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Vascular Dynamics and Peripheral Oxygen Uptake in Obese Individuals during Progressive Physical Exercise

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Keywords
Obesity · Exercise testing · Endothelin-1 · Nitric oxide · End-tidal O_2 pressure

Abstract
Background: Obese men show higher O_2 consumption than lean men during physical exercise, with a trend toward higher peripheral O_2 extraction; this is probably due to their larger muscle mass. ***Objectives:*** The aim of this study was to examine this phenomenon by measuring 2 vasoactive substances, endothelin-1 (ET-1) and nitric oxide (NO), during a progressive submaximal exercise. ***Methods:*** Seventeen obese (body mass index [BMI] 38.6) and 15 lean (BMI 22.5) men performed a maximal progressive cycle ergometer exercise to determine peak power output (PPO) and peak O_2 consumption ($\dot{V}O_{2peak}$); thereafter, they performed a submaximal cycle ergometer incremental test (every 6 min) at the same percentage of $\dot{V}O_{2peak}$ until they reached 57.5% PPO. Blood samples were collected at rest and at the end of every step to measure ET-1 and NO concentrations. ***Results:*** At rest, the ET-1 and NO concentrations in obese men and lean controls were the same. However, during exercise, the ET-1 concentration at each step was significantly lower ($p < 0.05$) in the obese group. There was no significant difference in NO concentration between the 2 groups, although the increase at the beginning of the exercise session was faster in obese individuals. During submaximal exercise, end-tidal O_2 pressure ($PETO_2$) was lower in the obese group, with a significant difference in the $PETO_2$/fat-free mass ratio at each step. ***Conclusions:*** ET-1 and NO levels during physical exercise are different in obese versus lean men. This may support the notion that increased O_2 consumption in obesity is due to different behaviors of the cardiorespiratory and circulatory systems.

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Introduction

Obesity is characterized by an increase in body mass, which requires greater metabolic energy exchange at rest and, particularly, during physical activity [1]. In general, obese subjects have a lower capacity for work than lean subjects [2], reaching an anaerobic threshold (AT) at

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workloads that are significantly lower [3–5]. Moreover, compared with nonobese individuals, obesity results in significantly higher O_2 consumption ($\dot{V}O_2$) at any levels of work output, leading to reduced economy of exercise [3, 5].

Previous studies show that smaller increases in ventilation ($\dot{V}_E$) during incremental exercise are not a factor that limits work capacity in obese individuals [6]. In general, increased transport and peripheral unloading of O_2 during progressive exercise is assured by increased cardiac output (Q) together with greater tissue extraction. The relationship between Q and $\dot{V}O_2$ appears to be the same for all individuals under most experimental conditions: an increase in $\dot{V}O_2$ together with an increase in the arterial-venous difference in O_2 concentration [7]. In obesity, this correlation shows a significantly shallower slope, meaning that during physical effort obese subjects show a lower Q for the same $\dot{V}O_2$ when compared with lean subjects; therefore, they must rely on a greater arterial-venous difference in O_2 concentration [6, 8, 9].

Obesity can be characterized by an increase in both fat and fat-free mass (FFM); this may justify a peripheral increase in O_2 extraction due to the heavier muscle mass at work. This hypothesis seems to agree with the lower values for end-tidal O_2 pressure ($PETO_2$) in these subjects [3, 9, 10]. $PETO_2$ is the lowest PO_2 measured during the alveolar portion of exhalation and is influenced by the partial pressure of O_2 in ambient air, arterial blood, and venous blood. In subjects without cardiopulmonary problems, a reduction in $PETO_2$ during exercise may be related to a reduction in the O_2 concentration in venous blood, whereas the O_2 concentration in arterial blood and ambient air remains steady [11].

In patients with central adiposity and metabolic syndrome, increased levels of intravascular endothelin-1 (ET-1) coexist alongside a reduction in nitric oxide (NO)-dependent vasodilator capacity [12], suggesting impaired blood flow in peripheral vessels. This is observed in obese individuals at rest [13]. Deranged vascular homeostasis in obesity is suggested, with an imbalance between NO and ET-1; indeed, NO is necessary for vascular relaxation, while ET-1 is the most potent vasoconstrictor [14, 15].

Physical exercise results in a greatly increased blood flow to the working muscles (both active skeletal muscle and heart muscle) and reduced blood flow to the visceral organs and nonworking muscle [16]. After exercise, Maeda et al. [17] observed a significant increase in the plasma concentrations of ET-1 in the femoral vein in the nonworking leg of young athletes, with no change in the working leg.

