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Mitochondrial capacity is affected by glycemic status in young untrained women with type 1 diabetes but is not impaired relative to healthy untrained women

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Item F, Heinz-Schweizer S, Wyss M, Fontana P, Lehmann R, Henning A, Weber M, Boesiger P, Bottelier U, Toigo M. Mitochondrial capacity is affected by glycemic status in young untrained women with type 1 diabetes but is not impaired relative to healthy untrained women. Am J Physiol Regul Integr Comp Physiol 301: R60–R66, 2011. First published April 13, 2011; doi:10.1152/ajpregu.00747.2010.—In this study, we examined whether glycemic status influences aerobic function in women with type 1 diabetes and whether aerobic function is reduced relative to healthy women. To this end, we compared several factors determining aerobic function of 29 young sedentary asymptomatic women (CON) with 9 women of similar age and activity level with type 1 diabetes [DIA, HbA1c range = 6.9–8.2%]. Calf muscle mitochondrial capacity was estimated by 31P-magnetic resonance spectroscopy. Capillarization and muscle fiber oxidative enzyme activity were assessed from vastus lateralis and soleus muscle biopsies. Oxygen uptake and cardiac output were evaluated by ergospirometry and N2O/SF6 rebreathing. Calf muscle mitochondrial capacity was not different between CON and DIA, as indicated by the identical calculated maximal rates of oxidative ATP synthesis [0.0307 (0.0070) vs. 0.0309 (0.0058) \( \mu \text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1} \), \( P = 0.930 \)]. Notably, HbA1c was negatively correlated with mitochondrial capacity in DIA (\( R^2 = 0.475, P = 0.040 \)). Although HbA1c was negatively correlated with cardiac output (\( R^2 = 0.742, P = 0.013 \)) in DIA, there was no difference between CON and DIA in maximal oxygen consumption [2.17 (0.34) vs. 2.21 (0.32) \( \text{L} \cdot \text{min}^{-1} \), \( P = 0.764 \)], cardiac output [12.1 (1.9) vs. 12.3 (1.8) \( \text{L} \cdot \text{min}^{-1} \), \( P = 0.783 \)], and endurance capacity [532 (212) vs. 471 (119) s, \( P = 0.475 \)]. There was also no difference between the two groups either in the oxidative enzyme activity or capillary-to-fiber ratio. We conclude that mitochondrial capacity depends on HbA1c in untrained women with type 1 diabetes but is not reduced relative to untrained healthy women.

AEROBIC FUNCTION DEPENDS on the transport of oxygen from ambient air to the mitochondrial respiratory chain and relies on a coordinated action between several processes (i.e., ventilation, blood flow, diffusion), which depends on distinct structures (i.e., lung, heart, capillaries, muscle fibers, mitochondria).

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Maximal oxygen uptake is determined by convective (perfusion) and diffusive oxygen transport capacity (30). Whereas convective oxygen transport capacity (i.e., oxygen delivery) is given by the product of arterial oxygen content and cardiac output, diffusive oxygen transport capacity is determined by arterial-mixed venous oxygen difference, which, in turn, relies on capillary supply and mitochondrial capacity (32). Aerobic function is reduced in several chronic diseases, e.g., chronic heart failure (11), chronic obstructive pulmonary disease (19), and possibly in type 2 diabetes (29).

However, the available data on the relationship between aerobic function and type 1 diabetes are contradictory. Some authors report decreased peak oxygen uptake and/or reduced submaximal cardiac output (15, 24, 27), yet, other investigators showed that peak oxygen uptake is not affected in patients with type 1 diabetes (13, 17, 37). However, none of these authors have concurrently investigated both skeletal muscle structure and in vivo (mitochondrial) function and combined these data with the functional assessment of peak oxygen uptake and peak cardiac output. Using 31P-magnetic resonance spectroscopy (MRS), Crowther et al. (8) found that mitochondrial capacity (i.e., the calculated maximal rate of oxidative ATP synthesis) is reduced in male patients with type 1 diabetes compared with healthy controls, and they suggested that reduced muscle mitochondrial capacity in conjunction with increased glycolytic flux represents a metabolic shift common to chronic metabolic diseases (obesity, type 1 and 2 diabetes). However, Holloszy (18) recently estimated that even if mitochondrial capacity were reduced, skeletal muscle still would contain sufficient mitochondria to allow a ~150-fold increase in oxygen uptake per kilogram of muscle under exercise conditions. Therefore, it is questionable whether and to what extent reduced mitochondrial capacity may limit aerobic function.

