Levothyroxine Improves Abnormal Cardiac Bioenergetics in Subclinical Hypothyroidism: A Cardiac Magnetic Resonance Spectroscopic Study

Asgar Madathil, Kieren G. Hollingsworth, Andrew M. Blamire, Salman Razvi, Julia L. Newton, Roy Taylor, and Jolanta U. Weaver

Institute of Cellular Medicine (A.M., K.G.H., A.M.B., S.R., J.L.N., R.T., J.U.W.) and Newcastle Magnetic Resonance Centre (K.G.H., A.M.B., R.T.), Newcastle University, Newcastle-upon-Tyne NE2 4HH, United Kingdom

Context: It is well established that subclinical hypothyroidism (SCH) is associated with mild cardiac dysfunction, but it is unknown whether there is an underlying impairment of cardiac bioenergetic function.

Objective: The objective of the study was to quantify the cardiac phosphocreatine to adenosine triphosphate ratio (PCr to ATP) in SCH, compared with healthy controls, and to measure the effect of 6 months of levothyroxine treatment.

Design and Setting: This was a 6-month, prospective, case-controlled interventional study.

Participants and Main Outcome Measures: The PCr to ATP ratio was measured using phosphorus-31 magnetic resonance spectroscopy in subjects with SCH at baseline and after levothyroxine therapy (1.6 μg/kg·d) and compared with age- and gender-matched euthyroid controls. All subjects were free of overt heart disease.

Results: Twenty-one subjects with SCH (normal free T_4 and serum TSH between 4.1 and 10 mIU/L) and 17 controls were matched for age (mean age 40.5 vs 43.3 y) and sex (females 81% vs 82%) but differed in mean TSH (6.5 vs 2.1 mIU/L, $P < .001$). At baseline the mean ($\pm$SD) PCr to ATP ratio in SCH was lower than in controls (1.80 ± 0.26 vs 2.07 ± 0.20, $P = .001$). In the 16 subjects studied after levothyroxine treatment, the PCr to ATP ratio improved (from 1.74 ± 0.24 to 1.91 ± 0.26, $P = .004$) and approached controls (borderline loss of significance, $P = .051$). On multivariate analysis, SCH was independently associated with a reduced PCr to ATP ratio, even after adjusting for confounding variables (body mass index and fasting glucose) ($P = .001$).

Conclusion: Our results demonstrate early cardiac bioenergetic impairment in SCH, which is reversible with levothyroxine therapy. This mechanistic insight provides justification for longitudinal trials to determine whether improvement in bioenergetic function improves cardiovascular outcome. **(*J Clin Endocrinol Metab* 100: E607–E610, 2015)**

Subclinical hypothyroidism (SCH) is defined as a condition in which serum TSH is raised with normal serum free thyroxine (FT4). It is an early manifestation of thyroid underactivity. SCH is a common medical condition, and its prevalence in the general population has been estimated at between 4% and 10%, depending on the population studied (1).

Thyroid hormones have a significant effect on cardiovascular function (2). SCH has been associated with reversible impairment in cardiac function (3). The mechanism leading to cardiac dysfunction in SCH is poorly understood. The cardiac phosphocreatine (PCr) acts as a shuttle between myocardial sarcoplasm and mitochondria for transferring energy, which is used for myocardial con-

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

Received July 16, 2014. Accepted December 19, 2014.
First Published Online December 26, 2014

Abbreviations: BMI, body mass index; BP, blood pressure; CI, confidence interval; FT4, free T_4; MR, magnetic resonance; MRS, MR spectroscopy; OR, odds ratio; PCr, phosphocreatine; PCr to ATP, ratio of phosphocreatine to ATP; SCH, subclinical hypothyroidism.

traction and relaxation (4). The cardiac phosphocreatine to ATP (PCr to ATP) ratio, as measured by phosphorus-31 magnetic resonance spectroscopy (MRS), has been shown to be a good indicator of myocardial bioenergetic status (5). Although largely used as a research modality, the PCr to ATP ratio has been shown to be predictive of mortality in dilated cardiomyopathy (6). Low PCr to ATP ratio has been associated with ischemic heart disease and heart failure (5), and this has been postulated as the mechanism for cardiac dysfunction in diseases affecting myocardium (7).

