Neuroendocrinology Letters Volume 37 No. 2 2016
ISSN: 0172-780X; ISSN-L: 0172-780X; Electronic/Online ISSN: 2354-4716
Web of Knowledge / Web of Science: Neuroendocrinol Lett
Pub Med / Medline: Neuro Endocrinol Lett

Thyroid-stimulating hormone acutely increases monocyte gene expression *in vivo*

AnneMarie Gagnon [1,2,3], Heather A. Lochnan [1,3], Christopher S. Tran [1], Alexander Sorisky [1,2,3]

1 Department of Medicine, University of Ottawa, Ottawa, Canada
2 Department of Biochemistry, Microbiology & Immunology, University of Ottawa, Ottawa, Canada
3 Chronic Disease Program, Ottawa Hospital Research Institute, Ottawa, Canada

Correspondence to: Dr. A. Sorisky
Ottawa Hospital Research Institute
501 Smyth Road, Ottawa, Ontario K1H 8L6, Canada.
tel: +1 613-737-8899 #73320; e-mail: asorisky@ohri.ca

Submitted: 2016-01-31 Accepted: 2016-02-26 Published online: 2016-04-29

Key words: **TSH; monocytes; inflammation; body mass index; atherosclerosis**

Neuroendocrinol Lett 2016; **37**(2):121–123 **PMID:** 27179574 NEL370216A06

Abstract

OBJECTIVES: Thyroid-stimulating hormone (TSH) acts in an extra-thyroidal fashion and induces a pro-inflammatory, pro-coagulant state. Blood monocytes can be activated by vascular stress, but it is not known if this occurs upon TSH administration. Our aim was to determine if recombinant human (rh) TSH, administered acutely to patients being screened for thyroid cancer recurrence, alters blood monocyte gene expression.

DESIGN AND SETTING: Patients (14 women, 1 man) had a mean (±SD) age of 48±10 years, a body mass index of 26±6 kg/m^2, a history of total thyroidectomy and radioablation for thyroid cancer, and were on L-thyroxine therapy at a university teaching hospital. They received 2 intramuscular doses of rhTSH (0.9 mg), administered on days 1 and 2. Blood samples were obtained at baseline on day1, and on days 3 and 5.

RESULTS: Monocyte MCP-1 mRNA (mean±SE) increased significantly by 1.7±0.3 fold on day 5 following rhTSH stimulation (p=0.03, n=15). IL-1β and CD36 mRNA expression also increased on day 5 (1.9±0.4 fold, p=0.07, n=14) and 2.5±0.4 fold, p=0.1, n=10), respectively, although did not quite reach statistical significance. Significant correlations were detected between the BMI of patients and their TSH-stimulated monocyte mRNA responses at day 5 for CD11a, (r=0.66, n=14, p=0.01); CD14 (r=0.638, n=13, p=0.019), and CD16, r=0.84, n=13, p=0.0003).

CONCLUSION: TSH administration increases pro-atherogenic monocyte gene expression.

Abbreviations:

BMI	- body mass index
CRP	- C-reactive protein
FFA	- free fatty acids
IL	- interleukin
rh	- recombinant human
RPMI	- Roswell Park Memorial Institute

ORIGINAL ARTICLE

INTRODUCTION

When recombinant human (rh)TSH is administered to patients previously treated for thyroid cancer (thyroidectomy and radioablation) to screen for recurrence, a brief period of metabolic and vascular stress ensues. This is due to extra-thyroidal action of TSH (Sorisky & Gagnon 2014). Activation of platelets, elevations in levels of interleukin (IL)-6, C-reactive protein (CRP), oxidative stress, microparticles, and free fatty acids (FFA), as well as a decrease in endothelium-dependent relaxation, occur (Sorisky & Gagnon 2014; Desideri *et al.* 2009; Burger *et al.* 2015; Kim *et al.* 2013). However, it is not known if circulating monocytes, immune cells implicated in inflammation and atherosclerosis, are altered in this context. Our aim was to determine if rhTSH alters monocyte gene expression.

