

Effects of thyroid hormone supplementation on oxidative stress after sleeve gastrectomy in rats

Suleyman Orman¹, Banu Isbilen Basok², Ucler Kisa³, Nevin Genc Kahraman⁴, Ferruh Kemal Isman⁴

¹Istanbul Medeniyet University Goztepe Training and Research Hospital, Department of Gastroenterology Surgery, Istanbul, Turkey

²University of Health Sciences, Tepecik Training and Research Hospital, Department of Medical Biochemistry, Izmir, Turkey

³Kirikkale University Faculty of Medicine, Department of Medical Biochemistry, Kirikkale, Turkey

⁴Istanbul Medeniyet University Goztepe Training and Research Hospital, Department of Medical Biochemistry, Istanbul, Turkey

Copyright © 2018 by authors and Annals of Medical Research Publishing Inc.

Abstract

Aim: Sleeve gastrectomy has been used for the surgical treatment of morbid obesity. The aim of the present study was to determine the effects of triiodothyronine supplementation on oxidative stress parameters in anastomotic tissue level.

Material and Methods: Twenty-four male Wistar albino rats were divided into control (n:12), and experimental (n:12) groups and underwent sleeve gastrectomy. Experimental group rats received a single dose of triiodothyronine (400 mg/100 g) in the operation day. Rats were sacrificed on postoperative day 7. Serum thyroid hormones were analysed. The supernatants were used to measure total oxidant status, total antioxidant status, nitric oxide and malondialdehyde levels. All tissue parameters were analysed by spectrophotometric methods. Oxidative stress index values were calculated.

Results: Thyroid stimulating hormone levels in both the control and triiodothyronine group did not significantly change on the 7th postoperative day ($p=0.663$). Free triiodothyronine levels were significantly higher in triiodothyronine group rats than in control group rats (triiodothyronine vs control: $p=0.004$). Although total oxidant status levels did not altered by thyroid hormone treatment ($p>0.05$), total antioxidant status levels significantly decreased ($p<0.05$). Oxidative stress index values were not statistically different in tissues ($p>0.05$). Tissue nitric oxide levels were also similar in both groups ($p>0.05$). Malondialdehyde levels increased in triiodothyronine given rats compared with the control group ($p<0.01$).

Conclusion: This study showed that total oxidant status levels and oxidative stress index values were similar in both groups. However, triiodothyronine supplementation induced lipid peroxidation by increasing tissue malondialdehyde levels that might deplete tissue antioxidant level.

Keywords: Sleeve Gastrectomy; Thyroid Hormone; Oxidative Stress.

INTRODUCTION

Morbid obesity is a well known cause of inflammatory changes and oxidative stress with many clinical complications (1,2). Sleeve gastrectomy is a surgical option that is used for achieving appropriate weight loss and improving the inflammatory profile in obesity (3,4).

Reactive oxygen species play role as an oxidizing agents and lead to detrimental effects on macromolecules in the cells which is known as oxidative stress (2,5-7). Reactive oxygen species production directly linked to metabolic effects of thyroid hormones which increase the rate of many reactions in the cells (8). Respiratory rate stimulation lead to more and more reactive oxygen species production (9,10). This effect of thyroid hormones results

in change of cell antioxidant status and promote reactive oxygen species production (11,12). The increase in thyroid hormone levels has been shown to convert the structure of membrane phospholipids (13,14). Unsaturation of fatty acids of membrane phospholipids makes them more susceptible to free radical attacks (15). However, iodine compounds of thyroid hormones can also reduce oxidative damage due to their molecular structure (16). The effects of thyroid hormones in stress response and sleeve gastrectomy has been demonstrated in previous studies (17).

The purpose of this study was to scrutinise the effects of triiodothyronine (T3) supplementation on oxidative stress parameters in anastomotic tissue level.