Thyroid hormones significantly influence cardiac mitochondrial function (8), and abnormal myocardial bioenergetics in hypothyroidism have been shown in animal studies (9). In humans, hypothyroidism has been associated with abnormal skeletal muscle energetic metabolism (10). The myocardial bioenergetics in patients with hypothyroidism have not been studied before. We hypothesized that patients with SCH would have abnormal cardiac bioenergetics that are reversible with treatment. This may explain the mechanism of cardiac dysfunction in SCH. The primary aim of our study was to quantify the cardiac PCr to ATP ratio in patients with SCH and compare this with age- and gender-matched euthyroid controls. Our secondary aim was to determine whether 6 months of levothyroxine treatment led to measurable changes in the cardiac PCr to ATP ratio in patients with SCH.

Materials and Methodology

The study design involved comparison of cardiac magnetic resonance (MR) spectroscopic data at baseline between patients with SCH and those of age- and gender-matched euthyroid controls. Patients were then treated with levothyroxine for 6 months to render them euthyroid, and repeat cardiac MR spectroscopy was performed, looking for changes in the cardiac PCr to ATP ratio.

Subjects

Patients with known SCH were recruited from primary and secondary care clinics. The inclusion criteria were age 18–65 years, SCH with TSH between 4.1 and 10.0 mIU/L, normal free T_4 (FT4 and TSH repeated 3 mo apart) and fatigue as one of the symptoms. The subjects with previous overt thyroid disease or known to have cardiovascular disease were excluded. The detailed exclusion criteria are given in the online Supplemental Materials. Age- and gender-matched euthyroid controls were recruited from staff members of the research institute. This study complied with the Declaration of Helsinki and had the approval of the local Research Ethics Committee. Written informed consent was obtained from all the participants.

Screening visit

After overnight fasting for 12 hours, the following blood samples were taken at the screening visit: free serum T_4, free serum T_3, serum TSH, fasting blood glucose, fasting serum cholesterol, liver function tests, creatinine, bone profile and full blood count. The serum TSH, free T4 and free T_3 were measured by Roche Cobas e601 with the coefficient of variation below 5%. The reference ranges for serum TSH, FT4, and free T_3 were 0.4–4.0 mIU/L, 9–25 pmol/L, and 2.5–7.5 pmol/L, respectively. Cardiac auscultation and 12-lead electrocardiography were performed to exclude subjects with overt cardiac dysfunction. Height, weight, and blood pressure (BP) were also measured.

Levothyroxine dosing and follow-up

After baseline MR scanning, all patients with SCH were started on levothyroxine at a dose of 1.6 μg/kg·d. The levothyroxine dose titration was done on a 6-weekly basis for 6 months with the aim to achieve a serum TSH in the range of 1–1.5 mIU/L.

Cardiac MR spectroscopy

Cardiac high-energy phosphate metabolism was assessed using ^{31}P MRS. Data were collected using a 3T Intera Achieva scanner (Philips) with a 10-cm diameter ^{31}P surface coil (Pulseteq) for transmission/reception of the signal. The detailed methodology is provided in the online Supplemental Methods and has also been previously published (11). The analysis of the spectra was performed by one analyst (K.G.H.) blinded to the thyroid status of the participants.

Statistics

A Student *t* test was used to compare data between patients and controls at baseline and for comparison of patients' data before and after levothyroxine treatment, respectively. Pearson correlation analysis was used to find association between variables. The age and body mass index (BMI) data were log transformed for correlation analysis because these were not normally distributed. After combining both SCH and HC groups, a linear regression analysis was performed using cardiac PCr to ATP ratio as the dependent variable and thyroid status, BMI, and fasting blood glucose as the covariates. The statistical software SPSS 19.0 for Windows (SPSS) was used for analysis.

Results

Of 21 patients who were recruited, two patients subsequently dropped out of the study (due to personal reasons), two had poor-quality spectra on the posttreatment MRS scan, and one patient was overreplaced with levothyroxine. Therefore, the data were successfully acquired for 16 patients on levothyroxine replacement (the posttreatment SCH group). Of 20 controls, 17 had good-quality spectra that were included in the final analysis, and the remaining three subjects had poor-quality spectra, which were excluded from the study. Rejection of spectra was based on inadequate signal to noise to fit any or all of PCr, the γ-resonance of ATP or 2,3-diphosphoglycerate.