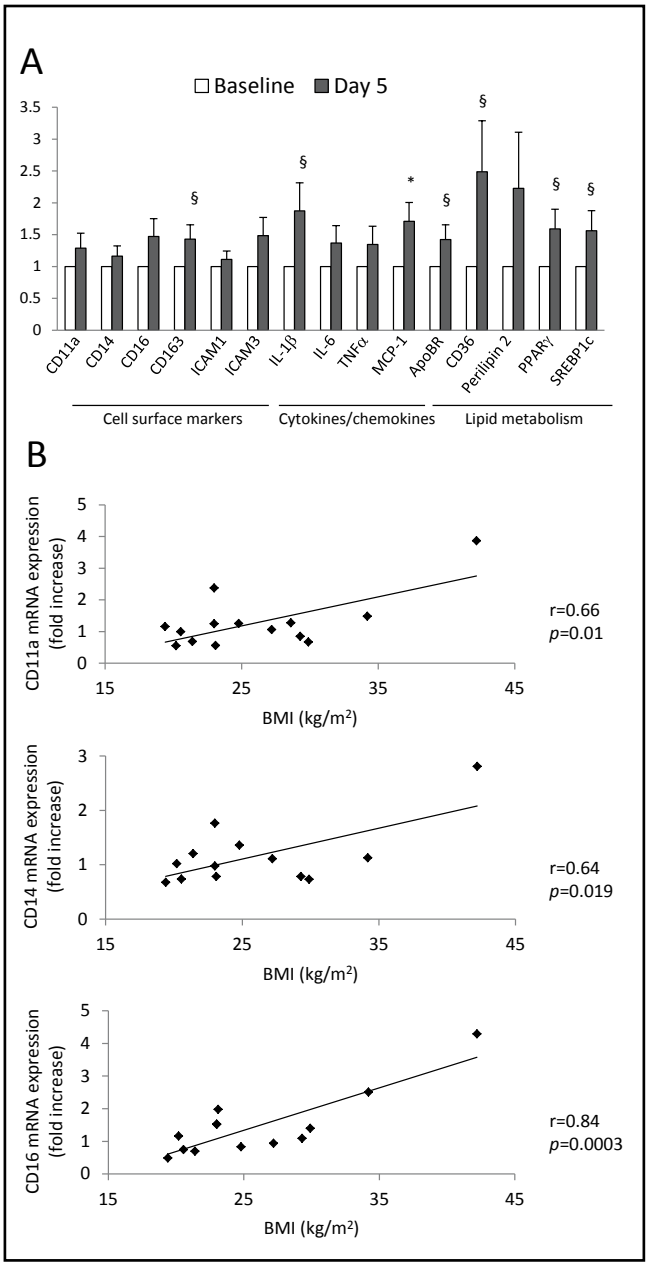

Fig. 1. Monocyte mRNA expression in response to rhTSH. Thyroidectomized patients were administered rhTSH on day 1 and day 2. Blood was drawn and monocytes were isolated at baseline, prior to rhTSH injection, and on day 5. RNA was extracted and quantified by real time PCR, using indicated primers. Levels were normalized to endogenous 18s rRNA, and expressed as a function of baseline levels. Results are the mean±SE of 10–15 separated patient samples. **A.** Gene expression for individual targets. * indicates $p<0.05$; § indicates $p\leq0.1$, as assessed by Student's t test. **B.** Pearson's correlations between fold increase in mRNA expression(CD11a, CD14 and CD16) at day 5 and BMI.

MATERIAL AND METHODS

Patients (14 women, 1 man) with a mean (±SD) age of 48±10 years and body mass index (BMI) of 26±6 kg/m^2, who were disease-free following treatment by thyroidectomy and radioablation (all papillary carcinoma), and on L-thyroxine therapy, were recruited (Ottawa Health Sciences Network Research Ethics Board, #2006558). They received 2 intramuscular doses of rhTSH (0.9 mg) on days 1 and 2 with ongoing L-thyroxine therapy. Blood was drawn on the mornings of days 1 (baseline; before rhTSH), 3, and 5. TSH and free thyroxine (reference ranges 0.3–5.6 mU/L and 7–17 pmol/L, respectively) were measured by AutoIA (Abbot DxI) in The Ottawa Hospital. For monocyte isolation, blood was diluted with PBS (1:2, v:v) supplemented with 2 mmol/L EDTA and layered onto Ficoll-Hypaque (GE Healthcare). Upon density-gradient centrifugation (400 × *g*, 30 min), peripheral blood mononuclear cells were isolated, washed, and seeded at a density of 1×10^5 cells/cm^2 in Roswell Park Memorial Institute (RPMI) medium supplemented with antibiotics (100 U/ml penicillin and 0.1 mg/ml streptomycin, all from Life Technologies) for 1 h. Adherent monocytes were washed, and then lysed with Qiazol (Qiagen). RNA was isolated as per manufacturer's instructions. Gene expression was determined by real-time PCR (LightCycler, Roche). Differences between means were assessed by Student's t test. Linear regression and Pearson's correlation were used to compare rhTSH responses where indicated. $p<0.05$ was considered significant.