Received: 03.09.2018 Accepted: 04.10.2018 Available online: 11.10.2018

Corresponding Author: Suleyman Orman, Istanbul Medeniyet University Goztepe Training and Research Hospital, Department of Gastroenterology Surgery, Istanbul, Turkey E-mail: suleymanorm@hotmail.com

MATERIAL and METHODS

Twenty-four male Wistar albino rats weighing from 300 to 350 g were used, according to a protocol reviewed and approved by the ethical board of Istanbul University School of Medicine, Istanbul. The study was performed in the Istanbul University Aziz Sancar Experimental Medicine Research Institute and Istanbul Medeniyet University Biochemistry Laboratories. All animals were managed in accordance with the recommendations of the National Institute of Health Guidelines for the Care and Use of Laboratory Animals.

Animals and Experimental Design

The rats were randomized into two groups: Control (n=12), Experiment (n=12) group.

Surgical Procedures and Postoperative Treatment

The animals were housed under controlled conditions. Surgical procedure and postoperative treatments were performed as described by Orman, et al. (18). Briefly; rats were kept in 6 hour fasting period. Antibiotherapy prophylaxis with Ceftriaxone 30 mg/kg was performed before incision. Aseptic conditions were provided before anesthesia. Stomach was exposed after midline incision. Sleeve gastrectomy was carried out after defining the area for resection (approximately, 70% of the stomach) with vascular forceps to include most of the fundus (Figure 1). The gastrorrhaphy was carried out using an invaginating continuous hand-sewn suture. The laparotomy was closed in single-plane. After postoperative treatment rats were sacrificed on the 7th postoperative day.



Figure 1. Image of sleeve gastrectomy; 70% of stomach was resected

Biochemical analyses

Manufacturers' protocols were followed for the measurement of samples. In T3 group, a single dose of

400 mg/100 g of T3 hormone (T2752; Sigma, St. Louis, MO, USA) was given subcutaneously in the operative day, whereas 400 mg/100 g 0.9 % NaCl was given to the control group in the same manner. To obtain serum samples for measuring TSH, FT3, and FT4, rat blood samples were collected. Serum thyroid stimulating hormone (TSH), free T3 (FT3), and free thyroxine (FT4) were analysed by using by a Rat TSH ELISA kit (Elabscience Biotechnology Co., Ltd, WuHan, P.R.C.). Each tissue was homogenized in ice-cold PBS (pH: 7.4) and centrifuged at 2,000 rpm for 20 minutes at 4°C to avoid contamination with cellular debris. The supernatants were used to measure total oxidant status, total antioxidant status, nitric oxide and malondialdehyde levels. All tissue parameters were analysed by spectrophotometric methods. Oxidative stress index values were calculated.

Statistical Analysis

The Number Cruncher Statistical System 2007 (NCSS, Kaysville, UT, USA) program was used for statistical analysis. When evaluating the study data, Mann Whitney U test was used to compare descriptive statistical methods (Mean, Standard Deviation, Median, Minimum, Maximum) as well as the two groups of variables that did not show normal distribution in the comparison of the quantitative data. A p value of <0.05 was considered statistically significant.

RESULTS

Although tissue total oxidant status levels did not altered by thyroid hormone treatment ($p=0.083$; $p>0.05$), total antioxidant status levels significantly decreased ($p=0.026$; $p<0.05$). Oxidative stress index values were not statistically different in tissues ($p=0.322$; $p>0.05$). Tissue nitric oxide levels were also similar in both groups ($p=0.137$; $p>0.05$). Malondialdehyde levels increased in T3 given rats compared with the control group ($p=0.002$; $p<0.01$) (Table 1).

Table 1. Comparison of TAS, TOS, NO, MDA ve OSI levels

		Control	Experiment	*P
TAS (nmol/mg)	Min-Max	1.25-1.61	1.28-2	0.026*
	Mean±SD	1.44±0.14	1.67±0.25	
TAS (nmol/mg)	Min-Max	78.75-294.3	183.46-325.07	0.083
	Mean±SD	194.38±73.06	250.31±47.4	
NO (nmol/mg)	Min-Max	10.02-31.27	9.48-23.99	0.137
	Mean±SD	20.34±7.61	15.4±3.96	
MDA (nmol/mg)	Min-Max	3.04-7.28	1.31-6.06	0.002**
	Mean±SD	5.43±1.69	2.66±1.35	
OSi	Min-Max	62.9-186.4	102.2-183.55	0.322
	Mean±SD	132.21±41.3	151.01±23.31	

*Mann Whitney U Test, TAS: Total Antioxidant Status, TOS: Total Oxidant Status, NO: Nitric Oxide, MDA: Malondialdehyde, OSI: Oxidative Stress Index, * $p<0.05$, ** $p<0.01$

TSH levels in both the control and T3 group did not significantly change on the 7th postoperative day ($p=0.663$). FT3 levels were significantly higher in T3 group rats than in control group rats (T3 vs control: $p=0.004$).