Taken together, it is currently not well understood 1) whether skeletal muscle structure and in vivo metabolic function are abnormal in patients with type 1 diabetes at all, 2) whether possible impairments in these “peripheral” factors coincide with possible impairments in maximal oxygen uptake, 3) whether and to what extent a reduction in maximal cardiac output also contributes to the decrease in maximal oxygen uptake, and 4) whether aerobic function is reduced in patients with type 1 diabetes.
uptake, and 4) whether glycemic status influences aerobic function. In this study we tested whether aerobic function and its determinants are reduced in young women with type 1 diabetes relative to healthy women of similar age and physical activity level. To this end, we compared maximal oxygen uptake, maximal cardiac output, calculated arterio-venous oxygen difference, $^{31}$P-MRS-derived calculated maximal rates of calf muscle oxidative ATP synthesis, vastus lateralis, and soleus muscle fiber metabolic phenotype and capillarization between the two groups. In addition, we assessed ventilatory threshold and endurance capacity to investigate differences in submaximal exercise capacity between the two groups.

We hypothesized that if peak stroke volume, cardiac output, mitochondrial capacity, capillarization, and oxidative enzymes were significantly reduced in women with type 1 diabetes relative to healthy controls, we should find a corresponding decrease in maximal oxygen uptake. Moreover, we hypothesized that if the possible impairments were confined to mitochondrial capacity, capillarization, and oxidative enzymes only (i.e., without concurrent reduction in maximum cardiac output), we still might observe a reduction in maximal oxygen uptake, and consequently a decreased calculated peak arterio-venous oxygen difference.

MATERIALS AND METHODS

Participants

We recruited 29 young sedentary asymptomatic women (CON) and 9 women of similar age and activity level with type 1 diabetes (DIA). Mean age was 24.1 (SD 4.2) years for CON and 26.9 (5.2) years for DIA without significant difference. The participants’ anthropometric data are presented in Table 1. Women with type 1 diabetes had no diabetic complications or coexisting cardiovascular diseases and were classified as C-peptide negative (<0.5 μU/ml). The duration of diabetes was 13.1 (6.6) years. Relative (per kg body mass) mean total daily insulin dose was 0.6 (0.3) units/kg. Glycosylated hemoglobin A1c (HbA1c) was 5.3 (0.2) (range 5.0–5.7%) and 7.6 (0.4) % (range 6.9–8.2%) for CON and DIA, respectively, and different between groups ($P<0.001$). Participants in the CON group showed no sign of either impaired glucose tolerance [fasting glucose concentration: 4.49 (0.26) mmol/l, blood glucose concentration 2 h post glucose load: 4.92 (1.19) mmol/l (36)] or calculated insulin resistance [homeostasis model assessment (HOMA-IR; Ref 20): 1.4 (0.4)]. All participants were untrained (less than 1 h of physical activity per week). After completing a routine health questionnaire the participants were informed about the applied procedures and the associated risks. Informed written consent was obtained from all participants. The experimental protocol was approved by the ethics committee of the canton of Zurich, and the study was performed in accordance with the ethical standards laid down in the Declaration of Helsinki for human experimentation.

