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Skeletal muscle alkaline Pi pool is decreased in overweight-to-obese sedentary subjects and relates to mitochondrial capacity and phosphodiester content

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Defects in skeletal muscle energy metabolism are indicative of systemic disorders such as obesity or type 2 diabetes. Phosphorus magnetic resonance spectroscopy (³¹P-MRS), in particular dynamic ³¹P-MRS, provides a powerful tool for the non-invasive investigation of muscular oxidative metabolism. The increase in spectral and temporal resolution of ³¹P-MRS at ultra high fields (i.e., 7T) uncovers new potential for previously implemented techniques, e.g., saturation transfer (ST) or highly resolved static spectra. In this study, we aimed to investigate the differences in muscle metabolism between overweight-to-obese sedentary (Ob/Sed) and lean active (L/Ac) individuals through dynamic, static, and ST ³¹P-MRS at 7T. In addition, as the dynamic ³¹P-MRS requires a complex setup and patient exercise, our aim was to identify an alternative technique that might provide a biomarker of oxidative metabolism. The Ob/Sed group exhibited lower mitochondrial capacity, and, in addition, static ³¹P-MRS also revealed differences in the Pi-to-ATP exchange flux, the alkaline Pi-pool, and glycerophosphocholine concentrations between the groups. In addition to these differences, we have identified correlations between dynamically measured oxidative flux and static concentrations of the alkaline Pi-pool and glycerophosphocholine, suggesting the possibility of using high spectral resolution ³¹P-MRS data, acquired at rest, as a marker of oxidative metabolism.

Obesity, resulting from an imbalance between energy intake and expenditure, is a worldwide epidemic associated with insulin resistance syndrome. Given that skeletal muscle accounts for almost half the total body mass and is responsible for the majority of glucose uptake and glycogen storage in response to insulin stimulus¹, the investigation of muscle energy expenditure is of particular importance with regard to the pathogenesis of obesity and metabolic syndrome. Recent studies showed that insulin resistance relates to abnormalities in energy metabolism, not only of skeletal muscle^{2–4}, but also of the heart⁵ and liver⁶. The contractile activity of skeletal muscle is primarily regulated by the ATP synthesis rate⁷, which, under aerobic conditions in exercised muscle, is determined mainly by the oxidative phosphorylation capacity of mitochondria. Changes in muscle energy metabolism related to mitochondrial dysfunction could indicate defects in lipid metabolism (i.e., fatty acid oxidation)⁸, potentially

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resulting in the progression of metabolic disease, such as type 2 diabetes, even in a young, overweight-to-obese, sedentary population^{9–11}.

The non-invasive detection of intramyocellular energy metabolites (i.e., phosphocreatine [PCr], ATP, and inorganic phosphate [Pi]) is possible through phosphorous magnetic resonance spectroscopy (³¹P-MRS), which provides an ideal tool for the *in vivo* monitoring of cellular energy status and metabolism^{7,12}. Dynamic ³¹P-MRS, during exercise and recovery, in particular, allows direct estimation of the oxidative ATP synthesis rate in challenged muscle^{12–15}, which reflects maximal mitochondrial capacity⁷. Altered mitochondrial metabolism is associated with obesity, elevated fasting glucose or insulin resistance^{16–20}. As the dynamic examinations require a complex setup, e.g., dedicated ergometers, and patient compliance throughout the whole exercise protocol, an alternative ³¹P-MRS technique for the assessment of energy metabolism at rest would constitute a significant advantage. The measurement of resting Pi-to-ATP flux (F_{ATP}) using ³¹P-MRS saturation transfer (ST), correlates with the findings of dynamic experiments^{21,22}. Although the absolute values of F_{ATP} do not provide a direct measure of oxidative metabolism²³, it has also been related to insulin resistance^{24,25}.

Recently, the use of ³¹P-MR spectra, measured in the equilibrium state, has been promoted to obtain similar information about muscle energy metabolism. In particular, the concentration of phosphodiester ([PDE]) was shown to correlate with the Pi-to-ATP flux²⁶. Moreover, an alkaline Pi pool (Pi_2) has been detected *in vivo* at ultra-high field (i.e., 7T)²⁷, and related to the PCr re-synthesis rate after exercise²⁸.

