Technetium-99m sestamibi retention in skeletal muscles, a potential indicator of mitochondrial function and anaerobic threshold in patients with type 2 diabetes

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Abstract In this study, we investigated the retention of technetium-99m sestamibi (MIBI), a radiopharmaceutical that accumulates in mitochondria, in patients with type 2 diabetes. We hypothesized that patients with type 2 diabetes had lower MIBI counts in their legs than non-diabetic volunteers, and that these abnormalities reflected a low anaerobic threshold (AT) during cardiopulmonary exercise testing (CPX). Eight non-diabetic volunteers (Group N) and 11 patients with type 2 diabetes (Group D) underwent CPX. Mitochondrial function was assessed using MIBI imaging of both legs. The MIBI counts in the legs were significantly lower in Group D than in Group N (D vs. N: 74.1 vs. 94.1 counts/pixel, p < 0.05). Similarly, peak oxygen uptake (peak\(\dot{V}_{\text{O}_2}\)) and AT were lower in Group D than in Group N (peak\(\dot{V}_{\text{O}_2}\): 19.8 vs. 26.5 ml/kg/min, p < 0.05; AT: 13.1 vs. 17.2 ml/kg/min, p < 0.05). A strong correlation was observed between MIBI counts and the peak\(\dot{V}_{\text{O}_2}\) and AT (peak\(\dot{V}_{\text{O}_2}\): r = 0.62, p < 0.01; AT: r = 0.76, p < 0.01). After the exclusion of an outlying subject, the correlation between peak\(\dot{V}_{\text{O}_2}\) and MIBI count in the legs was lost (r = 0.28, p = 0.26); however, the AT correlation was maintained (r = 0.59, p = 0.01). Patients with type 2 diabetes had reduced skeletal muscle MIBI counts, indicating reduced mitochondrial function. This abnormality may be linked to a low AT.

Keywords: type 2 diabetes, mitochondrial function, cardiopulmonary exercise testing, anaerobic threshold

Introduction Mitochondrial dysfunction in skeletal muscles is a promising therapeutic target in type 2 diabetes. Recent studies on humans suggest that defects in mitochondrial function play a role in the pathogenesis of insulin resistance in skeletal muscles. Petersen et al. found that insulin resistance in the elderly is related to increases in intramyocellular fatty acid metabolites that may be a result of an age-related reduction in mitochondrial oxidative capacity11. Petersen et al. also showed that insulin resistance in the skeletal muscle of insulin-resistant offspring of patients with type 2 diabetes is due to dysregulation of intramyocellular fatty acid metabolism, which may be caused by an inherited defect in mitochondrial oxidative capacity12. Biopsy studies have revealed that, compared to lean controls, patients with type 2 diabetes have smaller mitochondria with reduced bioenergetic capacity, and that these mitochondrial abnormalities are linked to insulin resistance13. Therefore, assessing muscle mitochondrial function is important for determining the mechanism underlyng insulin resistance and providing the physician with relevant information for clinical decision-making in patients with type 2 diabetes.

Technetium-99m sestamibi (\(^{99m}\text{Tc-MIBI}\))–delayed phase imaging has been widely used to diagnose myocardial ischemia, and the clearance rate of \(^{99m}\text{Tc-MIBI}\) has been reported as proportional to mitochondrial function in animal models and patients with heart disease14. Kawamoto et al. reported decreased retention of \(^{99m}\text{Tc-MIBI}\) in the hearts of patients with heart failure, which is associated with decreased mitochondrial membrane potential15. Several studies have reported that decreased retention of \(^{99m}\text{Tc-MIBI}\) in the heart reflects impaired myocardial con-
tractility due to mitochondrial dysfunction in patients with cardiomyopathy. Moreover, Crane et al. demonstrated that mitochondrial retention of $^{99m}$Tc-MIBI was not organ specific. In animal models, the calcium-induced release of $^{99m}$Tc-MIBI from the mitochondrial fraction collected from hepatic, pulmonary, renal, and skeletal muscle tissues was similar to that observed in the heart. The assessment of mitochondrial function using $^{99m}$Tc-MIBI imaging has also been applied to both animal and human skeletal muscles. However, data on the accumulation of $^{99m}$Tc-MIBI in the skeletal muscle of people with type 2 diabetes are currently lacking.

Cardiopulmonary exercise testing (CPX) provides information regarding the physiological responses of the cardiovascular and respiratory systems to exercise as these systems attempt to meet the metabolic demands of skeletal muscles. The anaerobic threshold (AT), also known as the lactate threshold, is considered an estimator of the onset of metabolic acidosis, caused predominantly by the increased rate in the rise of arterial lactate during incremental exercise. One small study identified a strong relationship between AT and insulin sensitivity, suggesting that insulin resistance via mitochondrial dysfunction leads to a low AT.

