

## Evaluation of Low Intensity Laser Effects on the Thyroid Gland of Male Mice

LUCIANE HIRAMATSU AZEVEDO, D.D.S., Ph.D.,<sup>1</sup>  
ANA CECÍLIA CORREA ARANHA, D.D.S., Ph.D.,<sup>2</sup>  
SANDRO FERNANDO STOLF, D.D.S., Ph.D.,<sup>3</sup>  
CARLOS DE PAULA EDUARDO, D.D.S., Ph.D.,<sup>2</sup>  
and MARTHA MARQUES FERREIRA VIEIRA, D.D.S., Ph.D.<sup>3</sup>

### ABSTRACT

**Objective:** The purpose of this study was to assess whether there were alterations in the thyroid hormone plasma levels under infrared laser irradiation, in the thyroid gland region. **Background Data:** Studies have demonstrated that infrared laser can cause alterations in thyroid glands. **Methods:** Sixty-five albino male mice were used and assigned to five groups ( $n = 13$ ), with differences in the times that they were sacrificed. Irradiation procedures consisted of an infrared diode laser emitting at 780 nm, at 4 J/cm<sup>2</sup> energy density, in contact mode, point manner. Blood was collected before irradiation (group 1), and then at 24 h (group 2), 48 h (group 3) and 72 h (group 4), and 1 week (group 5) after the third irradiation. The collected material was used for clinical analysis to evaluate the T<sub>3</sub> (triiodothyronine) and T<sub>4</sub> (thyroxin) hormones. Five animals were used for light microscopy analysis. **Results:** A statistically significant hormonal level alteration between the first day and 7 days after the last irradiation was found. **Conclusions:** It was concluded that low-level laser therapy (LLLT) of the thyroid gland may affect the level of thyroidal hormones.

### INTRODUCTION

LASER EQUIPMENT has already taken its place among the tools for oral medicine and dentistry.<sup>1–3</sup> Low-level laser therapy (LLLT) provides a post-operative period with less pronounced signs of inflammation, better tissue repair, and less discomfort. Studies have shown that the three effects of the laser apparatus (anti-inflammatory, biomodulator, and analgesic) may benefit patients.<sup>4,5</sup> However, precise diagnosis of the pathology to be treated is extremely important in choosing the most suitable technique and type of laser.

It is known that, in laser therapy, absorption of the laser should be considered at the surface as well as at a depth, because organs other than those targeted for irradiation might be affected.<sup>6,7</sup>

Early observations of the thyroid gland after direct infrared laser irradiation have demonstrated an increase in the mitotic activity of follicular cells, transitory hyperactivity in some follicles, epithelial necrosis, and lack of cellular masses.<sup>8,9</sup>

In adults, variations in triiodothyronine (T<sub>3</sub>) and thyroxin (T<sub>4</sub>) levels may promote changes in the organism, and in children, these alterations may be more exacerbated. Therefore, it is necessary to understand the effects of LLLT in the thyroid gland region.

Thus, the aim of this study was to assess the effects of LLLT on the thyroid gland, by measuring the quantity of T<sub>3</sub> and T<sub>4</sub> in the serum of mice by radio-immunoassay and to analyze the morphology of the irradiated thyroid gland by means of light microscopy.

### MATERIALS AND METHODS

#### *Selection of the animals*

Sixty-five male Swiss albino mice of similar ages with a mean weight of 20 g each were used in this study. The International and National Rules of Bioethics in Animals Research

<sup>1</sup>Department of Oral Pathology, School of Dentistry, University Hospital, University of São Paulo, São Paulo, SP, Brazil.

<sup>2</sup>Department of Restorative Dentistry, School of Dentistry, University of São Paulo, São Paulo, SP, Brazil.

<sup>3</sup>Lasers and Applications Center, Energy and Nuclear Research Institute (IPEN), São Paulo, SP, Brazil.

were followed during the study. The animals were randomly assigned to five groups ( $n = 13$ ):

- Group 1: Sacrificed before laser irradiation
- Group 2: Sacrificed 24 h after laser irradiation
- Group 3: Sacrificed 48 h after laser irradiation
- Group 4: Sacrificed 72 h after laser irradiation
- Group 5: Sacrificed 1 week after laser irradiation

The animals were exposed to the same ambient temperature, nutrition, and day–night cycle to avoid physiological changes in thyroid hormones.

#### *Laser device*

The laser beam employed was an infrared diode laser (Twin Laser, MMOptics, São Carlos, SP, Brazil), emitting at 780 nm with a power of 30 mW, and international safety rules were strictly followed.

