CLINICAL STUDY

Regular aerobic exercise training improves endothelium-dependent arterial dilation in patients with subclinical hypothyroidism

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Abstract

Objective: Impairment of flow-mediated endothelium-dependent arterial dilation (FMD) exists in patients with subclinical hypothyroidism (sHT). Several studies showed that exercise training can improve FMD in patients with type 1 and type 2 diabetes. Therefore, we hypothesized that exercise training can also improve FMD in subjects with sHT. The purpose of the study was to test this hypothesis.

Research design and methods: We selected 30 sedentary women with sHT and 27 sedentary healthy women with euthyroid. All individuals participated in an exercise training of 6 months. Before and after exercise training, high resolution ultrasound was used to measure FMD.

Results: At baseline, FMD among subjects with sHT was 3.87%, which was significantly lower than that in controls (5.98%; $P < 0.001$). After 6 months of exercise, there was a remarkable increase in FMD (31.3%) and VO_2 max (36.7%; $P < 0.01$), and significant decreases in total cholesterol (20%), low-density lipoprotein cholesterol (LDL; 29%), triglycerides (TG; 47.6%), and C-reactive protein (CRP; 61.5%; $P < 0.05$) were observed over the exercise in patients with sHT. The absolute changes in FMD showed significant correlation with changes in LDL ($r = -0.596$), TG ($r = -0.532$), and CRP ($r = -0.511$; $P < 0.01$), and multiple regression analysis showed changes of LDL, TG, CRP were significant determinants of changes of FMD in sHT patients during exercise course.

Conclusion: Regular aerobic exercise improves FMD in sHT patients, and changes of lipids and inflammation during the exercise period may partially contribute to the improvement of endothelial function.

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Introduction

The endothelium plays an important role in modulating vascular function and structure through the production of vasodilator and vasoconstrictor substances (1). Endothelial dysfunction reflects the disordered physiology of several endothelium-derived vasoactive factors, in particular nitric oxide (NO) (2), and is an early physiological event in atherosclerosis (1). Patients with subclinical hypothyroidism (sHT), a disorder characterized by elevated serum TSH levels despite normal free hormone (free tri-iodothyronine (fT_3) and free thyroxine (fT_4)) values, are associated with increased prevalence of atherosclerotic lesions and cardiovascular events (3, 4). Recently, many studies showed that impairment of flow-mediated endothelium-dependent arterial dilation (FMD) exists in patients with sHT (5, 6). Many studies suggested that treatment of sHT can improve cardiovascular health (5, 7). The crucial mechanism of this endothelial dysfunction remains unclear.

Exercise training can improve vascular endothelial function in patients with prediabetes (8), type 1 diabetes (9) and type 2 diabetes (10) as well as in the nondiabetic population (11). To date, no studies on endothelial function in subjects with sHT in response to exercise training have been reported. Therefore, we hypothesized that exercise training can also improve FMD in subjects with sHT. The purpose of the study was to test this hypothesis.

Subjects and methods

Subjects

From January 2002 to January 2006, 30 sedentary Chinese Han women with sHT (physical exercise less than once a week), referred to our hospital for healthy examination, between 46 and 65 years of age, mean age 53 ± 8 years, were studied. All patients with sHT were newly diagnosed, as were those with Hashimoto's

thyroiditis, who were positive for both antithyroid peroxidase (TPO-Ab) and antithyroglobulin (Tg-Ab) antibodies. The diagnosis of sHT was established on the basis of the elevated TSH levels, and normal fT_3 and fT_4 values. During the same period, 27 sedentary healthy women who were euthyroid (physical exercise less than once a week, from the medical staff in our hospital), aged 46–63 years, mean age 52 ± 9 years, were selected as control. All individuals participated in an exercise training program of 6 months. Of them, two patients and two healthy women discontinued the study after 1 month of training because of time constraints. All subjects including sHT and controls were postmenopausal women. Obese subjects, (body mass index (BMI) $> 30 \text{ kg/m}^2$) smokers, and those with hypertension, clinical detectable coronary artery disease, and other diseases were excluded from the study. Also, no patient was taking any drugs, such as estrogen supplements, T_4 , diuretics, antihypertensive and hypolipidemic drugs. All subjects gave informed consent. The study protocol was in agreement with the guidelines of the local ethics committee, and approved by the ethics committee at our hospital.

