


Function outperforms morphology in the assessment of muscular contribution to insulin sensitivity in premenopausal women

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Abstract

Introduction: Skeletal muscle contributes significantly to insulin sensitivity in humans. However, which non-invasive measurement best reflects this contribution remains unknown. Consequently, this paper compares morphologic and functional measurements.

Research methods and design: We conducted a cross-sectional analysis of 144 premenopausal women enrolled in the “Prediction, Prevention, and Sub-classification of Type 2 Diabetes” (PPSDiab) cohort study. For the analysis, we quantified insulin sensitivity by oral glucose tolerance testing and, in a subgroup of 30 women, euglycemic clamp. To assess skeletal muscle, we measured volume by magnetic resonance imaging, intramyocellular lipid content by magnetic resonance spectroscopy, and physical fitness by cardiopulmonary exercise testing.

Results: The mean age of the cohort was 35.7 ± 4.1 years and 94 participants (65%) had a history of gestational diabetes mellitus. Of the morphologic and functional muscle parameters, the maximum workload achieved during cardiopulmonary exercise testing associated most closely with insulin sensitivity (standardized beta = 0.39; $p < .001$). Peak oxygen uptake also demonstrated significant associations, whereas muscle volume and intramyocellular lipid content displayed none.

Conclusion: Functional measurements provided a better assessment of the muscular contribution to insulin sensitivity than morphologic measurements in premenopausal women. In particular, exercise testing rendered an easy and cost-effective method applicable in clinical settings and other human studies.

Keywords

type 2 diabetes, insulin resistance, exercise testing, magnetic resonance imaging, muscle volume

Introduction

Impaired insulin sensitivity maintains a central role in the pathogenesis of type 2 diabetes mellitus (T2DM). It precedes disease manifestation for up to 20 years and, therefore, is an early sign of metabolic dysfunction.¹ Thus, understanding insulin sensitivity remains important to improve T2DM prevention. Insulin sensitivity is mainly determined by a crosstalk between the liver, the adipose tissue, and skeletal muscle.² Skeletal muscle plays a

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particularly important role, because, in healthy individuals, it is responsible for approximately 80% of insulin-stimulated whole-body glucose uptake and disposal.³ On the other hand, T2DM is heterogeneous and its pathophysiology, in particular the contribution of liver and skeletal muscle, differs between the most common disease subtypes.⁴

Therefore, human studies of T2DM pathogenesis depend on non-invasive and feasible measurements that assess the contribution of skeletal muscle to an individual's whole-body insulin sensitivity. However, it remains unclear which non-invasive muscle measurement associates most closely with whole-body insulin sensitivity. In theory, this could be the quantification of muscle mass, as more muscle may absorb more glucose.⁵ Likewise, the intramyocellular lipid content may provide a strong non-invasive and workable measurement as muscular steatosis has been implicated as a cause of reduced insulin sensitivity.^{6,7} Yet, associations of intramyocellular lipid content with whole-body insulin sensitivity have been inconsistent in previous human studies.^{8–10} In addition, measurements of physical fitness may be superior to morphologic studies.^{11,12}

Here, we asked which muscle characteristic associates most closely with whole-body insulin sensitivity in a deeply phenotyped human cohort. Specifically, we compared magnetic resonance imaging of muscle volume, magnetic resonance spectroscopy of intramyocellular lipid content, and measures of physical fitness by cardiopulmonary exercise testing. The outcome parameters were the Matsuda insulin sensitivity index derived from an oral glucose tolerance test and, in a subgroup of participants, the M-value from a euglycemic clamp study. This analysis draws from a prospective study of premenopausal women with and without a history of gestational diabetes mellitus (GDM). By its design, the study cohort includes women with a wide range of insulin sensitivity, adiposity, and physical fitness. It is therefore well suited to address our research question.

