Role of Cardiorespiratory Fitness and Mitochondrial Oxidative Capacity in Reduced Walk Speed of Older Adults with Diabetes

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ABSTRACT

Cardiorespiratory fitness and mitochondrial oxidative capacity are associated with reduced walking speed in older adults. The impact of cardiorespiratory fitness and mitochondrial oxidative capacity on walking speed in older adults with diabetes has not been clearly defined. We examined differences in cardiorespiratory fitness and skeletal muscle mitochondrial oxidative capacity between older adults with and without diabetes as well as determine their relative contribution to slower walking speed in older adults with diabetes. Participants with diabetes (n=159) had lower cardiorespiratory fitness and mitochondrial respiration in permeabilized fiber bundles when compared to those without diabetes (n=717), following adjustments for covariates including BMI, chronic comorbid health conditions, and physical activity. 4-m and 400-m walking speeds were slower in those with diabetes. Mitochondrial oxidative capacity alone or combined with cardiorespiratory fitness mediated ~20-70% of the difference in walk speed between older adults with and without diabetes. Additional adjustments with BMI and co-morbidities further explained the group differences in walk speed. Cardiorespiratory fitness and skeletal muscle mitochondrial oxidative capacity contribute to slower walking speeds in older adults with diabetes.

ARTICLE HIGHLIGHTS

- The contributors to slower walking speed in older adults with diabetes remain unclear.
- This study was conducted to answer the question: How does mitochondrial oxidative capacity and cardiorespiratory fitness impact walking speed in older adults with diabetes?
- We found that mitochondrial oxidative capacity, cardiorespiratory fitness and walking speed were lower in older adults with diabetes compared to those without diabetes.
- In addition, mitochondrial oxidative capacity and cardiorespiratory fitness contributed to slower walking speed in those with diabetes.

Introduction

The US population is rapidly aging, and in 2022 the percentage of Americans aged 65 and older diagnosed with diabetes remains high at 30% [1]. This represents a healthcare challenge as older adults with diabetes have a greater risk of cardiovascular disease and mobility disability [2, 3]. The contributors to impaired mobility in older individuals with diabetes include the presence of co-morbidity, increased body mass index (BMI) [4], and lower muscle strength and quality [5-7]. However, the relationship between diabetes and loss of mobility measured by 4-m and 400-m remains only partially explained by muscle variables such as quality and strength[5]. Skeletal muscle mitochondrial oxidative capacity is an integral contributor to cardiorespiratory fitness, along with the ability of the cardiopulmonary system to supply oxygen to contracting muscles. Our group and others reported that cardiorespiratory fitness and skeletal muscle mitochondrial oxidative capacity are significantly associated and each linked to reduced walking speed in aging, which is a predictor of mobility disability [8-10]. For example, mitochondrial oxidative capacity assessed using ^{31}P-MRS was highly correlated with walking speed and partially explained age-related poorer performance in short (6-m) and long (400-m) walking tasks [10]. Several studies have found a close relationship between cardiorespiratory fitness and walking speed in older adults [11], which suggests that the decline in aerobic capacity also contributes to slower walking speed and mobility disability with age.

Muscle biopsies from individuals with diabetes have been shown to have lower oxidative phosphorylation (OXPHOS) capacity compared with muscle from healthy controls [12-14]. However, some studies [15, 16], but not all [12, 13, 17], indicate no differences between patients with diabetes and BMI-matched controls, indicating that obesity *per se* may underlie the lower muscle oxidative capacity. Indeed, other studies in people without diabetes have shown that BMI

is strongly related to muscle oxidative capacity [18]. In addition, reports assessing mitochondrial oxidative capacity in older adults with diabetes tend to have small sample size (n= ~8-12) and lack adjustment for critical confounding variables including physical activity and adiposity [13, 14, 17], and there are few reports focusing on older adults with diabetes specifically. Whether the presence of diabetes in a population of older adults further exacerbates declines in mitochondrial oxidative capacity and cardiorespiratory fitness remains unclear. Lower fitness is a predictor of insulin resistance and diabetes [19] and prevalence of diabetes is greater in individuals with low fitness [20]. Skeletal muscle mitochondrial oxidative capacity is an integral contributor to cardiorespiratory fitness, along with pulmonary function (ventilation, alveolar-capillary gas exchange), cardiac stroke volume, oxygen transport and delivery (Hgb concentration, arteries and arterioles, capillary density, diffusion capacity system) to supply oxygen to contracting muscles. While each of these factors could contribute to walking speed, our group and others reported that cardiorespiratory fitness and skeletal muscle mitochondrial oxidative capacity are significantly associated, and each linked to reduced walking speed in aging, which is a predictor of mobility disability [9, 11]. It is unknown whether diabetes associated changes in cardiorespiratory fitness and/or muscle energetics explain slower walking speed seen in older adults with diabetes, independent of other covariates.

In this analysis, we leveraged data from the Study of Muscle, Mobility and Aging (SOMMA) [21], to investigate the association of diabetes with cardiorespiratory fitness and mitochondrial oxidative capacity in a cohort of 879 older adults who were well phenotyped in terms of the free living physical activity behaviors and body composition. We assessed mitochondrial oxidative capacity using two approaches; ^{31}PMRS to measure muscle *in vivo* (ATPmax) and high-resolution respirometry to measure mitochondrial respiration in muscle

biopsies, a complementary approach that interrogates mitochondrial oxidative phosphorylation (OXPHOS) capacity at the myocellular level. Here, we tested the hypothesis that diabetes would be associated with lower cardiorespiratory fitness and muscle oxidative capacity in older adults independent of adiposity, physical activity levels, chronic comorbid health conditions, and oral hypoglycemic medication use. We also tested the extent to which cardiorespiratory fitness and mitochondrial respiration mediate slower walking speed of older adults with diabetes.

