

Effects of thyroid hormones on basal and stimulated gastric acid secretion due to histamine, carbachol and pentagastrin in rats

Fatemeh N. Rafsanjani, MS, PhD, Saleh Z. Asl, MS, PhD, Mohammad K. Naseri, PhD, Jalal Vahedian, MD, FRCS.

ABSTRACT

Objective: Thyroid hormones affect gastric acid secretion. As the mechanism of this effect has not been fully known, in this experimental study the isolated gastric acid secretion of hypothyroid and hyperthyroid rats were compared with control group by the administration of different doses of pentagastrin, histamine and carbachol as gastric acid secretion stimulators.

Methods: This study was carried out in Ahwaz University of Medical Sciences, Ahwaz, Iran in the year 2000. Each group were consisted of 8 rats (N-mari) of both sexes with a mean weight of 246 ± 5 grams. Hypothyroid and hyperthyroid states were induced respectively by adding methimazole (500 mg/liter) for 20 days and thyroxin (500 μ g/liter) for 35 days in animals drinking water. After general anesthesia, by intraperitoneal injection of sodium thiopental (50 mg/kg body weight), celiotomy was carried out quickly. The end of esophagus was tied and a silicon tube (2-2.5 mm) was entered into the stomach via duodenum and fasted in pylor region. The stomach was isolated by cutting the esophagus proximal to the tied region and the proximal part of duodenum and put into cold serous solution. After washing the serous and mucus surfaces by serous and mucus solutions, the stomach was

transferred immediately to a tissue bath containing warm serous solution ($V= 40$ ml, $T= 37^{\circ}\text{C}$). Gastric acid secretion in isolated stomach stimulated by pentagastrin, carbachol and histamine was measured by wash out technique and automatic titrator. Moreover, to study the effect of thyroid hormones on gastric acid secretion a number of dose-dependent experiments after the administration of different doses of histamine (50, 100, 150, 200 μ mol), carbachol (50, 100, 150, 200 μ mol), and pentagastrin (30, 60, 90, 120 μ g/kg body weight) were performed.

Results: Both basal and histamine, carbachol, pentagastrin stimulated-acid secretion decreased and increased in hypothyroid and hyperthyroid groups compared with control group.

Conclusion: It seems that thyroid hormones have not exert their effects by changing the cholinergic, gastrin and histamine receptors but probably by alerting the number or size of the secretory cells in stomach.

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Thyroid hormones affect most body organs and tissues such as cardiovascular system^{1,2} nervous system,³⁻⁵ liver⁶ and also growth, development and generation processes^{7,8}. Based on some evidences,

thyroid hormones affect gastric acid secretion.^{9,10} Moreover it has been found in a study that thyroid hormones alter the number of parietal cells, in a way that thyroidectomy decreases the number of parietal cells

From the Department of Physiology (Rafsanjani), Department of Surgery (Vahedian), Kerman University of Medical Sciences and Health Services, Kerman, Department of Endocrine Physiology (Asl), Shaheed Beheshti University of Medical Sciences, Tehran, and the Department of Physiology (Naseri), Ahwaz University of Medical Sciences and Health Services, Ahwaz, Iran.

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Address correspondence and reprint request to: Dr. Fatemeh N. Rafsanjani, Assistant Professor, Kerman University of Medical Sciences and Health Services, Kerman, Iran. Tel. +982 (1) 6431926. Fax. +982 (1) 2281561. E-mail: nabavizadeh2000@yahoo.com