Material and methods

Study design and participants

We performed a cross-sectional analysis within the mono-center, prospective “Prediction, Prevention, and Subclassification of type 2 Diabetes mellitus” (PPSDiab) cohort study.¹³ This study included women with a history of GDM during their last pregnancy and women following a normoglycemic pregnancy, enrolled between November 2011 and May 2016. In the PPSDiab study, the preceding GDM was employed to enrich the study cohort for individuals at risk for future T2DM.¹⁴ The study was evaluated by the ethics board of the Medical Faculty of the Ludwig-Maximilians-Universität München, Germany (study ID 300–311) and all study participants provided written informed consent.

For this analysis, we used data collected at the baseline visit of the PPSDiab study that took place 3–16 months after the pregnancy with either GDM or normoglycemia. Of the 304 women enrolled in the study, 299 had valid data from the baseline visit.¹¹ From these women, this analysis included only women who participated in a 5-point oral glucose tolerance test (OGTT), magnetic resonance imaging (MRI), as well as cardiopulmonary exercise. Therefore, the final study sample consisted of 144 women.

Clinical study procedures, glucose tolerance testing, and euglycemic clamp

A detailed medical history was obtained from all participants. The anthropometrics, a clinical examination, and laboratory chemistry were performed following standard operating protocols, as described previously.¹³ In addition, all participants completed a five-point OGTT with 75 g of oral glucose and measurements of plasma glucose (Glucose HK Gen.3, Roche Diagnostics, Mannheim, Germany) and serum insulin (CLIA, DiaSorin LIASON systems, Saluggia, Italy) at 0, 30, 60, 90, and 120 min, also as previously described.¹³ From the OGTT, the current (pre-) diabetic status was defined according to guidelines of the American Diabetes Association¹⁵ and the insulin sensitivity index (ISI) was calculated according to Matsuda and DeFronzo ($ISI = 10,000/\text{square root of } [\text{fasting glucose} \times \text{fasting insulin}] \times [\text{mean glucose} \times \text{mean insulin during OGTT}]$).¹⁶ We previously validated ISI against euglycemic clamp data in our cohort.¹³ In a subgroup of 30 women, the M-value was derived from a hyperinsulinemic euglycemic clamp test and divided by fat free body weight times steady-state plasma insulin, as described earlier.¹³ The resulting parameter, $M/(\text{ffm} \times I)$, was used in all analyses and had the unit ($\text{mmol}/\text{min}/\text{kg} \times \text{nmol}/\text{l}$). The baseline characteristics of the clamp subcohort were comparable to those of the whole study cohort ([Supplementary Table s1](#)).

Magnetic resonance imaging and spectroscopy

All study participants were invited for a second day of testing during the baseline study visit that included an MRI study (3 T system, Ingenia, or Achieva; Philips Health Care, Best, Netherlands). We also performed a whole-body axial sequence using the two-point Dixon technique. Here, the left musculus quadriceps was chosen for muscle quantification.¹⁷ By a semi-manual segmentation method using the sliceOmatic image analysis software (version 5.0 rev. 7, TomoVision, Magog, Canada), the cross-sectional area at 40% of the femur length was quantified.¹⁸ The femur length was defined as the distance between the femoral head and the lateral condyle. Muscle volume (MV) was estimated by multiplying the cross-sectional area by muscle length and shape factor.¹⁹ Besides absolute

volumes, we computed several indices from absolute volume of each compartment in MRI and height. For the muscle volume index (MVI), we divided MV by the square of height according to the guidelines of the European working group in sarcopenia.²⁰

For intramyocellular lipid content (IMCL), a single-voxel (¹H) magnetic resonance spectroscopy (MRS) of the left anterior tibial muscle was conducted by using a point resolved spectroscopy (PRESS) according to Torriani et al.²¹ The spectroscopy was analyzed by using the jMRUI software (version 4.0, jMRUI Consortium, Bmo, Czech Republic).

