Histological Effects of fasting and subsequent refeeding on thyroid follicles of rabbits morphometric analysis

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Abstract:
The effect of fasting and refeeding on thyroid gland had been studied histologically on (20) local breed rabbits. They were subdivided into (2) groups, (10) rabbits as control (fed) group, (10) rabbits as experimental(fasted) group. The control group was fed vegetables, while the experimental group was fasted for (48) hours, and then provided food for (4) hours, the results were : insignificant reduction in diameter of thyroid follicles, amount of colloid, & height of follicular epithelium in the experimental group, in which the diameter of thyroid follicles of control group was (21.1±4.7), while for experimental group was (19.5±4.42), the amount of colloid in control group was (18.2±3.8), and for experimental group was (15.6±5.2), the height of follicular epithelium in control group was (2.6±0.63), and for experimental group was (2.13±0.48). there was a significant increase in the number of C- cells in experimental group which was (3.31±1.9), while it was (1.2± 1.17) in the control group. The reduction in thyroid follicles, thyroid colloid, & height of follicular epithelium occur as a result of changing in feeding behavior which causes decrease in iodine food incorporation, and decrease in level of 5-monodeiodoinase a mediator of T3 and T4. The increase in the number of C cells happened as a protective mechanism to save the skeleton from excessive bone resorption.

Key words: Thyroid follicles, fasting, refeeding

1.Introduction:
The thyroid gland is the largest endocrine gland in the body, situated in the anterior cervical region below thyroid cartilage, consists of two lobes connected by narrow isthmus.

Thyroid function is to synthesize hormones : T3 (triiodothyronin) & T4 (thyroxine) these two hormones are important in stimulating enzymes involved in glucose oxidation, thereby controlling cellular temperature, metabolism of proteins, carbohydrate, & lipids. Thyroid hormones also raises the number of adrenergic receptors in blood vessels, thus playing a major role in the regulation of blood pressure (1).

Thyroid tissue is composed of 20-30 millions of microscopic spheres called follicles, which are lined by columnar to cuboidal epithelium, the central cavity of the follicle is filled with a gelatinous substance known as colloid: which represents the storage of secretion of thyroid hormones.

Another type of cells present in thyroid gland: parafollicular cells (c-cells) which is found in isolated clusters between thyroid follicles, these cells are larger than thyroid follicular cells, & are characterized by granular cytoplasm, these cells are responsible for the secretion of hormone Calcitonin, whose main effect is to lower the blood calcium level by inhibiting bone resorbtion (2).

The changes observed in humans & rodents are similar in many ways, although they may be more pronounced & more acute in rodents (3)

Alterations in the nutritional state of the mammals whether short or long term profoundly affect various aspects of metabolic, hormonal, physiologic, and histological structure of cells (4).

Fasting& subsequent refeeding belong to nutritional manipulations which reflect naturally occurring periodicity in nutrient supply, fasting evokes dramatic changes in carbohydrate, lipid, protein metabolism, & in turn these compounds are vital components in contribution of cellular structure of thyroid follicles (5).

Researchs have shown the effect of fasting& subsequent refeeding on thyroid gland: Zbignniew Kmiec et al., reported in a study: fasting decreases the levels of T3&T4 in the fasted group in comparison to the control group, & clarify that fasting causes reduction in thyroid follicular volume, & decrease in height of epithelial follicles in experimental rats(6).

Starvation was reported in an experiment done by Blum& Kunz results in decreasing plasma levels of T3& T4 hormones (7).

Kmiec et al., had explained that fasting makes the number of c-cells higher & as a sequence increases the levels of calcitonin in blood, in the fasted group of rats (8).

On the other hand Boelen et al., (9) investigated : fasting induces profound changes in hypothalamic-pituitary- thyroid axis (HPT) in a way increasing the level of T3 & decreasing the T4 level.

However : there are limited studies carried on the histological alterations of fasting & subsequent refeeding on the thyroid gland, most of these studies are concerned with the physiological effect of fasting on the thyroid gland, particularly on T3& T4 hormones.

The objective of this study is to investigate the effect of fasting& subsequent refeeding on histological structure of thyroid gland of rabbits.