while thyroxin administration increases that.¹¹ According to the mentioned study thyroid hormones stimulate the biochemical process involved in the cells growth and thyroxin increases the mitosis activity of cells especially in the crypts of digestional system in rat. Based on another study performed on rat, thyroxin has an important role in the gastric development.¹² In the mentioned study the gastric acid secretion decreased in those rats that their thyroxin level had been lowered by the administration of thiouracil and this effect was removed after the administration of thyroxin.¹² One of the gastric acid secretion stimulators that have been used in the present study is histamine. Histamine is released from the enterochromaffin cells of gastric mucous and has a pivotal role in the control of gastric acid secretion. Acetylcholine via activating histidine de-carboxylase increases the histamine release.¹³⁻¹⁵ This substance increases acid secretion via its receptors and cyclic adenosine monophosphate (cAMP) as a mediator in parietal cells. Sachs et al¹⁶ observed that in rat the released histamine from enterochromaffin cells activates the voltage dependent Ca^{2+}/Cl^{-} channels and consequently increases gastric acid secretion. Another gastric acid secretion stimulator that was used in this study was carbachol. Carbachol causes a rapid increase of gastric acid secretion in rat.¹⁷ Moreover, Negulescu and Machen¹⁸ reported that carbachol increases the intra-cellular calcium in isolated parietal cells and calcium as a secondary messenger increases gastric acid secretion. The 3rd gastric acid secretion stimulator in the present study was pentagastrin. Pentagastrin is a penta-peptide that acts such as gastrin hormone. It attaches to gastrin receptors and mimics its effect.¹⁹ According to some studies pentagastrin increases the parietal cells activity and consequently gastric acid secretion. It has been reported that pentagastrin exerts these effects both directly through its receptors in the parietal cells and indirectly through stimulating enterochromaffin cells and histamine release.^{19,20} Although it has been shown that thyroid hormones have an important role in gastric acid secretion, there has been no study on the effects of these hormones on acid secretion in the presence of histamine, carbachol and pentagastrin as stimulators. The aim of the present study was to compare basal and stimulated acid secretions of hypothyroid or hyperthyroid rats with those of control groups.

Methods. This study was carried out in Ahwaz University of Medical Sciences, Ahwaz, Iran in the year 2000. Each group were consisted of 8 rats (N mari) of both sexes with a mean weight of 246 ± 5 grams. As it has been no significant difference between 2 sexes in acid secretion,²¹ both sexes were used in the present study. Animals were being fed with standard food and kept in a 12/12 hour light/dark cycle and the temperature of $25 \pm 20^{\circ}C$. To induce hypothyroid state 500 mg methimazole (Loghman Co.) in each liter of drinking water for 20 days²² and to induce hyperthyroid state 500

μg thyroxin (Iran hormone Co.) in each liter of drinking water for 35 days¹¹ were used. Control group had access to normal water. Animals were deprived of food 24 hours before the experiment but had access to water.²³ After general anesthesia by intraperitoneal injection of thiopental sodium (Biochemie GmbH, Vienna-Austria) (50 mg/kg body weight), celiotomy was carried out and the end of esophagus was tied tightly by a silky string. A silicon tube (2-2.5 mm) was entered into the stomach via duodenum and fasted in the pylor region. The stomach was isolated by cutting the esophagus proximal to the tied region and the proximal part of duodenum. The isolated stomach was transferred to cold serous solution (temperature [T] = $4^{\circ}C$) that has sodium chloride (NaCl) (118), potassium chloride (KCl) (4.8), magnesium sulphate ($MgSO_4$) (1.2), phosphate dihydrogen potassium (KH_2PO_4) (15.9), calcium chloride ($CaCl_2$) (0.65) and glucose (31.6) mmol/liter.^{15,24} After washing the serous surface with serous solution and mucous surface with warm mucous solution (T = $37^{\circ}C$, pH = 7.4) that has NaCl (118), KCl (4.8), $MgSO_4$ (1.2), $CaCl_2$ (1.3) and glucose (31.6) mmol/liter^{15,24} the stomach was transferred immediately into the tissue bath containing warm serous solution (volume = 40 ml, T = $37^{\circ}C$).^{15,24} These were all carried out during 5-10 minutes, and to arrive at a stable state the tissue remained in the solution for 30 minutes. Acid secretion was measured by washout technique²⁵⁻²⁷ and automatic titrator (TTT80 radiometer from Denmark).²⁸ In order to provide ideal condition and nutrition for the isolated stomach, it was surrounded by serous solution from outside and mucous solution from inside. The inner solution had been oxygenated before, but the outer solution was being oxygenated continuously.^{22,24,29} First the basal acid secretion was measured 2 times with a 15 minutes interval in the 3 groups. Then 100- μmol histamine^{17,30} was added to the serous solution and acid secretion was measured every 15 minutes up to the basal rate. Fifteen minutes after adding of histamine, the tissue was washed by changing tissue bath solution. The same experiments were repeated with carbachol (100 μmol) and pentagastrin (60 $\mu g/kg$ body weight) in all 3 groups. To study the mechanism of thyroid hormones effect on gastric acid secretion a set of dose-dependent experiments with different doses of histamine, carbachol and pentagastrin was performed. For histamine, 50, 100, 150, 200 μmol were added to the tissue bath. In each dose after the measuring of acid secretion and the returning of acid secretion to the basal rate, the tissue was washed and the experiments were repeated for other doses. The same experiments were carried out for 50, 100, 150 and 200 μmol s of carbachol and 30, 60, 90, and 120 $\mu g/kg$ per body weight of pentagastrin in all 3 groups. The mean acid secretion for 3 substances was calculated into 3 groups. In order to be sure of hypothyroid and hyperthyroid states thyroxine (T₄) and thyroid stimulating hormones (TSH) in animals blood were measured by radio immuno assay method before and after drugs administrations. For this blood samples