Table 1. Body composition of study participants

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>DIA</th>
</tr>
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<tbody>
<tr>
<td>Body mass, kg</td>
<td>61.4 (6.5)</td>
<td>69.4 (8.6)†</td>
</tr>
<tr>
<td>Body height, m</td>
<td>1.68 (0.06)</td>
<td>1.68 (0.06)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>21.8 (2.1)</td>
<td>24.6 (3.0)†</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>31.6 (5.4)</td>
<td>35.0 (4.8)</td>
</tr>
<tr>
<td>Body fat mass, kg</td>
<td>19.0 (4.5)</td>
<td>23.9 (5.6)*</td>
</tr>
<tr>
<td>Lean mass, kg</td>
<td>40.6 (4.1)</td>
<td>43.7 (4.1)</td>
</tr>
<tr>
<td>Abdominal fat mass, kg</td>
<td>1.35 (0.54)</td>
<td>1.78 (0.71)</td>
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<tr>
<td>Abdominal lean mass, kg</td>
<td>2.46 (0.29)</td>
<td>2.70 (0.26)*</td>
</tr>
<tr>
<td>Gluteofemoral fat mass, kg</td>
<td>4.68 (0.98)</td>
<td>5.83 (1.30)*</td>
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<tr>
<td>Gluteofemoral lean mass, kg</td>
<td>5.53 (0.58)</td>
<td>5.92 (0.78)</td>
</tr>
<tr>
<td>Leg fat mass, kg</td>
<td>7.97 (1.68)</td>
<td>10.32 (2.47)*</td>
</tr>
<tr>
<td>Leg lean mass, kg</td>
<td>14.10 (1.60)</td>
<td>15.44 (1.83)*</td>
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<tr>
<td>Thigh fat mass, kg</td>
<td>6.21 (1.26)</td>
<td>8.18 (2.03)*</td>
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<td>Thigh lean mass, kg</td>
<td>10.62 (1.23)</td>
<td>11.70 (1.41)*</td>
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<td>1.67 (0.34)</td>
<td>2.15 (0.53)*</td>
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<tr>
<td>Lower leg lean mass, kg</td>
<td>3.39 (0.48)</td>
<td>3.74 (0.55)</td>
</tr>
</tbody>
</table>

Data are expressed as means (SD) for 29 healthy women (CON) and 9 women with type 1 diabetes (DIA). *$P<0.05$, †$P<0.01$.  

Magnetic Resonance Spectroscopy and Imaging Measurements

$^{31}$P-MRS scans were acquired at rest and during isometric muscle contraction using a 3 T whole body Philips Achieva Scanner (Philips Healthcare, Best, The Netherlands) with a transmit/receive surface coil (diameter: 0.14 m) tuned to 51.8 MHz. The MR-compatible ergometer setup was composed of a dynamometer with an integrated strain gauge (Sensory-Motor Lab, ETH Zurich, Zurich, Switzerland) for force measurements and a real-time visual feedback system for providing the participants with information on the level of exerted force relative to maximal voluntary force. $^{31}$P-MRS spectra of the triceps surae muscles were acquired using a pulse-acquire technique and an adiabatic hyperbolic secant excitation pulse. The test protocol consisted of isometric plantar flexion at 85% of the maximal force acting on the pedal (MVFP) for 30 s. Sequence parameters included repetition time = 1.5 s, 3 signal averages per time point, 2,048 sample points, 5 dummy scans; 20 spectra were recorded before the exercise for saturation correction; total number of spectra: 84. Recovery spectra were measured after cessation of the isometric contraction for 324 s. The reasons for this test protocol were that 1) pH remains close to 7.0, which is mandatory to derive valid phosphocreatine (PCr) recovery rates (4), 2) at 85% MVFP motor unit recruitment of large muscles is complete (1, 9), and 3) the obtained mean decrease in PCr concentration is sufficiently high to calculate PCr recovery rate ($k_{rc}$) (22).

Processing

Free-induction decays were zero-filled, line-broadened, Fourier-transformed into spectra, and fitted using *fitdfft* (35). Inorganic phosphate (Pi) and PCr concentrations were calculated using ATP as an internal reference standard, assuming a concentration of 8.2 mM (4). The saturation factor in the dynamic spectra was determined by the ratio of the relaxed spectra to the partially saturated spectra acquired prior to the plantar flexion exercise. We calculated $k_{rc}$, which is an indirect marker for the maximal rate of oxidative ATP synthesis (31), using a monoexponential fitting procedure. Intracellular pH values were calculated from the chemical shift of P according to the modified Henderson-Hasselbach equation (26).