RESEARCH DESIGN AND METHODS

Participant Recruitment

The Study of Muscle Mobility and Aging (SOMMA; http://sommaonline.ucsf.edu) is a prospective longitudinal cohort study of older adults and was designed to understand the biological basis of muscle aging [21]. Men and women (70+ yrs. old) were recruited between April 2019 to December 2021. Participants were enrolled at University of Pittsburgh and Wake Forest University School of Medicine if they were willing and able to complete a skeletal muscle biopsy and undergo Magnetic Resonance Imaging (MRI) and Spectroscopy (MRS). Exclusion criteria included an inability to walk one-quarter of a mile or climb a flight of stairs; had a BMI>40kg/m^2; had an active malignancy or dementia; or any medical contraindication to biopsy or MR. Finally, participants must have been able to complete the 400-m walk test; those who appeared as they might not be able to complete the 400-m walk at the in-person screening visit completed a short distance walk (4-m) to ensure their walking speed was ≥ 0.6m/s. Individuals with diabetes were identified by self-report, use of prescribed hypoglycemic medication, or having an HbA1c ≥6.5%. WIRB-Copernicus Group (WCG) Institutional Review Board

(WCGIRB, study number 20180764) approved the study as the single IRB and all participants provided written informed consent.

Baseline Assessments

Detail on study design and methodology have been published elsewhere [21]. The SOMMA Baseline Visit generally consisted of three days of assessments over several weeks: on "Day 1" the 400-m walk plus most other in-person assessments; "Day 2" cardiopulmonary exercise testing (CPET) and Magnetic Resonance Imaging (MRI) and Spectroscopy (MRS) were assessed; "Day 3" muscle biopsy specimen was collected. Age, sex, and race were collected by self-report. The self-reported medical conditions (heart disease, stroke, kidney disease/renal failure, and peripheral vascular disease) were assessed to determine if participants had those conditions or not. Participants were asked to bring all prescription medications they had taken in the 30 days prior to their "Day 1" clinic visit. If a participant forgot to bring one or more medications, clinic staff obtained this information over the telephone or at the return visit. The prescription medication use was reviewed and updated at the additional baseline visit days where CPET and tissue sampling were done. Body weight, height, and physical function assessments were also measured and accelerometry devices were provided. Most SOMMA participants completed cardiopulmonary exercise testing (CPET), magnetic resonance spectroscopy (MRS) and provided a percutaneous biopsy of the vastus lateralis muscle which was performed under fasted conditions. On the day of the muscle biopsy, participants prescribed oral hypoglycemic medication were asked to take their medications following tissue sampling.

Mobility Assessments

Mobility was assessed by 400m and 4m walking tests. For the 400-m walk test, participants were instructed to walk 10 complete laps around a set course at their usual pace and without overexerting themselves. 4m walking speed was measured in 2 trials and the participant's best time was used to calculate walking speed in m/sec.

Physical Activity Assessments

Two devices were used to assess activity: the thigh-worn activPal that recorded total daily step count and the wrist-worn ActiGraph GT9x. Devices were placed on the participants at the baseline "Day 1" visit with a data collection period of 7 full days. Data was included only if the device was worn over a 24-hour period and had ≥17hour per day wear time. Activity levels collected from ActiGraph were determined by cut points described by Montoye and colleagues [22], and included total daily physical activity time, and time spent in sedentary, light, and moderate to vigorous physical activity (MVPA).

Cardiopulmonary Exercise Testing Assessment

Participants walked for 5 minutes at a preferred walking speed, and progressive symptom-limited exercise protocol ensued with increases in speed (0.5 mph) and incline (2.5%) in 2-minute increments using a modified Balke protocol or a manual protocol [23, 24]. Cardiorespiratory fitness was determined as VO_2peak (mL/min) which was identified as the highest 30-second average of VO_2 (mL/min) achieved.

Magnetic Resonance (MR) Spectroscopy and Imaging

^{31}Phosphorous-MRS was used to assess *in-vivo* mitochondrial adenosine triphosphate (ATP) generation in the quadriceps muscle during an acute bout of knee isometric extension exercise [21, 24]. A detailed description of the ^{31}PMRS protocol can be found here [24]. A 3

Tesla magnetic resonance magnet (Siemens Medical Systems – Prisma [Pittsburgh] or Skyra [Wake Forest]) with a 12 cm $^{31}P/^{1}H$ dual tuned, surface RF coil (PulseTeq, Limited) was placed over the quadriceps with the shell of the coil consistently placed 10 cm superior to the patella, over the distal vastus lateralis muscle. Dynamic quantification of phosphocreatine (PCr), inorganic phosphate (Pi), phosphodiesterase (PDE) and ATP peak areas acquired using 100 microsecond block pulse targeting a pulse angle of 45^{o} with 2048 points and a sweep width of 5000Hz. Seventy-five dynamic spectra were acquired averaging 4 acquisitions with a 1.5 sec recycle time each for a spectral time resolution of 6 sec throughout rest, exercise, and recovery periods. Data was immediately processed to determine PCr breakdown and acidosis and then used to adjust exercise duration for a second or third trial to satisfy breakdown and acidosis criteria. Raw data were used to quantify peaks representing phosphorus metabolites using jMRUI v6.0. Temporal response of PCr area was used to calculate ATPmax (aka Qmax) from PCr recovery rates [25]. PCr area time course was referenced to the last 10 spectra of a 76-spectrum series. ATPmax was calculated using the recovery time constant as the inverse of the rate-constant as described in [26], and assuming a resting/recovered PCr concentration of 24.5 mM. Inorganic phosphate chemical shifts were followed relative to PCr [= -2.54 ppm] to calculate intracellular pH to monitor muscle acidosis. Quality control criteria for inclusion of dynamic ^{31}P MRS data in analyses were: 1) PCr breakdown between 33-50%; 2) pH throughout dynamic experiment greater than 6.8; and 3) monoexponential fit of PCr recovery data. Subject data not meeting all criteria were excluded from the analysis.

An AMRA scanning protocol with the Dixon water-fat imaging method was used to assess abdominal subcutaneous adipose tissue (ASAT), visceral adipose tissue (VAT), the ratio of VAT to ASAT (VAT/ASAT), and quadricep muscle fat infiltration (QFI). The entire body

was scanned and images were analyzed using AMRA Researcher® (AMARA medical AB, Linköping Sweden) [27].

