

Metabolic Response to Submaximal and Maximal Exercise in People with Severe Obesity, Prediabetes, and Diabetes

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Keywords

Cardiopulmonary exercise test · Functional evaluation · Metabolic flexibility · Morbid obesity · Respiratory Exchange Ratio

Abstract

Introduction: Metabolic adaptations to maximal physical exercise in people with obesity (PwO) are scarcely described. This cross-sectional study evaluates the metabolic response to exercise via the respiratory exchange ratio (RER) in PwO and different degrees of glycemic control. **Methods:** Eighty-five PwO (body mass index 46.0 [39.0–54.0] kg/m²), that is, 32 normoglycemic (Ob-N), 25 prediabetic (Ob-preDM), and 28 diabetic (Ob-T2DM) subjects and 18 healthy subjects performed an incremental, maximal cardiopulmonary exercise test. The RER was measured at rest (RERrest) and at peak exercise (RERpeak). **Results:** RERpeak was significantly higher in healthy subjects than that in PwO. Among those, RERpeak was significantly higher in Ob-N than that in Ob-preDM and Ob-T2DM (1.20 [1.15–1.27] vs. 1.18 [1.10–1.22] $p = 0.04$ and vs. 1.14 [1.10–1.18] $p < 0.001$, respectively). Accordingly,

Δ RER (RERpeak-RERrest) was lower in Ob-preDM and Ob-T2DM than that in Ob-N (0.32 [0.26–0.39] $p = 0.04$ and 0.29 [0.24–0.36] $p < 0.001$ vs. 0.38 [0.32–0.43], respectively), while no significant difference was found in Δ RER between Ob-preDM and Ob-T2DM and not even between Ob-N and healthy subjects. Moreover, Δ RER in PwO correlated with glucose area under curve ($p = 0.002$). **Conclusions:** PwO demonstrate restricted metabolic response during maximal exercise. Particularly, those with prediabetes already show metabolic inflexibility during exercise, similarly to those with type 2 diabetes. These findings also suggest a potential role of cardiopulmonary exercise testing in detecting early metabolic alterations in PwO.

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Introduction

Severe obesity is a disease tightly associated with metabolic syndrome, cardiovascular events, and all-cause mortality [1, 2]. The obesity epidemic is constantly expanding at all ages, due to a widespread lifestyle based on

sedentary habits and overfeeding [3]. The energy surplus stemming basically from physical inactivity and excessive caloric intake induces profound changes in metabolic pathways and raises the risk of type 2 diabetes. The natural history of the onset of type 2 diabetes, especially in people with obesity, involves different metabolic steps of impairment of glucose metabolism and metabolic flexibility [2, 4]. Indeed, metabolic flexibility is a pathophysiological concept concerning the capacity of shifting fuel selection in response to different metabolic requests (e.g., fasting, food intake, insulin stimulation) [5] and, in resting conditions, it can be assessed by indirect calorimetry [6]. Although metabolic flexibility becomes even more important during physical exercise to couple the substrate availability with the increasing metabolic demands, this has only been partially investigated in subjects affected by type 2 diabetes and obesity, and even less in those with prediabetes [5]. In people with obesity (PwO), it has been shown that already prediabetes is associated with early target organ damage, increased cardiovascular risk [7], and morphological modifications of their adipose tissue [8]. However, this condition has not been extensively studied since it is still frequently considered an initial stage of diabetes and not as disease itself. Subjects' substrate utilization and the associated metabolic response to physical exercise can be examined by cardiopulmonary exercise testing (CPET), which is also the gold standard test for the assessment of cardiorespiratory fitness, a strong prognostic marker, both for cardiovascular and all-cause mortality [9]. CPET is a non-invasive technique that relies primarily on the measurement of minute ventilation, oxygen consumption (VO_2) and carbon dioxide production (VCO_2) during exercise [10, 11]. The thereby registered respiratory exchange ratio (RER), simply calculated as VCO_2/VO_2 , not only indicates subjects' efforts and exercise intensities but also reflects the energy substrate utilization. When RER is low, fats represent the primary fuel for exercise, but with increasing intensity, the energy is mainly supplied by glucose resulting in higher RER values [12]. Indeed, the RER may represent an interesting noninvasive functional marker of metabolic adaptations and (in)flexibility during exercise, which could be useful also in clinical settings where CPET should be regularly performed [13]. Thus, the aim of this study was to investigate the metabolic response to incremental exercise in people with severe obesity and different degree of metabolic impairment.