Our aim was to compare the skeletal muscle metabolism of overweight-to-obese sedentary (Ob/Sed) subjects, who are prone to type 2 diabetes, and lean active (L/Ac) individuals, using static and dynamic ³¹P-MRS measurements in the quadriceps femoris muscle at 7 T. In addition, the interrelations between the derived parameters were investigated to determine possible alternatives to exercise-recovery experiments.

Results

Between groups comparison. In addition to a significantly higher BMI and lower VO_{2max} , the Ob/Sed individuals also differed from the L/Ac volunteers in the metabolic parameters derived from ³¹P-MRS. The concentration of the main muscular PDE (i.e., glycerophosphocholine [GPC]), as well as the total [PDE], were significantly higher, while the concentration of the alkaline Pi-pool (Pi_2) and its ratio to the main Pi concentration ($[Pi_1]$), i.e., (Pi_2/Pi), were significantly lower in the Ob/Sed group compared to the L/Ac group. In addition, the group of Ob/Sed subjects had significantly lower mitochondrial capacity (Q_{max}) and Pi-to-ATP exchange flux (F_{ATP}) values compared to the L/Ac group. Detailed information about the measured physiological and muscle energy metabolism parameters are listed in Table 1. In Fig. 1 are depicted representative ³¹P-MR spectra acquired at rest and during the exercise-recovery experiment and Fig. 2 depicts the comparison between the groups.

Correlations between the measured parameters. The measured concentration of PDE in the quadriceps muscle correlated positively with both age ($r = 0.45$, $p = 0.014$) and BMI ($r = 0.62$, $p = 0.0004$). BMI correlated negatively with the $[Pi_2]$ ($r = -0.56$, $p = 0.002$), as well as with the Pi_2/Pi ratio ($r = -0.44$, $p = 0.023$) and Q_{max} ($r = -0.39$, $p = 0.039$). The calculated F_{ATP} was also found to be negatively correlated with age ($r = -0.48$, $p = 0.009$) and BMI ($r = -0.51$, $p = 0.007$).

In addition, we have found correlations between the metabolic parameters extracted from the ³¹P-MRS measurements performed at rest and the oxidative metabolism markers measured in a dynamic exercise-recovery experiment. The [PDE] correlated negatively with Q_{max} ($r = -0.51$, $p = 0.005$), while both $[Pi_2]$ and Pi_2/Pi correlated with Q_{max} positively ($r = 0.68$, $p = 0.0001$ and $r = 0.65$, $p = 0.0002$, respectively). Q_{max} significantly correlated also with the k_{ATP} ($r = 0.51$, $p = 0.005$) and F_{ATP} ($r = 0.63$, $p = 0.0003$). Several correlations were also found between the different parameters of muscular energy metabolism measured at rest. The [PDE] correlated negatively with $[Pi_2]$ ($r = -0.63$, $p = 0.0003$), Pi_2/Pi ($r = -0.63$, $p = 0.0003$), k_{ATP} ($r = -0.54$, $p = 0.003$), and F_{ATP} ($r = -0.59$, $p = 0.001$). Both $[Pi_2]$ and Pi_2/Pi were correlated with k_{ATP} ($r = 0.41$, $p = 0.029$ and $r = 0.52$, $p = 0.005$), as well as with F_{ATP} ($r = 0.59$, $p = 0.001$ and $r = 0.45$, $p = 0.018$). All correlations of the evaluated metabolic parameters with the [PDE] were also significant for the [GPC]. Representative correlations are depicted in Fig. 3.

Multivariate stepwise regression analysis of Q_{max} including physiological and metabolic parameters derived from ³¹P-MRS data acquired at rest, identified $[Pi_2]$ ($r^2 = 0.46$, adjusted $r^2 = 0.44$, $p = 0.0001$) as the strongest and F_{ATP} ($r^2 = 0.54$, adjusted $r^2 = 0.50$, $p = 0.00001$) as the second-strongest independent predictor of Q_{max} . Detailed results are given in Table 2.