Little information is available about dynamic evaluation of ET-1 and NO in obese individuals during physical exercise. Under such conditions, peculiar responses might confirm alterations in vascular homeostasis and explain differences in peripheral O_2 extraction. Therefore, the aim of this study was to examine plasma concentrations of NO and ET-1 in obese individuals at rest and during a progressive submaximal cycle ergometer test. The goal was to see whether dysfunction of the endothelium, as measured by plasma concentrations of NO and ET-1, in obesity might improve during physical exercise, supporting other studies showing peculiar dynamics of O_2 transport and utilization in this group.

Materials and Methods

Subjects

Fifteen young sedentary healthy lean men (group L) and 17 young obese men (group O) were enrolled in the study from February to December 2014. The O group was recruited from the Istituto Auxologico Italiano (Piancavallo, Italy), whereas the L group comprised medical staff at the hospital. Subjects with hypertension (blood pressure >130/90 mm Hg), impaired fasting glucose levels (>6.1 mmoL × L^{-1}) [18], type 2 diabetes, and an abnormal electrocardiogram at rest were excluded. The study was approved by the Ethics Committee of the Istituto Auxologico Italiano, and all subjects provided written informed consent.

Experimental Design

All subjects underwent dual-energy X-ray absorptiometry (DEXA) to measure body composition (DPX-IQ X-ray bone densitometer version 4.7e; GE Healthcare, Chalfont St. Giles, UK). Body composition of obese individuals whose body weight exceeded 125 kg ($n = 7$) was assessed using a tetrapolar bioelectrical impedance method (BIA 101/S, Akern, Florence, Italy) [19]; the hydration status of each subject was controlled for bioimpedance vector analysis [20]. As BIA tends to overestimate FFM in obese individuals [21], linear regression analysis of DEXA and BIA results from a sample of 20 overweight/obese hospitalized male patients (body mass index [BMI] range 25–52) was used to transform the FFM BIA data derived from these 7 individuals. To check the accuracy of the linear regression, 1 participant was removed from the dataset, and the linear regression was redone with the remaining 16 subjects. The FFM predicted by the linear regression was then compared with the values measured for the removed subject. This was repeated for all participants; there was no significant difference between the predicted FFM and the measured value in any case ($p = 0.99$).

Maximal Exercise Protocol

Based on previous experience [22], peak O_2 uptake ($\dot{V}O_{2peak}$) and peak power output (PPO) were measured using a maximal ramp incremental test performed to exhaustion on a cycle ergometer (Ebike Basic BPlus, Niskayuna, NY, USA). After a 3-min rest period, the L group began with a 5-min warmup at 60 W (40 W for the O group), followed by a linear increase in PPO (by 30 W;

Table 1. Clinical characteristics and hormone concentrations in the study subjects under basal conditions, along with variables measured during the maximal incremental test

	Lean subjects (n = 15)	Obese subjects (n = 17)	p value
Basal conditions			
Age, years	33.3±6.5	34.6±8.2	ns
Body weight, kg	72.3±4.7	121.8±19.8	<0.001
Height, cm	178.5±5.4	177.9±8.0	ns
Body mass index	22.5±1.5	38.6±5.4	<0.001
Fat mass, kg	13.9±4.1	52.3±15.4	<0.001
Fat-free mass, kg	56.0±5.3	68.0±7.4	<0.001
Blood pressure, mm Hg			
Systolic	115.0±1.8	121.0±2.1	ns
Diastolic	78.0±2.0	85.0±2.6	ns
HR, beats/min	73.1±12.7	72.9±8.4	ns
Endothelin-1, pg/mL	1.2±0.6	1.5±0.8	ns
Nitric oxide, μmol/L	38.8±9.8	36.3±13.1	ns
Maximal exercise test			
PPO, W	262.9±52.7	211.5±28.3	<0.01
Peak blood pressure, mm Hg			
Systolic	141.0±4.2	143.0±3.5	ns
Diastolic	87.0±2.6	92.0±2.2	ns
HR_{max}, beats/min	182.9±8.7	168.6±8.6	<0.001
$\dot{V}O_{2peak}$, mL/min	3,056.8±527.2	3,037.2±449.3	ns
$\dot{V}O_{2peak}$/weight, mL/min/kg	42.3±6.6	25.2±3.5	<0.001
$\dot{V}O_{2peak}$/FFM, mL/min/kg	54.5±6.4	44.7±5.3	<0.001
$\dot{V}O_{2peak}/HR_{max}$, mL/min/beats/min	17.6±2.8	18.2±2.1	ns
Anaerobic threshold, % $\dot{V}O_{2peak}$	57.6±6.0	51.5±6.9	<0.01

Values are expressed as means ± standard deviations. ns, not significant; PPO, peak power output; HR, heart rate; FFM, fat-free mass.