RESULTS

At baseline, levels (mean±SD) of free thyroxine were 18±5 pmol/L (n=15) and of TSH were 0.46±0.85 mU/L (n=14). Following rhTSH injection, TSH levels increased to 111±36 mU/L (day 3; n=8), and 12.3±5.5 mU/L (day 5, n=15). Due to the low number of samples on day 3, gene expression data for monocytes were compared between days 1 and 5. Monocyte MCP-1 mRNA (mean±SE) increased significantly by 1.7±0.3 fold on day 5 following rhTSH stimulation (p=0.03, n=15) (Figure 1A). IL-1β and CD36 mRNA expression also increased on day 5 (1.9±0.4 fold, p=0.07, n=14) and 2.5±0.4 fold, p=0.1, n=10), respectively , although did not quite reach statistical significance. Significant cor-

relations were detected between the BMI of patients and their TSH-stimulated monocyte mRNA responses at day 5 for CD11a, (r=0.66, n=14, p=0.01); CD14 (r=0.638, n=13, p=0.019), and CD16, r=0.84, n=13, p=0.0003) (Figure 1B).

DISCUSSION

It is not known how rhTSH induces these changes in gene expression which are indicative of inflammatory and pro-atherogenic activation. Monocytes do not express TSH receptors, so they may be indirectly stimulated by cytokines released by non-thyroidal TSH-responsive cells, such as adipocytes. Consistent with this possibility of adipocyte involvement is that CD11a, CD14, and CD16 gene responses to rhTSH correlated positively with BMI, an indicator of adipose tissue accumulation.

rhTSH stimulation leads to an acute, isolated rise in circulating TSH. A chronic, milder isolated rise in TSH occurs with subclinical hypothyroidism. These patients are at higher risk for cardiovascular disease, but the mechanism is unknown (Sorisky & Gagnon 2014). Elevations in FFA, pro-inflammatory cytokines, microparticles, and platelet activation have been reported (Sorisky & Gagnon 2014; Burger *et al.* 2015; Kim *et al.* 2013). In patients undergoing endarterectomy for carotid stenosis, plaques from those with subclinical hypothyroidism had more macrophages and inflammation vs euthyroid patients (Marfella *et al.* 2011). In a rat model of subclinical hypothyroidism, blood monocytes had high toll-like receptor 4 levels, a receptor linked to inflammation (Yang *et al.* 2014). Future studies are warranted to determine if circulating monocytes are activated in subclinical hypothyroidism, and the role they may play in the elevated associated risk of cardiovascular disease.

ACKNOWLEDGMENTS

This study was funded through Canadian Institutes of Health Research operating grant MOP-102585 (to A.S.). We thank all study participants.

Authors' contributions

A. Sorisky and A Gagnon designed the study. H.A. Lochnan and C.S. Tran recruited and characterized of the research participants. Experimental work and data analysis were conducted by A. Gagnon. A. Sorisky and A. Gagnon wrote the manuscript, and all authors were involved in reviewing and editing the manuscript.

REFERENCES

1 Burger D, Gagnon A, Lochnan HA, Mahzari M, Sorisky A (2015). Thyroid-stimulating hormone acutely increases levels of circulating pro-coagulant microparticles. Clin Endocrinol **83**: 285–287.
2 Desideri G, Bocale R, Milardi D, Ghiadoni L, Grassi D, Necozione S, Taddei S, Di Orio F, *et al.* (2009). Enhanced proatherogenic inflammation after recombinant human TSH administration in patients monitored for thyroid cancer remnant. Clin Endocrinol **71**: 429–433.
3 Kim JH, Park JH, Kim SY, Bae HY (2013). The mean platelet volume is positively correlated with serum thyrotropin concentrations in a population of healthy subjects and subjects with unsuspected subclinical hypothyroidism. Thyroid **23**: 31–37.
4 Marfella R, Ferraraccio F, Rizzo MR, Portoghese M, Barbieri M, Basilio C, Nersita R, Siniscalchi LI, *et al.* (2011). Innate immune activity in plaque of patients with untreated and L-thyroxine-treated subclinical hypothyroidism. J Clin Endocrinol Metab **96**: 1015–1020.
5 Sorisky A, Gagnon A (2014). Freedom of expression beyond the thyroid: the thyroid-stimulating hormone receptor in the adipocyte. OA Biochemistry 2014 Feb22,2(1):2.
6 Yang SS, Tang L, Li RG, Ge GH, Qu XK, Ma JW, Qiao ZY, Zhang L, *et al.* (2014). The effects of subclinical hypothyroidism on serum lipid level and TLR4 expression of monocyte in peripheral blood of rats. Neuro Endocrinol Lett **35**: 80–86.