Muscle Biopsy and Mitochondrial Respiration

Muscle biopsy was completed with a Bergström trocar (5 or 6mm) as previously described [24]. Approximately 10 mg of muscle tissue was allocated for respirometry assays and were immediately submerged in ice cold BIOPS buffer. Fiber bundles of approximately 2-3 mg wet weight were gently teased apart and permeabilized as previously described [24] and weight was measured using an analytical balance (Mettler Toledo, Columbus, OH). Permeabilized fiber bundle (PmFB) oxygen consumption was measured using a high-resolution respirometer (Oxygraph-2k, Oroboros Instruments, Innsbruck, Austria). PmFB were placed into chambers containing MiR05 buffer supplemented with 25µM blebbistatin in the absence of light. Two protocols were completed in duplicate measuring carbohydrate supported (Protocol 1: 5mM pyruvate and 2mM malate) and fatty acid oxidation (FAO) supported (Protocol 2: 25µM palmitoyl-carnitine and 2mM malate) respiration. For protocol 1, state 3 respiration was stimulated with the addition of 4.2mM ADP ($OXPHOS_{CHO}$) followed by subsequent additions of 10mM glutamate and 10mM succinate sequentially ($maxOXPHOS_{CHO}$). Maximal uncoupled respiration was stimulated with consecutive 0.5uM titrations of 2-[2-[4-(trifluoromethoxy)phenyl] hydrazinylidene-propanedinitrile (FCCP) until maximum electron transport system (ETS) capacity was attained ($maxETS_{CHO}$). For protocol 2, state 3 respiration was stimulated with the addition of 4mM ADP ($OXPHOS_{FAO}$) followed by subsequent additions of 10mM glutamate and 10mM succinate to further stimulate mitochondrial complexes I and II ($maxOXPHOS_{FAO}$). PmFB eliciting an oxygen consumption response to cytochrome c oxidase >15% were not included in the final analysis. Respiratory control ratio (RCR) for protocol 1

(7.11 ± 2.86) and protocol 2 (3.47 ± 1.74) were assessed for quality control. Respiration was analyzed using DatLab 7.4.0.4. In SOMMA, not all participants completed both protocols due to time constraints. Protocol 1 (carbohydrates) was prioritized over Protocol 2 (fatty acids) when limited technician time was available, which left fewer participants with complete data for Protocol 2.

Statistical Analysis

Differences in participant characteristics between older adults with and without diabetes were compared using the T-test for continuous variables (or Kruskal-Wallis non-parametric test for skewed variables) and chi-square test for dichotomized variables. Linear regressions without adjustment were performed to determine differences in cardiorespiratory fitness, and mitochondrial oxidative capacity between those with and without diabetes, and additional adjustments for the following confounders: age, race, gender, site/technician, BMI, and chronic conditions (model 1). Model 1 was further adjusted for steps per day from ActiPal or volume of adipose tissue depots: Abdominal Subcutaneous Adipose Tissue (ASAT), Visceral Adipose Tissue (VAT), the proportion of VAT to ASAT (VAT/ASAT) and muscle Quadricep Fat Infiltration (QFI). Lastly, to determine the impact of hypoglycemic medication use within the group of older adults with diabetes, multivariate linear regression was performed adjusting for age, race, and site/technician. Those analyses were completed using JMP® Software, Version 16.

Linear regression was also used to determine the association of diabetes with walking speed. We evaluated the potential mediating influence of VO_2peak and/or mitochondrial respiration. We did this by comparing the beta coefficient for the association of diabetes status on walking speed from the 400m and 4m walk. The base model included the confounding variables: gender, age, and race. model 2: base model plus technician and max$OXPHOS_{CHO}$,

model 3: base model plus technician and maxOXPHOS$_{FAO}$, model 4: base model plus clinical site and VO_2peak, model 5: base model plus technician, VO_2peak and maxOXPHOS$_{CHO}$, model 6: base model plus technician, VO_2peak and maxOXPHOS$_{FAO}$. Fully adjusted models also included the potential mediators/confounders BMI, and chronic conditions. Finally, we compared the percent difference in the beta coefficient between the base model and subsequent models to understand how each adjustment impacted group differences in walking speed. Those analyses were performed using SAS version 9.4 (SAS institute Inc., Cary, NC).

Data and Resource Availability

All SOMMA data are publicly available via a web portal. Updated datasets are released approximately every 6 months (https://www.sommastudy.com/for-investigators).

RESULTS

Participant Characteristics

A total of 879 participants (59.2% women) with an average age of 76.5 ± 5.0 years were enrolled in SOMMA [21]. A summary of participants with available data are included in **Supplemental Figure 1.** Participant characteristics are summarized in **Table 1**. 18% of the SOMMA cohort were classified as having diabetes. The proportion of women to men was greater in the group without diabetes (p=0.02). The proportion of White participants compared to non-White was greater in the group without diabetes (p<0.001). Older adults with diabetes had a higher body weight, waist circumference, BMI, and HbA1c (all, p<0.001). Individuals with diabetes tended to have a higher prevalence of heart disease and hypertension, whereas those without diabetes more often had history of cancer diagnosis (p<0.001). Approximately 75% of participants with diabetes reported use of a hypoglycemic agent where 83% used metformin 19%

used insulin, and 2% used thiazolidinediones (TZD). Total daily steps, and time in MVPA was significantly lower ($p<0.001$ for both) and total sedentary time was significantly higher in those with diabetes ($p<0.001$). Quadriceps muscle fat infiltration (QFI) was higher in those with diabetes ($p=0.002$). In addition, both abdominal subcutaneous adipose tissue (ASAT) and visceral adipose tissue (VAT) volume and ratio of VAT/ASAT was significantly higher in older adults with diabetes ($p=0.005$ for ASAT and $p<0.001$ for both VAT and VAT/ASAT) (**Table 1**).

Cardiorespiratory Fitness and Muscle Oxidative Capacity in Diabetes

In the adjusted model (**Table 2**, model 1 + PA), VO_2peak was significantly lower in older adults with diabetes compared to those without ($p= 0.004$). State 3 (coupled) respiration in the presence of pyruvate + malate ($OXPHOS_{CHO}$, $p= 0.12$,) and with the addition of both glutamate and succinate ($maxOXPHOS_{CHO}$, $p= 0.10$) was similar between groups. Maximal electron transfer system (ETS) capacity measured by maximal uncoupled respiration with CHO substrates ($maxETS_{CHO}$, $p=0.03$) was significantly lower in those with diabetes (**Table 2**). State 3 (coupled) respiration in the presence of palmitoyl carnitine + malate was similar between those with and without diabetes ($OXPHOS_{FAO}$, $p= 0.10$). Maximal oxidative phosphorylation supported by fatty acid substrates ($maxOXPHOS_{FAO}$, $p=0.006$) remained significantly lower in older adults with diabetes in model 1 + PA. Sensitivity analyses completed in those with data for both Protocol 1 and Protocol 2 had similar results (data not shown) to ensure that differences between protocols were not due to differences in sample size.