Therefore, we hypothesized that patients with type 2 diabetes have a defect in their skeletal muscle mitochondrial accumulation of radiopharmaceuticals, as indicated by the retention of $^{99m}$Tc-MIBI. In addition, this abnormality was hypothesized to be associated with low AT.

**Patients and Methods**

Eight non-diabetic volunteers (Group N) and 11 patients with type 2 diabetes (Group D), who were matched for age, sex, and body mass index, were enrolled in this study (Table 1). All research volunteers underwent a screening medical examination before participating and were free of known cardiovascular diseases. Patients with type 2 diabetes enrolled in this study were referred by their personal physicians to visit our hospital for an exercise prescription and health counseling. Therefore, every patient should have performed daily walking exercise (20-30 min) at home. They were treated with a sulfonylurea, biguanide, or dipeptidyl peptidase-4 inhibitor, and these agents were maintained for at least 8 weeks before any measurements were taken. Non-diabetic volunteers did not have hyperglycemia. This group included one participant with hyperlipidemia, but none had had any major disease. Exclusion criteria included uncontrolled hyperglycemia, uncontrolled hypertension, insulin-dependent diabetes, prior respiratory disease, presence of any cancer, severe neurological disease, and age >80 years. Written consent was obtained from all subjects after they were informed of the study purpose, need for cardiovascular screening, and potential complications of the studies. The research protocol was approved by the Institutional Review Board of Kitano Hospital according to the ethical guidelines of the 1975 Declaration of Helsinki.

The results of $^{99m}$Tc-MIBI imaging and the CPX test were initially analyzed for all participants. On the day of imaging, all participants took their medication as usual and consumed a meal up to 1 hour before each test. The protocol for $^{99m}$Tc-MIBI administration was as follows: a dose of 740 MBq (20 mCi) of $^{99m}$Tc-MIBI was administered intravenously under resting conditions. After injection, planar images were obtained followed by single photon emission computed tomography (SPECT) images 3 hours. All images were acquired in the resting state us-

**Table 1. Characteristics of the study population**

| Variable                     | Non-diabetic volunteer | Patients with diabetes | p value |
|------------------------------|------------------------|------------------------|---------|
|                             | (n=8)                  | (n=11)                 |         |
| Age (years)                  | 57±7                   | 52±7                   | ns      |
| Gender (male/female)         | 5/3                    | 7/4                    | ns      |
| Body mass index (kg/m²)      | 23±2                   | 26±3                   | ns      |
| Systolic blood pressure (mmHg)| 130±15                | 125±11                 | ns      |
| Fasting plasma glucose       | -                      | 154±34                 | -       |
| HbA1c (%)                    | -                      | 6.8±0.9                | -       |
| Duration of diabetes (yr)    | -                      | 7.0±7.1                | -       |
| Drug therapy                 |                        |                        |         |
| No glucose-lowing drugs(n)   | -                      | 2                      | -       |
| Sulfonylurea(n)              | -                      | 6                      | -       |
| Biguanide(n)                 | -                      | 7                      | -       |
| DPP4 inhibitor(n)            | -                      | 8                      | -       |
| $^{99m}$Tc-MIBI counts of heart (counts/pixel) | 102±28              | 95.9±20                | ns      |
| $^{99m}$Tc-MIBI counts of legs (counts/pixel) | 94.1±18              | 74.1±18                | 0.03    |
| Peak VO$_2$ (ml/kg/min)      | 26.5±8                 | 19.8±3                 | 0.02    |
| Anaerobic threshold (ml/kg/min) | 17.2±4              | 13.1±2                 | 0.02    |
| Peak work load (w)           | 163±63                 | 125±25                 | ns      |
ing a dual-head rotating gamma camera (Toshiba, Tokyo, Japan)\textsuperscript{15-17}. Energy discrimination was provided by a 15\% window centered on the 140 keV photopeak. Data processing was performed on a nuclear medicine computer system. The $^{99m}$Tc-MIBI uptake of the heart and leg muscles was then assessed. First, the shapes of the heart\textsuperscript{18} and the upper thigh of the right leg were traced, and the regions of interest (ROIs) of the heart\textsuperscript{18} and leg were established (Fig. 1). Then, the total counts/pixel in the ROI was calculated and adjusted for body surface area.