DISCUSSION

Instability between reactive oxygen species and antioxidant systems results in oxidative stress and cell injury. Although reactive oxygen species plays physiological functions in the cell, they can also react with cell components and can cause oxidative damage (19). Circulating parameters of oxidative stress were evaluated in previous studies (20,21).

Obesity is a well known cause of oxidative stress (1,2). Adipocytes aggregate during morbid obesity and reduce blood flow. Hypoxia is inevitable under these circumstances. This process triggers the pro-inflammatory cycle between adipocytes and monocytes (22). The releases of cytokines induce more reactive oxygen species production. Many pathological conditions alongside with obesity, such as cardiovascular diseases and other inflammatory processes are the results of reactive oxygen species induced oxidative stress (5, 23).

Thyroid hormones play crucial role in stress response. Their metabolic effects on anastomotic healing were studied before (17-25). T3 activates respiratory genes in the cells and increase the production of reactive oxygen species which leads to activation of the transcription factors. As a response, cytokines trigger the expression of antioxidant enzymes (26). Another effect of thyroid hormones is improving the lipid peroxidation (27). Early and late recovery of cardiac functions after myocardial infarction and improvement after liver and renal surgery is strongly associated with correction of T3 levels (28, 29). The role of thyroid hormones in wound healing has been widely investigated. Although contradictory results have been reported, in general, its utility seems to be out-weighted (30,31). Both hypothyroidism and hyperthyroidism have role in oxidative stress (32). Thyroid hormones change the lipid composition of rat tissues and therefore results in oxidative stress (33). A slightly lower thyroid tone seems to exert some kind of protective effects, probably related to reduce oxidative stress (34). It is well known that hypothyroidism related oxidative stress in the clinical course of cardiovascular diseases. In a study; patients with primary hypothyroidism was with elevated nitric oxide and malondialdehyde levels, while superoxide dismutase was not different from controls (35-37).

In this study, we used 400 mg/100 g of T3 as the treatment dose (38,39). There was a significant decrease in FT4 levels in the T3 group compatible with other studies. This finding may be related to the overdose of exogenous T3, which may have a negative feedback mechanism on T4 (40,41).

We observed an improvement in the antioxidant capability and a decrease of total antioxidant status and increase of malondialdehyde levels after T3 usage in sleeve

gastrectomy surgery. Although total oxidant status levels and oxidative stress index values were similar in both groups, T3 supplementation induced lipid peroxidation by increasing tissue malondialdehyde levels that might deplete tissue antioxidant level. It seems thyroid hormone replacement therapy have strengthening and protecting affects on the sleeve gastrectomy cut surface line regarding the results of this experimental oxidative stress study's outcomes.

The present study has some limitations. The effect of thyroid hormone therapy on oxidative stress after long term results of weight loss could not be studied. T3 supplementation seems to play a crucial role in oxidative stress. Despite these confines, our results are encouraging and provide a new perspective in this field for future experimental studies.

CONCLUSION

Thyroid hormones have important role in the regulation of oxidative stress systems. It will be better to understand and clarify the molecular mechanisms associated with thyroid hormone therapies and oxidative stress. Appropriate treatments with best patient selection may be possible after replacement therapies.

Acknowledgements: The materials previously submitted at Federation of European Biochemical Societies (FEBS) as poster presentation in 2016.

Competing interests: The authors declare that they have no competing interest.