Cardiopulmonary exercise testing

Physical fitness was determined by maximum workload (W_{\max}), and achieved peak oxygen uptake ($VO_{2\text{peak}}$) in a cardiopulmonary exercise test to exhaustion by using MasterScreen CPX, a bicycle exercise testing system (Care Fusion, Höchberg, Germany), as previously described.¹¹ Briefly, a standardized stepwise ramp protocol to exhaustion was used that consisted of stepwise increments of 25 W every 3 min under monitoring of pedaling speed, oxygen uptake, carbon dioxide exhalation, and 12-channel ECG. At the end of each increment, capillary lactate was measured, and participants were asked to rate their perceived exertion according to a displayed Borg scale. The criteria for a valid test to exhaustion were a respiratory exchange ratio of at least 1.05 and subjective exhaustion (BORG scale ≥ 17). We determined the peak oxygen uptake ($VO_{2\text{peak}}$) as a close approximation of $VO_{2\text{max}}$,¹¹ before the termination of the workload. W_{\max} was calculated as [second-last workload + (seconds on last workload/180 s * 25 W)]. The stepwise ramp protocol was selected to accommodate the diverse fitness levels of the women in this study.¹¹

Statistical analysis

Normally distributed metric variables were expressed as mean \pm standard deviation, other metric variables as median (first quartile–third quartile). Categorical variables were displayed as absolute number (percentage). Univariate and multivariate linear regression models were performed for analysis of the associations between measured insulin sensitivity (logarithmized ISI or $M/(ffm \cdot I)$) as dependent variable and the different muscle parameters (MV, MVI, IMCL, W_{\max} , and $VO_{2\text{peak}}$) and BMI as independent variables. All regression models were further adjusted for age and time since delivery. All statistical calculations were performed using SAS statistical software package, version 9.4 (SAS Institute Inc., Cary, NC, USA) or SPSS version 28 (IBM, Armonk, NY, USA).

Results

The 144 women analyzed for this study had a mean age of 35.7 ± 4.1 years and a mean BMI of 25.0 ± 5.3 kg/m². Of these women, 109 (76%) were normoglycemic, 31 (21%) had prediabetes, and 4 (3%) had T2DM diagnosed at the study visit by oGTT. During the preceding pregnancy, 94 women (65%) had been diagnosed with GDM, and of these, 35 (37%) maintained some form of glucose dysregulation post-partum. The remaining 50 women (35% of the cohort) had had a normoglycemic pregnancy and none of these displayed glucose dysregulation post-partum. The full baseline characteristics are listed in Table 1.

In univariate linear regression analyses, MVI, W_{\max} , and $VO_{2\text{peak}}$ significantly associated with the dependent variable ISI, but MV and IMCL did not (Table 2). The association was strongest for W_{\max} , followed by $VO_{2\text{peak}}$. The negative association of MVI with ISI has to be considered to be a consequence of height in the denominator of the formula for MVI because height displayed a positive association with ISI but MV, the numerator in the MVI formula, displayed none (Table 2). Also, W_{\max} was associated with the dependent variable $M/(ffm \cdot I)$ in the smaller sample of women who also had a hyperinsulinemic, euglycemic clamp test (Table 3). Here, $VO_{2\text{peak}}$ did not reach significance despite standardized beta values comparable to the previous analysis in the larger sample with ISI as the dependent variable. Of all parameters examined, BMI demonstrated the strongest associations to both ISI and $M/(ffm \cdot I)$.

In multivariate models that included BMI and height, we further tested those muscle parameters that were significant in the univariate regression analyses. Here, W_{\max} and $VO_{2\text{peak}}$ remained significantly associated with ISI, but MVI and height did not show significance (Table 2; Figure 1(a) and (b)). With $M/(ffm \cdot I)$, W_{\max} displayed a p -value of 0.03, while the standardized beta coefficient remained comparable to that in the analysis with ISI as the dependent variable (0.325 and 0.255, respectively) (Table 3; Figure 1(c) and (d)). With both measures of insulin sensitivity, W_{\max} demonstrated the largest increase in the adjusted R^2 value over BMI alone, from 0.437 to 0.477 for ISI and from 0.469 to 0.542 for $M/(ffm \cdot I)$ (Tables 2 and 3).