Methods

Experimental Design and Participants

This is an observational cross-sectional study conducted in 85 PwO and 18 age- and gender-matched healthy subjects (HS) included as controls. Participants were consecutively recruited at the Centre for the Study and Integrated Treatment of Obesity of the Padua University Hospital between January 2014 and October 2019. PwO eligible for this study had a body mass index (BMI) ≥ 35 kg/m^2 with obesity-related comorbidities or a BMI ≥ 40 kg/m^2 with or without comorbidities, while the inclusion criteria for the HS were a BMI < 30 kg/m^2 and a normal fasting glucose level. Patients with cancer, chronic inflammatory diseases, drugs or alcohol abuse were excluded. The prevalence of the 3 principal obesity-related comorbidities (hypertension, dyslipidemia, and obstructive sleep apnea syndrome) was calculated on the basis of international criteria [14–16]. Each included participant provided written informed consent. The study has been performed in accordance with the principles of the Declaration of Helsinki (revised in 2008) and is presented following the STROBE checklist (see online suppl. Table 1; see www.karger.com/doi/10.1159/000517589 for all online suppl. material). The protocol was approved by the “Padua Ethical Committee for Clinical Research” (2892P, June 10, 2013).

Measurements

Each PwO underwent complete blood biochemical analyses after an 8-h fasting period, determining fasting plasma glucose (FPG), insulin, lipid profile, high-sensitivity C-reactive protein, interleukin-6, and leptin levels. Moreover, a 3-h 75 g oral glucose tolerance test was performed, measuring blood glucose and insulin plasma levels at baseline and 30, 90, 120, 150, and 180 min after glucose loading (180 mL of syrup with 82.5 g glucose monohydrate equal to 75 g of glucose). The oral glucose tolerance test was not performed in subjects with previous diagnosis of type 2 diabetes. Biochemical measurements were performed using diagnostic kits standardized according to the World Health Organization First International Reference Standard, as described in previous studies [8]. Insulin sensitivity and insulin resistance indices were calculated through the Matsuda index and the Homeostasis Model Assessment index (HOMA_{IR}), respectively [17, 18]. Moreover, the areas under the curve for glucose and insulin (G_{AUC} and I_{AUC}) were calculated using the trapezoidal method. In accordance with the American Diabetes Association, PwO were divided into 3 groups depending on their glycemic profile, that is normoglycemic (Ob-N), prediabetic (Ob-preDM), and diabetic (Ob-T2DM) PwO [7]. In subjects with type 2 diabetes, glycated hemoglobin A_{1c} (HbA_{1c}) was evaluated by high-performance liquid chromatography. Indirect calorimetry was performed in fasting condition and after 15 min of rest in a comfortable and thermo-neutral environment. A ventilated canopy calorimeter was used (Vmax; Sormedics, Milan, Italy). VO_2 and VCO_2 were measured continuously, and values were averaged in 1-min intervals. Fasting RER (RER_{fast}) was calculated by using the Weir [19] equation. Consecutively, body composition was analyzed by a single-frequency (300 μA , 50 kHz) electrical impedance analyzer (Soft Tissue Analyzer; Akern, Pontassieve, Italy). Moreover, the skeletal muscle mass (SM) was calculated using the equation proposed by Janssen et al. [20]: $\text{SM} (\text{kg}) = [(\text{Ht}^2/\text{R} \times 0.401) + (\text{gender} \times 3.825) + (\text{age} \times -0.071)] + 5.102$. Maximal isometric handgrip strength tests were performed 3 times for each hand, using a calibrated dynamometer (Baseline, Elms-