Financial Disclosure: There are no financial supports

Ethical approval: Study was started according to a protocol reviewed and approved by the ethical board of Istanbul University School of Medicine, Istanbul. The study was performed in the Istanbul University Aziz Sancar Experimental Medicine Research Institute and Istanbul Medeniyet University Biochemistry Laboratories

REFERENCES

1. Maury E, Brichard SM. Adipokine dysregulation, adipose tissue inflammation and metabolic syndrome. *Mol Cell Endocrinol* 2010;314:1-16.
2. Fernández-Sánchez A, Madrigal-Santillán E, Bautista M, et al. Inflammation, oxidative stress, and obesity. *Int J Mol Sci* 2011;12:3117-32.
3. Eisenberg D, Bellatorre A, Bellatorre N. Sleeve gastrectomy as a stand-alone bariatric operation for severe, morbid, and super obesity. *JLS* 2013;17:63-7.
4. Mallipedhi A, Prior SL, Barry JD, et al. Changes in inflammatory markers after sleeve gastrectomy in patients with impaired glucose homeostasis and type 2 diabetes. *Surg Obes Relat Dis* 2014;10:1123-8.
5. Sies H, Cadenas E. Oxidative stress: damage to intact cells and organs. *Philos Trans R Soc Lond B Biol Sci* 1985;311:617-31.
6. Bedard K, Krause KH. The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. *Physiol Rev* 2007;87:245-313.
7. Picklo M, Claycombe KJ, Meydani M. Adipose dysfunction, interaction of reactive oxygen species, and inflammation. *Adv Nutr* 2012;1:734-5.
8. Schwartz HL, Oppenheimer JH. Ontogenesis of 3,5,3'-triiodothyronine receptors in neonatal rat brain: dissociation between receptor concentration and stimulation of oxygen consumption by 3,5,3'-triiodothyronine. *Endocrinology* 1978;103:943-8.