The baseline characteristics of the SCH and controls groups are provided in Table 1. Both groups were matched for gender distribution, age, heart rate, BP, and fasting blood cholesterol and not for BMI and fasting blood glucose. The mean dose of levothyroxine was 102.3 (±28.5) μg/d. The

Table 1. Baseline Characteristics of Patients With SCH Compared With Controls

Characteristic	SCH (n = 21) Mean (±SD)	Controls (n = 17) Mean (±SD)	*P* Value
Age, y	40.5 (±12.0)	43.3 (±13.2)	.50
Women, n, % of n	17 (81)	14 (82)	.91
BMI, kg/m^2	28.9 (±5.8)	24.7 (±4.8)	.02
Blood pressure, mm Hg	122/74 (±17/9)	121/75 (±20/11)	.84
Heart rate, beats/min	68 (±7)	64 (±9)	.12
Fasting blood glucose, mmol/L	5.0 (±0.4)	4.7 (±0.5)	.02
Serum total cholesterol, mmol/L	5.5 (±1.1)	5.4 (±0.8)	.63
Serum TSH, mIU/L	6.5 (±1.7)	2.1 (±0.9)	.00
Serum free T4, pmol/L	13.6 (±1.3)	14.4 (±1.3)	.92
Serum free T_3, pmol/L	5.2 (±0.7)	4.4 (±0.8)	.04

mean serum TSH and FT4 levels at the end of the study were 2.0 ± 1.0 mIU/L and 19.0 ± 2.4 pmol/L, respectively.

The cardiac PCr to ATP ratio in patients with SCH at baseline was significantly lower than in controls (1.80 ± 0.26 vs 2.07 ± 0.2, $P = .001$) (Figure 1). After treatment with levothyroxine, the ratio improved significantly (n = 16) (1.74 ± 0.24 at baseline vs 1.91 ± 0.26, $P = .004$). Figure 1 also shows that the cardiac PCr to ATP ratio in SCH approached the level found in controls after levothyroxine treatment with borderline loss of significance (controls vs SCH after treatment, $P = .051$).

In the SCH group, the cardiac PCr to ATP ratio did not correlate with baseline FT4, free T_3, serum TSH, BMI, BP, serum cholesterol, or fasting blood glucose (data not shown). In a multiple regression analysis, subclinical hypothyroidism was a negative predictor for cardiac PCr to ATP ratio independent of other variables [odds ratio (OR) 0.30, 95% confidence interval (CI) 0.12–0.47, $P = .001$)]. The BMI and fasting blood glucose were not independent predictors of cardiac PCr to ATP ratio (OR 0.01, 95% CI 0.01–0.02, $P = 0.28$, and OR 0.17, 95% CI 0.02–0.036, $P = 0.08$, respectively).

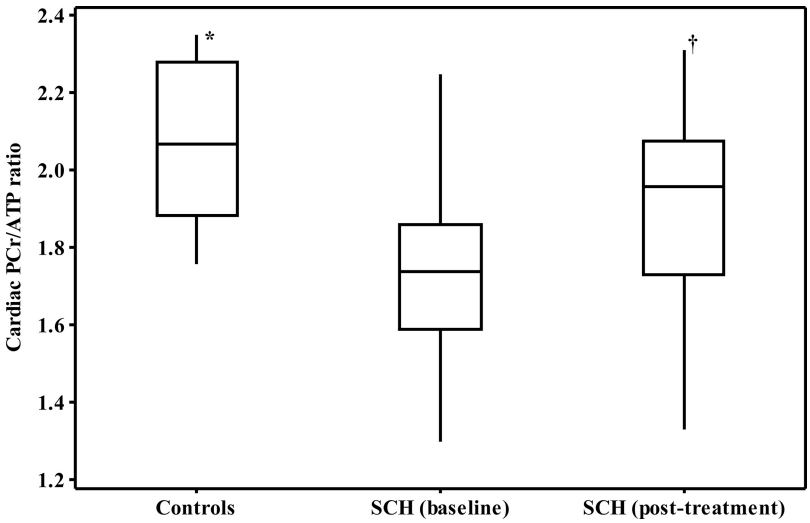

Figure 1. The comparison of cardiac PCr to ATP ratio between controls (n = 17) and SCH (n = 21) (baseline) groups (*, $P = .001$) and controls and SCH after treatment groups (n = 16) (†, $P = .051$).