Table 1. Clinical characteristics of the population

	HS (18)	PwO (85)	Ob-N (32)	Ob-preDM (25)	Ob-T2DM (28)	Ob-N vs. Ob-preDM	Ob-N vs. Ob-T2DM	Ob-preDM vs. Ob-T2DM
Age, years	47.5 (32–53)	46.0 (38.0–54.0)	40.5 (28.5–48.5)	46.0 (42.0–53.0)	50.5 (44.5–54)	Ns	Ns	0.001
BMI, kg/m ²	22.9 (21.4–24.6)	41.8 (38.1–45.1)***	41.0 (37.5–43.9)***	41.5 (36.7–44.5)***	43.2 (38.6–48.5)***	Ns	Ns	Ns
% Woman	14 (77.8%)	62 (72.9%)	81%	76%	61%	Ns	Ns	Ns
FM, kg	-	46.0 (28.8–55.2)	51.5 (44.9–59.5)	47.1 (34.2–51.1)	49.6 (41.9–52.1)	Ns	Ns	Ns
FFM, kg	-	58.2 (49.4–70.3)	60.1 (55.3–65.1)	63.8 (52.3–72.6)	70.9 (56.4–70.2)	Ns	Ns	Ns
FM, %	-	41.2 (24.6–47.7)	46.3 (41.1–50.8)	39.4 (33.4–46.6)	42.1 (41.2–44.8)	Ns	Ns	Ns
FFM, %	-	53.2 (44.9–59.1)	53.5 (49.2–58.5)	55.7 (52.3–61.4)	54.7 (53.2–55.6)	Ns	Ns	Ns
SM, kg	-	25.9 (21.1–32.1)	26.6 (24.4–29.7)	26.8 (21.7–29.7)	30.0 (24.4–32.1)	Ns	Ns	Ns
Waist, cm	-	121.5 (113–134.5)	117.5 (108.0–124.0)	121.0 (115.0–130.0)	129.0 (119.5–139.5)	Ns	0.005	Ns
Handgrip, kg	-	29 (19.5–34.5)	30.0 (24.7–33.0)	33.7 (29.0–40.0)	27.7 (23.0–29.0)	Ns	Ns	Ns
FPG, mmol/L	4.8 (4.5–5.2)*	5.7 (5.1–7)***	5.0 (4.8–5.3)	5.7 (5.5–5.9)***	7.0 (7.0–7.2)***	<0.001	<0.001	<0.001
G _{AUC} min, mmol/L	-	1,268 (1,128–1,449)	1,141.9 (1,026–1,237)	1,387.5 (1,316–1,497)	1,949 (1,641–2,216) [§]	<0.001	<0.001	Ns
I _{AUC} min, mU/L	-	12,945 (9,536–19,473)	10,104.8 (6,609–16,608)	15,898.5 (10,892–22,349)	17,602 (12,036–32,106) [§]	0.003	0.023	Ns
HOMA _{IR}	-	3.8 (2.3–5.2)	2.6 (1.7–4.4)	3.9 (3.4–5.6)	8.9 (5.1–11.9) [†]	Ns	<0.001	0.014
ISI _M	-	2.6 (1.9–3.9)	3.5 (2.4–4.6)	2.2 (1.8–2.5)	-	0.012	-	-
hs-CRP, mg/L	-	4.10 (2.0–8.83)	4.25 (1.61–8.69)	3.07 (1.79–6.0)	5.72 (2.63–11.0)	Ns	Ns	Ns
IL-6, ng/L	-	2.85 (1.90–4.07)	2.9 (1.9–4.0)	2.1 (1.9–3.7)	3.4 (2.2–5.0)	Ns	Ns	Ns
Leptin, µg/L	-	34 (22–46)	33 (22–47)	31 (24.5–41.5)	37 (18–46)	Ns	Ns	Ns
Dyslipidemia	0 (0)	44 (51.7%)***	11 (34.4%)	10 (40.0%)	23 (82.1%)	Ns	<0.001	0.002
Hypertension	0 (0)	44 (51.7%)***	9 (28.1%)	13 (52.0%)	22 (78.6%)	Ns	<0.001	0.041
OSAS	0 (0)	15 (17.6%)*	4 (12.5%)	2 (8.0%)	9 (32.1%)	Ns	Ns	0.031

Anthropometric and clinical parameters of HS, PwO and the 3 subgroups of PwO: Ob-N, Ob-preDM, and Ob-T2DM patients. Clinical data are presented as median (IQR) for continuous parameters and number (%) for dichotomous parameters. FM, fat mass; FFM, fat free mass; SM, skeletal muscle; FPG, fasting plasma glucose; G_{AUC}, glucose area under curve; I_{AUC}, insulin area under curve; ISI_M, insulin sensitivity Matsuda index; hs-CRP, high sensitivity C-reactive protein; OSAS, obstructive sleep apnea syndrome; HS, healthy subjects; PwO, people with obesity; Ob-N, people with obesity and normal glycemic status; Ob-preDM, people with obesity and prediabetes; Ob-T2DM, people with obesity and type 2 diabetes; BMI, body mass index; IQR, interquartile range; IL, interleukin; HOMA_{IR}, Homeostasis Model Assessment index. Statistical analysis was based on the Mann-Whitney test (HS vs. PwO) and Kruskal-Wallis test followed by Dunn's test for all other analyses. The statistical significance versus HS is expressed as * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$. [†] Data available for 16 HS. [§] Data available for 5 Ob-T2DM. [†] Data available in 18 patients; HOMA_{IR} was not calculated in patients assuming insulin.

ford, NY, USA) and taking the average value for analysis. ECG-monitored CPET was performed on a treadmill or in a small minority on a cycle ergometer, depending on the subjects' clinical characteristics. Ventilatory parameters were sampled breath by breath with the Jaeger-Masterscreen-CPX (Carefusion). The incremental modified Bruce ramp protocol was used for treadmill testing, while a 15-Watts/min ramp protocol was used on cycle ergometer [21]. Peak oxygen consumption (VO_2peak) and the oxygen uptake efficiency slope were determined as markers of aerobic exercise capacity. All tests were performed until subjects' exhaustion. Maximal effort was defined as a RER of at least 1.10 [10], and/or a maximal heart rate of $\geq 85\%$ of predicted by age, and/or a Borg rating of perceived exertion $\geq 18/20$ [22]. All people who did not reach the indicated criteria were excluded from the study. Functional exercise capacity was measured as metabolic equivalent of tasks (METs). The RER determined during CPET was measured at rest (RER_{rest}), after achieving stable breathing conditions, and throughout the test until reaching peak exercise (RER_{peak}). The RER at submaximal intensity (RER_{AT}) was determined by the mean of the RER values recorded 1 min before the anaerobic threshold. RER variations during exercise were calculated as RER_{peak}-RER_{rest} (ΔRER) and as a percentage of increase ($\Delta\text{RER}\%$) in order to correct for baseline.