#### *Irradiation method*

The animals were irradiated with the laser light being applied for 4 sec per point. The total time of application was 2 min and 13 sec in 1-cm<sup>2</sup>, in contact mode. The energy density applied to the thyroid gland was 4 J/cm<sup>2</sup> according to Hernandez et al.<sup>11</sup> and Parrado et al.<sup>8</sup>

LLLT was applied in a contact mode (using a point manner) on the first, second, and third day. After that, blood was collected according to the described groups.

For shaving, irradiation procedures, and blood collection, and animals were sedated under intraperitoneal injection of a standard solution of ketamine (Holliday) at 50 mg/Kg and xylazine (Rompun Injectable, Bayer), and bled by the retro-orbital plexus. Blood was collected before the laser application (group 1), and at 24 h (group 2), 48 h (group 3), 72 h (group 4), and 1 week (group 5) after the last (third) irradiation.

The collected material was frozen and sent for clinical laboratory analysis where a radio-immunoassay test was done to assess the quantity of T<sub>3</sub> and T<sub>4</sub> hormones.

#### *Radio-immunoassay test*

Twenty-five exams were carried out for the T<sub>3</sub> hormone and 25 for the T<sub>4</sub> hormone. The Coat-A-Count Total test was used (Los Angeles, CA) to measure the total circulating quantity of T<sub>3</sub> and T<sub>4</sub> hormones in serum and plasma.

#### *Light microscopy*

Five animals were sacrificed under anesthesia; one of them before laser irradiation, and the other three immediately after the first, second, and third days of irradiation, respectively, and the last one at 1 week after the third irradiation. Because so few animals were used, this aspect of the study is considered investigational. The thyroid glands were removed from five animals, and tissues were fixed in 10% buffered formol for 24 h and embedded in paraffin. After processing the material, historical cuts of 5 μm in thickness were stained with hematoxylin and eosin (Fig. 1).

#### *Statistical analysis*

For statistical analysis, the results were submitted to analysis of variance (ANOVA) separately (i.e., one test for T<sub>3</sub> and another for T<sub>4</sub>).

## RESULTS

#### *Radio-immunoassay*

Twenty-five values for T<sub>3</sub> and 25 values for T<sub>4</sub> relative to the five groups and five observation times (Table 1) were obtained.

#### *Triiodothyronine (T<sub>3</sub>)*

For T<sub>3</sub>, ANOVA showed that there was statistical significance at the level of 5% between observation times ( $F = 5.44$ ).

For a comparison among the means, the Tukey test ( $T, 12.05$ ) was carried out, which showed that there was a difference between the value at day 1 of irradiation and day 7 after the third irradiation, but there was no difference among the other times.

#### *Thyroxin (T<sub>4</sub>)*

For T<sub>4</sub>, ANOVA showed that there was statistical significance at the level of 3% among the observation times ( $F = 3.03$ ).

For a comparison among the means, the Tukey test ( $T, 1.70$ ) was carried out, which showed that there was a difference between the value at day 1 of irradiation and day 7 after the third irradiation, but there was no difference among the other times.