20 W for the O group) every minute until exhaustion. $\dot{V}O_2$, carbon dioxide production ($\dot{V}CO_2$), and $\dot{V}_E$ were measured continuously using a breath-by-breath online system (V_{max} 229, Sensor Medics, Yorba Linda, CA, USA). Before each test, a volume calibration and calibration of the gas analyzers using gases of known concentration were performed. Heart rate (HR) was recorded continuously using a HR monitor (Polar RS800, Kempele, Finland). $\dot{V}O_{2peak}$ was defined as the highest 10-s mean value recorded before a subject voluntarily terminated the test, whereas PPO was defined as the highest peak value reached during the maximal incremental ramp test.

Submaximal Exercise Protocol

Three days after a preliminary test, the experimental trial was performed in the morning (between 8:00 and 9:00 a.m.) after a minimum 12-h overnight fast. All participants were requested to refrain from exercise, alcohol, and caffeine for the 24 h preceding the test. After a 15-min seated resting period, the subjects remained seated for 15 min on the cycle ergometer and were connected to the metabolic system. Average HR and gas exchange data during the final 2 min of rest were used as the baseline. Thereafter, subjects performed a submaximal incremental test. After a standardized 10-min warmup at 20% PPO, the PO was increased by 7.5% PPO every 6 min (until 57.5% PPO or until the respiratory exchange ratio reached 1.0) as previously described [22].

Respiratory values ($\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}_E$) and $PETO_2$ were averaged over the final minute of each stage. The gas exchange threshold was analyzed by 2 blinded and independent investigators and calculated as described in the literature using the Wasserman ventilatory method: the point at which the $\dot{V}O_2$ respiratory equivalent ($\dot{V}_E/\dot{V}O_2$) increases and the $\dot{V}CO_2$ ventilatory equivalent ($\dot{V}_E/\dot{V}CO_2$) remains stable is assessed visually [23]. The estimated AT was checked using the Beaver ventilatory method, i.e., visually determining the inflection point of $\dot{V}CO_2$ on $\dot{V}O_2$ [24].

Blood Samples

During the second experimental protocol, blood samples were drawn at rest, during the last 3 min of the warmup, and during each step. Plasma NO and ET-1 concentrations were then measured. Briefly, blood samples were collected via an indwelling cannula inserted into the antecubital vein, which was kept patent by a continuous and slow saline infusion. Before the final step at the end of the exercise, all blood aliquots were placed on ice. After the final step, all blood samples were centrifuged at 4°C at 3,000 *g* for 10 min. Plasma or serum was transferred to storage tubes and frozen at –80°C until analysis.