Adjustment for levels of ASAT, VAT, VAT/ASAT and QFI did not impact group differences in mitochondrial respiration and VO_2peak measurements with an exception for $OXPHOS_{CHO}$ (**Table 2**). Adjustment for VAT tended to explain some variance in $OXPHOS_{CHO}$ between those with and without diabetes ($p=0.06$, **Table 2**). Within the diabetes group only,

ATPmax was lower in older adults taking any hypoglycemic medication (p=0.05) or metformin alone (p=0.01, **Supplemental table 1**). These findings make it difficult to distinguish the impact of medications versus diabetes on ATPmax and therefore, further analysis of mitochondrial oxidative capacity focused on respiration measurements. VO_2peak and mitochondrial oxidative capacity assessed by respiration were similar with the use of all hypoglycemic agents and metformin (**Supplemental Table 1**).

VO_2peak and Mitochondrial Oxidative Capacity Mediate the Relationship between Diabetes and Walking Speed

To determine the extent to which cardiorespiratory fitness and mitochondrial oxidative capacity were linked to clinically meaningful outcomes in older adults with and without diabetes, we next evaluated their relationship with 400-m and 4-m walking speeds. Both were significantly lower in those with diabetes compared to those without diabetes following adjustments for gender, age, and race (base model, **Table 3**). To assess whether VO_2peak and mitochondrial respiration mediated the relationship between diabetes and walk speed, we compared the β coefficient for mean 400-m and 4-m walking speed from the base linear regression model with the β coefficient of models that included VO_2peak and/or mitochondrial respiration variables. Additional individual adjustments for $maxOXPHOS_{CHO}$ (400-m, p=0.02, model 2), $maxOXPHOS_{FAO}$ (400-m, p=0.02; model 3) and VO_2peak (400-m p=0.01; model 4) resulted in no significant change in group differences in 400-m walking speed between those with and those without diabetes (**Table 3**). Alternatively, for 4-m walking speed, further adjustments for $maxOXPHOS_{CHO}$ (4-m, p=0.12; model 2) and $maxOXPHOS_{FAO}$ (4-m, p=0.51, model 3) significantly mediated differences between groups, whereas VO_2peak (4-m, p=0.04, model 4) did not. Examining the impact of combinations of VO_2peak and mitochondrial respiration revealed

that VO_2peak with max$OXPHOS_{CHO}$ (400-m p=0.07, 4-m p=0.27; model 5) or max$OXPHOS_{FAO}$ (400-m, p=0.20, 4-m, p=0.99) mediated differences between those with and without diabetes. Full adjustments including potential confounders/mediators BMI and chronic conditions explained the remaining variance in walking speed between groups. (**Table 3**). Comparing the β coefficient for mean 400-m and 4-m walking speed from the base linear regression model with the β coefficient of models that included both VO_2peak and mitochondrial respiration variables revealed that VO_2peak and mitochondrial respiration explained an additional ~46-100% of the variance in 400-m and 4-m walking speed between groups (**Figure 1**).

DISCUSSION

This is the first study investigate whether cardiorespiratory fitness and skeletal muscle oxidative capacity contribute to slow walking speed in older adults with diabetes in a unique well-phenotyped cohort of older adults that included 159 participants with diabetes. The main novel findings of the study are: 1) the association of diabetes status with slow walking speed was mediated by VO_2peak and skeletal muscle mitochondrial respiration; 2) VO_2peak and mitochondrial respiration was lower in older adults with diabetes while controlling for objectively assessed physical activity and adiposity and 3) Older adults taking hypoglycemic medications (insulin, TZDs, and/or metformin) or metformin alone had significantly lower ATPmax compared to older adults with diabetes but not taking medications, an observation that aligns with reports in the literature that metformin can impair mitochondrial oxidative capacity. Slower walking speed is indicative of poor health and has been shown to associate with survival in older adults [28]. Together, these findings are impactful as they are the first to link a biological quality of muscle (oxidative capacity) to explain slower walking speed in a particularly vulnerable patient population.

The well-phenotyped SOMMA cohort provided an opportunity to assess the impact of diabetes status on VO_2peak and mitochondrial oxidative capacity in older adults while controlling for objectively measured indices of physical activity, adiposity, and chronic conditions in addition to demographic variables. Many of the previous studies reporting lower muscle oxidative capacity measured in permeabilized fiber bundles from patients with diabetes have not rigorously assessed and controlled for the participants' physical activity [12-14, 17]. This is important as physical activity interventions (such as walking) can improve mitochondrial energetics in type 2 diabetes [29], during weight loss in obesity [30], and in older adults [31]. Here, we report that lower levels of objectively assessed physical activity partially explains lower respiration in older adults with diabetes. Interestingly, comparing our respirometry protocols with distinct substrate combinations, we found that CI+II FAO supported maxOXPHOS respiration remains lower in the diabetes group, while CI+II CHO only supported maxOXPHOS did not differ following adjustments for physical activity. This suggests that CHO supported coupled respiration may be more sensitive to levels of physical activity compared to FAO supported respiration. This is also in line with evidence indicating reduced rates of lipid oxidation in skeletal muscle from individuals with diabetes, potentially due to mitochondrial overload and incomplete fatty acid oxidation [32]. We also acknowledge that reduced mitochondrial oxidative capacity may be due to reduced mitochondria content. Future analysis will investigate this further.

The influence of adiposity on mitochondrial energetics has been considered in prior studies of diabetes, typically by matching control subjects for BMI [14]. Some studies [15, 16], but not all [12, 13, 17], indicate no differences between patients with diabetes and BMI-matched controls, suggesting that obesity *per se* may underlie the lower muscle oxidative capacity. Here,

we examined the influence that BMI and individual (ASAT, VAT, and QFI) and the proportion of VAT to SAT (VAT/ASAT) adipose depots had on muscle mitochondrial energetics in diabetes. However, regardless of whether adiposity was adjusted for BMI alone or combined with individually objectively measured adipose depots, mitochondrial respiration generally remained significantly lower in those with diabetes. Taken together, these findings indicate that adiposity and physical activity largely (there were some exceptions) do not entirely explain lower mitochondrial respiration in older adults with diabetes.