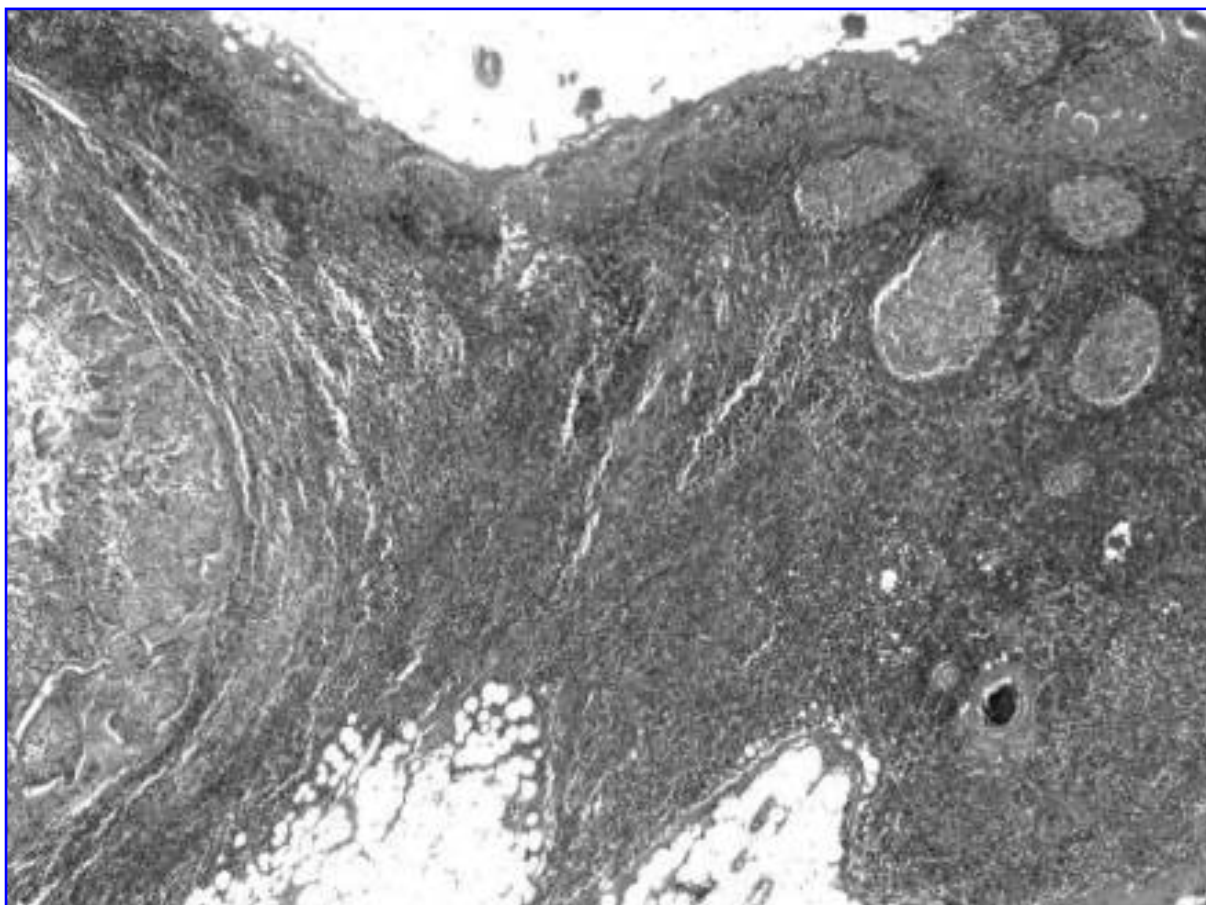
#### *Light microscopy*

Morphological alterations of the thyroid glands were not observed. All the histological cuts presented the thyroidal follicles with cuboid epithelium, disposed in only one layer, limiting spherical spaces filled by colloid. There was, therefore, neither an increase nor an accentuated decrease in the height of the follicular epithelium.

## DISCUSSION

The effects of low intensity laser biomodulation on endocrine glands have been studied by some authors.<sup>9–11</sup> Hernandez et al.<sup>11</sup> reported that the decrease of T<sub>3</sub> and T<sub>4</sub> hormones after infrared laser irradiation might be explained by the changes in the cyto-skeleton and/or thyroglobuline synthesis. This is in agreement with the accumulation of colloidal material in the cytoplasm found by electronic microscopy. The decrease of the thyroidal hormones in the blood after infrared laser should explain the increase in the TSH values.

Statistical analysis carried out in this study showed that the only statistically significant hormonal alteration for both T<sub>3</sub> and T<sub>4</sub>, was observed comparing the first day of application and seven days after the third application. This alteration is probably due to T<sub>3</sub> and T<sub>4</sub> level decrease after the first application and a small increase in the level of these hormones after 7 days.



**FIG. 1.** Histological features of the thyroid gland.

In 1999, Parrado et al.,<sup>8</sup> in an ultra-structural study of the thyroidal capillaries demonstrated, by light microscopy, a significant increase in the density of the capillary volume in mice irradiated with infrared laser; other authors also showed, by electron microscopy, an increase in the luminal area in the capillaries of irradiated mice, as well as an increase in the thickness of the endothelial cells.

Regarding the morphology, in the present study, no alteration of the thyroidal follicles were observed, differently from the study of Parrado et al.,<sup>9</sup> in 1989, in which, using different parameters (3.12 J/cm<sup>2</sup> and 9.36 J/cm<sup>2</sup>) with a laser of 904 nm for 15 days, alterations in the density of the follicles and the colloidal and epithelial volumes were observed; it should be pointed out that, in this case, all the animals were sacrificed

**TABLE 1.** VALUES OBTAINED BY MEANS OF THE RADIO-IMMUNOASSAY TEST AND THE CALCULATED SAMPLE MEANS

	<i>Period of time</i>				
	<i>Before Irradiation (G1)</i>	<i>1st day (G2)</i>	<i>2nd day (G3)</i>	<i>3rd day (G4)</i>	<i>7 days (G5)</i>
T <sub>3</sub> ng/dL (mean)	62.4	57	66	61	74
SD (±)	3.36	7.81	2.34	5.19	7.66
T <sub>4</sub> µg/dL (mean)	3.16	2.10	2.82	2.64	3.94
Standard deviation	0.87	4.26	0.24	0.30	1.05

after 15 days for histological assessment. In our study, five animals were sacrificed according to the following order: before the first irradiation, after the first, second, and third irradiation and 7 days after the last irradiation. Despite the few animals used, morphological alterations did not occur, perhaps because of the number of days of irradiation being reduced.