Statistical Analyses

Median values with interquartile range, and percentages were used to describe the continuous and dichotomous clinical parameters, respectively. All variables were tested for normality using the Shapiro-Wilk test and, based on their distribution, intergroup comparisons were performed by a parametric one-way ANOVA followed by Bonferroni post hoc analysis or nonparametric Kruskal-Wallis test followed by the Dunn's multiple comparison post hoc test. Student's *t* test or the Mann-Whitney test was used when 2 groups were compared. Pearson's χ^2 test was used to compare dichotomous variables. Two-sided *p* values < 0.05 were considered statistically significant. Pearson's correlation coefficient (*r*) and the respective *p* value were calculated to analyze correlations between variables. Multivariate stepwise regression analysis was performed to establish independent determinants. Statistical analyses were performed using Statistical Package for Social Science 20 (SPSS Inc., Chicago, IL, USA).

Results

Clinical Characteristics of Study Population

Demographic and anthropometric characteristics of the population are described in Table 1. Among all included PwO, 32 were Ob-N, 25 Ob-preDM, and 28 Ob-T2DM. The prevalence of comorbidities progressively increased in the 3 groups of PwO and Ob-T2DM groups displayed the highest percentage of subjects affected by hypertension and dyslipidemia. Inflammatory cytokines showed no substantial difference between the 3 groups of PwO. Furthermore, PwO were mostly sedentary (80.7%) without significant differences among subgroups (Ob-N

= 81.3%; Ob-preDM = 79.2%; Ob-T2DM = 81.5%). In PwO, CPET was mostly performed on a treadmill (89.4%), however, 11.6% were tested on a cycle ergometer with a similar subgroup distribution.

Metabolic Profile and Body Composition

HOMA_{IR} was higher in Ob-T2DM subjects than both Ob-N ($p < 0.001$) and Ob-preDM ($p = 0.014$), whereas it was not significantly different between Ob-N and Ob-preDM. However, G_{AUC} , I_{AUC} , and insulin sensitivity Matsuda index highlighted significant differences between Ob-N and Ob-preDM or Ob-T2DM subjects (Table 1). Fifty-two percent of the Ob-preDM subjects had impaired FPG, 24% had impaired glucose tolerance, and 24% had both. More than half (68%) of Ob-T2DM subjects had a diagnosis established < 5 years ago. Moreover, 29% had a poor diabetes control (HbA_{1c} higher than 69 mmol/mol), while 40% had an HbA_{1c} level lower than 59 mmol/mol (in 20% of Ob-T2DM subjects HbA_{1c} levels were not available). Microalbuminuria was detectable in 6 (21%) Ob-T2DM subjects, microvascular damage in 3 (10.7%) Ob-T2DM subjects and macrovascular damage only in 1 (3.6%) Ob-T2DM subject. No significant differences in fat mass (FM), fat free mass (FFM), and SM were observed among the 3 groups of PwO (Table 1). Last, handgrip strength was also similar in the 3 subgroups of PwO (Table 1) and significantly correlated with FFM ($r = 0.547$, $p = 0.001$).

CPET

PwO showed significantly lower exercise tolerance than HS, as well as lower submaximal and maximal aerobic capacity (METs, VO_2AT , and $\text{VO}_2\text{peak/kg}$, respectively; all $p < 0.001$). No difference was found in absolute aerobic power (Table 2). Among PwO, Ob-T2DM had lower exercise tolerance (METs) than Ob-preDM ($p = 0.039$) and Ob-N ($p = 0.024$), but no difference was found between Ob-preDM and Ob-N. Moreover, functional capacity expressed as $\text{VO}_2\text{peak/kg}$ followed the same trend, showing indeed similar outcomes between Ob-preDM and Ob-N. Regarding the metabolic response to exercise, data showed similar RER_{fast}, RER_{rest}, and RER_{AT} when people with and without obesity were compared, while RER_{peak} and the variation from rest to peak were significantly higher in HS than in PwO ($p < 0.001$). Moreover, RER_{peak} was significantly higher in Ob-N than both Ob-preDM and Ob-T2DM ($p = 0.04$ and $p = 0.001$, respectively). Accordingly, when analyzing ΔRER from rest to peak exercise, Ob-N showed a significantly greater increase in RER than Ob-preDM ($p = 0.04$) and Ob-