2.Materials & methods:
2.1.Animals:
Twenty local breed adult male rabbits aged twelve months were used, rabbits were subdivided into two groups, ten as control group, & ten as experimental group, then experimental group was further subdivided into five groups 2 rabbits in each, both

groups were housed in separated cages, & maintained on a controlled light schedule.

2.2.Dietary manipulation & sampling:

Control rabbits were fed normal vegetables & sampled correspondingly at the same time of the day as fasted animals . Other animals were fasted starting from 8:00 hour & sampled after 48 hours or fasted for 48 hours, then provided with food at 8:00 (start of feeding) and sampled after 4 hours.

2.3.Morphometry of rabbit thyroid gland:

Rabbits were killed by decapitation, thyroid gland were removed and fixed in 10% formalin solution for 24 hours, then embedded in paraffin wax & serially cut at 10Mm.. Sections were stained with hematoxylene & eosin,

- **Counting technique:**

In each specimen slides of thyroid tissue of both control (fed), & experimental (fasted-reefed) group fifteen microscopic fields had been taken in order to estimate & count the different histological parameters.

A calibrated stage micrometer type (Leitz), & a calibrated ocular lens were used, all measurements were done under (40X) objective lens in which each line of caliberated ocular micrometer lens is equal to (3) lines of calibrated stage, so each line of calibrated ocular micrometer lens is equal to (3.3 µm.).

2.4. Statistical analysis:

Results are expressed as the mean ± SD. Analysis of Variance(ANOVA), was used for comparison of mean values for both control& experimental groups.

3.Results:

3.1. Effect of Fasting & refeedng on body weight of rabbits:

Fasting for 48 hours decrease the body mass of experimental group by 11.3%, as revealed in table (1), in which the mean of body weight of control group of rabbits was (1264gm ± 37.95), while that of experimental group was (1121.2±33.3 gm).

3.2. Qualitative morphological changes of thyroid tissue of fed& fasted-refed group:

Thyroid specimen slides of fed (control) group appear thyroid follicles filled with colloid material, which show few resorptive lacunae, lined by follicular epithelial cells of columnar – cuboidal in shape, as seen in Figures (1)& (2).

After 48 hours of fasting, thyroid specimen slide of experimental group reveal thyroid tissue similar to that of control group, but with slight smaller follicles, there are many, frequent large resorptive lacunae appear in the colloidal material, the follicular epithelial cells are flattened- cuboidal in shape, as shown in Figures (3)& (4).

3.3 Morphometric analysis of thyroid tissues in control& experimental groups:

Fasting for 48 hours did not significantly influence circumference, surface area, & volume of follicles in thyroid tissue of experimental group.

The mean of follicular diameter was 18% smaller in the experimental group as compared with the control one as noted in table (1), the mean of follicular volume of fasted- fed group was (19.5 ± 4.42 µm), while the diameter of follicles of fed group was (21.3± 4.7 µm), the reduction in the follicular diameter in the experimental group was not significant.

Table (1) clarify the colloidal volume of both control& experimental groups, the mean colloid volume of control group was (18.2±3.8µm), the mean of colloid volume in experimental group was (15.6±5.2 µm), the difference in the colloid volume between the control& experimental group was not significant.

Also table (1) reveals the height of follicular epithelia in which the mean of height of epithelial cells of control group was (2.6±0.63 µm), and the mean of height of follicular epithelial cells of the experimental group was (2.13±0.48 µm).

The number of parafollicular cells (C-cells)as noted in Table (1), had been increased in the experimental group, which was (3.31±1.9) , & in the control group was (1.2±1.17), as revealed in figures (4)& (5), this increase in the number of parafollicular cells in the experimental group was significant.

4. Discussion:

In this study fasting& subsequent refeeding of rabbits causes reduction in the diameter of thyroid follicles, follicular colloid, height of follicular epithelial cells, & increase in the number of C-cells.

Since the two major hormones (T3&T4) are unique, unlike other types of hormones, are neither protein nor cholesterol based, they are iodine incorporated as an active component, the amount of iodine is dependent on ingested& type of food(10).

There is much debate about the physiological differentiation between the two hormones (T3& T4), it is currently thought that (T4) may act as the reserve form, having more direct role in the hypothalamus-pituitary negative feedback loop, while (T3) has more a dynamic physiological effect in the body (11).