Skeletal Muscle Biopsy Analyses

Sample preparation. We obtained percutaneous biopsies from the middle region of the nondominant soleus and vastus lateralis muscles, using a ProMag Ultra device and 14 gauge needles (Angiotech Pharmaceuticals, Gainesville, FL). Muscle tissue was mounted in an embedding medium (Tissue-Tek, Sakura, Zoeterwoude, The Netherlands), snap frozen in isopentane cooled to −160°C with liquid nitrogen. We stained the serial cryocut cross sections using the myofibrillar ATPase (mATPase) method according to Guth and Sama (16) with minor modifications (23). Muscle fibers were classified according to their myosin heavy chain (MYH) isoform into MYH-1 and MYH-2. For the analysis of oxidative enzyme activity, we incubated consecutive sections in media containing cytochrome c oxidase. The monoclonal mouse anti-human CD31 endothelial cell antibody (1:600 dilution; DAKO, Carpinteria, Canada) was used as a marker for muscle capillaries, and capillary-to-fiber ratio was calculated by dividing the number of CD31-positive cells by the number of...
muscle fibers. For all histochemical and immunohistochemical analyses, we used the National Institutes of Health (NIH) Image J software (1.41o; NIH, Bethesda, MD). Cytochrome c activity was determined from the measured mean optical density pixel values of the muscle fibers normalized to the background pixel values on the same section, and reported in arbitrary numbers. For all fiber analyses, only fibers fully encircled by adjacent fibers were evaluated, and measurements were made for at least 50 of each of the main fiber types (i.e., MYH-1 and MYH-2). Previous studies investigating the skeletal muscle fiber sample size required for a reliable, valid representation of an individual’s average fiber area and capillary-to-fiber ratio, showed that 50 fiber measurements per individual for type 1 and 2 fibers and capillary contacts are sufficient to characterize type 1 and 2 fiber areas and capillary-to-fiber ratio of an individual (21, 28).

Cardiac Output and Oxygen Consumption Measurements

We used Innocor (Innovision, Odense, Denmark) to estimate cardiac output by inert gas rebreathing and oxygen consumption by breath-by-breath ergospirometry during a graded cycling exercise test (GXT), as previously described (12). Participants started cycling at 50 W, and power was increased by 25 W every 2 min until volitional exhaustion. Arterio-venous oxygen difference was calculated by dividing oxygen consumption by cardiac output. Ventilatory threshold was determined as the power corresponding with a disproportionate increase in minute ventilation during the GXT.

Endurance Capacity

On a separate day, we used a constant-load cycling exercise test (CLT) to assess time to exhaustion at submaximal power as an indicator of endurance capacity. Power of the initial warm-up stage was 40% maximal power of the first GXT. After 1 min, we increased power to 60% peak power (for 2 min) and then to 85% maximal power. 85% maximal power was sustained until volitional exhaustion, i.e., the point in time at which the participants stopped pedaling or were no longer able to maintain pedal rate within the required limits.

Dual-Energy X-Ray Absorptiometry

We performed dual-energy X-ray absorptiometry (DXA) measurements using Lunar iDXA (GE Healthcare, Madison, WI).

Blood Analyses

Plasma glucose concentrations were determined by an automated hexokinase method (HK Unit-Kit III, Roche, Basel, Switzerland), and serum insulin concentrations were determined by radioimmunoassay (Insulin ct-kit, Cisbio Bioassays, Bagnols-sur-Cèze, France). HbA1c was immunochemically determined with a DCA 2000 device (Bayer, Leverkusen, Germany). Furthermore, CON participants performed a standard 75-g oral glucose tolerance test in the morning after an overnight (12 h) fast.

Statistical Analyses

Data are presented as means and SD. Group differences between CON and DIA were tested for statistical significance by t-tests for independent samples, and equality of variances was checked using Levene’s test. The level of significance was set to $P < 0.05$. For all statistical analyses, SPSS 16.0 statistical software (SPSS, Chicago, IL) was used.

RESULTS

Calf Muscle Mitochondrial Capacity

As indicated by the identical postexercise $k_{PCr}$ for CON and DIA [0.0307 (0.0070) and 0.0309 (0.0058) s$^{-1}$, respectively], calculated from the PCr recovery over time (Fig. 1A), calf muscle mitochondrial capacity was not different ($P = 0.930$) between the two groups. pH did not affect PCr recovery, as shown by the independency of $k_{PCr}$ from pH (Fig. 1B). For both, CON and DIA, the end-exercise pH values were close to 7.0 and not different from the values at rest [CON: 7.03 (0.06) vs. 7.03 (0.02), $P = 0.846$; DIA: 7.02 (0.10) vs. 7.03 (0.01), $P = 0.707$]. Between groups, there were no differences in either resting ($P = 0.262$) or end-exercise pH ($P = 0.845$, Fig. 1C). The resting P$_{i}$ concentrations [4.87 (0.75) vs. 4.67 (0.57) mM, $P = 0.467$] and resting PCr concentrations ($P = 0.669$, Fig. 1A) were similar between CON and DIA. Force increased during the initial ~4 s and remained steady during the following ~26 s of isometric plantar flexion (Fig. 1D).