Table 2. CPET-derived functional parameters

	HS (18)	PwO (85)	HS vs. PwO	Ob-N (32)	Ob-preDM (25)	Ob-T2DM (28)	Ob-N vs. Ob-preDM	Ob-N vs. Ob-T2DM	Ob-preDM vs. Ob-T2DM
METs	15.9 (13.3–18.0)	9.1 (7.8–10.6)	<0.001	9.6 (8.2–11.8)***	9.7 (8.3–11.7)***	8.2 (7.6–9.1)***	Ns	0.024	0.039
VO ₂ peak/kg, mL/min/kg	35.9 (32.2–40.1)	21.4 (18.9–25.2)	<0.001	21.7 (20.6–25.6)**	22 (21–25.1)**	19.4 (17.9–21.9)***	Ns	0.022	0.046
VO ₂ peak, L/min	2.4 (2.1–2.9)	2.4 (2.1–2.9)	Ns	2.6 (2.1–2.9)	2.4 (2.1–2.8)	2.4 (1.9–2.7)	Ns	Ns	Ns
VO ₂ AT, mL/min/kg	22.3 (18.6–27.7)	15.1 (13–17.4)	<0.001	16.1 (14.4–17.5)**	14.8 (12.7–17.3)***	14.0 (12.7–17.1)***	Ns	Ns	Ns
VO ₂ AT (%peak)	68 (53–71)	70 (65–78)	0.05	72 (62–76)	68 (62–74)	75 (68–80)**	Ns	Ns	0.02
OUES	2,185 (1,797–2,734)	2,379 (1,993–2,756)	Ns	2,356 (1,923–2,725)	2,262 (2,048–2,756)	2,407 (2,014–3,019)	Ns	Ns	Ns
HRpeak (% predicted)	98.5 (94–103)	92 (87–98)	0.003	93 (88–98)	93 (88–101)	89.5 (87–98)	Ns	Ns	Ns
RERlast	–	0.74 (0.68–0.81)	–	0.73 (0.67–0.80)	0.73 (0.69–0.81)	0.77 (0.70–0.83)	Ns	Ns	Ns
RERrest	0.82 (0.79–0.86)	0.83 (0.79–0.88)	Ns	0.83 (0.78–0.87)	0.83 (0.79–0.88)	0.84 (0.80–0.88)	Ns	Ns	Ns
RERpeak	1.26 (1.22–1.32)	1.17 (1.11–1.22)	<0.001	1.20 (1.15–1.27)*	1.18 (1.10–1.22)***	1.14 (1.10–1.18)***	0.04	<0.001	Ns
RER _{AT}	0.94 (0.90–0.98)	0.91 (0.88–0.94)	Ns	0.92 (0.89–0.94)	0.92 (0.88–0.94)	0.91 (0.87–0.95)	Ns	Ns	Ns
ΔRER	0.42 (0.38–0.52)	0.33 (0.28–0.41)	<0.001	0.38 (0.32–0.43)	0.32 (0.26–0.39)**	0.29 (0.24–0.36)***	0.04	<0.001	Ns
ΔRER%	51.2 (43.6–65.6)	41.0 (30.7–50.6)	0.002	46.0 (38.0–55.3)	41.0 (30.2–50.0)**	35.8 (28.9–46.3)***	0.06	0.002	Ns

CPET-derived functional parameters of HS, PwO and the 3 subgroups of PwO: Ob-N, Ob-preDM, and Ob-T2DM patients. Data are presented as median (IQR). METs, peak metabolic equivalent of tasks; VO₂ATI, volume of oxygen consumption at submaximal intensity of exercise; OUES, oxygen uptake efficiency slope; HR, heart rate; RERlast, respiratory exchange ratio at rest in fasting condition; RERrest, respiratory exchange ratio at rest; RERpeak, respiratory exchange ratio at peak of exercise; RER_{AT}, respiratory exchange ratio at submaximal exercise; ΔRER, RERpeak-RERrest; PwO, people with obesity; HS, healthy subjects; Ob-N, people with obesity and normal glycemic status; Ob-preDM, people with obesity and prediabetes; Ob-T2DM, people with obesity and type 2 diabetes; IQR, interquartile range. The statistical significance versus HS is expressed as * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$. Statistical analysis was based on Mann-Whitney test (HS vs. PwO) and Kruskal-Wallis test followed by Dunn's test for all other analyses.

T2DM ($p < 0.001$), respectively (Fig. 1). Data further showed that ΔRER and ΔRER% were neither significantly different between Ob-preDM and Ob-T2DM nor between HS and Ob-N. In univariate correlation analyses applied for PwO (Table 3), RERpeak was found positively and significantly correlated with FM, FFM and VO₂/kg ($p = 0.02$, $p = 0.005$, $p = 0.011$, respectively), but inversely with G_{AUC} ($p = 0.014$). ΔRER was directly and significantly correlated with FM ($p = 0.04$) and VO₂/kg ($p = 0.024$), while it inversely correlated with age ($p = 0.006$), HOMA_{IR} ($p = 0.038$), FPG ($p = 0.048$), and G_{AUC} ($p = 0.002$). ΔRER% correlated directly with VO₂/kg ($p = 0.048$), and inversely with age ($p = 0.006$), waist circumference ($p = 0.028$), G_{AUC} ($p = 0.002$), and HOMA_{IR} ($p = 0.046$). In multivariate stepwise regression analyses (Table 4) RERpeak was found independently and directly determined by FFM and inversely by G_{AUC}, while ΔRER and ΔRER% were independently determined by G_{AUC} only. Also, when excluding Ob-T2DM and after adjusting for BMI, ΔRER, and ΔRER% still remained independently determined by G_{AUC} only.