Metformin is one of the most commonly prescribed medications for diabetes, and although its mechanism of action remains highly debated, there is evidence of an inhibitory effect on complex I of the electron transport chain [33, 34]. Both pre-clinical and clinical studies report maladaptation of mitochondrial function following metformin treatment, and blunting of exercise induced increases in muscle hypertrophy and cardiorespiratory fitness, specifically in older adults [33, 35, 36]. Conversely, others have reported that metformin can improve mitochondrial function via effects on mitophagy, autophagy [37] or AMPK activation [38]. Our current understanding of how metformin impacts skeletal muscle mitochondrial function of older adults is reviewed in detail elsewhere [39, 40]. Here, we reveal lower ATPmax in older adults with diabetes taking hypoglycemic medication or metformin alone, compared to older adults with diabetes not taking medications. Interestingly, we did not observe an association of metformin with diabetes status in the respiration assays, suggesting different sensitivities to metformin based on whether mitochondrial energetics are assessed in vivo (ATPmax) compared to permeabilized fiber bundle preparations ex vivo. However, an important caveat is that participants were asked to withhold medication prior to the muscle biopsy and respirometry assays but not on the day of ^{31}PMRS assessments. In addition, others have reported no effect on

mitochondrial respiration after a two-week metformin treatment compared to control [41], or in long term diabetes patients [42]. Given that metformin is water soluble, it is also plausible that the inhibitory effect of metformin is lost when the muscle fiber bundles are washed in preparation for the respirometry assay. Further work is needed to decipher the impact metformin has on skeletal muscle mitochondrial energetics in older adults with diabetes.

Zhao and colleagues reported that adults with diabetes were 31-34% less likely to participate in physical activity, which in turn may contribute to lower VO_2peak [43]. In line with these reports, SOMMA participants with diabetes were significantly less active and significantly more sedentary. Additional adjustments for physical activity did not resolve differences in VO_2peak between groups, suggesting that lower VO_2peak in older adults with diabetes is independent of physical activity in this cohort. In addition, independent of adiposity measured by BMI or combined BMI with ASAT, VAT, VAT/ASAT and QFI, VO_2peak remained significantly lower in those with diabetes compared to those without. Taken together, our work suggests that physical activity and adiposity may not be implicated in lower cardiorespiratory fitness in older adults with diabetes and that other factors including perhaps genetics and heritability are more important determinants to low cardiorespiratory fitness in diabetes.

Previous work from our laboratory and others revealed a significant relationship between walking speed with both VO_2peak and mitochondrial energetics in older adults [9, 28, 44]. Here, we explored the contribution of both mitochondrial respiration and cardiorespiratory fitness to mobility in older adults with diabetes. We report that mitochondria respiration, both independently (~40-70%) and combined with VO_2peak (~55-100%), mediated the variance in 4-m walking speed in those with diabetes. Differences between groups are further explained by additional adjustments for potential confounders/mediators, including BMI and comorbidities.

Reports indicate that muscle mass is lost at an accelerated rate in older adults with diabetes [7], contributing to reductions in muscle strength and quality [6], and ultimately reducing physical function and mobility [5, 45]. We extended these findings by highlighting how mitochondrial respiration may independently and combined with cardiorespiratory fitness also contribute to lower walk speed in older adults with diabetes. This finding is of particular interest because slower walking speed is an important indicator of health and has been shown to associate with survival in older adults [28]. Further assessments of specific aspects of the cardiorespiratory system (e.g., pulmonary function, oxygen transportation, muscle capillarization) and mitochondrial function (e.g., H_2O_2 emission, calcium retention capacity, membrane potential) may reveal novel targets to prevent mobility loss in older adults with diabetes.

Our study has several strengths but also a few notable weaknesses that we are well positioned to address in the future. First, the duration of diabetes status and the length of time that participants have been taking oral hypoglycemic agents were not recorded. However, this cohort is being followed longitudinally, so there is opportunity to capture that data in the future and for powerful analysis of changes over time in the same individuals. In addition, participants were predominantly (85.6%) non-Hispanic White limiting our ability to generalize to other race/ethnic groups where mobility disability is more prevalent [46] . The participants recruited were able to complete 4-m walk test at a gait speed of greater than or equal to 0.6m/s, which may be faster than those with more advanced diabetes who are unable to complete mobility related tasks [2]. However, longitudinal assessments will provide an opportunity to study decline in mobility and incident mobility disability in those with diabetes in the future. The strengths of our study design include the collection of muscle biopsies and use of two measurements of mitochondrial oxidative capacity in a large cohort of older individuals combined with rigorous

assessment of objectively measured physical activity, fitness and body composition. This unique study design allows us to link a fundamental biological process in muscle biopsies to a key clinical outcome. Furthermore, the collection of these biological resources, and the foundational work completed in this manuscript will support future analysis focused on further understanding the biological qualities of muscle, including measures of mitochondrial content, that contribute to slower walking speed in older adults with diabetes.

In summary, our findings highlight that mitochondrial respiration independently and combined with cardiorespiratory fitness contribute to slower 400-m and 4-m walk speed in older adults with diabetes. Additionally, future work should aim to decipher the impact of diabetes medication on mitochondrial function as this remains to be a gap in the literature.

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Conflict of Interest – SRC and PMCa are consultants to Bioage Labs. PMCa is a consultant to and owns stock in MyoCorps. All other authors report no conflict of interest.

Author Contributions and Guarantor Statement – SVR and LYL completed statistical analysis with final review from the statistical team at the coordinating center. SVR and PMCo conceived the idea and co-wrote the manuscript. LYL, GD, SM, PMCo and PK completed experiments or completed quality control, validation, and interpretation of data. LYL, GD, PMCa, PK, TM, SM, MJJ, AJM, EEK, DJM, FGST, SRC, ABN, RTH, SBK, BHG edited the manuscript. SRC, PMCa, ABN, SBK, RTH, and BHG enabled the study with either funding acquisition, project administration, and/or conceptualization to the study.