were obtained from caudal vein under anesthesia with ether before the drug administration and from kidney vein under general anesthesia after drug administration. Acid secretion in 3 groups was calculated by mean \pm SE and to compare the results analysis of variance and Tukey test were used. $P < 0.05$ was considered as significant.

Results. In hypothyroid group (**Table 1**) the amount of T₄ hormone after the administration of methimazole had significant decrease in comparison to that before drug administration ($p < 0.0005$). In hyperthyroid group the amount of T₄ after the administration of T₄ had significant increase in comparison to that before drug administration ($p < 0.004$). In regard to TSH hormone, in hypothyroid group it had significant increase after methimazole administration ($p < 0.002$) and in hyperthyroid group it had significant decrease after T₄ administration ($p < 0.0001$). There were no significant differences in thyroxin and TSH levels before and after the study in control group. Comparison of results obtained in the 3 groups showed, that basal acid secretion in hypothyroid and hyperthyroid groups were significantly less and more than control group ($p < 0.0001$) (**Figures 1-3**). Histamine stimulated acid secretion (100 μ mol, final concentration in tissue bath, 15 minutes) had gradual increase during 30th-45th minutes. Histamine stimulated acid secretion at 75th minute was 7.9 ± 0.14 μ mol in control group, but it was 5 ± 0.07 μ mol in hypothyroid group that is significantly lower ($p < 0.0001$). The amount of acid secretion during returning to basal stage was significantly less in hypothyroid group in comparison to control group ($p < 0.0001$) (**Figure 1**). Histamine stimulated acid secretion at 75th minute showed significant increase in hyperthyroid group comparing with control group ($p < 0.0001$). The amount of acid secretion during returning to basal rate was more in hyperthyroid group in comparison to control group ($p < 0.0001$) (**Figure 1**). Carbachol stimulated acid secretion (100 μ mol, final concentration in tissue bath, 15 minutes) continued

during 30th-45th minutes. At 45th minute it was 9.5 ± 0.01 μ mol/15 minute in control group while it was 6.7 ± 0.1 μ mol/15 minute in hypothyroid group that is significantly lower ($p < 0.0001$). Carbachol stimulated acid secretion in hyperthyroid group (12.6 ± 0.1 μ mol per 15 minutes) was significantly more than that in control group (9.5 ± 0.01 μ mol per 15 minutes) ($p < 0.0001$). Acid secretion during returning to the basal state was more in hyperthyroid group compared with control group ($P < 0.0001$) (**Figure 2**). Pentagastrin stimulated acid secretion (60 μ g/kg body weight, final concentration in tissue bath, 15 minute) at 45th minute was significantly less in hypothyroid group (5.3 ± 0.1 μ mol/15 minute) in comparison to that in control group (8.02 ± 0.1 μ mol/15 minute) ($p < 0.0001$). Acid secretion during returning to basal rate in hypothyroid group was continuously less than that in control group ($p < 0.0001$). Pentagastrin stimulated acid secretion was significantly more in hyperthyroid group (10.6 ± 0.09 μ mol/ 15 minute) compared with control group (8.02 ± 0.1 μ mol/15 minute) ($p < 0.0001$). Acid secretion during returning to basal rate was continuously more in hyperthyroid group in comparison to that in control group ($p < 0.0001$) (**Figure 3**). As it was mentioned before, in order to determine the effects of thyroid hormones on acid secretion a set of dose-dependent experiments was performed on isolated stomach of rats, with different doses of pentagastrin, histamine and carbachol. Then the maximum stimulated acid secretion was determined for each stimulator. Comparison of control group with hypothyroid group showed that the maximum acid secretion with 50, 100, 150 and 200 μ mol histamine in hypothyroid group was significantly less than that in control group ($p < 0.0001$) (**Figure 4**). As it is shown in hyperthyroid group, with the same doses of histamine the maximum acid secretion was significantly more than that in control group ($p < 0.0001$) (**Figure 4**). **Figure 5** shows that the maximum acid secretion with 50, 100, 150 and 200 μ mol carbachol was less in hypothyroid group than that in control group. Whereas in hyperthyroid group it was significantly more than that in