Skeletal Muscle Fiber Properties

For both, vastus lateralis and soleus muscles, no differences in capillary-to-fiber ratio and MYH-1 fiber percentage were found between CON and DIA (Fig. 2, A and B). The oxidative fiber phenotype was not different between CON and DIA, as indicated by the similar cytochrome c oxidase activity in MYH-1 and MYH-2 fibers (Fig. 2C). In general, muscle fiber diameters tended to be smaller for CON relative to DIA (Fig. 2D). However, only the vastus lateralis muscle MYH-2 mean fiber diameter was significantly ($P = 0.006$) smaller in CON than in DIA (Fig. 2D).

Oxygen Consumption, Cardiac Output, and Arterio-Venous Oxygen Difference

Oxygen consumption, cardiac output, and calculated arteriovenous oxygen difference were not different between CON and DIA at maximal and submaximal power during a GXT (Fig. 3).

Exercise Performance, Ventilatory Threshold, and Endurance Capacity

We found no difference in maximal power output [163 (26) vs. 171 (25) W, $P = 0.486$] and ventilatory threshold [124 (18) vs. 125 (17) W, $P = 0.942$] between CON and DIA during a GXT. Furthermore, cycling endurance capacity was not different between the two groups, as indicated by the similar cycling time to exhaustion [CON: 532 (212) s, DIA: 471 (119) s, $P = 0.475$] during a CLT at submaximal power.

Effect of Glycemic Control on Mitochondrial Capacity and Cardiac Output

HbA1c was negatively correlated with $k_{PCr}$ ($R^2 = 0.475$, $P = 0.040$, Fig. 4A) and peak cardiac output ($R^2 = 0.742$, $P = 0.013$; Fig. 4B) in women with type 1 diabetes.

Body Composition

Both total and segmental lean and fat masses were lower in CON relative to DIA, while % body fat was the same in both groups (Table 1).

DISCUSSION

The aim of the present study was to determine whether aerobic function is reduced in women with type 1 diabetes and whether their glycemic status influences mitochondrial capacity. We found that in women with type 1 diabetes, glycemic status affected mitochondrial capacity and cardiac output but
that maximal oxygen uptake, maximal cardiac output, calculated arterio-venous oxygen difference, in vivo skeletal muscle mitochondrial capacity, muscle fiber metabolic phenotype, and capillarization were not different between women with type 1 diabetes and healthy women of similar age and physical activity level. Analogous to maximal aerobic function, we found that ventilatory threshold and endurance capacity were similar between the two groups. Our results indicate that glycemic status affects mitochondrial capacity in women with type 1 diabetes, but that neither maximal nor submaximal aerobic function is reduced in young women with type 1 diabetes relative to healthy controls.

To obtain maximal rates of oxidative ATP synthesis, we applied a test protocol that decreased skeletal muscle PCr concentration but left intracellular pH close to 7.0 (Fig. 1), the latter being crucial to derive valid PCr recovery rates (4). Our finding that the calculated maximal rate of oxidative ATP synthesis (mitochondrial capacity) was similar between women with type 1 diabetes compared with healthy controls (Fig. 1) conflicts with that of Crowther et al. (8), who reported slower PCr recovery rates in men with type 1 diabetes relative to age-matched controls. Three reasons could possibly help to explain the divergent findings. A first reason might be that Crowther et al. (8) determined mitochondrial capacity from isometric dorsiflexions, while we used isometric plantar flexions. Thus, differences in oxidative metabolism between dorsiflexion and plantar flexion muscles might lead to divergent findings. But given the fact that MYH-1 fiber type proportion of tibialis anterior muscle lies between that of soleus and gastrocnemius muscles (33), it seems unlikely that differences in fiber type distribution represent a reason for discrepancy. A second possible reason might be that in the study by Crowther et al. (8), the participants were not able to sustain the target force of 70% MVFP for 30 s (Fig. 2A in (8)). Therefore, the drop in pH might have varied between participants, affecting the calculation of PCr recovery rates. We avoided this possible drawback by providing the participants with a real-time visual feedback on Fp relative to MVFP. This way, all of our study participants sustained the target force of 85% MVFP for 30 s (Fig. 1D). Finally, the discrepancy between our results and those of Crowther et al. (8) might originate from sex-specific differences in the calculated maximal rate of oxidative ATP synthesis.