Discussion In this comparative analysis of functional and morphologic muscle parameters, we identified W_{\max} , the maximal workload reached during cardiopulmonary exercise testing, as the variable most closely associated with whole-body insulin sensitivity. Although $VO_{2\text{peak}}$ and MVI also displayed associations, they were weaker. The regression of muscle characteristics with both variables to describe insulin sensitivity (ISI and $M/(ffm \cdot I)$) yielded similar results although the association to $M/(ffm \cdot I)$ was not statistically reliable. We attribute this difference primarily to the much smaller size of the clamp cohort,

Table 1. Baseline characteristics of the study cohort ($n = 144$).

Clinical parameters	Age, yrs	35.7 ± 4.1
	Height, cm	167.3 ± 6.5
	Weight, kg	69.8 ± 13.9
	BMI, kg/m ²	25.0 ± 5.3
	Waist circumference, cm ($n = 143$)	80.6 ± 11.3
	Time after delivery, months	9.2 ± 2.8
Glucose tolerance category	NGT	109 (76%)
	IFG	14 (10%)
	IGT	12 (8%)
	IFG + IGT	5 (3%)
	T2DM	4 (3%)
	Metabolic status in preceding pregnancy	Normoglycemia
Insulin sensitivity	GDM	94 (65%)
	ISI	5.8 (3.5–7.8)
MRI/MRS	M/(ffm*1) ($n=30$)	137.8 (96.4–173.5)
	MV, dm ³	1.32 (1.16–1.42)
	MVI, dm ³ /m ²	0.47 (0.43–0.50)
	IMCL, % ($n = 117$)	0.94 (0.62–1.43)
Cardiopulmonary exercise testing	VO _{2peak} , ml/min	1852 (1664–2080)
	VO _{2peak} /kg, ml/kg/min	27.6 (23.1–31.2)
	W _{max} , W	130.6 (111.0–148.5)

Normally distributed variables are given as mean ± SD, non-normally distributed variables as median (Q1, Q3), categorical variables as n (percent). BMI (body mass index), ISI (insulin sensitivity index), M/(ffm*1) (M-value derived from hyperinsulinemic, euglycemic clamp test, divided by fat free body mass times steady-state plasma insulin), MV (muscle volume), MVI (muscle volume index), IMCL (intramyocellular lipid content) VO_{2peak} (peak oxygen uptake), and W_{max} (maximum workload). OGTT (oral glucose tolerance test), pGDM (post gestational diabetes), controls (euglycemic pregnancy), IFG (impaired fasting glucose), IGT (impaired glucose tolerance), T2DM (type 2 diabetes mellitus).

Table 2. Linear regression analyses; dependent variable: insulin sensitivity index ($n = 144$).

Independent variable(s)	Adj. R ²	Std. beta	p-value
<i>Univariate</i>			
MV	−0.018	−0.050	.56
MVI	0.013	−0.187	.03
Height	0.031	0.23	.007
IMCL	−0.017	−0.074	.43
W_{max}	0.134	0.390	<.001
VO_{2peak}	0.016	0.190	.02
BMI	0.437	−0.662	<.001
<i>Multivariate</i>			
MVI	0.431	0.127	.08
BMI		−0.703	<.001
Height		0.042	.53
W_{max}	0.477	0.255	<.001
BMI		−0.609	<.001
Height		−0.025	.70
VO_{2peak}	0.447	0.175	.009
BMI		−0.660	<.001
Height		−0.013	.85

ISI (insulin sensitivity index), MV (muscle volume), MVI (muscle volume index), IMCL (intramyocellular lipid), W_{max} (maximum workload), and VO_{2peak} (peak oxygen uptake); BMI (body mass index); all models adjusted for time after delivery and age; significant associations are marked in bold.

Table 3. Linear regression analyses; dependent variable: M/(ffm*1) ($n = 30$).