Methods

Laboratory methods In patients with sHT, vascular and laboratory examinations were performed 1–3 days before and 6 months after the initiation of the exercise training program. After a 12-h fasting period, blood was obtained for measuring plasma lipids, glucose, thyroid function, and other parameters. Serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL), triglycerides (TG), and high-density lipoprotein cholesterol (HDL) were measured enzymatically. Apolipoprotein A1 (ApoA₁) and ApoB were measured by immunoturbidimetry. Serum lipoprotein (a) (Lp(a)) levels were measured by an ELISA method. Fast blood glucose (FBG) was measured by a glucose oxidase procedure. C-reactive protein (CRP) concentrations were measured using the CRP (Latex) ultrasensitive assay. The concentrations of fT_3 (normal range, 3.20–9.20 pmol/l) and fT_4 (normal range, 9.10–25.60 pmol/l) were measured by RIA, and TSH (normal range, 0.3–5.5 mU/l) was determined with an ultrasensitive IRMA method. Tg-Ab was measured by specific IRMA (normal range, $< 50 \text{ } \mu\text{g/ml}$); TPO-Ab was measured by specific RIA (normal range, $< 10 \text{ } \mu\text{g/ml}$). The coefficients of variation (CVs) for these assays were 1–2% (TC, HDL, TG, FBG, CRP, fT_3 , fT_4 , TSH), 2–3% (LDL), 2–4% (ApoA₁ and ApoB), 4–7% (Lp(a), Tg-Ab, and TPO-Ab).

Brachial artery ultrasonography The vascular studies of the brachial artery were performed non-invasively, as we have previously described (8, 12). High resolution ultrasound was used to measure changes in arterial diameter in response to reactive hyperemia

(with increased flow producing an endothelium-dependent stimulus to vasodilation; FMD) and to glyceryl trinitrate (GTN, an endothelium-independent vasodilator; GTN-induced dilation) (128XP/10 with a 7.0 MHz linear array transducer; Acuson, Mountain View, CA, USA). The intra- and interobserver variability in our laboratory for repeated measurements of artery diameter are 0.09 ± 0.10 and $0.08 \pm 0.13 \text{ mm}$ respectively. The CV for FMD measurements over time is 7.8–9.2%; the power of the study to justify the selected sample size is 83.7%, and the resolving power of the method tested on 'phantom arteries' is $8.6 \text{ } \mu\text{m}$.

The subjects rested in the supine position for 10 min before the first scan and remained supine throughout the study. The target artery (the brachial 2–10 cm above the elbow) was scanned in longitudinal section, and the center of the vessel was identified when the clearest images of anterior and posterior walls of the artery were obtained. The transmit zone was set to the level of the anterior vessel wall. Depth and gain settings were optimized to identify the lumen to vessel wall interface. Images were magnified with the resolution box function leading to a television line width of $\sim 0.05 \text{ mm}$. Machine settings were kept constant during each study.

Flow increase was induced by inflation of a blood pressure tourniquet placed around the forearm distal to the target artery to 300 mmHg. The cuff was released after 5 min and, after cuff deflation, the artery was scanned continuously for 90 s, 15 min was allowed for vessel recovery, sublingual GTN (400- μg spray) was then administered and, 5 min later, the last scan was done. The electrocardiogram was monitored continuously.

Vessel diameter was measured by two observers, unaware of clinical details and the stage of the experiment. The arterial diameter was measured at a fixed distance from an anatomical marker, such as a bifurcation, with ultrasonic calipers. Measurements were taken from the anterior to the posterior 'm' line at end diastole, incident with the R-wave on the electrocardiogram. The mean diameter was calculated from four cardiac cycles. For the hyperemia scan, vessel diameter was measured 45–60 s after cuff release. Diameter changes were derived as percent change relative to the first baseline scan (100%). Baseline blood flow (measured during the first baseline scan) was estimated by multiplying angle-corrected, pulsed Doppler recordings of the flow velocity integral by π and the square of the radius of the artery. Reactive hyperemia was calculated as the maximum flow recorded in the first 15 s after cuff deflation divided by the flow during the resting (baseline) scan.