Discussion

The main finding of this study is that people with severe obesity and those with type 2 diabetes (Ob-T2DM) and prediabetes (Ob-preDM) were found to have a similar, significantly lower, increase in the RER during maximal CPET than those with obesity and normal glucose levels (Ob-N). To the best of our knowledge, this is the first study to analyze the metabolic response during maximal CPET in a large population with a high degree of obesity, evaluated within a standardized clinical setting. This study compares subjects with different metabolic phenotypes, thus providing an overview of a hypothetical continuum from health to disease with increasing metabolic risk. Previous literature already documented that individuals with obesity and various degrees of metabolic alterations had an impaired metabolic flexibility during exercise [23–25]. However, all these studies evaluated submaximal exercise and were performed on small populations, characterized by overweight and/or mild to moderate obesity, providing partially contrasting results. Malin et al. [26] studied a population with overweight/moderate obesity and found that those with both glucose intolerance and fasting hyperglycemia had lower RER during continuous, submaximal exercise. Moreover, Prior et al. [27] described that subjects with glucose intolerance had a

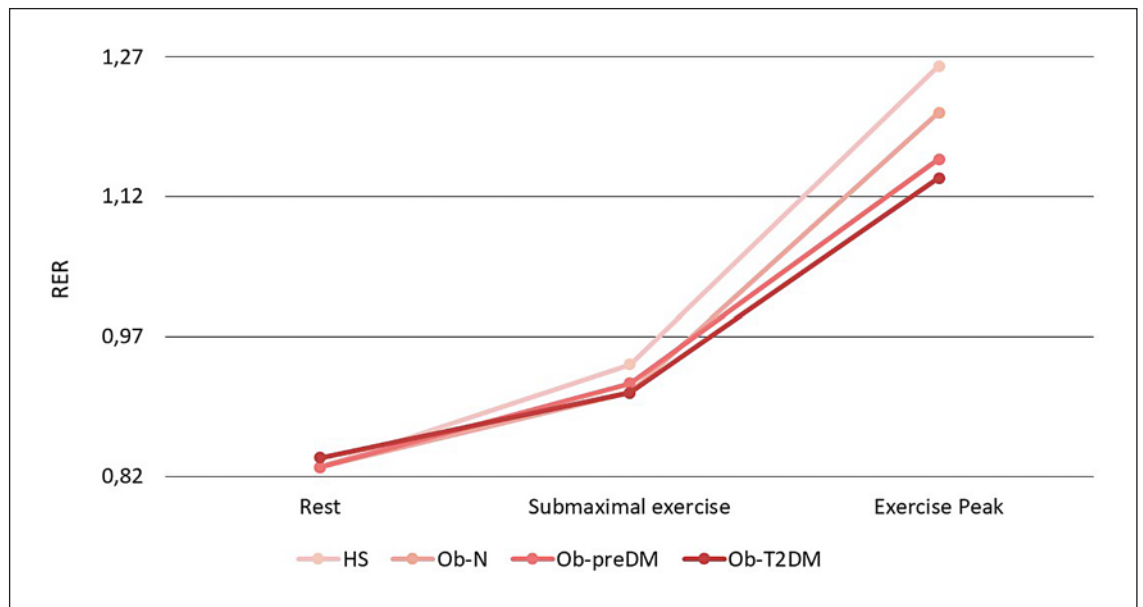


Fig. 1. Metabolic response to exercise in the different subpopulations examined in this study. RER measured at rest, during submaximal exercise, and at peak of exercise. Passing progressively from health to severe obesity, up to obesity complicated by prediabetes or diabetes, the ability to use glucose during exercise seems increasingly impaired. However, in this study population,

Ob-preDM and Ob-T2DM showed comparable metabolic response during exercise. RER, respiratory exchange ratio; HS, healthy subjects; Ob-N, people with obesity and normal glycemic status; Ob-preDM, people with obesity and prediabetes; Ob-T2DM, people with obesity and type 2 diabetes.