Our findings that cytochrome c oxidase activity, capillary-to-fiber ratio, and MYH-1 fiber proportion of vastus lateralis and soleus muscles were not different between the two groups support our result that in vivo mitochondrial capacity was not impaired in women with type 1 diabetes and lend further credence to the notion that the oxidative metabolism is not impaired in type 1 diabetes. In line with our results, other
authors have found no difference in vastus lateralis muscle succinate dehydrogenase activity (13), skeletal muscle capillary-to-fiber ratio, and fiber-type distribution in vastus lateralis muscle (38) between patients with type 1 diabetes and healthy controls. However, there are also reports showing that MYH-1 fiber proportion in women and men with type 1 diabetes (13) is reduced. Besides muscle fiber metabolism, mitochondrial capacity also depends on muscle capillarization. In fact, the size of the capillary network determines mean red blood cell transit time and surface area for gas exchange and may also influence the exchange of substrates and metabolites and removal of heat (34). However, capillary density relates more to submaximal (e.g., endurance capacity and ventilatory threshold) than to maximal measures (32). In this study, neither capillarization nor endurance capacity or ventilatory threshold was reduced in women with type 1 diabetes relative to healthy controls, indicating that submaximal aerobic function was not impaired in women with type 1 diabetes.

It has been unclear up to now, whether maximal oxygen uptake and exercise performance (i.e., maximal power output during a GXT) are reduced in type 1 diabetes. While some authors reported that adolescents with type 1 diabetes display both reduced oxygen uptake (15, 24) and peak power (24), other investigators showed that these measures are unchanged in adults with type 1 diabetes.

Fig. 2. Capillary-to-fiber ratio (A), myosin heavy chain isoform type 1 (MYH-1) fiber percentage (B), cytochrome c (cyt c) oxidase activity (C), and fiber diameter (D) of soleus (SOL) and vastus lateralis (VL) muscle fibers. Bars and error bars represent mean values and SD, respectively. Only data sets meeting the described quality criteria were included: SOL muscle MYH-1 fiber percentage: 9 CON and 5 DIA; vastus lateralis muscle MYH-1 fiber percentage: 13 CON, and 6 DIA; cyt c oxidase activity and fiber diameter: 14 CON and 6 DIA; soleus muscle capillary-to-fiber ratio: 15 CON and 5 DIA; VL capillary-to-fiber ratio: 14 CON and 6 DIA]. AU, arbitrary units; MYH-2, myosin heavy chain isoform type 2. *P < 0.05.

Fig. 3. Oxygen consumption (A), cardiac output (B), and calculated arterio-venous oxygen difference (C) at submaximal and maximal power during a graded cycling exercise test (GXT) to exhaustion. Bars and error bars represent mean values and standard deviations, respectively, for 29 CON women and 7 DIA women. Two individuals in the DIA group did not agree to participate in a GXT.
Here, we showed that neither maximal oxygen uptake (Fig. 3A) nor maximal power during a GXT was different between patients with type 1 diabetes and healthy controls. Furthermore, the similar maximal oxygen uptake between the two groups relied on similar perfusive (cardiac output, Fig. 3B) and diffusive (calculated arterio-venous oxygen difference, Fig. 3C) oxygen transport capacities, indicating that submaximal and maximal oxygen uptake, delivery, and extraction are not reduced in women with type 1 diabetes.