Table 1 - Thyroxin and thyroid stimulating hormone levels in euthyroid, hypothyroid and hyperthyroid rats

Mean + SE group	Before the intervention T ₄ (mg/dl)	After the intervention T ₄ (mg/dl)	Before the intervention TSH (μ u/ml)	After the intervention TSH (μ u/ml)
Control euthyroid N = 8	1.4 ± 0.5	1.4 ± 0.5	1.18 ± 0.005	0.18 ± 0.005
Hypothyroid N = 8	2.3 ± 0.3	$0.02 \pm 0.001^*$	0.18 ± 0.006	$1.4 \pm 0.4^\dagger$
Hyperthyroid N = 8	2.2 ± 0.5	$11.7 \pm 2.7^\dagger$	0.19 ± 0.002	$0.16 \pm 0.001^*$
* $p < 0.05$, $^\dagger p < 0.001$ T ₄ - thyroxine, TSH - thyroid stimulating hormone				

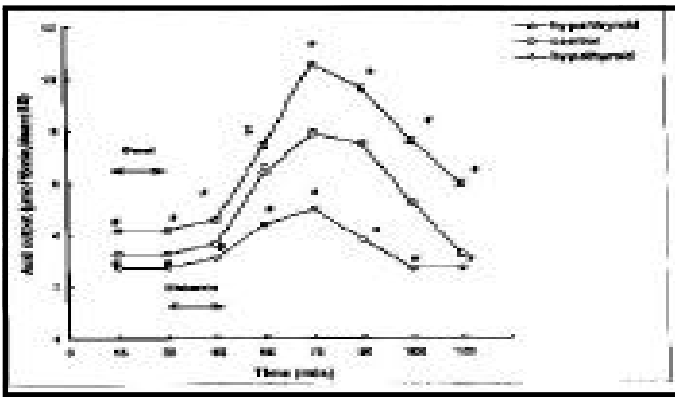


Figure 1 - Mean variation of basal and histamine-induced acid output in rat.

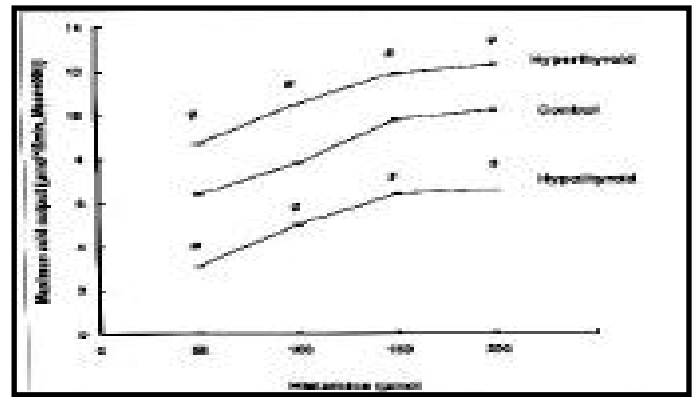


Figure 4 - Mean variation of maximal acid output in response to different doses of histamine in rat.

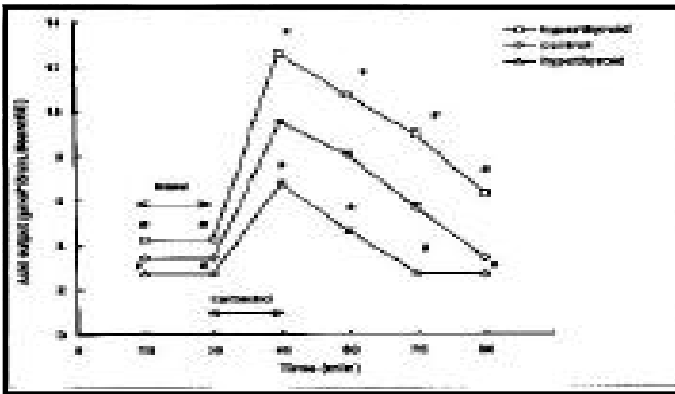


Figure 2 - Mean variation of basal and carbachol-induced acid output in rat.