Exercise intervention All subjects underwent a supervised orientation and thereafter performed exercise on their own. Initially, subjects walked 25–30 min/day, 3–4 days/week, at a relatively low intensity of exercise ($\sim 60\%$ of their individually determined maximal heart rate obtained during the

Table 1 Clinical and biochemical characteristics in control subjects and in patients with subclinical hypothyroidism (sHT) before and 6 months after exercise training intervention.

Variables	Patients with sHT		Control subjects	
	Before exercise (n=30)	After exercise (n=28)	Before exercise (n=27)	After exercise (n=25)
Age (years)	53.0±8	53.5±9	52.0±7	52.5±10
BMI (kg/m ²)	24.7±1.5	24.0±1.3	23.2±1.6	22.8±2.7
Fasting plasma glucose (mg/dl)	86.2±1.1	85.3±1.3	85.1±1.5	86.0±1.9
Mean arterial pressure (mmHg)	102±5	97±7	99±8	98±7
Total cholesterol (mmol/l)	5.33±0.62	4.26±0.68*	4.42±0.49*	4.29±0.52*
LDL (mmol/l)	3.47±0.65	2.55±0.65†	2.38±0.46†	2.22±0.44†
HDL (mmol/l)	1.15±0.29	1.28±0.34	1.33±0.37	1.30±0.35
Triglycerides (mmol/l)	2.87±1.07	1.76±0.94†	1.46±0.71†	1.51±0.68†
Apolipoprotein A1 (g/l)	1.21±0.33	1.20±0.41	1.33±0.36	1.28±0.40
Apolipoprotein B (g/l)	1.22±0.38	1.19±0.32	1.12±0.26	1.09±0.32
C-reactive protein (mg/l)	3.22±0.13	1.63±0.08†	1.71±0.09†	1.65±0.11†
VO ₂ max (ml/kg per min)	25.6±2.8	35.0±3.8†, ‡	30.4±3.3*	39.7±3.1†, ‡
fT ₃ (pmol/l)	5.52±1.60	6.34±1.58	6.27±1.63	6.08±1.46
fT ₄ (pmol/l)	13.77±3.58	14.12±3.12	14.45±2.78	15.14±3.12
TSH (mU/l)	7.62±1.46§	7.59±1.52§	2.95±1.42	2.66±1.29
TPO-Ab (U/ml)	428.5±211.2§	395.7±210.4§	20.6±11.35	24.3±13.1
Tg-Ab (U/ml)	297.8±123.7§	266.3±105.6§	17.4±9.2	16.2±10.3

* $P<0.05$, † $P<0.01$ compared with sHT before exercise training; ‡ $P<0.05$, § $P<0.01$ compared with control group before and after exercise; ‖ $P<0.05$ compared with before exercise.

measurement of maximal oxygen consumption). As their exercise tolerance improved, the intensity and duration of walking were increased to 40–45 min/day, 4–6 days/week, at an intensity of 70–75% of maximal heart rate (30–50% subjects were advised to walk/jog or jog continuously to reach their target heart rate range). The patients were allowed to carry out an additional exercise training at home, but compliance to training session had to be >60% for eligibility.

Maximal oxygen consumption Maximal oxygen consumption (VO₂ max) was assessed with online computer-assisted open-circuit spirometry during incremental treadmill exercise as previously described in detail (13). Heart rate (electrocardiograph) was also measured throughout the protocol.

Statistical methods

Data are reported as the mean±s.d. Data among different groups were compared with ANOVA. Linear regression analyses were used to assess the relation

between the exercise training-induced changes in plasma lipids, glucose, CRP, and FMD. Association between the absolute changes of endothelium-dependent or -independent arterial dilation and the absolute changes of other variables, such as TC, TG, CRP, was assessed by multiple regression analysis. A P value <0.05 was considered significant. Lp(a) concentrations were log transformed before analysis. All analyses were carried out by using the statistical package SPSS 11.5 (Chicago, IL, USA).