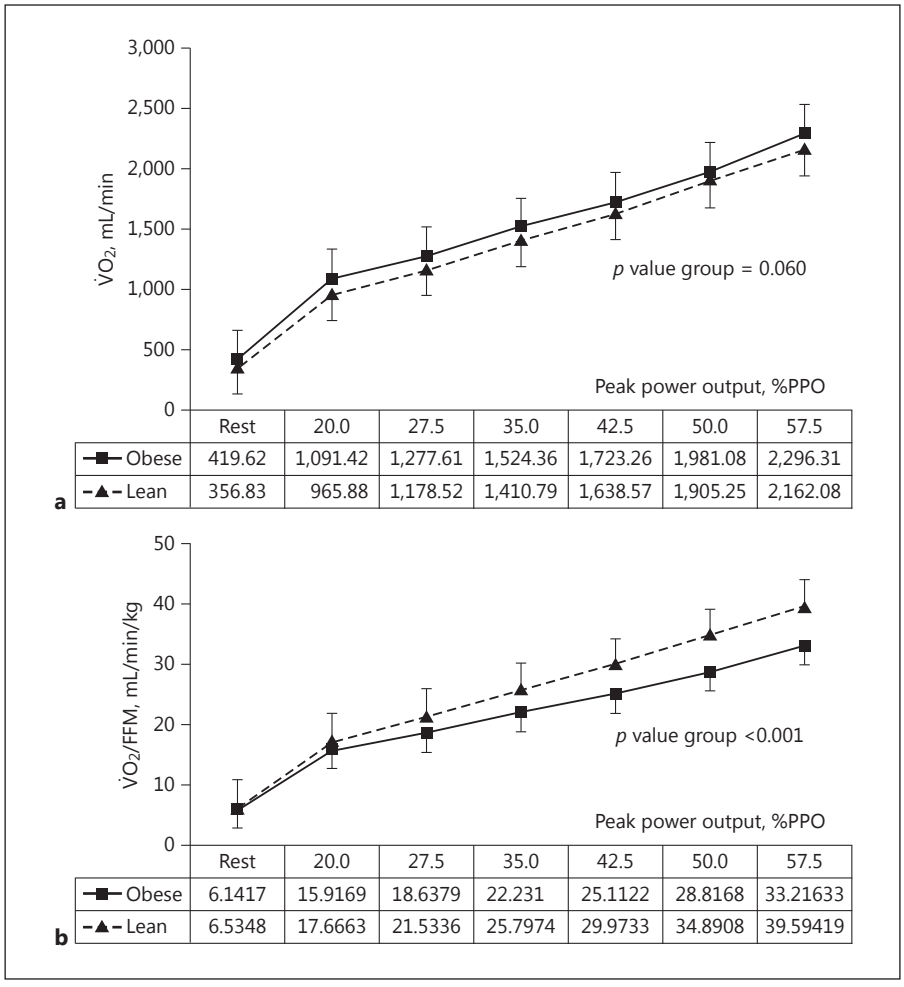

Fig. 1. Mean absolute values of O_2 consumption ($\dot{V}O_2$) (**a**) and $\dot{V}O_2$ adjusted for fat-free mass (FFM) (**b**) during submaximal exercise test in lean and obese subjects. Values are means ± standard errors.

NO plasma concentrations were determined using a nitrate/nitrite assay (Kamiya Biomedical Company, Seattle, WA, USA), which combines high sensitivity and fast processing in a 2-step assay. The first step used nitrate reductase to convert nitrate to nitrite. The second used Griess reagents to convert nitrite to a deep purple azo compound. The amount of the azo chromophore accurately reflects NO levels in a sample. The detection limit of the assay is approximately 0.1 nmoL NO/well, or 1 $\mu M \times L^{-1}$. The inter- and intra-assay coefficients of variation were 3.4 and 2.7%, respectively. ET-1 plasma concentrations were measured in an ET-1 immunoassay (R&D Systems Europe, Ltd., Abingdon, UK). The sensitivity of the assay was 0.207 pg × mL^{-1}, with an assay range of 0.39–25 pg × mL^{-1}. Inter- and intra-assay coefficients of variation were 5.3 and 1.9%, respectively.

Statistical Analysis

Clinical variables were compared between groups using the Wilcoxon Mann-Whitney test. Repeated-measures ANCOVA was used to compare $\dot{V}O_2$, $\dot{V}O_2$/FFM, $PETO_2$, and $PETO_2$/FFM between the 2 groups at different exercise intensities. Differences between ET-1 and NO concentrations at different exercise intensities and at baseline were calculated, and repeated-measures ANCOVA was used to determine whether differences were associated with group type (O or L). The ANCOVA models included groups, exercise intensity, and the interaction between group and exercise intensity. All models were adjusted for age. All models, except $\dot{V}O_2$ and $\dot{V}O_2$/FFM, were adjusted for $\dot{V}O_2$ at baseline. Models of differences between ET-1 and NO were adjusted for the respective baseline values. All analyses were performed using SAS version 9.4 software (SAS Institute, NC, USA). Significance was set at $p \leq 0.05$.