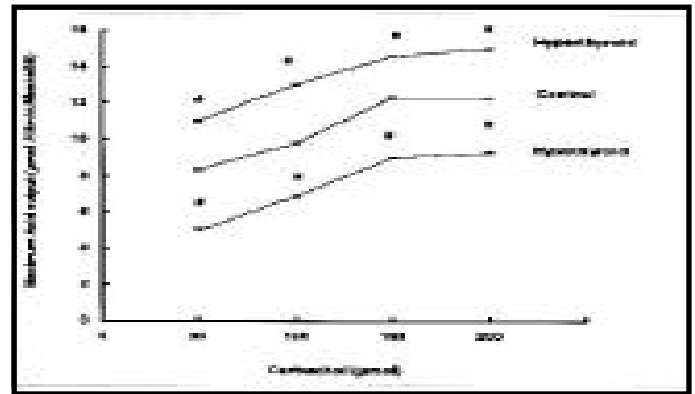


Figure 5 - Mean variation of maximal acid output in response to different doses of carbachol in rat.

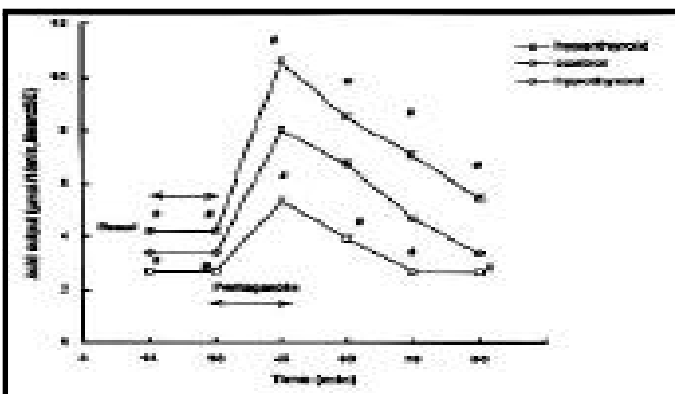


Figure 3 - Mean variation of basal and pentagastrin-induced acid output in rat.

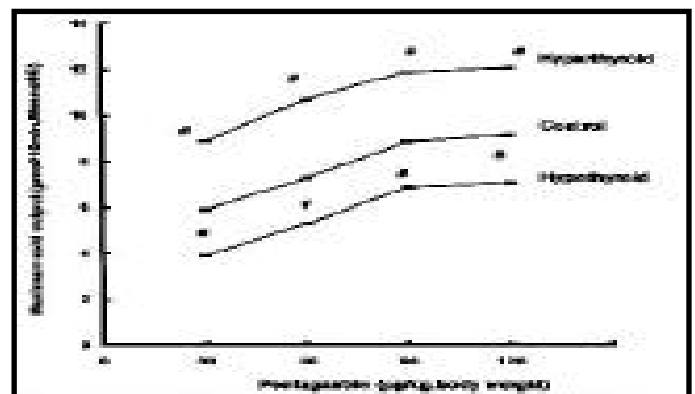


Figure 6 - Mean variation of maximal acid output in response to different doses of pentagastrin in rat.

control group ($p < 0.0001$). Comparison of hypothyroid and control group showed that maximum acid secretion with 30, 60, 90 and 120 $\mu\text{g}/\text{kg}$ body weight pentagastrin was significantly less in hypothyroid group than that in control group ($p < 0.0001$) (**Figure 6**). In hyperthyroid group the maximum acid secretion with the same doses of pentagastrin was significantly more than that in control group (**Figure 6**).