Results

The clinical characteristics and biochemical results of the control subjects and subjects with sHT before and 6 months after the exercise training were given in Table 1. At baseline, TC, TG, LDL, CRP, TSH, TPO-Ab, and TG-Ab concentrations were significantly higher in patients with sHT than those in controls ($P<0.05$). In both groups, other parameters, i.e. mean arterial pressure, fT₃, fT₄, did not differ between the two groups

Table 2 The results of brachial artery studies in control subjects and in patients with subclinical hypothyroidism (sHT) before and 6 months after exercise training intervention.

Variables	Patients with sHT		Control subjects	
	Before exercise (n=30)	After exercise (n=28)	Before exercise (n=27)	After exercise (n=25)
Baseline vessel (mm)	3.79±0.58	3.80±0.55	3.82±0.63	3.79±0.54
Baseline flow (ml/min)	82.68±33.43	84.32±35.21	82.54±35.28	83.15±34.72
Reactive hyperemia (%)	438.6±132.4	441.8±127.0	439.1±116.5	442.4±122.8
Flow-mediated dilation (%)	3.87±0.69	5.08±0.73* †	5.98±0.97*	6.13±0.87*
GNT-induced dilation (%)	20.62±1.38	22.17±1.44	20.92±1.50	21.76±1.28

* $P<0.001$ compared with patients with sHT before exercise training; † $P<0.001$ compared with control before and after training.

Figure 1 Changes of FMD before and after exercise training in 28 patients with sHT who completed the exercise training program.

($P > 0.05$). Vascular characteristics of the groups were listed in Table 2. FMD among subjects with sHT was 3.87%, which was significantly lower than that in controls (5.98%; $P < 0.001$). The baseline vessel size (diameter), GNT-induced arterial dilation, and baseline flow were not significantly different between the two groups ($P > 0.05$).

After 6 months of exercise training, there was a remarkable increase in FMD (reaching 5.08%, and Δ FMD 31.3%) and VO_2 max (36.7%) in subjects with sHT ($P < 0.01$). As shown in Fig. 1, most of the patients who completed the exercise training program showed a marked increase in FMD during the course of exercise intervention. Other vascular parameters such as baseline vessel and baseline flow, did not change markedly (Table 2). Some of metabolic control and other parameters improved with exercise training, and significant decreases in TC (20%), LDL (29%), TG (47.6%), CRP (61.5%) were observed over the exercise training period ($P < 0.05$). Other clinical parameters, such as thyroid function, TPO-Ab and Tg-Ab, did not significantly change during the exercise training period in patients with sHT (Table 1). In the control group, only VO_2 max was found to increase significantly during the exercise training ($P < 0.05$), other variables, including FMD and GNT-induced dilation, failed to emerge as significant changes ($P > 0.05$).

To reveal possible causes of exercise training-induced FMD improvement, linear correlation coefficients were calculated between changes in metabolic and inflammatory parameters (TC, TG, LDL, CRP, VO_2 max) and those of vascular reactivity (FMD and GNT-induced arterial dilation). The absolute changes in FMD showed significant correlation with the changes in LDL ($r = -0.596$, $P = 0.001$), TG ($r = -0.532$, $P = 0.004$) and CRP ($r = -0.511$, $P = 0.005$; Figs 2–4). No significant correlations were found between changes in FMD and those of TC and VO_2 max in patients with sHT. There were no significant correlations between changes of FMD and changes of metabolic and inflammatory parameters in control

subjects. Multiple regression analysis showed that changes of LDL, TG, and CRP were found to be significant determinants of changes of FMD in patients with sHT, and this association was not found in healthy women.

Discussion

Endothelial dysfunction is an important early event in atherogenesis (1). Both spatial and temporal correlations between endothelial dysfunction and coronary atherosclerosis have been shown in animal models and in humans (14–16). Impairment of endothelial function in early life could result in abnormal reactions between the vessel wall and platelets, neutrophils, and macrophages, and thus could contribute to the initial stages of atherogenesis (1). Impaired FMD has been observed in patients with coronary artery disease (1), type 2 diabetes mellitus (17), type 1 diabetes mellitus (18), and impaired fasting glucose (IFG) (8), hypertension (19), hypercholesterolemia (20), overt hypothyroidism, and sHT patients (5).