CPX was performed in the upright position on a bicycle ergometer with an initial workload of 0 W and subsequent increments of 20 W (male) or 15 W (female) every minute until exhaustion was reached. A 12-lead electrocardiography was performed to monitor the heart rate throughout the test using a stress test system (ML-6500, Fukuda Denshi). Oxygen consumption and carbon dioxide production were measured with a metabolic cart (AE810s, Minato). The exercise test variables analyzed in the study included oxygen consumption at AT and peak oxygen uptake (peak$\bar{\text{V}}_{\text{O}_2}$). AT was determined from the gas exchange data using the V-slope method\textsuperscript{19}. At low exercise intensities, muscle cells that are primarily oxidative are recruited; but as intensity increases, cells that rely primarily on glycolytic pathways are recruited, thus increasing the output of lactic acid. The additional acid produced causes an increase in the elimination of carbon dioxide (V$\text{CO}_2$) by buffering the CO$_2$ in the blood. In the V-slope calculation of AT, the best fit line is drawn parallel to the plot of V$\text{CO}_2$ versus $\bar{\text{V}}_{\text{O}_2}$. The point at which the $\bar{\text{V}}_{\text{O}_2}$ versus V$\text{CO}_2$ line departs from the best fit line is the AT\textsuperscript{20}.

All values were expressed as means $\pm$ standard deviations. Differences between the groups were assessed using a t-test or $\chi^2$ test, as appropriate (SPSS 16.0 for Windows, SPSS Inc., Chicago, USA). We then determined Pearson correlation coefficient (PCC) between the $^{99m}$Tc-MIBI counts and the CPX parameters. Multiple linear regression analysis was then performed using a forward stepwise approach to assess the combined effects of the parameters of CPX in predicting the $^{99m}$Tc-MIBI counts. Values of $p < 0.05$ were regarded as significant.

**Results**

The baseline characteristics of the study population are included in Table 1. No differences were observed between the D and N groups with regard to age, gender distribution, or body mass index.

In all 19 subjects, the mean $^{99m}$Tc-MIBI retention count in the heart was 98.4 $\pm$ 23 counts/pixel. The mean $^{99m}$Tc-MIBI retention count in both legs was 82.5 $\pm$ 20 counts/pixel, the mean peak$\bar{\text{V}}_{\text{O}_2}$ was 22.8 $\pm$ 6.4 ml/min/kg, and the mean AT was 14.9 $\pm$ 3.8 ml/min/kg.

The $^{99m}$Tc-MIBI count in the heart did not differ between the two groups (D vs. N: 95.9 vs. 102 counts/pixel, ns). In contrast, the $^{99m}$Tc-MIBI count in the legs was significantly lower in Group D than in Group N (74.1 vs. 82.5 counts/pixel, $p < 0.05$).

![Fig. 1](image-url) The Assessing of $^{99m}$Tc-MIBI uptake of the heart and leg muscles. The shapes of the heart (A) and the upper thigh of the right leg (B) were traced, and the regions of interest (ROIs) of the heart and leg were established.
94.1 counts/pixel, \( p < 0.05 \)). Similarly, peak\( \dot{V}O_2 \) and AT were lower in Group D than in Group N (peak\( \dot{V}O_2 \): 19.8 vs. 26.5 ml/kg/min, \( p < 0.05 \); AT: 13.1 vs. 17.2 ml/kg/min, \( p < 0.05 \)).

The \(^{99m}\)Tc-MIBI counts in the legs did not correlate with fasting plasma glucose levels, HbA1c, or duration of diabetes. However, a strong correlation was observed between \(^{99m}\)Tc-MIBI count in the legs and peak\( \dot{V}O_2 \) and AT (peak\( \dot{V}O_2 \): \( r = 0.62, p < 0.001 \); AT: \( r = 0.76, p < 0.001 \), Fig. 2). After excluding an outlying subject, the correlation between MIBI count in the legs and peak\( \dot{V}O_2 \) was lost (\( r = 0.28, p = 0.26 \)). Nevertheless, the correlation with AT was maintained (\( r = 0.59, p = 0.01 \)). In stepwise multiple linear regression analysis, AT emerged as the only predictor of \(^{99m}\)Tc-MIBI count in the legs when age, gender, and peak\( \dot{V}O_2 \) were entered into the final model (joint \( R = 0.76, p < 0.001 \)).

**Discussion**

The present study demonstrated that patients with type 2 diabetes have lower \(^{99m}\)Tc-MIBI counts in their legs, an indicator of radiopharmaceutical accumulating in the mitochondria, compared to that in non-diabetic volunteers. These abnormalities may reflect a low AT.