Results

Baseline characteristics, including ET-1 and NO, were comparable between the L and O groups (Table 1). As shown, there were no significant differences between the groups in terms of age, height, blood pressure, HR, ET-1 and NO levels, and $\dot{V}O_{2peak}$ and $\dot{V}O_{2peak}/HR_{max}$. Body weight, BMI, fat mass, and FFM were significantly great-

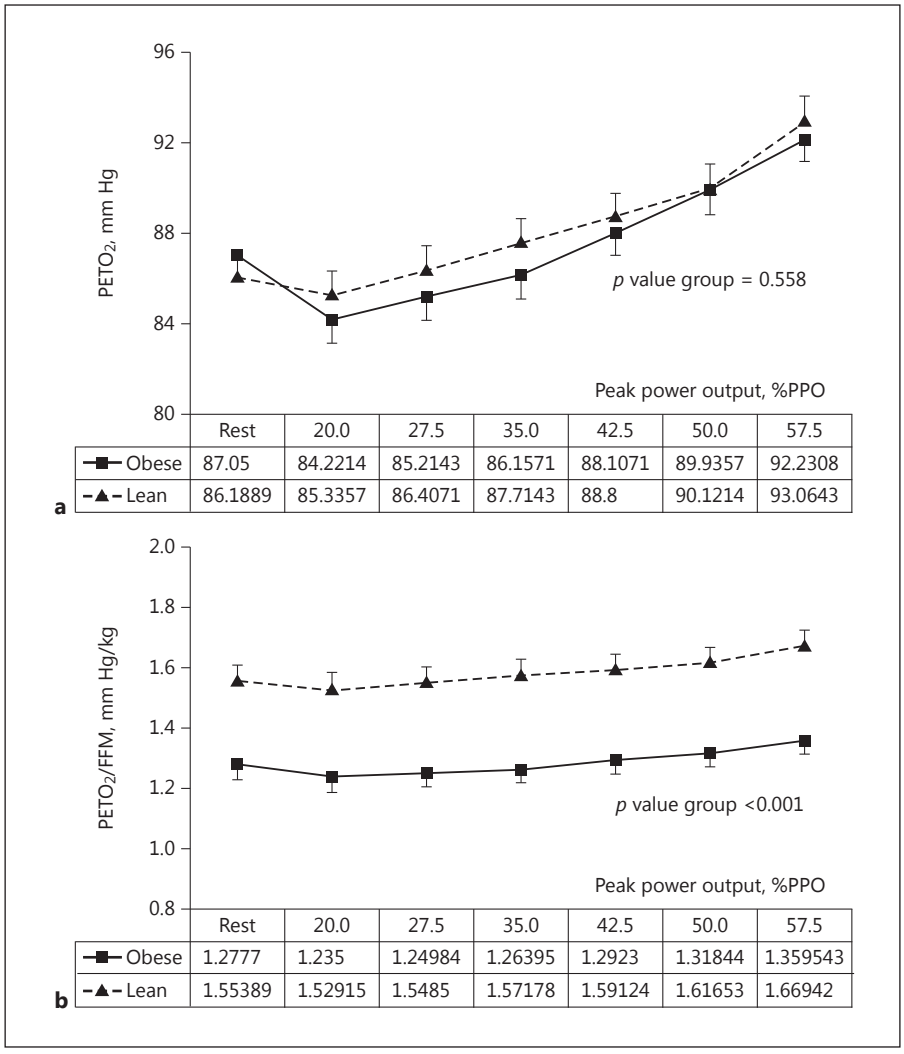

Fig. 2. Mean absolute values of end-tidal O_2 pressure ($PETO_2$) (**a**) and $PETO_2$ adjusted for fat-free mass (FFM) (**b**) during submaximal exercise test in lean and obese subjects. Values are means ± standard errors.

er in the O group than in the L group (BMI 38.6 ± 5.4 vs. 22.5 ± 1.5, respectively; $p < 0.001$). By contrast, PPO, HR_{max}, $\dot{V}O_2$/weight, $\dot{V}O_2$/FFM, and AT were significantly greater in the L group.

As shown in Figure 1a, the $\dot{V}O_2$ values for each group were not different ($p = 0.0596$) at baseline, and the trend in $\dot{V}O_2$ was similar ($p = 0.175$). Only exercise intensity was significantly different ($p < 0.001$) when considering $PETO_2$ as a response variable (Fig. 2a); however, there were also significant differences between groups when $\dot{V}O_2$/FFM ($p < 0.001$) (Fig. 1b) and $PETO_2$/FFM ($p < 0.001$) (Fig. 2b) were considered as response variables.

Figure 3 shows falls in ET-1 concentration (vs. baseline) in each group. There was a significant difference in the mean value between the groups ($p = 0.0306$), although the overall trend and the differences at different exercise intensities were not statistically significant ($p = 0.9579$ and $p = 0.2567$, respectively). By contrast, ANCOVA revealed no significant difference in plasma NO concentrations ($p = 0.5800$) or exercise intensity ($p = 0.0532$) between groups, while the interaction between group and exercise was significant ($p = 0.0458$) (Fig. 4).