Independent variable(s)	Adj. R ²	Std. beta	p-value
<i>Univariate</i>			
MV	−0.011	−0.235	0.24
MVI	0.044	−0.333	0.10
Height	−0.058	0.086	0.66
IMCL	−0.040	−0.173	0.40
W_{max}	0.236	0.524	0.004
VO _{2peak}	−0.013	0.220	0.25
BMI	0.469	−0.703	<.001
<i>Multivariate</i>			
W _{max}	0.542	0.325	0.03
BMI		−0.622	<.001
Height		−0.189	0.17

M/(ffm*1) (M-value derived from clamp test), MV (muscle volume), MVI (muscle volume index), IMCL (intramyocellular lipid), W_{max} (maximum workload), and VO_{2peak} (peak oxygen uptake); all models adjusted for time after delivery and age; significant associations are marked in bold.

because the standardized beta coefficients and the adjusted R² values were comparable to those for ISI.

We measured maximum workload and VO_{2peak} on a bicycle ergometer using a rather long protocol with step-wise increases in workload. Thus, these measurements

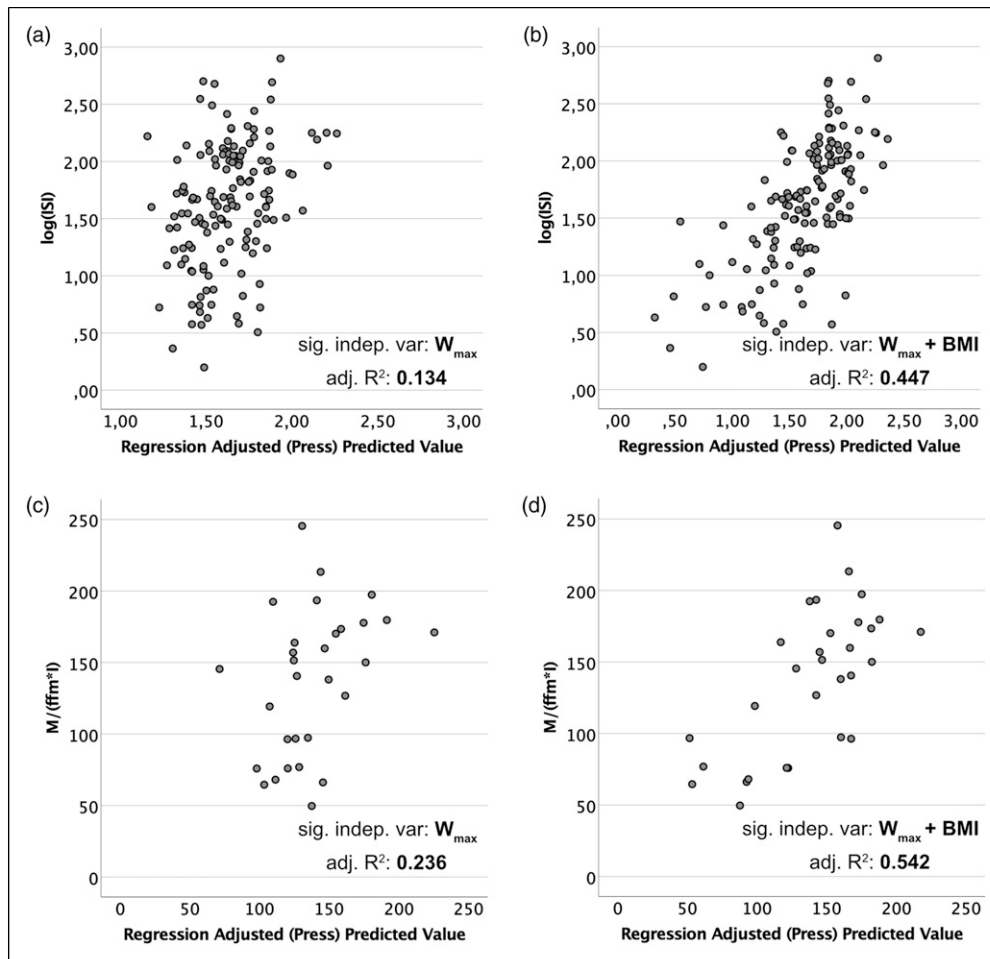


Figure 1. Graphical representation of the most important linear regression models from Tables 2 and 3. The dependent variables are displayed on the y-axes. In addition to the significant independent variables listed in the graphs, the models underlying A. and C. also included time after delivery and age, B. and D. time after delivery, age and height.