Discussion

In this study, we compared parameters of skeletal muscle metabolism, measured by static and dynamic ³¹P-MRS methods, between a group of overweight-to-obese sedentary subjects, who are prone to diabetes, and a group of lean active individuals. We have found that the combination of increased BMI and sedentary lifestyle leads to significant differences in the alkaline Pi pool in skeletal muscle, as well as in other metabolic ³¹P-MRS parameters, such as the concentration of PDE, the Pi-to-ATP metabolic flux, and mitochondrial capacity. In addition, significant correlations were found between the concentration of PDE, the alkaline Pi_2/Pi ratio, and the resting Pi-to-ATP exchange rate and flux, measured by ³¹P-MRS techniques at rest, and the maximal mitochondrial oxidative flux, measured by an exercise-recovery experiment.

Dynamic ³¹P-MRS provides a parameter closely related to training status, i.e., the mitochondrial capacity (Q_{max}) of the muscle tissue¹⁵. This was also demonstrated in our study, as the Q_{max} of the overweight-to-obese sedentary subjects was significantly lower when compared to active, lean individuals. The correlation between Q_{max} and BMI found in this study can be explained by the decreased physical activity in more obese individuals, as our regression analysis showed a primary connection of Q_{max} with other parameters of muscle metabolism and not with BMI. This is in good agreement with a recent *in vitro* study, which found no differences in mitochondrial

Variable	Overweight Obese/Sedentary	Lean/Active
N (female)	14 (5) ^o	15 (5)
Age (years)	34.6 ± 7.1	29.3 ± 5.5
BMI (kg.m ⁻²)	30.4 ± 2.3	23.1 ± 2.6 [*]
Body fat (%)	35.2 ± 7.1	18.3 ± 6.1 [*]
LBM (kg)	62.4 ± 10.9	63.0 ± 15.6
VO _{2max} (mL.min ⁻¹ .kg ⁻¹)	36.8 ± 5.3	45.9 ± 3.1 [*]
Steps per 24 hours	6052 ± 1166	11093 ± 4074 [*]
static MRS		
[PDE] (mM)	4.21 ± 1.12	2.82 ± 1.00 [†]
[GPC] (mM)	3.95 ± 1.04	2.47 ± 0.98 [†]
[GPE] (mM)	0.26 ± 0.27	0.23 ± 0.17
[Pi ₂] (mM)	0.18 ± 0.07	0.28 ± 0.06 [*]
Pi ₂ /Pi	0.05 ± 0.02	0.08 ± 0.02 [*]
pH _{rest}	7.06 ± 0.04	7.05 ± 0.03
[ADP] _{rest} (μM)	10.1 ± 0.9	9.8 ± 0.6
ST		
k _{ATP} (s ⁻¹)	0.07 ± 0.02	0.08 ± 0.01
F _{ATP} (mM.s ⁻¹)	0.25 ± 0.06	0.31 ± 0.04 [†]
k _{CK} (s ⁻¹)	0.27 ± 0.05	0.25 ± 0.05
F _{CK} (mM.s ⁻¹)	9.26 ± 2.36	8.66 ± 2.40
Dynamic		
PCr drop (% signal)	38.4 ± 19.4	40.4 ± 13.9
τ _{PCr} (s)	40.9 ± 14.0	42.6 ± 15.8
V _{PCr} (mM.s ⁻¹)	0.29 ± 0.10	0.32 ± 0.07
Q _{max} (mM.s ⁻¹)	0.50 ± 0.08	0.58 ± 0.07 [†]
pH _{end exercise}	6.97 ± 0.14	6.90 ± 0.16
[ADP] _{end exercise} (μM)	47.8 ± 32.0	40.9 ± 15.5

Table 1. Characteristics of the studied groups and results of muscle energy metabolism measurements via static ³¹P-MRS, saturation transfer, and dynamic experiments. Data are given as mean ± standard deviation. ^oFor one volunteer from the overweight-to-obese sedentary group, only dynamic experiment data are available. Significant differences (unpaired t-test) between the groups are depicted as follows: ^{*}*p* < 0.01; [†]*p* < 0.05.

respiratory capacity and mitochondrial content in myocellular tissue samples between lean and obese subjects with similar training status²⁹. Similarly, a different *in vivo* study did not find any changes in mitochondrial capacity in humans after weight reduction stimulated by diet-only; however, if combined with increased physical activity, an improvement in aerobic capacity was observed³⁰.