The present study showed that FMD using non-invasive measurement in sedentary patients with sHT decreased significantly at baseline, and multiple regression analysis showed that FMD was associated with TG, LDL, CRP, and TSH (data was not shown). This is in agreement with our previous results (5). After 6 months exercise training program, the impaired FMD in sedentary patients with sHT was significantly improved. This result is in good agreement with other training

Figure 2 Linear regression analyses to evaluate correlation of change in FMD with change in LDL before and after exercise training program in 28 patients with sHT who completed the exercise training program.

Figure 3 Linear regression analyses to evaluate correlation of change in FMD with change in triglyceride before and after exercise training program in 28 patients with sHT who completed the exercise training program.

studies in different patient cohorts (9–12). As far as we know, this is the first report in sHT patients' cohort.

There is no direct evidence of which mechanisms contribute to the functional improvement of the vasculature in patients with sHT in our study. Most authors have discussed the role of increased shear stress, which affects the vascular NO system in many ways (1, 21). Endothelial L-arginine uptake, the substrate of NO production, is increased (22). Further, NO synthase gene expression in endothelial cells is augmented (23). Training could have increased the antioxidative capacity by increased expression of the potent antioxidative extracellular enzyme superoxide dismutase, as shown in animal studies (24). This may partially contribute to the improvement of endothelial function during the exercise training period in patients with sHT.

Endothelial injury has been induced experimentally by hypercholesterolemia, particularly the LDL component (25). In clinical studies using invasive techniques, hypercholesterolemic adults have been shown to have impaired endothelium-dependent responses to a variety of pharmacologic stimuli, including infusions of acetylcholine (26, 27). In type 2 diabetes, FMD was negatively associated with LDL (12). Besides, FMD was inversely related to plasma TG in healthy individuals (28). In the present study, after 6 months of exercise training period, a significant decrease in LDL levels and TG was observed, and their changes are in a significant correlation with the change in FMD. These can also contribute to the improvement of endothelial function in patients with sHT.

CRP has been recently considered as a potential contributor to inflammatory diseases including atherosclerosis as well as a marker of cardiovascular risk (29). More recently, one study showed that low-grade systemic inflammation caused endothelial dysfunction in sHT patients with Hashimoto's thyroiditis (30). In the present study, our results showed that CRP decreased markedly, and the absolute change of CRP was negatively associated with change of FMD during the exercise training course in patients with sHT. Therefore, this may partially explain the improvement of endothelial function during exercise training course in patients with sHT.

In this study, we only found that exercise training can improve endothelial function in patients with sHT, but not in healthy subjects. The mechanisms are not clear completely. The possible explanations are as follows: i) the baseline FMD is significantly lower in patients with sHT than that in healthy women, ii) the endothelial function may be benefit from the improvement of lipid metabolism and inflammation during the exercise training period in sHT patients, iii) a longer period of training may be required to have a detectable improvement of endothelial function.

Consistent with several previous findings in prediabetes (8) and diabetes as well as nondiabetic patients (31), we did not find any association between VO_2 max and endothelial function. The mechanism is not clear. This may be related to the narrow range of differences in VO_2 max among individuals.

Some limitations of the present study should be mentioned. First, we did not measure the plasma insulin level, the relation between insulin or insulin resistance,

Figure 4 Linear regression analyses to evaluate correlation of change in FMD with change in CRP before and after exercise training program in 28 patients with sHT who completed the exercise training program.

and endothelium-dependent arterial dilation before and after exercise training could not be evaluated. Insulin resistance is associated with endothelial dysfunction and cardiovascular risk (32). Secondly, the number of study subjects is relatively small. It is difficult to exclude completely the bias of the results, which should be confirmed in larger studies.

In summary, these results showed that regular aerobic exercise training can improve FMD in patients with sHT, although the mechanism for the improvement of FMD has not been explained completely.

Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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