reflected cardiorespiratory capacity and muscular fitness with an endurance component, in contrast to steep ramp protocols that primarily test acute maximum capacity.²² Our study protocol has proven useful for a wide range of fitness levels and, thus, is suitable for metabolic studies in the general population. Why maximum workload exhibited an even stronger association with insulin sensitivity than VO_{2peak} remains unclear, but may show that maximum workload is an indicator for anaerobic muscle capacity and muscle function that influences insulin sensitivity stronger than cardiorespiratory fitness itself. Another possible explanation is that the stepwise exercise protocol we used in this study may underestimate the predictive capacity of VO_{2peak} , which would have benefited from a steep ramp protocol.

Of the morphologic measurements in our analysis, only the muscle volume adjusted for body surface area (MVI) displayed a weak, negative association with insulin ISI, which resulted from height in the denominator of the formula for MVI, not from muscle volume. This result

agrees with some previous studies that could not predict insulin sensitivity from DXA-measured muscle mass divided by the square of height (MMI)²³ or MRI-measured muscle volume.²⁴ Yet, other researchers that have determined muscle mass by BIA and computed its percentage of body weight (%MM)^{25–28} interpret that a direct association exists while others that have calculated DXA-measured MMI^{29–31} portray that an inverse association with insulin sensitivity occurs.

An association with insulin sensitivity in our cohort could not be determined in the intramyocellular lipid content measured by MRS in the tibialis anterior muscle, the muscle most often examined in this context. This result contrasts to early studies of intramyocellular lipids^{6,7} but mirrors more recent work. In particular, increased intramyocellular lipid content has also been found after exercise training and in athletes with a high muscular oxidative capacity and increased insulin sensitivity.^{10,32} These findings suggest that healthy IMCL accumulation can

occur^{8,9} and that only specific lipid intermediates interfere with insulin signaling.³³

The extensive capacity of skeletal muscle to utilize glucose is primarily provided by mitochondria. Thus, altered gene expression and signaling pathways in mitochondria are believed to play a primary role in the development of low muscular insulin sensitivity and, consequently, the pathogenesis of diabetes.³⁴ Because maximum work load and VO_{2peak} are indirect measures also of muscle mitochondrial capacity,^{35,36} an association of the measurements with insulin sensitivity may exist. Furthermore, these results further highlight that exercise programs that increase the mitochondrial capacity of skeletal muscle are beneficial for diabetes prevention.

The strengths of this study include the combination of several methods to assess skeletal muscle, which permitted a comparative analysis. We also analyzed the dependent parameter, insulin sensitivity, with two different methods, ISI derived from oral glucose tolerance testing and, as a sensitivity analysis, the M-value from euglycemic clamp studies. In addition, our study cohort depicted a broad spectrum of insulin sensitivity but otherwise was homogeneous, as it included only premenopausal women in a narrow age range and without major comorbidities. The later point can also be seen as a weakness of this work because we remain uncertain if our results also apply to the general population. External validation of our findings in other cohorts would therefore be beneficial. We also could not prove causality as we conducted a cross-sectional observational study. Furthermore, the euglycemic clamp procedure, the gold-standard for determining insulin sensitivity, was only performed in a subgroup of the study participants. This may have limited the accuracy of the determination of insulin sensitivity in the whole cohort, although we had validated ISI, the surrogate parameter we used, for this cohort in a previous study.¹³

Conclusions

This analysis identified a simple exercise test on a bicycle ergometer as a suitable method to assess the muscular contribution to insulin sensitivity in premenopausal women. Together with BMI, the exercise test has strong predictive capacity and, even more importantly, both measurements point directly to possible interventions in an at-risk individual; fitness training and weight loss, as required. The two measurements, followed by individualized advice, can therefore be applied clinically in early diabetes prevention, such as in post-partum counseling after GDM. Additionally, other clinical studies may benefit from this method when examining the metabolic role of the musculature.

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Supplemental material

Supplemental material for this article is available online.

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