9. Horrum MA, Tobin RB, Ecklund RE. Thyroxine-induced changes in rat liver mitochondrial ubiquinone. *Biochem Biophys Res Commun* 1986;138:381-6.
10. Venditti P, Puca A, Di Meo S. Effects of thyroid state on H2O2 production by rat heart mitochondria: sites of production with complex I- and complex II-linked substrates. *Horm Metab Res* 2003;35:55-61.
11. Fernández V, Barrientos X, Kipreos K, Valenzuela A, et al. Superoxide radical generation, NADPH oxidase activity, and cytochrome P-450 content of rat liver microsomal fractions in an experimental hyperthyroid state: relation to lipid peroxidation. *Endocrinology* 1985;117:496-501.
12. Ueta Y, Levy A, Chowdrey HS, et al. Hypothalamic nitric oxide synthase gene expression is regulated by thyroid hormones. *Endocrinology* 1995;136:4182-7.
13. Bangur CS, Howland JL, Katyare SS. Thyroid hormone treatment alters phospholipid composition and membrane fluidity of rat brain mitochondria. *Biochem J* 1995;305:29-32.
14. Gredilla R, López Torres M, Portero-Otín M, et al. Influence of hyper- and hypothyroidism on lipid peroxidation, unsaturation of phospholipids, glutathione system and oxidative damage to nuclear and mitochondrial DNA in mice skeletal muscle. *Mol Cell Biochem* 2001;221:41-8.
15. Kanner J, German JB, Kinsella JE. Initiation of lipid peroxidation in biological systems. *Crit Rev Food Sci Nutr* 1987;25:317-64.
16. Oziol L, Faure P, Vergely C, et al. In vitro free radical scavenging capacity of thyroid hormones and structural analogues. *J Endocrinol* 2001;170:197-206.
17. Orman S, Karaman K, Basok BI, et al. Effects of thyroid hormone therapy on cut-surface healing of the remnant stomach with short-term weight loss alterations after sleeve gastrectomy. *J Invest Surg* 2018;31:153-62.
18. Orman S, Yol S, Uzun H, et al. Effect of acellular amniotic membrane matrix patch on healing of cut surface after sleeve gastrectomy in rats. *J Invest Surg* 2018;29:1-9.
19. Kalyanaraman B. Teaching the basics of redox biology to medical and graduate students: Oxidants, antioxidants and disease mechanisms. *Redox Biol* 2013;1:244-57.
20. Mentese A, Güven S, Demir S, et al. Circulating parameters of oxidative stress and hypoxia in normal pregnancy and HELLP syndrome. *Adv Clin Exp Med* 2018;21:
21. Picu A, Petcu L, Ștefan S, et al. Markers of oxidative stress and antioxidant defense in romanian patients with type 2 diabetes mellitus and obesity. *Molecules* 2017;22:E714.
22. Deng Y, Scherer PE. Adipokines as novel biomarkers and regulators of the metabolic syndrome. *Ann N Y Acad Sci* 2010;1212:1-19.
23. Donmez-Altuntas H, Sahin F, Bayram F, et al. Evaluation of chromosomal damage, cytostasis, cytotoxicity, oxidative DNA damage and their association with body-mass index in obese subjects. *Mutat Res Genet Toxicol Environ Mutagen* 2014;771:30-6.
24. Mourouzis I, Politi E, Pantos C. Thyroid hormone and tissue repair: new tricks for an old hormone? *J Thyroid Res* 2013;2013:312104.
25. Karaman K, Bostanci EB, Dincer N, et al. Effects of thyroid hormone supplementation on anastomotic healing after segmental colonic resection. *J Surg Res* 2012;176:460-7.
26. Videla LA. Hormetic responses of thyroid hormone calorogenesis in the liver: Association with oxidative stress. *IUBMB Life* 2010;62:460-6.
27. Ferreyra C, O'Valle F, Osorio JM, et al. Effect of preconditioning with triiodothyronine on renal ischemia/reperfusion injury and poly(ADP-ribose) polymerase expression in The rats. *Transplant Proc* 2009;41:2073-5.
28. Erkan E, Sakarcan A, Haklar G, et al. Thyroxine prevents reoxygenation injury in isolated proximal tubule cells. *Pediatr Nephrol* 2003;18:636-43.
29. Lymvaivos I, Mourouzis I, Cokkinos DV, et al. Thyroid hormone and recovery of cardiac function in patients with acute myocardial infarction: a strong association? *Eur J Endocrinol* 2011;165:107-14.
30. Ashton IK, Dekel S. Fracture repair in the Snell dwarf mouse. *Br J Exp Pathol* 1983;64:479-86.
31. Safer JD. Thyroid hormone and wound healing. *J Thyroid Res* 2013;2013:124538.
32. Resch U, Hesel G, Tatzber F, et al. Antioxidant status in thyroid dysfunction. *Clin Chem Lab Med* 2002;40:1132-4.
33. Hoch FL. Lipids and thyroid hormones. *Prog Lipid Res* 1988;27:199-270.
34. da Rosa Araujo AS, Silva de Miranda MF, de Oliveira UO, et al. Increased resistance to hydrogen peroxide-induced cardiac contracture is associated with decreased myocardial oxidative stress in hypothyroid rats. *Cell Biochem Funct* 2010;28:38-44.
35. Baskol G, Atmaca H, Tanriverdi F, et al. Oxidative stress and enzymatic antioxidant status in patients with hypothyroidism before and after treatment. *Exp Clin Endocrinol Diabetes* 2007;115:522-6.
36. Elahi MM, Kong YX, Matata BM. Oxidative stress as a mediator of cardiovascular disease. *Oxid Med Cell Longev* 2009;2:259-69.
37. Keller H, Dreyer C, Medin J, et al. Fatty acids and retinoids control lipid metabolism through activation of peroxisome proliferator-activated receptor-retinoid X receptor heterodimers. *Proc Natl Acad Sci U S A* 1993;90:2160-4.
38. Inan M, Koyuncu A, Aydin C, et al. Thyroid hormone supplementation in sepsis: An experimental study. *Surg Today* 2003;33:24-9.
39. Malik R, Habib M, Tootle R, et al. Exogenous thyroid hormone induces liver enlargement, whilst maintaining regenerative potential--a study relevant to donor preconditioning. *Am J Transplant* 2005;5:1801-7.
40. Mebis L, van den Berghe G. The hypothalamus-pituitary-thyroid axis in critical illness. *Neth J Med* 2009;67:332-40.
41. Short KR, Nygren J, Nair KS. Effect of T(3)-induced hyperthyroidism on mitochondrial and cytoplasmic protein synthesis rates in oxidative and glycolytic tissues in rats. *Am J Physiol Endocrinol Metab* 2007;292:642-7.