Discussion

Our results show for the first time that the cardiac PCr to ATP ratio is reduced in patients with SCH and improves significantly after 6 months of treatment with levothyroxine. Our findings strengthen the results of previous studies assessing cardiac function in SCH by furthering our understanding of the bioenergetic basis of myocardial dysfunction.

Thyroid hormones affect cardiac function in many ways. They increase cardiac output by improving cardiac ionotropy and chronotropy (12). At a molecular level, thyroid hormones positively regulate gene encoding for α-myosin heavy chain and β-adrenergic receptors via genomic actions (12). Work on the role of thyroid hormone in cardiac mitochondrial function has shown that thyroid hormones stimulate cardiac mitochondrial biogenesis and oxidative phosphorylation (8). Therefore, it is possible that hypothyroidism directly lead to low cardiac PCr to ATP ratio, which might contribute toward abnormal cardiac function. Abnormal myocardial energetics have been correlated with impaired ventricular function in patients with type 2 diabetes, and proposed mechanisms include impaired relaxation of cardiac myocytes due to reduced calcium sequestration in cardiac sarcoplasmic reticulum (7). It is also possible that peripheral hemodynamic changes in hypothyroidism cause abnormal cardiac bioenergetics, which improved with levothyroxine treatment. Hence, it is unclear at this stage whether abnormal bioenergetics was the cause or consequence of hemodynamic changes in hypothyroidism.

In SCH patients with serum TSH between 4.1 and 10 mIU/L, the treatment benefits are uncertain (13), but surrogate markers of cardiac dysfunction like isovolumic relaxation time have been shown to improve with

levothyroxine treatment (14). In addition, a recent retrospective data analysis has shown that levothyroxine therapy improved cardiovascular outcomes in younger patients with SCH (15). Our study adds to the evidence that even with serum TSH below 10 mIU/L, reversible cardiac dysfunction as manifested by abnormal PCr to ATP ratio can be demonstrated.

Our patients with SCH had higher fasting blood glucose and free T_3 levels than controls, but these were well within normal ranges. Although the SCH subjects had higher BMI than the **controls**, previous work has shown no effect of this degree of BMI difference on PCr to ATP ratio (16, 17). At baseline, we found no relationship between BMI, fasting glucose, serum FT4, and PCr to ATP ratio in our study. The BMI and fasting blood glucose were not measured at the end of the study and hence unable to clarify whether this would have influenced the PCr to ATP ratio at the end of the study. The lack of correlation between thyroid hormones and cardiac PCr to ATP ratio may be due to small number of participants in the study. However, a regression analysis showed that SCH is a negative predictor of PCr to ATP ratio after adjusting for other potential confounding variables. The limitations of the study include small sample size, lack of assessment of ventricular functional parameters, and lack of clinical outcomes. Because this study was designed as a proof of concept, we did not include a placebo arm for the SCH group. However, a previous animal study with placebo-controlled design have shown improvement in cardiac PCr to ATP ratio after iv T_3 infusions in the hypothyroid state (9). In that study, the cardiac PCr to ATP ratio did not increase after placebo infusions in hypothyroid state or T_3 infusions in the euthyroid state. These findings show that the thyroid hormones modulate myocardial energetics in hypothyroidism in vivo.

In summary, our study has shown that the cardiac PCr to ATP ratio is low in SCH, and it has increased with levothyroxine treatment. This might be due to the direct effect of mild thyroid hormone deficiency. This proof-of-concept study may provide further mechanistic insight into the bioenergetic basis of cardiac dysfunction seen in SCH. It is important to measure hemodynamic parameters and clinical end points to evaluate the clinical significance of abnormal bioenergetics in SCH. Larger randomized controlled studies with hemodynamic measurements and clinical end points are required to investigate whether an improvement in myocardial energetic function translates into the clinical benefits in SCH.