Protein malnutrition or food restriction does impair T4& T3 conversion by 5- monodeidinase activity, in which the starvation or food restriction increased corticosterone levels which in turn decreases 5-monodeidinase one of the mediators of T3& T4(12)

From above, fasting & subsequent refeeding causes alterations in physiological function of thyroid gland which based on changes in histological features of thyroid follicles: the atrophy of thyroid follicles, decrease in diameter of colloid,& these findings of this study are coincident with the results of experiment carried by Kimec et al., (6).

But diet can effect thyroid function as a high caloric / high carbohydrate diet can lead to increase conversion of T4 to T3, a mechanism that likely assists in keeping an organism's body weight stable, meanwhile prolonged (chronic) fasting can result in a decrease of T3 production which may be adaptive for conditions of food scarcity slowing down the body's metabolism & energy consumption (13).

Prolonged (chronic) fasting explains the results of a research done by Numazawa & Osania (14), they noted that prolonged fasting for three days or more causes enlargement in thyroid follicles of mice as a matter of adaptation to fasting.

In this experiment : the acute elevation in the number of c-cells in fasted group support the concept of gastero- entero-thyroid C-cells system(15) , which serves to stimulate calcitonin secretion & thus protect the skeleton from excessive bone resorption during period of dietary insufficiency (16).

The C-cells findings are agreed with a study investigated by Kmiec et al., (8), & are confirmed by the results of experiment examined by R. Swaminathan et al., (16) on pigs, who stated: calcitonin secretion is increased when pigs were fasted for more than 48 hours & then fed, the elevation in calcitonin secretion assists in control of post prandial hypercalemia.

Table (1) : Shows the different parameters between the control (fed) and experimental (fasted) groups

Group	Body weight (gm)	Epithelial cell height expressed in Micrometer (µm)	Follicular diameter expressed in Micrometer (µm)	Colloid diameter expressed in Micrometer (µm)	Number of parafollicular cells (per microscopic field)
Control group	1264±37.95	2.6±0.63	21.1±4.7	18.2±3.8	1.2± 1.17
Experimental group	1121.3±33.63	2.13±0.48	19.5±4.42	15.6±5.2	3.31±1.9

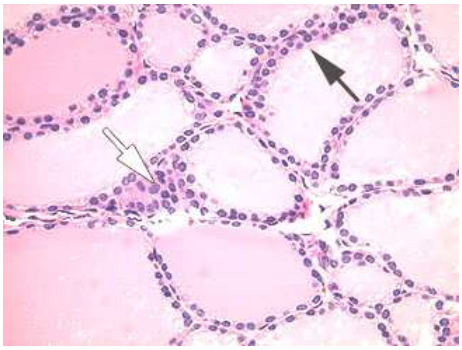


Figure (1): thyroid follicles of the control group (fed), note the black arrow points on follicular cells, white arrow on parafollicular cell.(objective 40X)

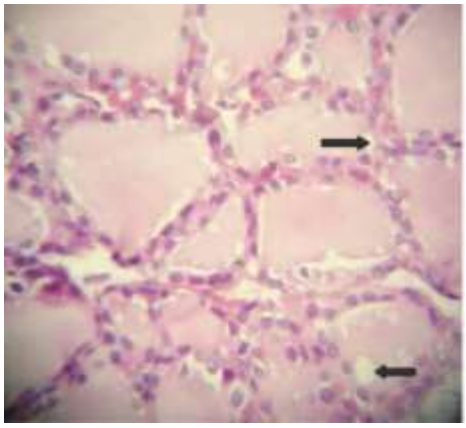


Figure (2): Thyroid tissue of control (fed) group: Note the small resorptive lacunae of colloid material of thyroid follicle, pointed by black arrows (40X).