Significantly higher myocellular PDE levels were found in our Ob/Sed subjects when compared to the L/Ac group. This was further supported with the positive correlation found between [PDE] and BMI. This is in good agreement with the finding of a previous report by Szendroedi *et al.*²⁶ in subjects with a comparable physical activity index. A correlation of [PDE] and age was also reported, in the current study and in^{26,31}; however, our regression analysis showed that physical activity and BMI, rather than age, primarily predict the PDE levels (data not shown). The increased spectral resolution of the 7 T MR system, used in our study, reveals that the measured [PDE] is mainly attributable to [GPC], with only a small contribution from glycerophosphoethanolamine ([GPE]), and that, in fact, it is the [GPC] that is responsible for the differences between the two groups. This separation in PDE signals was not visible in the previous study performed at 3 T²⁶.

The ratio of alkaline Pi₂ to cytosolic Pi (Pi₂/Pi) was lower in the Ob/Sed group in comparison to the L/Ac group. Recently, van Oorschot *et al.* reported a dependence of Pi₂/Pi, measured in the vastus lateralis, on the training status, when they compared highly trained runners with normally active individuals²⁸. The potential influence of BMI was, however, not considered in the aforementioned study. The results of our study suggest such a dependence of the Pi₂/Pi ratio in skeletal muscle on BMI, as a linear correlation between BMI and Pi₂/Pi was found. Nevertheless, the results of our regression analysis identified only [GPC] and Q_{max} as the primary predictors of the Pi₂/Pi (data not shown). The differences in Pi₂/Pi found between the groups can be directly attributed to the changes in [Pi₂], which was also significantly higher in the L/Ac group in comparison to the Ob/Sed group.

Although the mean F_{ATP} values of our Ob/Sed group (F_{ATP} = 0.25 ± 0.06 mM.s⁻¹) are still above the decreased values reported previously in patients with type 2 diabetes (F_{ATP} = 0.21 ± 0.05 mM.s⁻¹)²⁵ the physical inactivity together with the overweight of our volunteers caused a significant reduction in the myocellular Pi-to-ATP metabolic flux, when compared to L/Ac individuals (F_{ATP} = 0.31 ± 0.04 mM.s⁻¹).

Interrelations between metabolic parameters measured by dynamic and static ³¹P-MRS. We report several correlations between the parameters of static ³¹P-MR spectra, exchange rates and metabolite fluxes