In spite of the vast literature on low intensity laser, information on its effects on biological tissues are not conclusive, often clinical cases being reported with little scientific basis.<sup>5</sup> Many papers did not indicate the correct parameters, preventing them from being reproduced.<sup>12</sup>

Parrado et al.,<sup>9</sup> in 1989, reported the use of 46.8 and 140.4 J/cm<sup>2</sup> in the region of the thyroid gland in mice, when in truth they used 3.12 and 9.36 J/cm<sup>2</sup> for 15 days. There are no cumulative doses, and the daily dose cannot be multiplied by the number of days of laser use, as was described.

Recently, Vidal et al.<sup>4</sup> showed that infrared laser irradiation stimulated the growth and maturation of thyroidal endothelial cells in young mice, while in adult mice, it could cause thickening of the endothelium and reduction of capillary lumen.

Probable alteration in thyroid glands after irradiation with low intensity infrared emission lasers should be investigated, varying the wavelength, energy density and power, with the purpose of being able to affirm with confidence, either the deleterious effects, or even benefits on the thyroid gland. Furthermore, a difficulty found in this study was the low amount of serum obtained for carrying out the radio-immunoassay; this being so, it is suggested that further investigations should be done on larger animals. Measurements of TSH and other indices of inflammation should be important to analyze the data collected. The acute rise in thyroid hormone after 7 days suggested possible thyroiditis. Finally, there is a need for further investigation to evaluate more protocols and energy densities.

## CONCLUSION

The hormonal level of mice was altered, being statistically significant between the first day of application and seven days after the last application. Despite the few animals used in this study, there was no morphological alteration in the histological cuts of the thyroid gland.

## ACKNOWLEDGMENTS

We would like to thank FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo); the Special Laboratory of Laser in Dentistry (LELO), which provided the equipment to

perform this research; and Nanci do Nascimento, Helena Costa, and Martha Simões from IPEN and also Mirian Turbino for statistical support.

## REFERENCES

1. Howell, R.M., Cohen, D.M., Powell, G.L., et al. (1988). The use of low energy laser therapy to treat aphthous ulcers. *Ann. Dent.* 47, 16–18.
2. Bosatra, M., Jucci, A., Olliaro, P., et al. (1984). *In vitro* fibroblast and dermis fibroblast activation by laser irradiation at low energy. *Dermatologica* 168, 157–162.
3. Mester, E., Mester, A.F., and Mester, A. (1985). The biomedical effects of laser application. *Lasers Surg. Med.* 5, 31–39.
4. Vidal, L., Ortiz, M., and Perez de Vargas, I. (2002). Ultrastructural changes in thyroid perifollicular capillaries during normal postnatal development and after infrared laser radiation. *Lasers Med. Sci.* 17, 187–197.
5. Wilder-Smith, P. (1988). The soft laser: therapeutic tool or popular placebo? *Oral Surg. Med. Oral Pathol.* 66, 654–658.
6. Karu, T. (1989). Photobiology of low-power laser effects. *Health Physics* 56, 691–704.
7. Longo, L., Evangelista, S., Tinacci, G., et al. (1987). Effect of diodes-laser silver arsenide-aluminium (Ga-Al-As) 904 nm on healing of experimental wounds. *Lasers Surg. Med.* 7, 444–447.
8. Parrado, C., Carrillo de Albornoz, F., Vidal, L., et al. (1999). A quantitative investigation of microvascular changes in the thyroid gland after infrared (IR) laser radiation. *Histol. Histopathol.* 14, 1067–1071.
9. Peláez, A., Vidal, L., and Vargas, I. (1989). Quantitative study of the morphological changes in the thyroid gland following IR laser irradiation. *Lasers Med. Sci.* 5, 77–80.
10. Goldman, L., and Richfield, D.F. (1964). The effect of repeated exposure to laser beams. *Acta Derm. Venereol.* 44, 264–268.
11. Hernández, L.C., Santisteban, P., Valle-Soto, M., et al. (1989). Changes in mRNA of thyroglobulin, cytoskeleton of thyroid cells and thyroid hormone levels induced by IR-laser irradiation. *Laser Ther.* 4, 203–208.
12. Mezawa, S., Iwata, K., and Naito, H. (1988). The possible effect of soft-laser irradiation on heat nociceptors in the cat tongue. *Arch. Oral Biol.* 33, 693–694.

Address reprint requests to:  
 Dr. Carlos de Paula Eduardo  
 Departamento de Dentística  
 Faculdade de Odontologia de São Paulo  
 USP  
 Av. Prof. Lineu Prestes, 2227  
 Cidade Universitária  
 São Paulo, SP, Brazil 05508-900

E-mail: cpeduard@usp.br