It is well established that reduced insulin sensitivity is a prominent feature of type 2 diabetes. However, reduced insulin sensitivity can also be found in individuals with type 1 diabetes (10, 17). We did not assess insulin sensitivity in this study and thus cannot quantify to which degree the type 1 diabetes participants were insulin resistant. Nevertheless, according to evidence indicating that type 1 diabetes patients are insulin resistant to some degree, and assuming that the type 1 individuals who participated in this study were not different in this regard, our data might be interpreted to support the suggestion of other scientists (18) that insulin resistance is not the consequence of reduced mitochondrial capacity. Our finding that skeletal muscle mitochondrial capacity was not impaired in women with type 1 diabetes despite higher total and segmental fat masses (Tab. 1) supports this speculation, i.e., that reduced mitochondrial capacity is not instrumental to insulin resistance, especially in light of the negative correlation between both abdominal (7) and thigh fat (14) and insulin sensitivity.

HbA1c levels above 7% are indicative of poor glycemic control in type 1 diabetes (2). In this regard, it has recently been shown that endurance athletes with type 1 diabetes and high (>7%) HbA1c have lower peak oxygen consumption, peak stroke volume, and peak cardiac output relative to athletes with low (<7%) HbA1c (5), and that HbA1c negatively correlates with peak stroke volume. Although we did not find a reduced mitochondrial capacity and aerobic function in women with type 1 diabetes with a mean HbA1c of 7.6% (range 6.9–8.2%) relative to healthy female participants, linear regression revealed that women with type 1 diabetes, exhibiting higher HbA1c have a lower $k_{PCr}$ and peak cardiac output (Fig. 4). On the one hand, the level of hyperglycemia might thus affect the function of the heart. In fact, it has been shown in humans that the increase in glycemic control due to insulin pump therapy positively correlated with the increase in systolic function of the left ventricle (3). On the other hand, hyperglycemia might affect mitochondrial function by increasing the input of reducing equivalents into the mitochondrial electron transport chain, which leads to an increase in the production of reactive oxygen species (ROS) (6, 25). Furthermore, in vitro data revealed that extended exposure to high glucose conditions leads to increased ROS production and a concomitant change in mitochondrial morphology (39). Taken together, our data might be interpreted to support the notion that hyperglycemia impairs mitochondrial capacity and cardiac function, but that in patients with type 1 diabetes, only at higher HbA1c levels (>7.6%) does the impairment become significant relative to untrained healthy women.

We do acknowledge some limitations with the current investigation. For example, we calculated arterio-venous oxygen difference from the estimated oxygen uptake and cardiac output according to the Fick principle, instead of calculating arterio-venous oxygen difference and oxygen extraction from measured arterial and venous oxygen content and arterial oxygen concentration. A further limitation is that we did not directly estimate insulin sensitivity from euglycemic hyperinsulinemic clamps nor hepatic glucose production. However, the primary aim of this study was to determine the relationship between type 1 diabetes and aerobic function, irrespective of the degree of insulin resistance. We thus feel that our approach of combining noninvasive inert gas rebreathing with gas exchange measurements (to assess maximal oxygen uptake and cardiac output), to subsequently calculate stroke volume and arterio-venous oxygen difference according to the Fick principle, and to relate these systemic measures with in vivo calculated maximal mitochondrial ATP synthesis rate and oxidative markers from muscle biopsies, provides novel information about the relationship between aerobic function and its underlying components in type 1 diabetes.

**Perspectives and Significance**

The available data concerning the relationship between aerobic function and type 1 diabetes are contradicting. Furthermore, there are no studies that have concurrently investigated skeletal muscle structure as well as in vivo metabolic function and their relationship to maximal oxygen uptake and cardiac output. In the present study, we used an integrative approach to concurrently investigate several elements determining aerobic function. The novelty of this study is that in young untrained women with type 1 diabetes and a mean HbA1c of 7.6% (range 6.9–8.2%), glycemic status affected mitochondrial capacity and peak cardiac output, but that neither maximal nor submaximal aerobic function was reduced relative to healthy women of similar age and activity level. These findings point toward an important role for glycemic status in influencing aerobic func-
tion. Therefore, exercise effects in type 1 diabetes should be investigated as a function of glycemic status. Furthermore, to improve aerobic function, patients with type 1 diabetes may be advised to improve their individual glycemic status before or while engaging in a regular exercise training program to maximize aerobic function.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES