A Sub-Maximal Exercise-Based Assessment of Metabolic Flexibility Using a Lactate Biomarker in an Outpatient Rehabilitation Setting: Feasibility and Safety in an Adult Cohort

A Sub-Maximal Exercise-Based Assessment of Metabolic Flexibility Using a Lactate Biomarker in an Outpatient Rehabilitation Setting: Feasibility and Safety in an Adult Cohort

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Abstract

Metabolic flexibility can be described as the sum of an organism's adaptive responses for preferential oxidization of fuels relative to anatomical supply (ingestion and storage) and physiological demand (physical activity and inactivity). Fuel selection and oxidation occurs at the level of the mitochondria. Mitochondria are intracellular organelles with a primary role in oxidative metabolism. Mitochondrial adaptability to substrate availability and physical activity/inactivity may be considered the functional components of metabolic flexibility. Currently, metabolic flexibility is observed with two methods: Respiratory Quotient (RQ) via Indirect Calorimetry and a Euglycemic-Hyperinsulinemic clamp. Given the robust mitochondrial response to increases or decreases in physical activity, an additional method for observing metabolic flexibility may be through observation of a metabolic intermediate that bridges glycolytic and oxidative metabolism: lactate. Recently, lactate has been described as a proxy for mitochondrial function and a surrogate of glycolytic and oxidative function. Moreover, current methods that utilize blood lactate thresholds (turn-points, transitions, etc.) to determine power to mass ratios (W/kg) in cyclists may afford a useful application for lactate in quantifying metabolic flexibility within the general population. One iteration on the W/kg concept is the MetFlex Index™, a score of metabolic flexibility and fitness, defined as the power (Watts) an individual produces at the first lactate threshold relative to that individual's body mass index. Here we describe a novel method for using blood lactate during a graded exercise cycling test for identifying and describing metabolic flexibility and fitness in an adult outpatient rehabilitation cohort.

1 Introduction

Metabolic Syndrome affects 1 of every 3 U.S. adults within the United States (1). Additional prevalence data demonstrates that 40% of adults are living with obesity and 10% of adults are living with diabetes (2-3). Recently, severity of illness during a Covid-19 infection and higher mortality rates have been associated with adults living with metabolic syndrome (4), with accumulating risk relative to an increased number of components of metabolic syndrome (5). Higher mortality rates were observed in those with lower cardiorespiratory fitness (CRF) (6). Physical inactivity and sedentary behavior, inaction that directly contribute to a low CRF, often factor into the severity of

metabolic dysfunction (7-13). Alternatively, physical activity and exercise directly contribute to increased CRF and are effective attenuators of metabolic disease via skeletal muscle. Additionally, increased fitness demonstrates effects on tissues and organs beyond skeletal muscle, such as the brain, liver, and heart (14). Physical activity and exercise can directly affect metabolic health via changes to mitochondrial structure and function (15 -16). Furthermore, it may be that CRF can modulate the risks associated with obesity and disease progression by mitigating metabolic dysfunction (17), also described here as the "obesity paradox" relative to CRF (18). Given that physical activity and fitness are essential for health, the utility of a quantifiable metabolic biomarker during exercise testing to describe metabolic flexibility and fitness could prove useful in metabolic health promotion, disease prevention, and chronic condition management.

2 Metabolic Flexibility

Metabolic Flexibility is the ability of an organism to adapt fuel availability with energy demands. Terminology initially describing energy and metabolism as "skeletal muscle adaptability" (19-20) appears to be transitioning toward "metabolic flexibility" and "metabolic inflexibility" (21-22). Explanations of these concepts and related methodologies have been developed and described in detail elsewhere (10,16, 23-32). The primary methodology for determining metabolic flexibility has been by calculating changes in the RQ via indirect calorimetry during a euglycemic-hyperinsulinemic clamp. Observations with this method demonstrated a reduced ability, or "inflexibility", to switch from fat to glucose oxidation in the transition from fasting to insulin with glucose stimulation. Individuals with metabolic health impairments including insulin resistance, obesity, and diabetes also had a higher preference for glucose relative to fat as an energy source when fasted, again demonstrating metabolic inflexibility. Gaps persist in understanding the relationship between the onset and development of metabolic health impairments and metabolic flexibility, particularly the association between metabolic inflexibility and ectopic lipid accumulation (32). It may be useful to explore less invasive and scalable, movement-based methods for quantifying metabolic flexibility (10) to assess the efficacy of metabolic health and fitness interventions within clinical and nonclinical populations. One potential biomarker is lactate, a metabolic intermediate serving as a metabolic bridge between glycolysis and oxidation.

3 Lactate

Historically, lactate has been described as a waste product associated with hypoxia and fatigue (33). More recently, lactate has been reframed as the "fulcrum of metabolism" (34), demonstrating increased significance as a biomarker in metabolism (35-36), yet remaining under-developed as a metabolic marker of health and disease (37). Lactate may be a useful metabolic biomarker (in comparison to other markers, for example, like the waist:hip ratio, skeletal muscle fatty acid oxidation, and the HDL: LDL ratio), by measuring resting fasting plasma lactate (38-39) or by observing lactate clearance capacity through a graded exercise test (40). Relationships between lactate and substrate oxidation (RQ) were observed during a graded exercise test comparing professional cyclists and less fit individuals, including a group of very low fit adults with metabolic syndrome (40). Exercise testing in this study was performed on a lower extremity ergometer with lower to higher power increments at standardized stage durations while blood lactate was sampled at the end of each stage duration as ventilatory data (VCO2/VO2) was acquired throughout. There was a significant difference in the relationship between lactate metabolism and substrate oxidation between individuals with different levels of fitness as endurance athletes were able to prolong fat oxidation and delay substantial carbohydrate oxidation relative to their lactate curves or lactate clearance capacities. Less fit individuals, most pronounced in individuals with metabolic syndrome,

demonstrated a significantly impaired capacity for fat oxidation with greater reliance on carbohydrate oxidation at very low workloads including the early onset of lactate accumulation (poor clearance capacity). Early lactate accumulation may have an impact on adipose metabolism and vice-versa (41) as the "early onset of lactate accumulation signifies poor capacity to mediate metabolic strain resulting in significant organismal stress" (37). Lactate thresholds demonstrate observable and informative relationships with substrate oxidation where lactate and fat oxidation were strongly negatively correlated, and lactate and carbohydrate oxidation were strongly positively correlated (40). Since lactate metabolism and fat oxidation occur at the level of the mitochondria, observing lactate behavior may additionally afford an indirect observation of mitochondrial function. Lactate may further demonstrate its utility as a surrogate marker for substrate oxidation considering that fat oxidation decreases at or near the first lactate threshold. This relationship of reduced fat oxidation as lactate accumulates may provide an additional method for quantifying metabolic flexibility.

Lactate metabolism relative to power output (Watts) is commonly used to compare cyclists at different stages in training or across a career (42) and, in conjunction with additional metabolic data, may predict high performance and outcome prior to a competitive season (43). Power to mass ratios (W/kg) are traditionally derived from the ability to sustain power over a given duration, typically at or near the second lactate threshold, maximal lactate steady state, maximal metabolic steady state, etc. At the second lactate threshold, fat oxidation is at or near its lowest rates and carbohydrate oxidation continues to increase. Since fat oxidation begins to trend downward at or near the first lactate threshold, this first threshold may demonstrate utility for measuring and managing metabolic flexibility in the general population.

4 Body Mass Index

Body Mass Index (BMI) was developed over 150 years ago but was not integrated within medical literature until the early 1970's (44). BMI persists as a statistical tool within the National Institutes of Health and World Health Organization for comparisons within and between populations (44), albeit not without limitations. Recently, integrating results from cardiopulmonary exercise testing to determine CRF (e.g. VO2peak) relative to body mass (kg) yielded a fitness to mass ratio (VO2peak/kg) which provided utility for an 'obesity staging system', identifying higher at-risk individuals for mortality (17). Here, accounting for physical capacity demonstrates a useful elaboration on the BMI metric for further identifying and monitoring metabolically at-risk and low fit individuals.

5 MetFlex Index™

The MetFlex Index™ is a power to weight ratio like the W/kg metric, yet with two differences: 1) the Index identifies the power attained at the first lactate threshold rather than the second lactate threshold or another point of interest and 2) power is relative to BMI rather than an individual's mass in kilograms. Recall that the first lactate threshold indicates a transition in substrate utilization from peak or maximal fat oxidation toward predominantly carbohydrate oxidation as lactate begins to accumulate due to production exceeding clearance capacity or oxidation. The accumulation of lactate may directly or indirectly inhibit, down-regulate, or out-compete fat oxidation (41) and potentially lipolysis. The lower the lactate threshold and clearance capacity relative to power, the lower the fat oxidization, the lower the metabolic flexibility, and vice versa. Additionally, using BMI instead of kg, as in the Watts/kg performance model, affords integration of the long-standing BMI biometric with physical capacity, providing further information on risk and risk stratification in healthcare, consistent with individualization and precision trends in health and fitness. Here, defining

physiological capacity as the power produced at the first lactate threshold, one can also compare the efficacy of, and responsiveness to, any intervention claiming to improve oxidative capacity, including an exercise program, nutrition program, or a pharma-based program. The MetFlex Index™ could monitor a gain or loss in metabolic flexibility over time, regardless of the intervention, or be used to monitor the stability, progression, or regression of metabolic flexibility related to a disease process or chronic condition. The ability to quantify the relationship between an external load (power as Watts) to an internal load (lactate clearance capacity and heart rate) has tremendous value as a movement-based health and fitness metric (48).

6 Methods

Seventy-seven (41 F/36 M) volunteers participated in this observational study. Informed consent was received from all participants. The testing conformed to the Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects. Potential risks from participation in testing included sore muscles, sore finger or earlobe from the safety spring lancet, possible bruising and infection. The testing utilized submaximal effort protocols and all participants were allowed to cease testing at any time.

Single participant testing occurred in a rolling format between November 2020 and June 2021 at an Orthopedics specialty practice located within the Midwestern Region of the US. All testing was performed between the hours of 0800 and 1800, based on subject preference, within the Outpatient Rehabilitation Department of the practice. Testing was provided by staff (rehab technicians, Physical Therapist Assistants, and Physical Therapists) who were trained by OVAL (formerly elexr) representatives.

Orthopedic medical staff were informed of the testing interest of their respective participants and provided approval to proceed with testing given any associated medical diagnoses. Participants received the Physical Activity Readiness Questionnaire (PARQ) prior to testing to screen for appropriateness to participate. Biometrics including height, weight, waist circumference, blood pressure, resting heart rate, and pulse oximetry were measured prior to exercise testing (Table 1). Consistent with ACSM/AHA guidelines, any participant with a vital sign measurement beyond established parameters was excluded from the testing protocol. Medication use and chronic diseases were not considered exclusionary for participation if they could tolerate a sustained, upright cycling position and pedal without pain. Participants on routine prescription medications were instructed to maintain their medication regimen as prescribed. The temperature within the building remained at 72 degrees Fahrenheit with normal controlled humidity. All relevant data was entered into a digital-based testing platform (OVAL Admin app) on an iOS or Android-based tablet.

	WC (cm)	BMI	SBP	DBP	RHR	Rest La	Age	SpO2	Weight (lbs)	Height (in)	Metflex Index
mean	110.6	34.6	127.4	79.4	77.2	1.2	47.5	97.3	225.3	67.7	18.6
standard error	2.6	0.9	1.2	0.7	1.4	0.0	1.6	0.2	6.1	0.5	1.3
median	107.0	33.1	128.0	80.0	75.0	1.1	48.0	98.0	220.0	68.0	16.7
mode	81.0	27.8	124.0	80.0	99.0	1.0	44.0	98.0	210.0	68.0	9.3
standard deviation	21.4	8.1	10.8	6.2	12.0	0.4	13.9	1.5	53.4	4.2	11.1
sample variance	456.5	65.6	117.2	38.3	143.8	0.2	193.8	2.2	2853.3	17.4	122.4
kurtosis	-0.8	-0.1	1.3	0.4	-0.8	0.5	-0.5	0.0	-0.4	-0.3	0.3
skewness	0.3	0.6	0.2	-0.4	0.3	0.8	-0.2	-0.8	0.2	-0.2	0.8
range	82.0	34.3	62.0	30.0	44.0	2.0	59.0	6.0	234.0	19.5	47.7
minimum	74.0	20.7	98.0	62.0	55.0	0.5	18.0	93.0	126.0	56.0	2.8
maximum	156.0	55.0	160.0	92.0	99.0	2.5	77.0	99.0	360.0	75.5	50.5

Table 1. Summary distribution and descriptive statistics relative to each biometric. WC, waist circumference; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; RHR, resting heart rate; Rest La, resting lactate; SpO2, pulse oxygenation.

7 Testing Protocols

Participants were scheduled for a test after exclusion and inclusion criteria were met. Participants were instructed not to perform vigorous exercise or activity at least 24 hrs. prior to the test, maintain normal and adequate hydration, maintain normal sleeping patterns, avoid alcohol, maintain a normal medication regimen, maintain a normal eating pattern, ingest a small snack 2 hours before the test, wear comfortable clothing, and bring a towel and water bottle. Upon arrival, participants were escorted to a private room for vital signs and biometric assessments. Participants were asked about their current exercise and activity patterns and if they were experiencing any new onset of symptoms associated with poor tolerance to movement including shortness of breath, chest pain, dizziness, nausea, etc. Following vital signs assessments, participants were explained the testing protocol and consented to proceed with the exercise test. Participants were informed they could stop testing at any time at their discretion. Participants were fitted with a heart rate monitor (Polar H10, chest strap) per manufacturer guidelines prior to testing.

For testing comfort, participants were fitted on an upright exercise cycle (commercial fitness model) maintaining knee extension at down stroke between approximately 5 to 15 degrees from full extension minimizing pelvic compensations at the saddle. The heart rate monitor was paired and connected to the OVAL Admin app on the tablet for testing. Resting blood lactate was sampled from the participant's left earlobe using standard precautions and appropriate PPE. The earlobe was prepped with an alcohol swab and allowed to dry prior to 23G safety spring lancet introduction. After successfully establishing access, a single blood drop was expressed and then removed with tissue (or gauze) and a second drop was expressed and sampled with a lactate strip and meter (Nova Biomedical Lactate Plus Meter and Strips). After thirteen seconds the results were available and entered in the OVAL Admin app. Following the resting lactate entry, a testing protocol was determined with a digital-based algorithm. The protocol selection criteria include resting heart rate, BMI, resting lactate level, exercise frequency, and yes/no taking medications for a chronic condition. Based on the values entered, the algorithm assigned the participant to either a 5-, 15-, or 30-watt progression.

Testing commenced once a protocol was determined. The participant was instructed to maintain a specific level of starting Watts on the cycle as displayed in the Watts section on the cycle's dashboard. The participant was instructed to maintain between 60-80 rpms the entire test except in the early stages during very low wattage where rpms maintained were typically below 60. The OVAL Admin app maintained a timer set to three minutes for each stage duration with a thirty second alert for the tester to prepare for the next lactate sample. The Watts progressed and maintained at each stage were monitored throughout testing by the participant and the tester. Stages were advanced every three minutes with progressive increases in Watts as per the protocol for the individual (protocol selection criteria). The participant's heart rate was automatically recorded throughout the test as the monitor was paired and connected to the OVAL Admin app. The lactate level was entered into the OVAL Admin app manually by the tester following successful blood sampling and lactate meter analysis. The testing ceased based on an algorithm indicating that a specific level of lactate was attained or surpassed. Following completion of the test, the OVAL Admin app transitioned into a recovery screen where the participant "cooled-down" for ten minutes. During recovery, the participant's heart rate was automatically entered in the app every minute on the minute. Additional data was collected during the recovery phase including the first- and second-

minute heart rate recovery rates and five- and ten-minute post-test lactate samples. Recovery data was not part of the analysis in this report.

8 Results and Interpretation

No adverse events were observed during or after the exercise testing protocols. No infections were reported within 7 days after the testing.

Figure 1., below demonstrates the distribution of MetFlex Index™ scores across the sample. The lower the score, the lower the metabolic flexibility and fitness, and vice-versa.

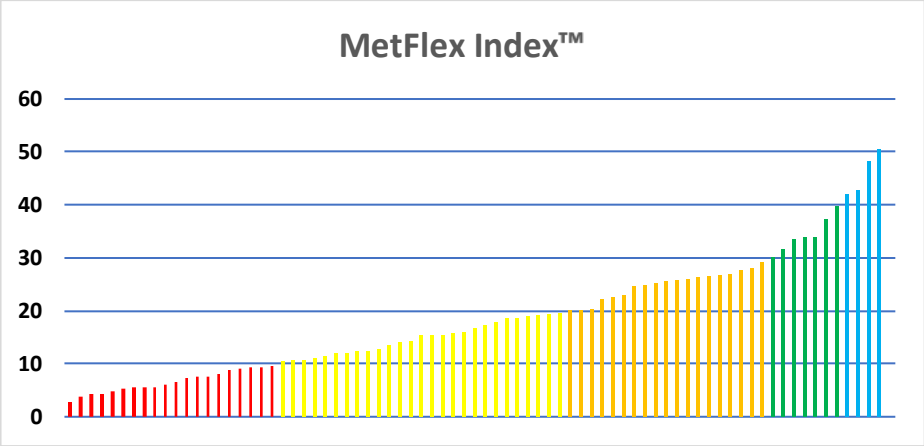

Figure 1. Summary of MetFlex Index™ scores. Red indicates MetFlex Indexes between 0-9.99. Yellow indicates MetFlex Indexes between 10 and 19.99. Orange indicates MetFlex Indexes between 20 and 29.99. Green indicates MetFlex Indexes between 30 and 39.99. Blue indicates MetFlex Indexes of 40 and above. The MetFlex Index affords stratification of fitness relative to lower (in red) to higher fitness levels (in blue).

Table 2. below displays the associations between the MetFlex Index™ and secondary variables collected.

Variable	R	R2	Adj. R2	coef var	std err	p-value
WC (cm)	-0.45	0.20	0.19	-0.22	0.06	0.00
BMI	-0.52	0.27	0.26	-0.71	0.13	0.00
SBP	-0.26	0.07	0.05	-0.26	0.11	0.02
DBP	-0.20	0.04	0.03	-0.35	0.20	0.08
RHR	-0.33	0.11	0.10	-0.30	0.10	0.00
Rest La	-0.35	0.13	0.11	-9.37	2.86	0.00
Age	-0.40	0.16	0.15	-0.32	0.08	0.00
Sp02	0.18	0.03	0.02	1.36	0.85	0.11
Weight (lbs)	-0.33	0.11	0.09	-0.07	0.02	0.00
Height (in)	0.38	0.14	0.13	1.00	0.28	0.00

Table 2. Summary of associations between MetFlex Index™ and other collected secondary variables.

Table 3., below displays the summary of means and standard deviations between four MetFlex Index™ decile groupings and 40+ grouping. Decile groupings were provided to observe the scaling of the MetFlex Index™ and each secondary variable.

	WC (cm)		BMI		SBP		DBP		RHR		Rest La		Age		Sp02		Weight (lbs)		Height (in)	
	mean	std	mean	std	mean	std	mean	std	mean	std	mean	std	mean	std	mean	std	mean	std	mean	std
MetflexGroups																				
0-9.99	117.9	18.3	39.6	7.7	131.7	8.3	80.8	5.4	81.1	11.4	1.4	0.5	54.8	11.5	96.8	1.7	238.5	52.3	65.0	3.7
10-19.99	117.8	22.2	36.1	8.0	129.6	12.1	81.6	6.9	79.0	10.9	1.3	0.4	51.4	11.3	97.1	1.5	238.9	59.3	68.1	4.7
20-29.99	99.3	19.1	31.3	6.7	120.9	10.0	75.1	5.3	73.5	10.2	1.0	0.3	38.2	13.9	97.8	1.3	208.0	44.7	68.4	2.6
30-39.99	95.6	9.4	27.7	2.8	124.3	7.5	78.6	2.2	75.1	14.6	1.0	0.3	37.7	15.3	97.4	1.3	196.7	33.9	70.4	4.1
40+	94.0	28.3	27.9	4.9	127.5	9.8	80.5	4.7	68.0	20.2	1.0	0.4	46.8	6.6	97.8	1.0	198.5	50.7	70.3	3.3

Table 3. Summary of means and standard deviations between MetFlex Index™ groups.

Age and MetFlex Index™

The mean age was 47.5 years old with a range from 18 to 77 years. The pattern observed was inverse and negative as the greater the age of the participant, the lower the MetFlex Index™ and, reciprocally, the lower the age of the participant, the higher the MetFlex Index™ (Figure 2.). This pattern is consistent with historical VO2max findings where higher VO2max (aerobic capacity) is associated with younger to middle-aged adults and lower VO2max is associated with older adults. Noteworthy, there was variation observed in fitness levels within each age group suggesting that consistent exposure to movement via exercise and physical activity affords and sustains higher levels of fitness across the lifespan. Additionally, this is a small sample with mixed age and sex at various stages of acute and chronic rehabilitation, which could affect the age and metabolic fitness relationship.

Figure 2. Regression between Age and MetFlex Index™

Table 4. displays the effect sizes (ES) between the lowest decile of the MetFlex Index™, 0-9.99, which we established as our control for comparison, to four other MetFlex Index™ groups relative to Age. Patterning suggests a greater effect size with higher levels of fitness relative to age. The 40+

MetFlex Index™ group has a smaller number of participants and may be affecting the trend in the effect size.

Variable	Control	Exp	ES
Age	0-9.99	10-19.99	0.29
Age	0-9.99	20-29.99	1.30
Age	0-9.99	30-39.99	1.36
Age	0-9.99	40+	0.73

Table 4. Effect Sizes between Age and MetFlex Index™ groups.

Table 5. compares our lowest MetFlex Index™ decile group to four other groups with progressively higher fitness. Confidence Intervals, T-stats, and P-values are displayed for comparison. The 40+ MetFlex Index™ group has a smaller sample size which may explain the statistical discrepancies in trending in that comparison group.

Variable	Group1	Group2	Mean Diff	Pooled SD	Std Er	CI (95%)	T-stat	P-value
Age	0-9.99	10-19.99	3.36	11.42	3.38	(-3.3, 10)	1.00	0.32
Age	0-9.99	20-29.99	16.64	12.76	4.11	(8.6, 24.7)	4.07	0.00
Age	0-9.99	30-39.99	17.09	12.55	6.34	(4.7, 29.5)	3.10	0.00
Age	0-9.99	40+	8.05	10.99	4.19	(-0.2, 16.3)	1.34	0.19

Table 5. Confidence Intervals and T-stats between Age and MetFlex Index™ groups.

Body Mass Index and MetFlex Index™

The mean BMI was 34.6 with a range from 20.7 to 55. The pattern observed was inverse and negative as the greater the MetFlex Index™ of the participant, the lower the BMI and, reciprocally, the lower the MetFlex Index™, the higher the BMI (Figure 3.). This pattern is consistent with historical findings of higher VO2max levels associated with lower BMIs. However, there was variation in fitness levels across each BMI group suggesting that consistent movement as exercise and physical activity affords higher levels of fitness across BMI levels. Additionally, this study only quantifies BMI and not body composition such as skeletal muscle mass and fat mass. BMI is also the denominator within the MetFlex Index™ equation.

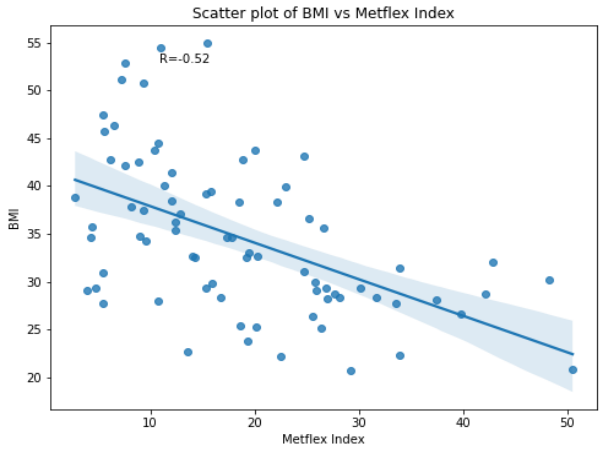

Figure 3. Regression between BMI and MetFlex Index™

Table 6. displays the effect sizes between the lowest decile of MetFlex Index™, 0-9.99, which we established as our control for comparison, to four other MetFlex Index™ groups relative to BMI. Patterning suggests greater effect size with higher levels of fitness relative to BMI. The 40+ MetFlex Index™ group has a smaller number of participants and may be affecting the pattern.

Variable	Control	Exp	ES
BMI	0-9.99	10-19.99	0.45
BMI	0-9.99	20-29.99	1.16
BMI	0-9.99	30-39.99	1.73
BMI	0-9.99	40+	1.58

Table 6. Effect Sizes between BMI and MetFlex Index™ groups.

Table 7. compares our lowest MetFlex Index™ group to four other groups with progressively higher fitness relative to BMI. Confidence Intervals, T-stats, and P-values are displayed for comparison. The 40+ MetFlex Index™ group has a smaller sample size which may explain the discrepancies in that comparison group. Additionally, BMI is the denominator in our power to weight ratio so BMI could be confounding here.

Variable	Group1	Group2	Mean Diff	Pooled SD	Std Er	CI (95%)	T-stat	P-value
BMI	0-9.99	10-19.99	3.56	7.87	2.31	(-1, 8.1)	1.53	0.13
BMI	0-9.99	20-29.99	8.34	7.22	2.30	(3.8, 12.9)	3.61	0.00
BMI	0-9.99	30-39.99	11.91	6.87	2.03	(7.9, 15.9)	3.95	0.00
BMI	0-9.99	40+	11.70	7.40	3.01	(5.8, 17.6)	2.89	0.01

Table 7. Confidence Intervals and T-stats between BMI and MetFlex Index™ groups.

Waist Circumference and MetFlex Index™

The mean Waist Circumference in centimeters was 110.6 with a range from 74 to 156. The pattern observed was inversely and negatively correlated as the greater the MetFlex Index™ of the participant, the lower the Waist Circumference and, reciprocally, the lower the MetFlex Index™, the greater the Waist Circumference (Figure 4.). This pattern is consistent with historical VO2max findings where a higher VO2max is associated with lower Waist Circumference, an indirect measure of visceral fat. However, there was variation in fitness levels across each Waist Circumference group indicating that it may be that consistent movement as exercise and physical activity affords higher levels of fitness across Waist Circumference levels.

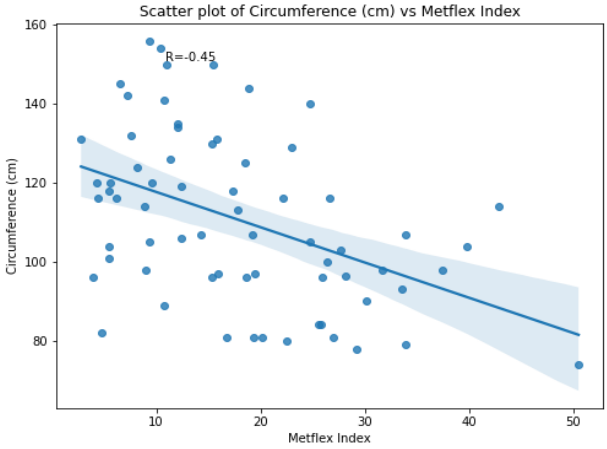

Figure 4. Regression between Waist Circumference and MetFlex Index™

Table 7. displays the effect sizes between the lowest decile of MetFlex Index™, 0-9.99, which we established as our control for comparison, to four other MetFlex Index™ groups relative to Waist Circumference. Patterning suggests greater effect size with higher levels of fitness relative to Waist Circumference. The 40+ MetFlex Index™ group has a smaller number of participants and may be affecting the pattern.

Variable	Control	Exp	ES
WC	0-9.99	10-19.99	0.01
WC	0-9.99	20-29.99	1.00
WC	0-9.99	30-39.99	1.35
WC	0-9.99	40+	1.26

Table 7. Effect Sizes between Waist Circumference and MetFlex Index™ groups.

Table 8. compares our lowest MetFlex Index™ group to four other groups with progressively higher fitness relative to Waist Circumference. Confidence Intervals, T-stats, and P-values are displayed for

comparison. The 40+ MetFlex Index™ group has a smaller sample size which may explain the statistical discrepancies in that comparison group.

Variable	Group1	Group2	Mean Diff	Pooled SD	Std Er	CI (95%)	T-stat	P-value
WC (cm)	0-9.99	10-19.99	0.10	20.59	6.18	(-12, 12.2)	0.02	0.99
WC (cm)	0-9.99	20-29.99	18.59	18.66	6.48	(5.9, 31.3)	2.88	0.01
WC (cm)	0-9.99	30-39.99	22.32	16.51	5.49	(11.6, 33.1)	3.06	0.01
WC (cm)	0-9.99	40+	23.89	18.95	20.44	(-16.2, 63.9)	1.70	0.11

Table 8. Confidence Intervals and T-stats between Waist Circumference and MetFlex Index™ groups.

Height and MetFlex Index™

The mean Height in inches was 67.7 with a range from 56 to 75.5. The pattern observed was direct and positive as the greater the MetFlex Index™ of the participant, the greater the participant's Height and, reciprocally, the lower the MetFlex Index™, the lower the Height. (Figure 5.). However, there was variation in fitness levels across each Height group indicating that it may be that consistent movement as exercise and physical activity affords higher levels of fitness across all Height levels.

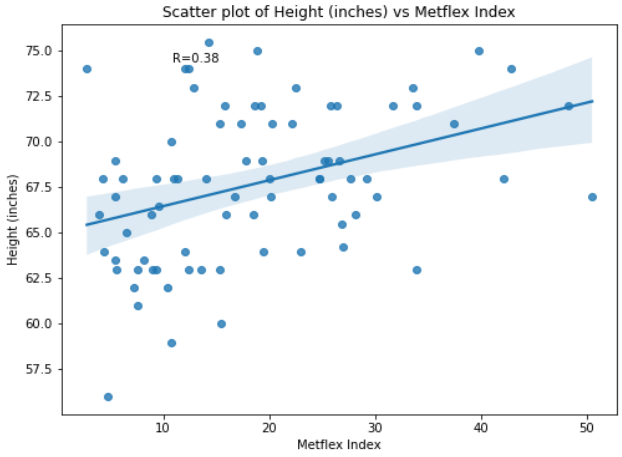

Figure 5. Regression between Height and MetFlex Index™

Table 9. displays the effect sizes between the lowest decile of MetFlex Index™, 0-9.99, which we established as our control for comparison, to four other MetFlex Index™ groups relative to Height. Patterning suggests greater effect size with higher levels of fitness relative to Height. The 40+ MetFlex Index™ group has a small number of participants and may be affecting the pattern.

Variable	Control	Exp	ES
Height (in)	0-9.99	10-19.99	0.73
Height (in)	0-9.99	20-29.99	1.08
Height (in)	0-9.99	30-39.99	1.44
Height (in)	0-9.99	40+	1.45

Table 9. Effect Sizes between Height and MetFlex Index™ groups.

Table 10. compares our lowest MetFlex Index™ group to four other groups with progressively higher fitness relative to Height. Confidence Intervals, T-stats, and P-values are displayed for comparison. The 40+ MetFlex Index™ group has a smaller sample size which may explain the statistical discrepancies in that comparison group.

Variable	Group1	Group2	Mean Diff	Pooled SD	Std Er	CI (95%)	T-stat	P-value
Height (in)	0-9.99	10-19.99	-3.12	4.30	1.22	(-5.5, -0.7)	-2.46	0.02
Height (in)	0-9.99	20-29.99	-3.43	3.19	1.01	(-5.4, -1.4)	-3.36	0.00
Height (in)	0-9.99	30-39.99	-5.45	3.79	1.75	(-8.9, -2)	-3.28	0.00
Height (in)	0-9.99	40+	-5.28	3.65	1.85	(-8.9, -1.7)	-2.64	0.01

Table 10. Confidence Intervals and T-stats between Height and MetFlex Index™ groups.

Weight and MetFlex Index™

The mean Weight in pounds was 225.3 with a range from 126 to 360. The pattern observed was inverse and negative as the greater the MetFlex Index™ of the participant, the lower the participant's Weight and, reciprocally, the lower the MetFlex Index™, the greater the Weight. (Figure 6.). However, there was variation in fitness levels across each Weight group indicating that it may be that consistent movement as exercise and physical activity affords higher levels of fitness across all Weight levels.

Figure 6. Regression between Weight and MetFlex Index™

Table 11. displays the effect sizes between the lowest decile of MetFlex Index™, 0-9.99, which we established as our control for comparison, to four other MetFlex Index™ groups relative to Weight. Patterning suggests greater effect size with higher levels of fitness relative to Weight. The 40+ MetFlex Index™ group has a small number of participants and may be affecting the pattern.

Variable	Control	Exp	ES
Weight (lbs)	0-9.99	10-19.99	-0.01
Weight (lbs)	0-9.99	20-29.99	0.63
Weight (lbs)	0-9.99	30-39.99	0.86
Weight (lbs)	0-9.99	40+	0.77

Table 11. Effect Sizes between Weight and MetFlex Index™ groups.

Table 12. compares our lowest MetFlex Index™ group to four other groups with progressively higher fitness relative to Weight. Confidence Intervals, T-stats, and P-values are displayed for comparison. The 40+ MetFlex Index™ group has a smaller sample size which may explain the discrepancies in that comparison group.

Variable	Group1	Group2	Mean Diff	Pooled SD	Std Er	CI (95%)	T-stat	P-value
Weight (lbs)	0-9.99	10-19.99	-0.37	56.48	16.35	(-32.4, 31.7)	-0.02	0.98
Weight (lbs)	0-9.99	20-29.99	30.54	48.75	15.55	(0.1, 61)	1.96	0.06
Weight (lbs)	0-9.99	30-39.99	41.83	48.54	17.35	(7.8, 75.8)	1.96	0.06
Weight (lbs)	0-9.99	40+	40.04	52.09	27.90	(-14.6, 94.7)	1.40	0.17

Table 12. Confidence Intervals and T-stats between Weight and MetFlex Index™ groups.

Resting Lactate and MetFlex Index™

The mean Resting Lactate in mmol was 1.2 with a range from 0.5 to 2.5. The pattern observed was inverse and negative as the greater the MetFlex Index™ of the participant, the lower the participant's Resting Lactate level and, reciprocally, the lower the MetFlex Index™, the greater the Resting Lactate level (Figure 7.). However, there was variation in fitness levels across each Resting Lactate group suggesting that consistent movement as exercise and physical activity affords higher levels of fitness across all Resting Lactate levels.

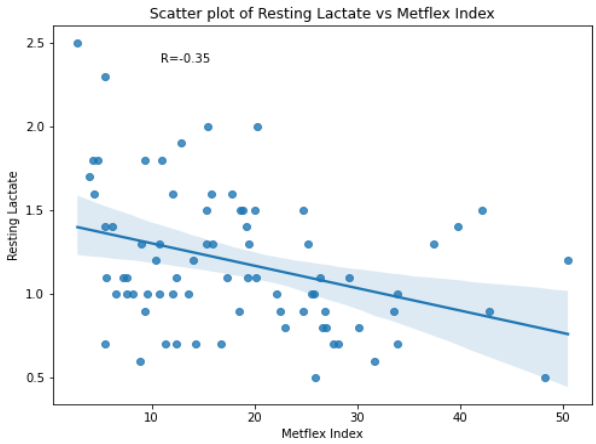

Figure 7. Regression between Resting Lactate and MetFlex Index™

Table 13. displays the effect sizes between the lowest decile of MetFlex Index™, 0-9.99, which we established as our control for comparison, to four other MetFlex Index™ groups relative to Resting Lactate. Patterning suggests greater effect size with higher levels of fitness relative to Resting Lactate. The 40+ MetFlex Index™ group has a small number of participants and may be affecting the pattern.

Variable	Control	Exp	ES
Rest La	0-9.99	10-19.99	0.22
Rest La	0-9.99	20-29.99	0.74
Rest La	0-9.99	30-39.99	0.85
Rest La	0-9.99	40+	0.66

Table 13. Effect Sizes between Resting Lactate and MetFlex Index™ groups.

Table 14. compares our lowest MetFlex Index™ group to four other groups with progressively higher fitness relative to Resting Lactate. Confidence Intervals, T-stats, and P-values are displayed for comparison. The 40+ MetFlex Index™ group has a smaller sample size which may explain the statistical discrepancies in that comparison group.

Variable	Group1	Group2	Mean Diff	Pooled SD	Std Er	CI (95%)	T-stat	P-value
Rest La	0-9.99	10-19.99	0.10	0.43	0.13	(-0.2, 0.4)	0.75	0.46
Rest La	0-9.99	20-29.99	0.32	0.44	0.14	(0.1, 0.6)	2.31	0.03
Rest La	0-9.99	30-39.99	0.40	0.47	0.16	(0.1, 0.7)	1.94	0.06
Rest La	0-9.99	40+	0.33	0.50	0.24	(-0.1, 0.8)	1.21	0.24

Table 14. Confidence Intervals and T-stats between Resting Lactate and MetFlex Index™ groups.

Resting Heart Rate and MetFlex Index™

The mean Resting Heart Rate in beats per minute was 77.2 with a range from 55 to 99. The pattern observed was inverse and negative as the greater the MetFlex Index™ of the participant, the lower the participant's Resting Heart Rate and, reciprocally, the lower the MetFlex Index™, the greater the Resting Heart Rate (Figure 8.). However, there was variation in fitness levels across each Resting Heart Rate group suggesting that consistent movement as exercise and physical activity affords higher levels of fitness across all Resting Heart Rate levels.

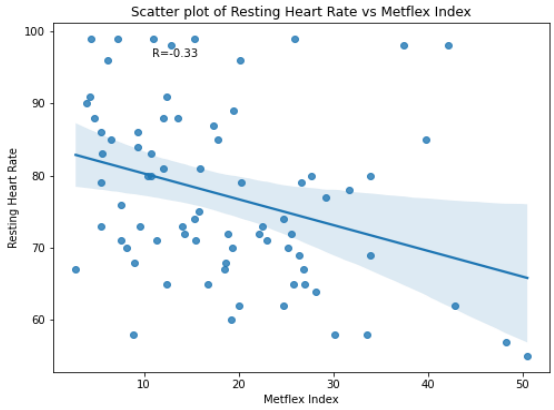

Figure 8. Regression between Resting Heart Rate and MetFlex Index™

Table 15. displays the effect sizes between the lowest decile of MetFlex Index™, 0-9.99, which we established as our control for comparison, to four other MetFlex Index™ groups relative to Resting Heart Rate. Patterning suggests greater effect size with higher levels of fitness relative to Resting Heart Rate. The 40+ MetFlex Index™ group has a small number of participants and may be affecting the pattern.

Variable	Control	Exp	ES
RHR	0-9.99	10-19.99	0.19
RHR	0-9.99	20-29.99	0.71
RHR	0-9.99	30-39.99	0.49
RHR	0-9.99	40+	1.01

Table 15. Effect Sizes between Resting Heart Rate and MetFlex Index™ groups.

Table 16. compares our lowest MetFlex Index™ group to four other groups with progressively higher fitness relative to Resting Heart Rate. Confidence Intervals, T-stats, and P-values are displayed for comparison. The 40+ MetFlex Index™ group has a smaller sample size which may explain the discrepancies in that comparison group.

Variable	Group1	Group2	Mean Diff	Pooled SD	Std Er	CI (95%)	T-stat	P-value
RHR	0-9.99	10-19.99	2.14	11.10	3.30	(-4.3, 8.6)	0.65	0.52
RHR	0-9.99	20-29.99	7.63	10.79	3.45	(0.9, 14.4)	2.21	0.03
RHR	0-9.99	30-39.99	5.96	12.22	6.07	(-5.9, 17.9)	1.11	0.28
RHR	0-9.99	40+	13.10	12.93	10.42	(-7.3, 33.5)	1.85	0.08

Table 16. Confidence Intervals and T-stats between Resting Heart Rate and MetFlex Index™ groups.

Systolic Blood Pressure and MetFlex Index™

The mean Systolic Blood Pressure in mmHg was 127.4 with a range from 98 to 160. The pattern observed was inversely and negative as the greater the MetFlex Index™ of the participant, the lower the participant's Systolic Blood Pressure and, reciprocally, the lower the MetFlex Index™, the greater the Systolic Blood Pressure (Figure 9.). However, there is variation in fitness levels across each Systolic Blood Pressure group suggesting that consistent movement as exercise and physical activity affords higher levels of fitness across all Systolic Blood Pressure levels.

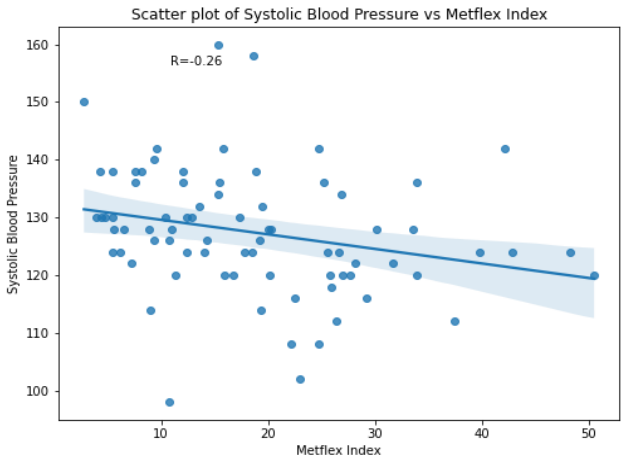

Figure 9. Regression between Systolic Blood Pressure and MetFlex Index™

Table 17. displays the effect sizes between the lowest decile of MetFlex Index™, 0-9.99, which we established as our control for comparison, to four other MetFlex Index™ groups relative to Systolic Blood Pressure. Patterning suggests greater effect size with higher levels of fitness relative to Systolic Blood Pressure. The 40+ MetFlex Index™ group, and the overall smaller sample size, may explain the discrepancies in that comparison group and overall trending.

Variable	Control	Exp	ES
SBP	0-9.99	10-19.99	0.19
SBP	0-9.99	20-29.99	1.18
SBP	0-9.99	30-39.99	0.92
SBP	0-9.99	40+	0.49

Table 17. Effect Sizes between Systolic Blood Pressure and MetFlex Index™ groups.

Table 18. compares our lowest MetFlex Index™ group to four other groups with progressively higher fitness relative to Systolic Blood Pressure. Confidence Intervals, T-stats, and P-values are displayed for comparison. The 40+ MetFlex Index™ group, and the overall smaller sample size, may explain the discrepancies in that comparison group and overall trending.

Variable	Group1	Group2	Mean Diff	Pooled SD	Std Er	CI (95%)	T-stat	P-value
SBP	0-9.99	10-19.99	2.07	10.67	2.98	(-3.8, 7.9)	0.66	0.51
SBP	0-9.99	20-29.99	10.75	9.14	2.94	(5, 16.5)	3.67	0.00
SBP	0-9.99	30-39.99	7.41	8.09	3.39	(0.8, 14.1)	2.09	0.05
SBP	0-9.99	40+	4.20	8.50	5.26	(-6.1, 14.5)	0.90	0.38

Table 18. Confidence Intervals and T-stats between Systolic Blood Pressure and MetFlex Index™ groups

Diastolic Blood Pressure and MetFlex Index™

The mean Diastolic Blood Pressure in mmHg was 79.4 with a range from 62 to 92. The pattern observed was inverse and negative as the greater the MetFlex Index™ of the participant, the lower the participant's Diastolic Blood Pressure and, reciprocally, the lower the MetFlex Index™, the higher the Diastolic Blood Pressure (Figure 10.). However, there was variation in fitness levels across each Diastolic Blood Pressure group suggesting that consistent movement as exercise and activity affords higher levels of fitness across all Diastolic Blood Pressure levels.

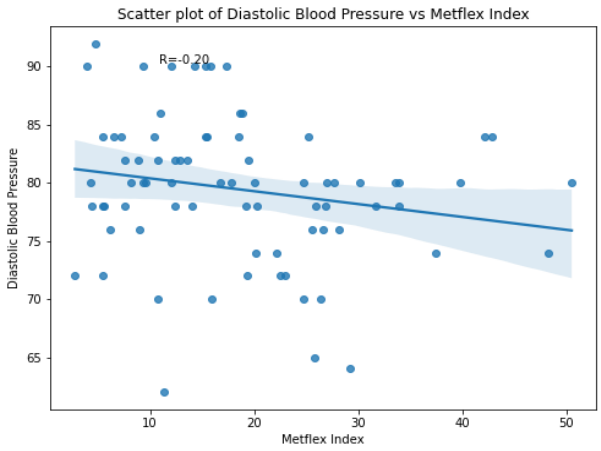

Figure 10. Regression between Diastolic Blood Pressure and MetFlex Index™

Table 19. displays the effect sizes between the lowest decile of MetFlex Index™, 0-9.99, which we established as our control for comparison, to four other MetFlex Index™ groups relative to Diastolic Blood Pressure. Patterning suggests greater effect size with higher levels of fitness relative to Diastolic Blood Pressure. The 40+ MetFlex Index™ group, and the overall smaller sample size, may explain the discrepancies in that comparison group and overall trending.

Variable	Control	Exp	ES
DBP	0-9.99	10-19.99	-0.12
DBP	0-9.99	20-29.99	1.06
DBP	0-9.99	30-39.99	0.46
DBP	0-9.99	40+	0.06

Table 19. Effect Sizes between Diastolic Blood Pressure and MetFlex Index™ groups.

Table 20. compares our lowest MetFlex Index™ group to four other groups with progressively higher fitness relative to Diastolic Blood Pressure. Confidence Intervals, T-stats, and P-values are displayed for comparison. The 40+ MetFlex Index™ group, and the overall smaller sample size, may explain the discrepancies in that comparison group and overall trending.

Variable	Group1	Group2	Mean Diff	Pooled SD	Std Er	CI (95%)	T-stat	P-value
DBP	0-9.99	10-19.99	-0.76	6.31	1.80	(-4.3, 2.8)	-0.41	0.69
DBP	0-9.99	20-29.99	5.69	5.37	1.72	(2.3, 9.1)	3.31	0.00
DBP	0-9.99	30-39.99	2.23	4.87	1.48	(-0.7, 5.1)	1.04	0.31
DBP	0-9.99	40+	0.30	5.35	2.66	(-4.9, 5.5)	0.10	0.92

Table 20. Confidence Intervals and T-stats between Diastolic Blood Pressure and MetFlex Index™ groups.

Pulse Oxygenation and MetFlex Index™

The mean Pulse Oxygenation in percent was 97.3 with a range from 93 to 99. The pattern observed was direct and positive as the greater the MetFlex Index™ of the participant, the greater the participant's Pulse Oxygenation and the lower the MetFlex Index™, the lower the Pulse Oxygenation (Figure 11.). However, there is variation in fitness levels across each Pulse Oxygenation group suggesting that consistent movement as exercise and physical activity affords higher levels of fitness across all Pulse Oxygenation levels.

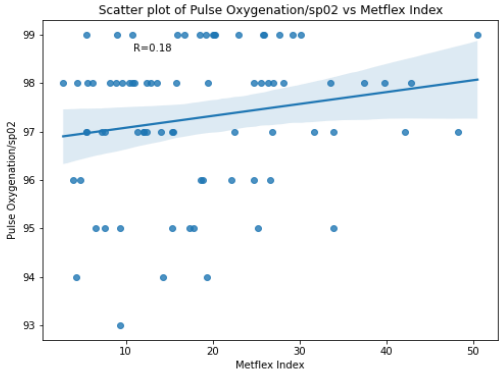

Figure 11. Regression between Pulse Oxygenation and MetFlex Index™

Table 21. displays the effect sizes between the lowest decile of MetFlex Index™, 0-9.99, which we established as our control for comparison, to four other MetFlex Index™ groups relative to Pulse Oxygenation. Patterning suggests greater effect size with higher levels of fitness relative to Pulse Oxygenation. The 40+ MetFlex Index™ group and a smaller number of participants in the sample may be affecting the pattern.

Variable	Control	Exp	ES
SpO2	0-9.99	10-19.99	0.22
SpO2	0-9.99	20-29.99	0.69
SpO2	0-9.99	30-39.99	0.40
SpO2	0-9.99	40+	0.60

Table 21. Effect Sizes between Pulse Oxygenation and MetFlex Index™ groups.

Table 22. compares our lowest MetFlex Index™ group to four other groups with progressively higher fitness relative to Pulse Oxygenation. Confidence Intervals, T-stats, and P-values are displayed for comparison. The 40+ MetFlex Index™ group has a smaller sample size which may explain the discrepancies in that comparison group.

Variable	Group1	Group2	Mean Diff	Pooled SD	Std Er	CI (95%)	T-stat	P-value
SpO2	0-9.99	10-19.99	-0.35	1.58	0.47	(-1.3, 0.6)	-0.75	0.46
SpO2	0-9.99	20-29.99	-1.04	1.50	0.48	(-2, -0.1)	-2.16	0.04
SpO2	0-9.99	30-39.99	-0.63	1.59	0.61	(-1.8, 0.6)	-0.90	0.38
SpO2	0-9.99	40+	-0.95	1.59	0.61	(-2.1, 0.2)	-1.09	0.29

Table 22. Confidence Intervals and T-stats between Pulse Oxygenation and MetFlex Index™ groups.

9 Discussion

This observational study evaluated a sub-maximal exercise-based assessment using a lactate biomarker for describing metabolic flexibility. Initial findings support a safe and feasible application in adults in an outpatient rehabilitation setting. The MetFlex Index™ demonstrated patterns with common metrics of metabolic health providing utility of the Index for describing metabolic fitness in adult populations. Although a small sample size, we observed trending in various metrics with either increasing or decreasing MetFlex Index™ scores (in deciles). A higher MetFlex Index™ was associated with improved or normal values of metabolic health whereas a lower MetFlex Index™ was associated with worse or outside normal values of metabolic health. Larger samples will provide further clarification on the relationships between the MetFlex Index™ and other secondary variables of interest. Future studies that incorporate body composition may provide additional patterns of the MetFlex Index™ including metabolic flexibility and fitness relative to body fat mass, visceral fat, and skeletal muscle mass.

10 Conclusion

Historically, lactate was only assessed in critical care populations and in elite athletic performance. Our findings support an application for using lactate for assessing fitness in the general population. Additional applications for quantifying metabolic flexibility in clinical and nonclinical settings are needed as current methods of cardiopulmonary exercise testing (VO2) are not easily

scalable due to poor accessibility to testing labs. This is partly due to: current staffing limitations and extensive education and training requirements; the technical aspects related to the management of lab equipment; and the facility-related costs of managing and maintaining a lab. Even if a lab were accessible (mostly in urban environments), one requires considerable ill health to participate in a cardiopulmonary exercise test (or stress test). Given the current state and trajectory of poor metabolic health and low fitness in the U.S., fitness tests should be scaled for access across health care and established as a standard of care, i.e., an "active" vital sign. Accessibility, safety, ease of testing, cost effectiveness, and capacity to scale within urban and rural outpatient settings are some potential benefits to using a lactate-based metabolic biomarker for examining metabolic flexibility in health, fitness, and potentially, disease and chronic condition management.

11 References

1. Hirode G, Wong RJ. Trends in the Prevalence of Metabolic Syndrome in the United States, 2011-2016. JAMA. 2020 Jun 23;323(24):2526-2528. doi: 10.1001/jama.2020.4501. PMID: 32573660; PMCID: PMC7312413.
2. Hales CM, Carroll MD, Fryar CD, Ogden CL. Prevalence of obesity and severe obesity among adults: United States, 2017–2018. NCHS Data Brief, no 360. Hyattsville, MD: National Center for Health Statistics. 2020
3. Centers for Disease Control and Prevention. National Diabetes Statistics Report, 2020. Atlanta, GA: Centers for Disease Control and Prevention, U.S. Dept of Health and Human Services; 2020.
4. Liu D, Zhang T, Wang Y, Xia L. The Centrality of Obesity in the Course of Severe COVID-19. Front Endocrinol (Lausanne). 2021 Mar 11;12:620566. doi: 10.3389/fendo.2021.620566. PMID: 33776917; PMCID: PMC7992974
5. Alamdari NM, Rahimi FS, Afaghi S, Zarghi A, Qaderi S, Tarki FE, Ghafouri SR, Besharat S. The impact of metabolic syndrome on morbidity and mortality among intensive care unit admitted COVID-19 patients. Diabetes Metab Syndr. 2020 Nov-Dec;14(6):1979-1986. doi: 10.1016/j.dsx.2020.10.012. Epub 2020 Oct 13. PMID: 33080538; PMCID: PMC7550894.
6. Burtscher J, Burtscher M, Millet GP. The central role of mitochondrial fitness on antiviral defenses: An advocacy for physical activity during the COVID-19 pandemic. Redox Biol. 2021 Apr 23;43:101976. doi: 10.1016/j.redox.2021.101976. Epub ahead of print. PMID: 33932869; PMCID: PMC8062414.
7. Cunningham C, O' Sullivan R, Caserotti P, Tully MA. Consequences of physical inactivity in older adults: A systematic review of reviews and meta-analyses. Scand J Med Sci Sports. 2020 May;30(5):816-827. doi: 10.1111/sms.13616. Epub 2020 Feb 4. PMID: 32020713.
8. Le Roux E, De Jong NP, Blanc S, Simon C, Bessesen DH, Bergouignan A. Physiology of physical inactivity, sedentary behaviours and non-exercise activity: insights from the space bedrest model. J Physiol. 2021 Jan 27. doi: 10.1113/JP281064. Epub ahead of print. PMID: 33501660.
9. Tabozzi SA, Di Santo SG, Franchini F, Ratto F, Luchi M, Filiputti B, Ardigò LP, Lafortuna CL. Sedentary Behaviors and Physical Activity Habits Independently Affect Fat Oxidation in Fasting Conditions and Capillary Glucose Levels After Standardized Glucose-Rich Meal in Healthy Females. Front Physiol. 2020 Jul 7;11:710. doi: 10.3389/fphys.2020.00710. PMID: 32733264; PMCID: PMC7358445.

10. Rynders CA, Blanc S, DeJong N, Bessesen DH, Bergouignan A. Sedentary behaviour is a key determinant of metabolic inflexibility. J Physiol. 2018 Apr 15;596(8):1319-1330. doi: 10.1113/JP273282. Epub 2017 Jul 4. PMID: 28543022; PMCID: PMC5899985.
11. Pillon NJ, Gabriel BM, Dollet L, Smith JAB, Sardón Puig L, Botella J, Bishop DJ, Krook A, Zierath JR. Transcriptomic profiling of skeletal muscle adaptations to exercise and inactivity. Nat Commun. 2020 Jan 24;11(1):470. doi: 10.1038/s41467-019-13869-w. PMID: 31980607; PMCID: PMC6981202.
12. Rudwill F, O'Gorman D, Lefai E, Chery I, Zahariev A, Normand S, Pagano AF, Chopard A, Damiot A, Laurens C, Hodson L, Canet-Soulas E, Heer M, Meuthen PF, Buehlmeier J, Baecker N, Meiller L, Gauquelin-Koch G, Blanc S, Simon C, Bergouignan A. Metabolic Inflexibility Is an Early Marker of Bed-Rest-Induced Glucose Intolerance Even When Fat Mass Is Stable. J Clin Endocrinol Metab. 2018 May 1;103(5):1910-1920. doi: 10.1210/jc.2017-02267. PMID: 29546280; PMCID: PMC7263792.
13. Booth FW, Roberts CK, Thyfault JP, Ruegsegger GN, Toedebusch RG. Role of Inactivity in Chronic Diseases: Evolutionary Insight and Pathophysiological Mechanisms. Physiol Rev. 2017 Oct 1;97(4):1351-1402. doi: 10.1152/physrev.00019.2016. PMID: 28814614; PMCID: PMC6347102.
14. Thyfault JP, Bergouignan A. Exercise and metabolic health: beyond skeletal muscle. Diabetologia. 2020 Aug;63(8):1464-1474. doi: 10.1007/s00125-020-05177-6. Epub 2020 Jun 11. PMID: 32529412; PMCID: PMC7377236.
15. Krako Jakovljevic N, Pavlovic K, Jotic A, Lalic K, Stoiljkovic M, Lukic L, Milicic T, Macesic M, Stanarcic Gajovic J, Lalic NM. Targeting Mitochondria in Diabetes. Int J Mol Sci. 2021 Jun 21;22(12):6642. doi: 10.3390/ijms22126642. PMID: 34205752; PMCID: PMC8233932.
16. Tsilingiris D, Tzeravini E, Koliaki C, Dalamaga M, Kokkinos A. The Role of Mitochondrial Adaptation and Metabolic Flexibility in the Pathophysiology of Obesity and Insulin Resistance: an Updated Overview. Curr Obes Rep. 2021 Apr 10. doi: 10.1007/s13679-021-00434-0. Epub ahead of print. PMID: 33840072.
17. Bettini S, Quinto G, Neunhaeuserer D, Battista F, Belligoli A, Milan G, Gasperetti A, Vettor R, Ermolao A, Busetto L. Edmonton Obesity Staging System: an improvement by cardiopulmonary exercise testing. Int J Obes (Lond). 2021 May 14. doi: 10.1038/s41366-021-00856-9. Epub ahead of print. PMID: 33990701.
18. Khan A, Van Iterson EH, Laffin LJ. The obesity paradox in heart failure: What is the role of cardiorespiratory fitness? Cleve Clin J Med. 2021 Aug 2;88(8):449-458. doi: 10.3949/ccjm.88a.20098. PMID: 34341029.
19. Saltin B., Gollnick P. D. Skeletal muscle adaptability: significance for metabolism and performance. Handbook of Physiology. Skeletal Muscle.1983Am. Physiol. Soc.Bethesda, MD, sect. 10, chapt. 19, p. 555–632.
20. Egan B, Zierath JR. Exercise metabolism and the molecular regulation of skeletal muscle adaptation. Cell Metab. 2013 Feb 5;17(2):162-84. doi: 10.1016/j.cmet.2012.12.012. PMID: 23395166.
21. Kelley DE, Goodpaster B, Wing RR, Simoneau JA. Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. Am J Physiol. 1999 Dec;277(6):E1130-41. doi: 10.1152/ajpendo.1999.277.6.E1130. PMID: 10600804.
22. Mandarino LJ, Consoli A, Jain A, Kelley DE. Interaction of carbohydrate and fat fuels in human skeletal muscle: impact of obesity and NIDDM. Am J Physiol 270: E463–E470, 1996. doi:10.1152/ajpendo.1996. 270.3.E463.
23. Harper ME, Patti ME. Metabolic terminology: what's in a name? Nat Metab. 2020 Jun;2(6):476-477. doi: 10.1038/s42255-020-0216-7. PMID: 32694727.

24. Goodpaster BH, Sparks LM. Metabolic Flexibility in Health and Disease. Cell Metab. 2017 May 2;25(5):1027-1036. doi: 10.1016/j.cmet.2017.04.015. PMID: 28467922; PMCID: PMC5513193.
25. Smith RL, Soeters MR, Wüst RCI, Houtkooper RH. Metabolic Flexibility as an Adaptation to Energy Resources and Requirements in Health and Disease. Endocr Rev. 2018 Aug 1;39(4):489-517. doi: 10.1210/er.2017-00211. PMID: 29697773; PMCID: PMC6093334.
26. Muoio DM. Metabolic inflexibility: when mitochondrial indecision leads to metabolic gridlock. Cell. 2014 Dec 4;159(6):1253-62. doi: 10.1016/j.cell.2014.11.034. PMID: 25480291; PMCID: PMC4765362.
27. Galgani JE, Fernández-Verdejo R. Pathophysiological role of metabolic flexibility on metabolic health. Obes Rev. 2021 Feb;22(2):e13131. doi: 10.1111/obr.13131. Epub 2020 Aug 19. PMID: 32815226.
28. Tareen SHK, Kutmon M, de Kok TM, Mariman ECM, van Baak MA, Evelo CT, Adriaens ME, Arts ICW. Stratifying cellular metabolism during weight loss: an interplay of metabolism, metabolic flexibility and inflammation. Sci Rep. 2020 Feb 3;10(1):1651. doi: 10.1038/s41598-020-58358-z. PMID: 32015415; PMCID: PMC6997359.
29. Bosch M, Parton RG, Pol A. Lipid droplets, bioenergetic fluxes, and metabolic flexibility. Semin Cell Dev Biol. 2020 Dec;108:33-46. doi: 10.1016/j.semcdb.2020.02.010. Epub 2020 Mar 4. PMID: 32146030.
30. Karwi QG, Uddin GM, Ho KL, Lopaschuk GD. Loss of Metabolic Flexibility in the Failing Heart. Front Cardiovasc Med. 2018 Jun 6;5:68. doi: 10.3389/fcvm.2018.00068. PMID: 29928647; PMCID: PMC5997788.
31. Fritzen AM, Lundsgaard AM, Kiens B. Tuning fatty acid oxidation in skeletal muscle with dietary fat and exercise. Nat Rev Endocrinol. 2020 Dec;16(12):683-696. doi: 10.1038/s41574-020-0405-1. Epub 2020 Sep 22. PMID: 32963340.
32. Galgani JE, Fernández-Verdejo R. Pathophysiological role of metabolic flexibility on metabolic health. Obes Rev. 2021 Feb;22(2):e13131. doi: 10.1111/obr.13131. Epub 2020 Aug 19. PMID: 32815226.
33. Ferguson BS, Rogatzki MJ, Goodwin ML, Kane DA, Rightmire Z, Gladden LB. Lactate metabolism: historical context, prior misinterpretations, and current understanding. Eur J Appl Physiol. 2018 Apr;118(4):691-728. doi: 10.1007/s00421-017-3795-6. Epub 2018 Jan 10. PMID: 29322250.
34. Brooks GA. Lactate as a fulcrum of metabolism. Redox Biol. 2020 Aug;35:101454. doi: 10.1016/j.redox.2020.101454. Epub 2020 Feb 9. PMID: 32113910; PMCID: PMC7284908.
35. Glancy B, Kane DA, Kavazis AN, Goodwin ML, Willis WT, Gladden LB. Mitochondrial lactate metabolism: history and implications for exercise and disease. J Physiol. 2021 Feb;599(3):863-888. doi: 10.1113/JP278930. Epub 2020 May 27. PMID: 32358865.
36. Broskey NT, Zou K, Dohm GL, Houmard JA. Plasma Lactate as a Marker for Metabolic Health. Exerc Sport Sci Rev. 2020 Jul;48(3):119-124. doi: 10.1249/JES.0000000000000220. PMID: 32271180; PMCID: PMC7311283.
37. Poole DC, Rossiter HB, Brooks GA, Gladden LB. The anaerobic threshold: 50+ years of controversy. J Physiol. 2021 Feb;599(3):737-767. doi: 10.1113/JP279963. Epub 2020 Nov 19. PMID: 33112439.
38. Broskey NT, Pories WJ, Jones TE, Tanner CJ, Zheng D, Cortright RN, Yang ZW, Khang N, Yang J, Houmard JA, Lynis Dohm G. The association between lactate and muscle aerobic substrate oxidation: Is lactate an early marker for metabolic disease in healthy subjects? Physiol Rep. 2021 Feb;9(3):e14729. doi: 10.14814/phy2.14729. PMID: 33527749; PMCID: PMC7851428.

39. Jones TE, Pories WJ, Houmard JA, Tanner CJ, Zheng D, Zou K, Coen PM, Goodpaster BH, Kraus WE, Dohm GL. Plasma lactate as a marker of metabolic health: Implications of elevated lactate for impairment of aerobic metabolism in the metabolic syndrome. Surgery. 2019 Nov;166(5):861-866. doi: 10.1016/j.surg.2019.04.017. Epub 2019 Jun 25. PMID: 31253418; PMCID: PMC7142375.
40. San-Millán I, Brooks GA. Assessment of Metabolic Flexibility by Means of Measuring Blood Lactate, Fat, and Carbohydrate Oxidation Responses to Exercise in Professional Endurance Athletes and Less-Fit Individuals. Sports Med. 2018 Feb;48(2):467-479. doi: 10.1007/s40279-017-0751-x. PMID: 28623613.
41. Lagarde D, Jeanson Y, Portais JC, Galinier A, Ader I, Casteilla L, Carrière A. Lactate Fluxes and Plasticity of Adipose Tissues: A Redox Perspective. Front Physiol. 2021 Jun 30;12:689747. doi: 10.3389/fphys.2021.689747. PMID: 34276410; PMCID: PMC8278056.
42. Angelo D'Alessandro (ed.), High-Throughput Metabolomics: Methods and Protocols, Methods in Molecular Biology, vol. 1978, *Blood Biomarkers in Sports Medicine and Performance and the Future of Metabolomics.* Iñigo San-Millán. https://doi.org/10.1007/978-1-4939-9236-2_26,
43. San-Millán I, Stefanoni D, Martinez JL, Hansen KC, D'Alessandro A, Nemkov T. Metabolomics of Endurance Capacity in World Tour Professional Cyclists. Front Physiol. 2020 Jun 5;11:578. doi: 10.3389/fphys.2020.00578. PMID: 32581847; PMCID: PMC7291837.
44. Gutin I. In BMI We Trust: Reframing the Body Mass Index as a Measure of Health. Soc Theory Health. 2018 Aug;16(3):256-271. doi: 10.1057/s41285-017-0055-0. Epub 2017 Oct 25. PMID: 31007613; PMCID: PMC6469873.
45. Weir CB, Jan A. BMI Classification Percentile And Cut Off Points. 2021 May 9. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan–. PMID: 31082114.
46. Sedlmeier AM, Baumeister SE, Weber A, Fischer B, Thorand B, Ittermann T, Dörr M, Felix SB, Völzke H, Peters A, Leitzmann MF. Relation of body fat mass and fat-free mass to total mortality: results from 7 prospective cohort studies. Am J Clin Nutr. 2021 Mar 11;113(3):639-646. doi: 10.1093/ajcn/nqaa339. PMID: 33437985.
47. Whelton SP, McAuley PA, Dardari Z, Orimoloye OA, Brawner CA, Ehrman JK, Keteyian SJ, Al-Mallah M, Blaha MJ. Association of BMI, Fitness, and Mortality in Patients With Diabetes: Evaluating the Obesity Paradox in the Henry Ford Exercise Testing Project (FIT Project) Cohort. Diabetes Care. 2020 Mar;43(3):677-682. doi: 10.2337/dc19-1673. Epub 2020 Jan 16. PMID: 31949085.
48. Herold F, Müller P, Gronwald T, Müller NG. Dose-Response Matters! - A Perspective on the Exercise Prescription in Exercise-Cognition Research. Front Psychol. 2019 Nov 1;10:2338. doi: 10.3389/fpsyg.2019.02338. PMID: 31736815; PMCID: PMC6839278.