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Table 1: Participant Characteristics

Basic Characteristics	**Without Diabetes**	**With Diabetes**	**p-value**
N	717	159	
Age (year)	76.47 ± 5.05	75.79 ± 4.84	0.12
Female	61(438)	51(81)	**0.02**
Race (white)	88(631)	70(111)	**<0.001**
Hispanic ethnicity	1(7)	1(2)	0.76
Weight (kg)	74.74 ± 14.75	82.41 ± 15.58	**<0.001**
Height (m)	1.66 ± 0.10	1.67 ± 0.10	0.16
Waist circumference (cm)	92.69 ± 12.98	100.90 ± 12.57	**<0.001**
BMI (kg/m^2)	27.17 ± 4.44	29.55 ± 4.63	**<0.001**
HbA1c (%)	5.51 ± 0.31	6.73 ± 0.89	**<0.001**
Systolic blood pressure (mmHg)	129.71 ± 15.80	133.72 ± 16.08	**0.004**
Co-morbidities			
Lung disease	13(93)	14(22)	0.77
Arthritis	57(409)	52 (83)	0.25
Stroke	2(16)	3(5)	0.51
Heart disease	6(42)	11(18)	**0.02**
Cancer	43(308)	24(38)	**<0.001**
Hypertension	26(187)	37(59)	**0.006**
Kidney disease/renal failure	3(24)	6(9)	0.19
Peripheral vascular disease	1(4)	1(2)	0.37
Hypothyroidism	19(133)	17(27)	0.64
Glaucoma	9(65)	11(18)	0.39

Macular degeneration	9(61)	5(8)	0.12
Cataracts	66(472)	62(99)	0.37
Hypoglycemic Medications			
All	--	75(118)	
• Insulin	--	19(23)	
• Metformin	--	84(99)	
• Thiazolidinediones	--	3(3)	
Physical Activity			
Total daily steps	7066.68 ± 3233.95	5848.70 ± 2791.12	**<0.001**
Total sedentary time (min)	610.10 ± 112.28	659.40 ± 108.65	**<0.001**
Time in light physical activity (min)	98.20 ± 26.54	92.84 ± 29.16	**0.027**
Time in moderate to vigorous physical activity (min)	192.95 ± 86.40	155.66 ± 76.74	**<0.001**
Body Adiposity			
Abdominal adipose tissue volume (ASAT) (L)	7.50 ± 3.14	8.30 ± 3.31	**0.005**
Visceral adipose tissue volume (VAT) (L)	3.96 ± 2.21	5.36 ± 2.31	**<0.001**
VAT/ASAT	0.57 ± 0.34	0.72 ± 0.38	**<0.001**
Quadricep fat infiltration (%)	0.066 ± 0.022	0.072 ± 0.020	**0.002**

Data presented as means ± standard deviation or % (N). Hypoglycemic medications is calculated only for those taking medications. Significance accepted at **p<0.05** and indicated with bold font.

Table 2. Cardiorespiratory fitness and mitochondrial oxidative capacity in older adults with and without diabetes

Model	Diabetes Status	VO_2peak (mL/min)	$OXPHOS_{CHO}$ (pmol/mg/ww)	$_{max}OXPHOS_{CHO}$ (pmol/mg/ww)	$_{max}ETS_{CHO}$ (pmol/mg/ww)	$OXPHOS_{FAO}$ (pmol/mg/ww)	$_{max}OXPHOS_{FAO}$ (pmol/mg/ww)
Unadjusted	WOD	1532.10 ± 16.67	**31.08 ± 0.45**	**60.63 ± 0.74**	**81.43 ± 0.97**	12.94 ± 0.22	**58.22 ± 0.84**
	WD	1513.75 ± 36.19	**28.10 ± 0.97**$^{\#}$	**55.11 ± 1.61**$^{\#}$	**73.80 ± 2.11**$^{\#}$	11.93 ± 0.47	**53.31 ± 1.78**$^{\#}$
Model 1	WOD	**1486.68 ± 19.07**	**30.14 ± 0.91**	**59.02 ± 1.45**	**77.53 ± 1.90**	**12.58 ± 0.47**	**59.25 ± 1.64**
	WD	**1386.69 ± 25.66***	**27.88 ± 1.11**$^{\#}$	**54.98 ± 1.80**$^{\#}$	**71.25 ± 2.37**$^{\#}$	**11.49 ± 0.55**$^{\#}$	**54.12 ± 1.93**$^{\#}$
Model 1 + PA (steps/day)	WOD	**1487.30 ± 19.53**	30.15 ± 0.91	58.65 ± 1.47	**77.02 ± 1.98**	12.60 ± 0.50	**59.21 ± 1.61**
	WD	**1412.11 ± 26.03**$^{\#}$	28.51 ± 1.11	55.89 ± 1.78	**71.83 ± 2.44**$^{\#}$	11.70 ± 0.59	**54.29 ± 1.92**$^{\#}$
Model 1 + ASAT (L)	WOD	**1492.37 ± 19.52**	**30.34 ± 0.94**	**59.50 ± 1.49**	**77.64 ± 1.95**	**12.67 ± 0.48**	**59.83 ± 1.67**
	WD	**1376.07 ± 26.56***	**27.83 ± 1.15**$^{\#}$	**55.07 ± 1.82**$^{\#}$	**70.92 ± 2.45**$^{\#}$	**11.37 ± 0.57**$^{\#}$	**53.85 ± 1.98**$^{\#}$
Model 1 + VAT (L)	WOD	**1488.92 ± 19.56**	30.16 ± 0.94	**59.16 ± 1.49**	**77.33 ± 1.96**	**12.62 ± 0.49**	**59.57 ± 1.68**
	WD	**1386.86 ± 26.60***	28.04 ± 1.15	**55.46 ± 1.81**$^{\#}$	**71.29 ± 2.45**$^{\#}$	**11.50 ± 0.57**$^{\#}$	**54.66 ± 1.98**$^{\#}$
Model 1 + VAT/ASAT	WOD	**1491.43 ± 19.47**	**30.29 ± 0.94**	**59.39 ± 1.49**	**77.53 ± 1.95**	**12.67 ± 0.49**	**59.90 ± 1.68**
	WD	**1391.58 ± 26.76***	**28.02 ± 1.16**$^{\#}$	**55.44 ± 1.83**$^{\#}$	**71.33 ± 2.46**$^{\#}$	**11.44 ± 0.57**$^{\#}$	**54.01 ± 2.00**$^{\#}$
Model 1 + QFI (%)	WOD	**1486.37 ± 19.00**	**30.28 ± 0.93**	**59.38 ± 1.48**	**77.78 ± 1.94**	**12.68 ± 0.48**	**59.74 ± 1.68**
	WD	**1386.10 ± 25.75***	**27.97 ± 1.14**$^{\#}$	**55.33 ± 1.81**$^{\#}$	**71.06 ± 2.43**$^{\#}$	**11.37 ± 0.57**$^{\#}$	**54.22 ± 1.98**$^{\#}$

Mean ± standard error with significance accepted at ***p<0.001, #p<0.05** and represented with bold font**.** Model 1 is adjusted for age, race, gender, site/technician, BMI, and comorbidities. PA = physical activity, ASAT = abdominal subcutaneous adipose tissue, VAT = visceral adipose tissue, QFI = muscle quadricep fat infiltration, WOD = without diabetes, WD = with diabetes, $OXPHOS_{CHO}$ = carbohydrate supported respiration, $maxOXPHOS_{CHO}$ = carbohydrate supported maximal complex I+II stimulated respiration, $maxETS_{CHO}$ = carbohydrate supported maximal electron transport system, $OXPHOS_{FAO}$ = fatty acid oxidation (FAO) supported respiration, $maxOXPHOS_{FAO}$ = fatty acid oxidation supported maximal complex I+II stimulated respiration.