Discussion

Here, we measured dynamic ET-1 and NO concentrations at rest and during/after progressive submaximal exercise in obese and lean men. Based on previous observations of lower $PETO_2$ values in obesity during physical stress, the aim was to look for any connection between

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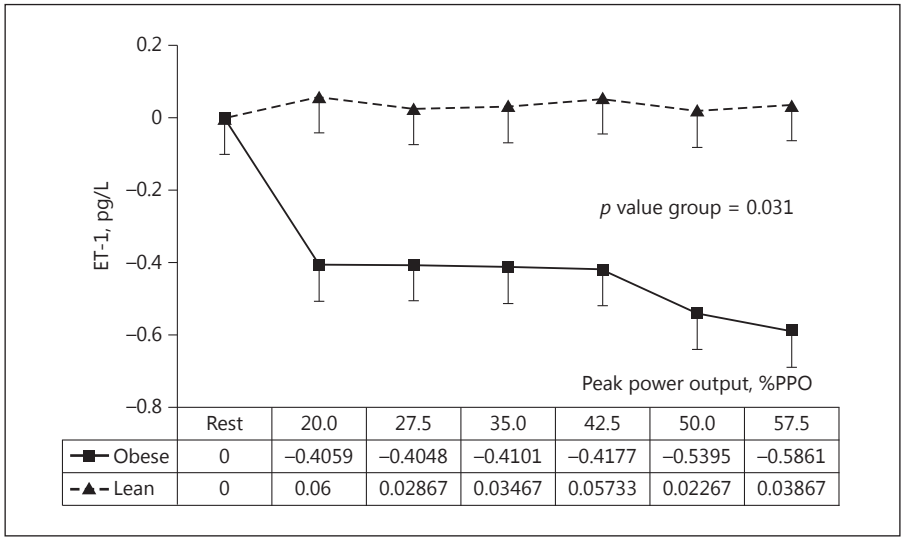
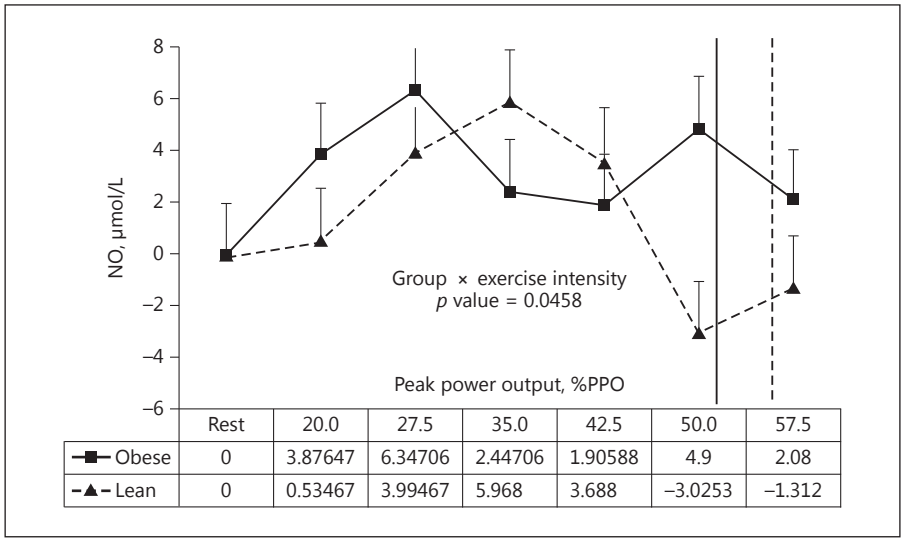


	Rest	20.0	27.5	35.0	42.5	50.0	57.5
Obese	0	–0.4059	–0.4048	–0.4101	–0.4177	–0.5395	–0.5861
Lean	0	0.06	0.02867	0.03467	0.05733	0.02267	0.03867

Fig. 3. Mean difference of endothelin-1 (ET-1) versus baseline during submaximal exercise in lean and obese subjects. Values are means ± standard errors.