There is clinical evidence that type 2 diabetes occurs in conjunction with an acquired decline in the amount and function of mitochondria in skeletal muscles. Phielix et

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**Fig. 2** Regression scatterplots of \(^{99m}\)Tc-MIBI counts in the legs against peak \( \dot{V}O_2 \) (A) and anaerobic threshold (B). ●: non-diabetic volunteers, ▲: patients with type 2 diabetes, r: Pearson correlation coefficient.
al. reported that patients with type 2 diabetes have lower ex vivo intrinsic mitochondrial respiratory capacity, independent of mitochondrial content, than non-diabetic patients. Kelley et al. also reported lower mitochondrial oxidative capacity in muscle biopsy samples from patients with type 2 diabetes than in those from healthy individuals. Our finding supports the results of prior research and provides new insight into the nature of mitochondrial dysfunction in patients with type 2 diabetes.

Impaired exercise capacity is commonly found in patients with type 2 diabetes. Nyholm et al. demonstrated that patients with type 2 diabetes, and possibly their relatives, have a lower peak VO₂ than non-diabetic subjects. Thamer et al. also observed that subjects with a family history of type 2 diabetes had significantly lower maximal oxygen uptake (VO₂max) than control subjects after adjusting for gender, age, BMI, and habitual physical activity. However, the factors associated with reduced exercise performance have not been fully characterized. A limitation in oxygen delivery, such as a cardiac output response abnormality, is one explanation for lower VO₂max in patients with diabetes, but Macanney et al. confirmed that diminished exercise capacity occurs in the absence of any reduction of the cardiac output response in these patients.

In the present study, a significant association was demonstrated between mitochondrial membrane potential in the legs and CPX parameters of exercise capacity. This finding indicates a potential pathogenic relationship between muscle mitochondrial dysfunction and impaired exercise capacity in patients with type 2 diabetes. The findings of Thamer et al. showing that reduced VO₂max precedes skeletal muscle insulin resistance in subjects with a familial predisposition for type 2 diabetes partially support our results.

AT, also known as the lactate threshold, has been widely used for many years as an index for the development of individualized exercise prescriptions in patients with cardiovascular disease. Nevertheless, the physiological mechanisms underlying the increases in muscle lactate levels that occur at the AT remain controversial. One possible explanation is that the imbalance between oxygen delivery and oxidative capacity contributes to the onset of lactic acid production. Hence, in patients with type 2 diabetes, the limited muscle mitochondrial oxidative capacity may accelerate the production of lactic acid during exercise, and this abnormality is linked to a low AT.

Our results extend the clinical applicability of CPX to patients with type 2 diabetes. CPX is a noninvasive technique and requires only 30 minutes for the entire procedure. Importantly, mitochondrial dysfunction is a significant factor in the pathogenesis of type 2 diabetes, and has the potential for reversal with lifestyle intervention. Therefore, to improve prevention policies, allied health professionals who are responsible for assessing and treating patients with type 2 diabetes should be aware of the importance of CPX as a screening tool for mitochondrial dysfunction and, when appropriate, should advocate its implementation.

In the present study, patients were selected if they had no complications or comorbidities (CC) that would interfere with oxygen delivery systems in exercise, such as cardiovascular and respiratory diseases. Therefore, our findings apply only to patients with type 2 diabetes who have normal cardiovascular and respiratory systems; and how this information applies to the more general population of patients with type 2 diabetes that we see in practice remains to be determined.

This study also has several limitations. ⁹⁹mTc-MIBI imaging is now accepted as an indicator of radiopharmaceutical accumulation in mitochondria. However, there is no generally accepted standardized method for measuring mitochondrial function. In addition, we adjusted the ⁹⁹mTc-MIBI counts by body surface area. Adjustment based on lean body mass may have been more helpful in clarifying the difference between the amount and function of mitochondria. We did not obtain relevant clinical data for insulin resistance such as fasting plasma insulin, which is likely to affect mitochondrial function. Furthermore, we did not obtain specific data concerning habitual activity or exercise levels in the subjects. The impact of medication on mitochondrial function is also important. In the present study, we did not identify an association between any drugs and mitochondrial function, but this issue should be considered in a more detailed investigation. Finally, the number of patients was small. Only a large-scale clinical trial can provide definitive evidence to support this important clinical topic.

Conclusions

In summary, type 2 patients with diabetes have lower skeletal muscle ⁹⁹mTc-MIBI counts, which is an indicator of mitochondrial function. This abnormality may be linked to low AT, although more studies with larger sample sizes are necessary to validate these results.

Disclosure

This manuscript has been read and approved by all authors. This paper is unique, is not under consideration by any other publication, and has not been published elsewhere. The authors and peer reviewers of this paper report no conflicts of interest. The authors confirm that they have permission to reproduce any copyrighted material. This study was supported by grants from Tazuke Kofukai Medical Research Institute.

Human rights statement and informed consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human
experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions. Informed consent or substitution for it was obtained from all patients and volunteers included in the study.

**Conflict of Interests**

The authors declare that there is no conflict of interests regarding the publication of this article.

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