Table 3: Multivariable linear regression analysis for the association of diabetes with 400m and 4m walk speed

	Without Diabetes	With Diabetes	
Multivariable model	Means ± SE	β ± SE	P
400m walk speed (m/sec)			
Base Model: adjusted for gender, age and race	1.06 ± 0.01	-0.05 ± 0.01	**0.00**
Model 2: base model plus maxOXPHOS$_{CHO}$	1.06 ± 0.01	-0.04 ± 0.02	**0.02**
Model 3: base model plus maxOXPHOS$_{FAO}$	1.06 ± 0.01	-0.04 ± 0.02	**0.02**
Model 4: base model plus VO_2peak	1.07 ± 0.01	-0.04 ± 0.01	**0.01**
Model 5: base model plus VO_2peak and maxOXPHOS$_{CHO}$	1.07 ± 0.01	-0.03 ± 0.02	0.07
Model 6: base model plus VO_2peak and maxOXPHOS$_{FAO}$	1.07 ± 0.01	-0.02 ± 0.02	0.20
Fully adjusted: model 5 plus BMI and comorbidities	1.06 ± 0.01	0.003 ± 0.01	0.87
Fully adjusted: model 6 plus BMI and comorbidities	1.06 ± 0.01	-0.005 ± 0.02	0.78
4m walk speed (m/sec)			
Base Model: adjusted for gender, age, and race	1.05 ± 0.01	-0.04 ± 0.02	**0.01**
Model 2: base model plus maxOXPHOS$_{CHO}$	1.04 ± 0.01	-0.03 ± 0.02	0.12
Model 3: base model plus maxOXPHOS$_{FAO}$	1.05 ± 0.01	-0.01 ± 0.02	0.51
Model 4: base model plus VO_2peak	1.05 ± 0.01	-0.04 ± 0.02	**0.04**
Model 5: base model plus VO_2peak and maxOXPHOS$_{CHO}$	1.05 ± 0.01	-0.02 ± 0.02	0.27
Model 6: base model plus VO_2peak and maxOXPHOS$_{FAO}$	1.05 ± 0.01	0.00 ± 0.02	0.99
Fully adjusted: model 5 plus BMI and comorbidities	1.05 ± 0.01	0.003 ± 0.02	0.84

Fully adjusted: model 6 plus BMI and comorbidities	1.05 ± 0.01	0.01 ± 0.02	0.50

β estimate ± standard error with significance accepted at ***p<0.05**. $maxOXPHOS_{CHO}$ = carbohydrate supported maximal complex I+II stimulated respiration, $maxOXPHOS_{FAO}$ = fatty acid oxidation supported maximal complex I+II stimulated respiration. Models including $maxOXPHOS_{CHO}$ and $maxOXPHOS_{FAO}$ are adjusted for technician, and models with VO_2peak are adjusted for site.

Figure Titles

Figure 1. Percent reduction in the association between diabetes and 400-m and 4-m walking speed after adjusting for both individual and combined carbohydrate and fatty acid supported respiration, and cardiorespiratory fitness. Bars depict the reduction in β coefficient from linear regression models (models 2-6 compared to the base model: [1-(β adjusted/β base adjusted)]. Base model: age, gender, and race, model 2: base model + $maxOXPHOS_{CHO}$, model 3: base model + $maxOXPHOS_{FAO}$, model 4: base model + VO_2peak, model 5: base model + VO2peak + $maxOXPHOS_{CHO}$, model 6: base model + VO_2peak + $maxOXPHOS_{FAO}$.

Supplemental Figure 1. SOMMA cohort sample size for analysis of muscle function, mitochondrial oxidative capacity, CRF, PA and body composition. Participants missing data for ex-vivo mitochondrial energetics is due exclusions samples passing quality control. Missing ATPmax measures were due to safety reasons or exclusion due to technical conditions during testing.