Table 3. Impact of anthropometric and functional parameters on metabolic response to exercise

	RER _{peak}		Δ RER		Δ RER%	
	<i>R</i>	<i>p</i> value	<i>r</i>	<i>p</i> value	<i>r</i>	<i>p</i> value
Age, years	Ns		-0.299	0.006	-0.294	0.006
BMI, kg/m ²	Ns		Ns		Ns	
Waist, cm	Ns		Ns		-0.246	0.028
FM, kg	0.254	0.02	0.219	0.04		Ns
FFM, kg	0.305	0.005	Ns		Ns	
G_{AUC} min, mmol/L	-0.311	0.014	-0.387	0.002	-0.378	0.002
I_{AUC} min, mU/L	Ns		Ns		Ns	
HOMA _{IR}	Ns		-0.240	0.038	-0.232	0.046
ISI _M	Ns		Ns		Ns	
FPG, mmol/L	Ns		-0.215	0.048	Ns	
MET _s	Ns		Ns		Ns	
VO ₂ , mL/min/kg	0.276	0.011	0.244	0.024	0.216	0.048

Univariate correlation analyses, performed for PwO, analyzing the metabolic response to maximal exercise. RER_{peak}, Δ RER, and Δ RER% were tested for those parameters that showed significant differences between Ob-N, Ob-preDM, and Ob-T2DM. Correlation is expressed as Pearson's *R* coefficient; *p* value is considered significant when <0.05. FM, fat mass; FFM, fat free mass; FPG, fasting plasma glucose; G_{AUC} , glucose area under curve; I_{AUC} , insulin area under curve; ISI_M, insulin sensitivity Matsuda index; MET_s, peak metabolic equivalent of tasks; RER, respiratory exchange ratio; RER_{peak}, respiratory exchange ratio at peak of exercise; Δ RER, RER_{peak}-RER_{rest}; PwO, people with obesity; Ob-N, people with obesity and normal glycemic status; Ob-preDM, people with obesity and prediabetes; Ob-T2DM, people with obesity and type 2 diabetes; HOMA_{IR}, Homeostasis Model Assessment index.

Table 4. Multivariate stepwise regression analyses evaluating metabolic response to exercise in PwO

	RERpeak*		Δ RER*		Δ RER%*	
	beta	<i>p</i> value	beta	<i>p</i> value	beta	<i>p</i> value
All PwO						
FFM, kg	0.271	0.026	–		–	
G_{AUC} min, mmol/L	–0.295	0.016	–0.387	0.002	–0.328	0.013
Ob-N and Ob-preDM						
FFM, kg	0.363	0.006	–		–	
G_{AUC} min, mmol/L	Ns		–0.307	0.020	–0.309	0.019

Multivariate correlation analyses performed for all PwO and by excluding Ob-T2DM, evaluating the impact of those parameters that showed significant correlations in univariable analysis (FFM and G_{AUC}) on parameters of metabolic response to exercise, that is, RERpeak, Δ RER, and Δ RER%. Correlation is expressed as beta coefficient; *p* value is considered significant in <0.05. FFM, fat free mass; G_{AUC} , glucose area under curve; RER, respiratory exchange ratio; RERpeak, respiratory exchange ratio at peak of exercise; Δ RER, RERpeak-RERrest; PwO, people with obesity; Ob-T2DM, people with obesity and type 2 diabetes; BMI, body mass index. * Adjusted for BMI.

significant impairment of carbohydrate metabolism during submaximal exercise. They found also that metabolic inflexibility during exercise was related to glucose intolerance [27]. In line with these findings, we found that RERpeak was significantly lower in Ob-preDM and Ob-T2DM than Ob-N and HS. Indeed, no statistical difference was found between Ob-preDM and Ob-T2DM, confirming, once again, the high metabolic similarity between prediabetes and type 2 diabetes in PwO (Fig. 1). The majority of patients with diabetes did not display micro- or macrovascular complications, and most of them had a recent diagnosis of diabetes (<5 years), despite not all demonstrated optimal glycemic control. This homogeneous study population allows studying the role of diabetes itself by limiting the potential confounding factors of associated long-term complications. Nevertheless, the metabolic response to exercise is clearly different between Ob-N and both Ob-preDM and Ob-T2DM. Moreover, metabolic flexibility seems to be crucial for the metabolic response and substrate selection during exercise. Evidence of such metabolic inflexibility during incremental/maximal exercise implies a number of possible pathophysiological mechanisms [26, 28, 29]. The reduction in metabolic flexibility in individuals with altered fasting glucose has been explained by a selective impairment of insulin-stimulated glucose oxidation and a reduced insulin inhibition of lipid oxidation. This suggests a crucial role of the glucose-fatty acid cycle in regulating the glucose flux, and an impaired regulation of the lipolysis even among pre-diabetic individuals [28]. Furthermore, the dysreg-

ulation in homeostasis of free fatty acids and glucose in prediabetic subjects could lead to ectopic fat distribution [8, 30]. These elevated levels of plasma free fatty acids [31] can in turn inhibit glycogen synthase and pyruvate dehydrogenase activity, obtaining a lower glucose disposal and a restriction in glucose oxidation for skeletal muscles [32, 33], energetic substrates needed particularly at higher intensities. Indeed, we found that the G_{AUC} independently correlated with the metabolic response during exercise (Δ RER), while RERpeak after excluding Ob-T2DM subjects was determined by FFM only. It has been well-described that skeletal muscles, via their insulin independent glucose oxidative capacity during exercise, play a key role in subjects at risk of type 2 diabetes, suggesting the hypothesis that metabolic inflexibility may precede the development of insulin resistance [34, 35]. Furthermore, it can be assumed that mitochondrial function and metabolic pathways involved in metabolic flexibility may be influenced by physical activity levels [36]. In the present study, the levels of physical activity were not assessed quantitatively, but anamnestic information was collected about the active or sedentary lifestyle. Healthy subjects were defined as such also on the basis of their active lifestyle [37], while PwO showed a high prevalence of sedentary behavior (80.7%). Nevertheless, among subgroups of PwO, the prevalence of sedentary habits was similar. Thus, it seems unlikely that this issue could have affected the study outcomes on metabolic differences in our population. Furthermore, the continuous exposure to excess of substrates that occurs in severe obesity re-