Acknowledgments

We thank Louise Morris and Tim Hodgson for their radiographer support.

Address all correspondence and requests for reprints to: Dr J. U. Weaver, Senior Lecturer, Institute of Cellular Medicine, Newcastle University, Framlington Place, Newcastle-upon-Tyne NE2 4HH, United Kingdom. E-mail: jolanta.weaver@newcastle.ac.uk.

This work was supported by the BUPA Foundation, The Medical Research Charity. K.G.H. is supported by the UK Medical Research Council (Grant G1100160).

Disclosure Summary: The authors have nothing to disclose.

References

1. **Biondi B, Cooper DS.** The clinical significance of subclinical thyroid dysfunction. *Endocr Rev*. 2008;29:76–131.
2. **Biondi B.** Cardiovascular effects of mild hypothyroidism. *Thyroid*. 2007;17:625–630.
3. **Cooper DS, Biondi B.** Subclinical thyroid disease. *Lancet*. 2012; 379:1142–1154.
4. **Meyer RA, Sweeney HL, Kushmerick MJ.** A simple analysis of the "phosphocreatine shuttle." *Am J Physiol*. 1984;246:C365–C377.
5. **Hudsmith LE, Neubauer S.** Magnetic resonance spectroscopy in myocardial disease. *JACC Cardiovasc Imaging*. 2009;2:87–96.
6. **Neubauer S, Horn M, Cramer M, et al.** Myocardial phosphocreatine-to-ATP ratio is a predictor of mortality in patients with dilated cardiomyopathy. *Circulation*. 1997;96:2190–2196.
7. **Diamant M, Lamb HJ, Groeneveld Y, et al.** Diastolic dysfunction is associated with altered myocardial metabolism in asymptomatic normotensive patients with well-controlled type 2 diabetes mellitus. *J Am Coll Cardiol*. 2003;42:328–335.
8. **Marin-Garcia J.** Thyroid hormone and myocardial mitochondrial biogenesis. *Vasc Pharmacol*. 52:120–130.
9. **Portman MA, Qian K, Krueger J, Ning XH.** Direct action of t3 on phosphorylation potential in the sheep heart in vivo. *Am J Physiol Heart Circ Physiol*. 2005;288:H2484–H2490.
10. **Kaminsky P, Robin-Lherbier B, Brunotte F, et al.** Energetic metabolism in hypothyroid skeletal muscle, as studied by phosphorus magnetic resonance spectroscopy. *J Clin Endocrinol Metab*. 1992;74:124–129.
11. **Bates MG, Hollingsworth KG, Newman JH, et al.** Concentric hypertrophic remodelling and subendocardial dysfunction in mitochondrial DNA point mutation carriers. *Eur Heart J Cardiovasc Imaging*. 2013;14(7):650–658.
12. **Klein I, Ojamaa K.** Thyroid hormone and the cardiovascular system. *N Engl J Med*. 2001;344:501–509.
13. **Surks MI, Ortiz E, Daniels GH, et al.** Subclinical thyroid disease: scientific review and guidelines for diagnosis and management. *JAMA*. 2004;291:228–238.
14. **Monzani F, Di Bello V, Caraccio N, et al.** Effect of levothyroxine on cardiac function and structure in subclinical hypothyroidism: a double blind, placebo-controlled study. *J Clin Endocrinol Metab*. 2001; 86:1110–1115.
15. **Razvi S, Weaver JU, Butler TJ, Pearce SH.** Levothyroxine treatment of subclinical hypothyroidism, fatal and nonfatal cardiovascular events, and mortality. *Arch Intern Med*. 2012;172:811–817.
16. **Rider OJ, Francis JM, Ali MK, et al.** Effects of catecholamine stress on diastolic function and myocardial energetics in obesity. *Circulation*. 2012;125:1511–1519.
17. **Rider OJ, Francis JM, Tyler D, Byrne J, Clarke K, Neubauer S.** Effects of weight loss on myocardial energetics and diastolic function in obesity. *Int J Cardiovasc Imaging*. 2013;29(5):1043–1050.