	Rest	20.0	27.5	35.0	42.5	50.0	57.5
Obese	0	3.87647	6.34706	2.44706	1.90588	4.9	2.08
Lean	0	0.53467	3.99467	5.968	3.688	–3.0253	–1.312

Fig. 4. Mean difference of nitric oxide (NO) versus baseline during submaximal exercise in lean and obese subjects. Values are means ± standard errors. Anaerobic threshold for lean and obese subjects in vertical lines.

arterial regulation and patterns of peripheral O_2 transport.

As observed in previous studies, for any given level of O_2 uptake obese subjects tend to exhibit lower $PETO_2$ values and a greater arterial-venous difference in O_2 concentration than lean subjects, which may result in increased peripheral O_2 extraction. This may be due to a higher body mass, particularly muscle mass. In fact, differences in $PETO_2$ and FFM between obese and lean subjects become significant when expressed as a ratio ($PETO_2$/FFM). These differences may indicate that each kilogram of FFM (during physical stress, this mainly means each kilogram of muscle) in an obese person extracts more O_2 than that in a lean person [3].

Here, we focused on the hypothesis that a greater difference in arterial-venous O_2 concentrations might be linked to differences in peripheral mechanisms that regulate blood flow, such as ET-1 (a vasoconstrictor) and NO (a vasodilator). In humans, chronic exercise increases basal NO levels and reduces ET-1 levels [25]. A similar result is noted in overweight and obese subjects [26] in terms of an increase in central arterial relaxation after a period of aerobic exercise.

Under basal conditions, obese subjects generally have higher ET-1 levels [27] and lower NO levels [28, 29] than lean subjects, favoring arterial vasoconstriction. Here, we found that resting ET-1 levels were slightly higher and resting NO levels slightly lower in obese subjects than in

lean subjects; this may be due to factors such as the lack of hypertensive patients in the cohort. During exercise, we noted a rapid and significant reduction in venous ET-1 levels in obese subjects but no significant change in lean subjects. Plasma NO showed a parallel increase in the 2 groups, with an earlier decrease in obesity.

We cannot exclude the possibility that rapid shunting of ET-1 blood pools might be caused by an increase in enzymatic degradation; however, an animal study showed that this may be a possible metabolic effect of insulin. Indeed, ET-1 secretion reduces both insulin-mediated skeletal muscle capillary recruitment and skeletal muscle glucose uptake by 50% [30]. Furthermore, the pattern of ET-1 concentrations (along with the consequent vasodilation) seems to agree with previous observations that differences in arterial-venous O_2 concentrations and $PETO_2$ play a role in ensuring increased peripheral O_2 extraction and normal glucose uptake. For this reason, the significant difference between the 2 groups in terms of $PETO_2$ (adjusted for FFM to take into consideration the large overall mass of obese subjects) appears important. NO concentrations showed a similar trend, at least in the context of lower power outputs. At higher power outputs, both obese and lean subjects experienced a prompt reduction in NO (although the reduction was less in obese subjects, probably to maintain adequate vasodilation). This reduction in obese subjects was detectable at lower power outputs than in lean subjects but, curiously, at a similar time distance from the subsequent exceeding of AT in both groups. We believe that one possible interpretation of these dynamic responses is the need to maintain an adequate blood flow to ensure the most optimal homeostasis. For this reason, a fall in ET-1 levels, particularly in obese subjects with more muscle mass, is necessary; an increase in NO secretion probably maintains appropriate blood flow to the muscles (also to the heart and brain), as reported in animals studies [16].

This study has some limitations because we did not measure blood flow directly, nor plasma ET-1 and NO concentrations in the veins of the working muscles. Flow-mediated dilatation is probably linked directly to peripheral O_2 extraction. A recent study suggested that this dilator response is likely endothelium dependent and largely NO mediated [31]. Furthermore, we need to issue a word of caution about using the nitrite/nitrate assay to measure NO concentrations in blood because it is a surrogate marker that might be influenced by non-NO synthase pathways. However, despite these limitations, the results reported herein underline the role of ET-1 and NO in obesity during physical exercise.

In conclusion, these data show that dynamic secretion of vasoactive substances (ET-1 and NO) differs between obese and lean subjects. In obesity, ET-1 and NO appear to play an important role in regulating peripheral O_2 delivery. Further studies are needed to confirm these effects and their possible vascular implications. In particular, studies should look for differences in NO and ET-1 concentrations in working and nonworking muscles in obese subjects.

Financial Disclosure and Conflicts of Interest

The authors declare that there are no conflicts of interest.

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