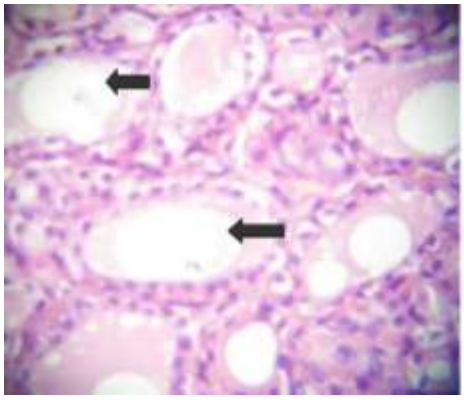
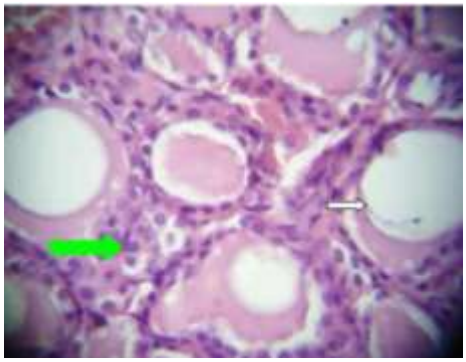


Figure (3): throid tissue of experimental group : observe the large resorptive lacunae of thyroid follicles to that of control group, pointed by black arrows (40X).



Figure(4): thyroid follicles of experimental group: Note large resorptive colloid lacunae pointed by white arrow, green arrow points on clusters of parafollicular cells(C-cells) (40X).

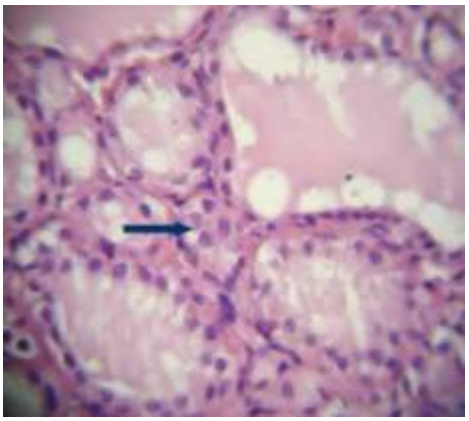


Figure (5): thyroid tissue of experimental group: note cluster of C-cells (shows the increased number of C-cells, 40X).

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التأثيرات النسيجية للصيام وما يليه من الإفطار في الحويصلات الدرقية للأرانب: تحليل شكلي

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الملخص

تأثير الصيام وما يليه من أطعام على الغدة الدرقية تمت دراسته نسيجيا على (٢٠) أرنباً محلياً، حيث تمَ تقسيم الأرانب الى مجموعتين، مجموعة السيطرة إذ تضم (١٠) ارنباَ، ومجموعة التجربة وتضم (١٠) أرنباً، وأخضعت مجموعة التجربة للصيام لمدة (٤٨) ساعة، ثم أطعمت لمدة أربع ساعات، وكانت النتائج: نقصان في أقطار الحويصلات الدرقية إذ أصبحت (4.42±19.5)، وكانت في مجموعة السيطرة (4.7±21.1) ، ونقصان في أقطار المادة الغروانية الحويصلية إذ بلغت في مجموعة التجربة (5.2±15.6) ، وكانت في مجموعة السيطرة(3.8±18.2) ، ونقصان في ارتفاع الخلايا الطلائية الحويصلية (الجرابية)، إذ بلغت في مجموعة التجربة(0.48±2.13) ، بينما في مجموعة السيطرة (0.63±2.6) ، وكان هذا النقصان في كل هذه المقاسات غير معنوي، وشهدت مجموعة التجربة زيادة في عدد الخلايا خارج حويصلية (الجرابية) إذ سجل عددها في مجموعة التجربة (1.9±3.31) ، بينما كان عددها في مجموعة السيطرة (1.17 ±1.2) ، وكانت هذه الزيادة معنوية. وكان سبب النقصان في أقطار الحويصلات (الجراب) الدرقية، ونقصان كمية المادة الغروانية، ونقصان أرتفاع الخلايل الطلائية الحويصلية هو التغير في سلوك التغذية ونقصان اليود في الطعام، والذي يؤدي الى نقصان في إنزيم الخامس أحادي اليود المسؤول في التغيير في تحولات هرمون الثايروكسين (T3) والترايودوثايرونين(T4)، أما سبب زيادة عدد الخلايا خارج الحويصلية هو كعملية وقائية لحماية الجهاز الهيكلي من زيادة امتصاص أو اذابة العظم للحصول على الكاليسيوم.