Discussion. Histamine is released from enterochromaffin cells of mucous surface and has a pivotal role in the control of gastric acid secretion. Acetylcholine, too, by activating histidin de-carboxylase enzyme involved in histamine synthesis exerts some of its effects via increasing the amount of histamine.^{13,15,31} Histamine acts via its specific receptors in the surface of parietal cells and cyclic adenosine mono-phosphate as a mediator.^{17,20} Therefore, it takes time until histamine arrives at its receptor and produces cAMP and finally exerts its effect. Therefore in the present study the maximum stimulatory effect of histamine on acid secretion was with some delay (**Figure 1**). Carbachol was another gastric acid secretion stimulator used in the present study. Carbachol caused a significant increase in acid secretion in all three groups. Some reports have shown that carbachol causes a rapid increase in acid secretion in rat.¹⁶ Moreover carbachol causes alterations in permeability of cell membrane to calcium and increases calcium influx in isolated parietal cells. This effect remains until carbachol is present.³⁰ It has been observed that carbachol causes an increase in the amount of intracellular calcium and calcium as a 2nd messenger increases gastric acid secretion in isolated parietal cells of stomach.^{13,18} As calcium is an important factor in acid secretion, it seems that in isolated stomach, carbachol increases acid secretion with the same mechanism. Based on present evidences, carbachol attaches to M^3 acetylcholine receptor in the surface of parietal cells, and it is not affected by acetylcholine esterase enzyme. Therefore it leads to a stable effect.¹³ This fact was observed in the present study too. Pentagastrin was used as another gastric acid secretion stimulator in our study. Pentagastrin is a pentapeptid similar to gastrin hormone. It exerts its effect via gastrin or cholecystokinin receptor type B.^{19,29} Gastrin receptors related to the regulation of gastric secretions are located on parietal cells, enterochromaffin and chief cells.³² Therefore gastrin or pentagastrin increase gastric acid secretion via two mechanisms: 1) Directly through exerting effect on receptors on the surface of parietal cells. 2) Indirectly through stimulating enterochromaffin cells and histamine release and like gastrin and acetylcholine affects gastric acid secretion by increasing the amount of intracellular calcium and histamine via secondary messenger of cAMP. Two secondary messengers of calcium and cAMP are important in gastric acid secretion and activation of cells. However, it is probable that in the present study, pentagastrin has increased the

gastric acid secretion via the mentioned mechanisms. Results of the present study that in the presence of all three stimulators basal and stimulated acid secretions had significant decrease in hypothyroid group and significant increase in hyperthyroid group comparing with control group. Although the mentioned stimulators increase the stimulated acid secretion in comparison to basal state in all three groups, it seems that the difference of the three groups in the amount of thyroid hormones is responsible for significant decrease of both basal and stimulated acid secretions in hypothyroid group and significant increase of them in hyperthyroid group comparing with control group. Other studies have shown that thyroid hormones increase parietal and gastrin producing cells and consequently acid and gastrin secretions.³³ Adeniyi and his coworkers concluded that increase in the amount of thyroxin leads to an increase in the number of parietal cells in rat.¹¹ Moreover it has been reported that these hormones stimulate the biochemical processes involved in cells growth. Thyroxin administration increases the cell mitosis activity of gastrointestinal system, especially gastric fundus region cells in rat. This leads to increased parietal cell mass and consequently an increase in acid secretion. The opposite state has been observed following thyroidectomy in rat.^{11,34} Moreover alterations in thyroid hormones change the number of alpha and beta-adrenergic receptors in the most tissues of rat and influence them with this mechanism too. Therefore it is probable that in digestional system especially stomach, too, thyroid hormones alterations affect the number of important receptors, especially acetyl-choline, histamine and gastrin receptors.

The main objective of our study was to evaluate the effects of thyroid hormones on gastric acid secretion via their effects on the number of cholinergic, histamine and gastrin receptors. Therefore, the gastric acid secretion in hypothyroid and control groups were determined separately in the presence of different doses of pentagastrin, carbachol and histamine. Based on our findings (**Figures 4-6**) thyroid hormones have not affected the number or sensitivity of receptors. In fact responsiveness has changed but sensitivity has not. It seems that increase of acid secretion in hyperthyroid group and decrease of it in hypothyroid group in comparison to control group is due to the difference of two groups in regard to the amount of thyroid hormones. In other words increase of these hormones has increased the number of parietal cells in hyperthyroid group and the versus in hypothyroid group. It is also probable that thyroid hormones exert their effects via affecting the parietal cells size or affecting the metabolic activity of these cells which both requires further studies.

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