (1987) and Olaleye and Elegbe (2005) respectively, unlike the study of Matty & Seshadri (1965) that reported decreased glucose uptake by hyperthyroid intestine and marked loss of glucose from the serosal side. However, Matty & Seshadri could not explain the reason for the serosal glucose transfer inspite of the decreased uptake of glucose; their result might have been affected by the toxic dose of thyroxine (100ug/100g b/w) administered to the rats and the experimental methods used. Althausen (1949) reported accelerated uptake of glucose in the intact intestine of hyperthyroid rats and in this invitro study thyroxine increases the amount of glucose passing across each centimeter length of the intestine; this increase is more significant in the jejunum than in the ileum.

These findings show that exogenous thyroxine increases mucosal glucose transfer and thus glucose

absorption, increases glucose gradient, gut glucose uptake and metabolism with the increase in the jejunum greater than that of the ileum. Disappearance from the intestinal lumen in the whole animal corresponds to mucosal transfer in vitro, and entry into the blood stream is analogous to serosal transfer (Barry & Smyth, 1960; Smyth, 1963); Therefore hypothyroidism (achieved by thyroidectomy) causes reduced glucose absorption from the intestine while thyroxine increases absorption of glucose but in excess thyroxine may cause reverse glucose transfer in the small intestine. Also thyroxine did not alter the variational differences in glucose transfer capacity that exist in the different parts of the small intestine

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