duces metabolic flexibility affecting also mitochondrial oxidative capacity in humans [33]. In particular, mitochondria select the most appropriate energy source for each physiological condition, but in the context of chronic overfeeding, competition between substrates increases and the mitochondria are stuck in a state of indecision (mitochondrial “gridlock”). In this condition, insulin should exert the fundamental role to direct the systemic flux of substrates, but when insulin resistance has been developed, metabolic consequences of the substrate excess are amplified, by unleashing a storm of nutrients that are distributed following abnormal interaction between hormonal and metabolic mechanisms. Thus, metabolic inflexibility can be considered as both a cause and marker of mitochondrial congestion, which in turn influences insulin response and thus cellular glucose uptake [2]. Moreover, it is known that skeletal muscles of subjects with obesity have an increased content of triglycerides and poor glycogen storage inducing the choice of lipids as energy substrate [38–40]. During exercise also, insulin-independent mechanisms are activated, which trigger the translocation of GLUT4 and glucose utilization [41]. This is even more pronounced at vigorous intensity exercise, when glucose is the preferred fuel to sustain effort. Indeed, our data are in line with those of a recent study, where the metabolic response to submaximal exercise was similar when overweight and obese subjects were compared with healthy controls [42]. However, the distinctive metabolic response among subgroups of PwO became more pronounced at maximal exercise intensities, where glucose is more and more needed as higher efficient energetic substrate (Fig. 1). Although the physiological response to exercise may be influenced by fat distribution [43], in our study, the waist circumference was not independently related to metabolic inflexibility during exercise, and the difference in waist circumference was significant only between Ob-T2DM and Ob-N. Furthermore, it has been previously observed that the physiological response to exercise in patients with obesity and chronic obstructive pulmonary disease was different if performed on a bike and compared to a treadmill [44]. Nevertheless, in the present study, the little percentage of PwO that performed CPET on a cycle ergometer was similarly distributed among subgroups of PwO and should thus not significantly affect study outcomes.

Data of our study have also highlighted a significant progressive impairment in functional capacity from HS to those with obesity and comorbidities, while absolute

VO₂peak did not show any significant difference between PwO. These results confirm previous findings and underline the importance of early functional evaluation in these subjects [45]. Future studies might specifically investigate if exercise and other therapeutic/lifestyle interventions could improve metabolic (in)flexibility during exercise. This work pointed out that people with obesity and prediabetes already have functional metabolic changes that characterize those with diabetes. Therefore, more clinical attention should be paid to PwO and prediabetes. Indeed, metabolic impairment can be unmasked early by CPET, which is of important clinical relevance, allowing a timely and more tailored multidisciplinary intervention. Future investigations should examine the trend of metabolic inflexibility in a prospective experimental design and evaluate the specific impact of the anaerobic metabolism in subjects with different metabolic impairments.

In conclusion, this study shows that people with severe obesity, especially when complicated by prediabetes or diabetes, are characterized by metabolic inflexibility measured by RER during exercise. Moreover, Δ RER is independently determined by G_{AUC} . Our findings suggest that in a population with severe obesity, subjects with prediabetes and diabetes present similar metabolic inflexibility. CPET seems indeed useful for an early detection of metabolic inflexibility in this population. This study provided further characterization of an obesity phenotype at higher metabolic risk, aiming to pave the way for subsequent studies that focus on the appropriate diagnostic assessment, therapeutic goals, and timing for treatment.

Statement of Ethics

The study complied with the guidelines for human studies and was conducted in accordance with the World Medical Association Declaration of Helsinki. All study participants provided their written informed consent to participate in the study. The protocol was approved by the “Padua Ethical Committee for Clinical Research” (2892P, June 10, 2013).

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

F.B., A.B., D.N., A.G., S.B., G.Q., R.V., L.B., and A.E. contributed to the conception and/or design of the work. F.B., A.B., D.N., A.G., S.B., C.C., R.M., G.Q., M.B., R.V., L.B., and A.E. contributed to the acquisition and/or analysis, and/or interpretation of data for the work. F.B. and A.B. drafted the manuscript. All authors critically revised the manuscript and gave final approval.

Data Availability Statement

Data that support the findings of this study are available on reasonable request from the corresponding author.

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