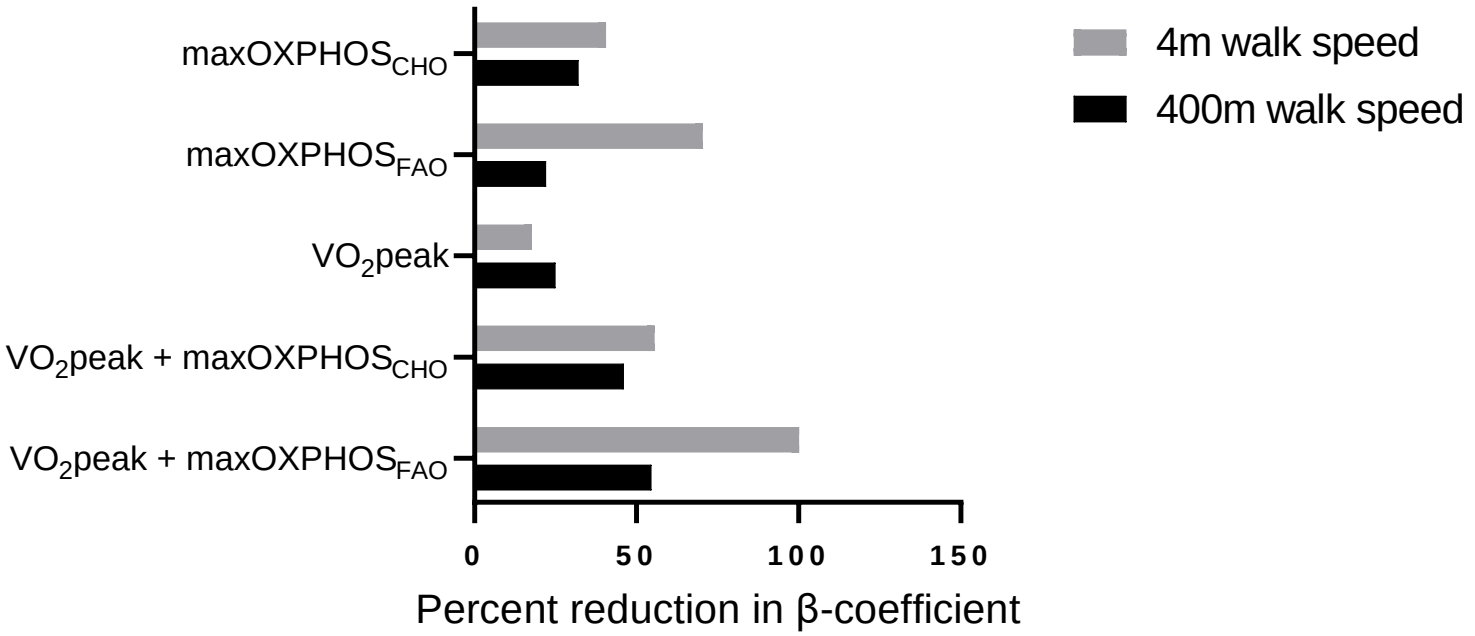
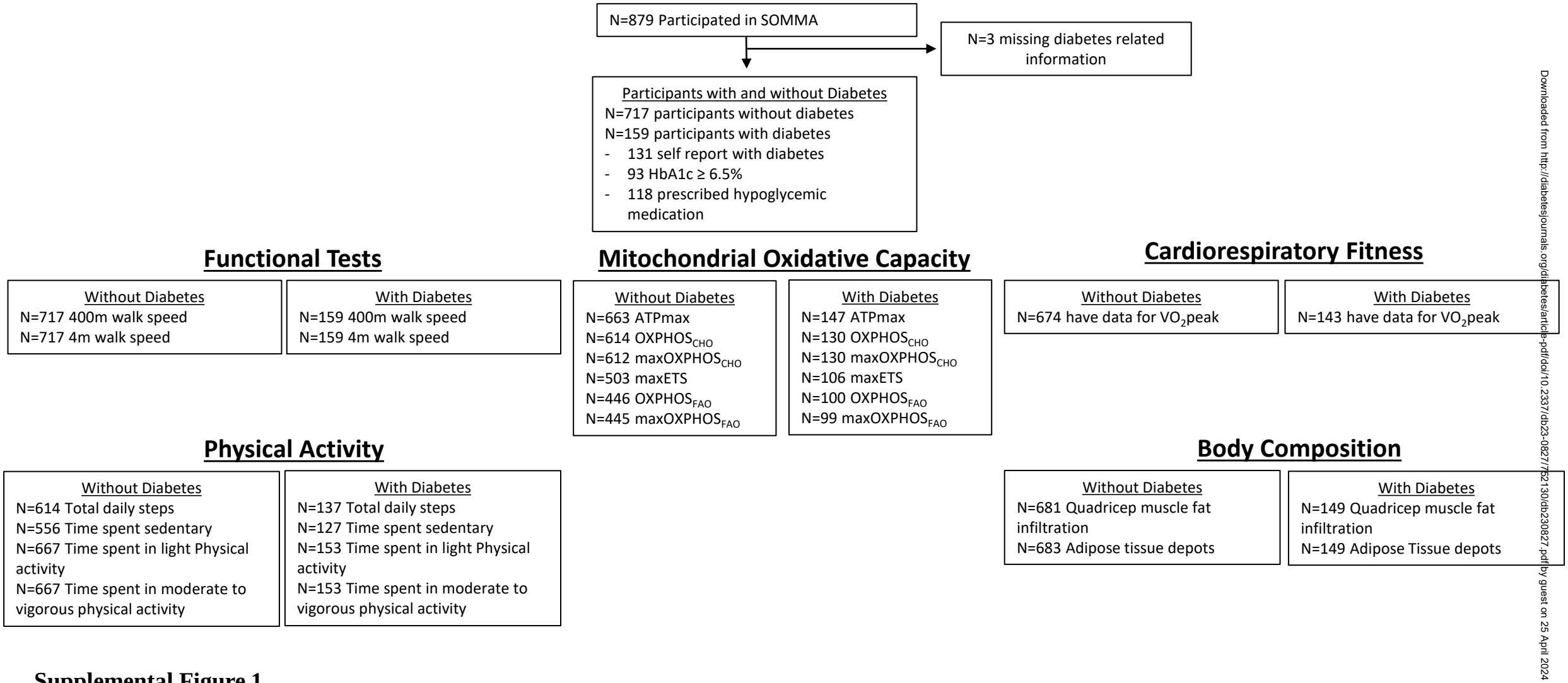


Figure 1.



Supplemental Figure 1.

Supplemental Table 1. Association of hypoglycemic drugs and metformin with cardiorespiratory fitness and mitochondrial oxidative capacity

	All hypoglycemic agents		Metformin	
	β estimate	p-value	β estimate	p-value
VO_2peak	-82.27 ± 62.24	0.19	-13.08 ± 55.47	0.81
ATPmax	**-0.05 ± 0.03**	**0.05**	**-0.06 ± 0.02**	**0.01***
$OXPHOS_{CHO}$	-1.03 ± 2.01	0.61	-0.71 ± 1.81	0.70
$maxOXPHOS_{CHO}$	-3.52 ± 3.20	0.27	-3.11 ± 2.89	0.28
$maxETS_{CHO}$	-2.95 ± 4.45	0.51	-4.07 ± 4.04	0.32
$OXPHOS_{FAO}$	-1.35 ± 1.06	0.21	-0.68 ± 1.00	0.50
$maxOXPHOS_{FAO}$	-2.57 ± 3.69	0.49	-5.30 ± 3.43	0.13

β estimate ± standard error with significance accepted at **p<0.05**. Data adjusted for age, race, and site/technician. ATPmax = maximal adenosine triphosphate production, $OXPHOS_{CHO}$ = carbohydrate supported respiration, $maxOXPHOS_{CHO}$ = carbohydrate supported maximal complex I+II stimulated respiration, $maxETS_{CHO}$ = carbohydrate supported maximal electron transport system, $OXPHOS_{FAO}$ = fatty acid oxidation (FAO) supported respiration, $maxOXPHOS_{FAO}$ = fatty acid oxidation supported maximal complex I+II stimulated respiration.