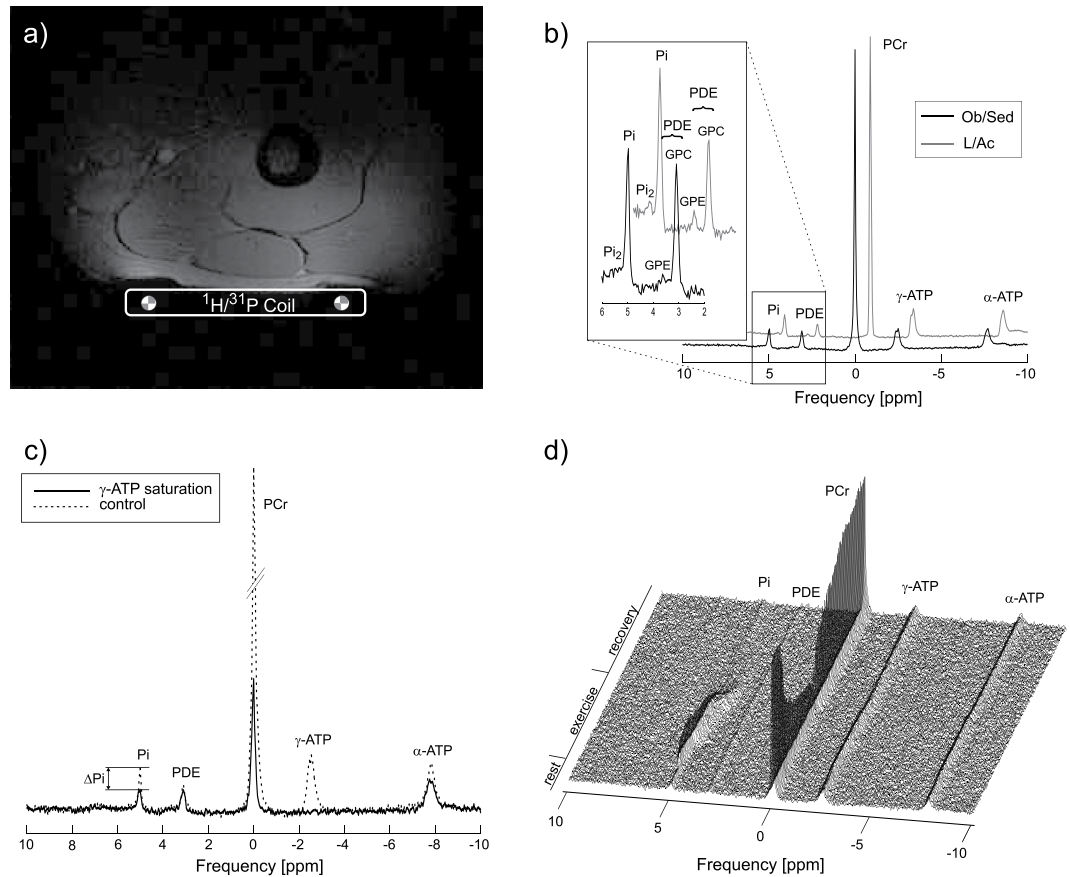


Figure 1. (a) An *in vivo* localizer image of the human thigh with the depicted coil position. (b) Highly spectrally resolved representative ^{31}P -MR spectra from an obese sedentary and lean active subject, scaled to PCr signal intensity. The area of Pi and PDE peaks is enlarged. Note higher Pi_2 and lower PDE signal intensity in the L/Ac subject. (c) Saturation transfer spectra showing the effect of γ -ATP saturation (solid line) on its chemical exchange partner, Pi, compared with the control experiment (dashed line). (d) Time course of the ^{31}P spectra during a dynamic ^{31}P -MRS experiment. Note the PCr signal depletion during exercise and its re-synthesis during recovery.

measured by ST at rest, and oxidative metabolism markers measured by exercise-recovery experiments. The alkaline Pi_2 resonance is suspected to represent mitochondrial density in the muscle tissue and depends on the amount of regular physical activity²⁸. Its relation to training status was also confirmed in our study, as the $[\text{Pi}_2]$, as well as Pi_2/Pi , positively correlated with the maximal oxidative flux (Q_{\max}), determined during the dynamic experiment, and also with the k_{ATP} and F_{ATP} defining the Pi-to-ATP exchange rate and metabolic flux. Linear correlations between Q_{\max} and metabolic parameters measured by ST experiments at rest (i.e., k_{ATP} and F_{ATP}) reported in our previous study on overweight-to-obese subjects²², were also found in this study combining the two different population groups. In addition, the multivariate regression analysis identified $[\text{Pi}_2]$ and F_{ATP} as independent predictors of Q_{\max} , suggesting the potential use of highly spectrally resolved static ^{31}P -MRS at 7 T and ST as alternative techniques to dynamic exercise-recovery experiments.

The identified correlation between [PDE] and measured Pi-to-ATP metabolite flux (F_{ATP}) is in good agreement with a recent report by Szendroedi *et al.*²⁶. Significant correlations were found also between [PDE] and mitochondrial capacity (Q_{\max}), as well as other ^{31}P -MR parameters of muscle metabolism measured at rest, i.e., $[\text{Pi}_2]$, Pi_2/Pi , and k_{ATP} . In addition, the main contributors to total [PDE] were analyzed and all [PDE] correlations were also significant for [GPC]. Our results suggest the potential of using [PDE], or, if distinguishable (i.e., at 7 T) directly, the [GPC], as a surrogate biomarker of skeletal muscle energy metabolism. Although it is not perfectly clear what links GPC to muscle energy metabolism, previous studies support this finding^{32–37}. In particular, Farber *et al.*, studying a model of membrane defect of Alzheimer's disease, reported that an inhibition of oxidative phosphorylation causes accumulation of GPC through accelerated PC turnover³⁴. Impaired oxidative metabolism and elevated PDE levels have been also reported in patients with spinal cord injury³⁵ and congenital lipodystrophy³⁶. Muscular PDE content was also related to glucometabolic control in type II diabetes²⁶. Furthermore, excessive amounts of PDE have been reported in fibromyalgia³³, Duchenne muscular dystrophy³², or Becker muscular dystrophy³⁷, connecting abnormal membrane metabolism with muscle dysfunction. Nonetheless, further investigations of this relation are still necessary.

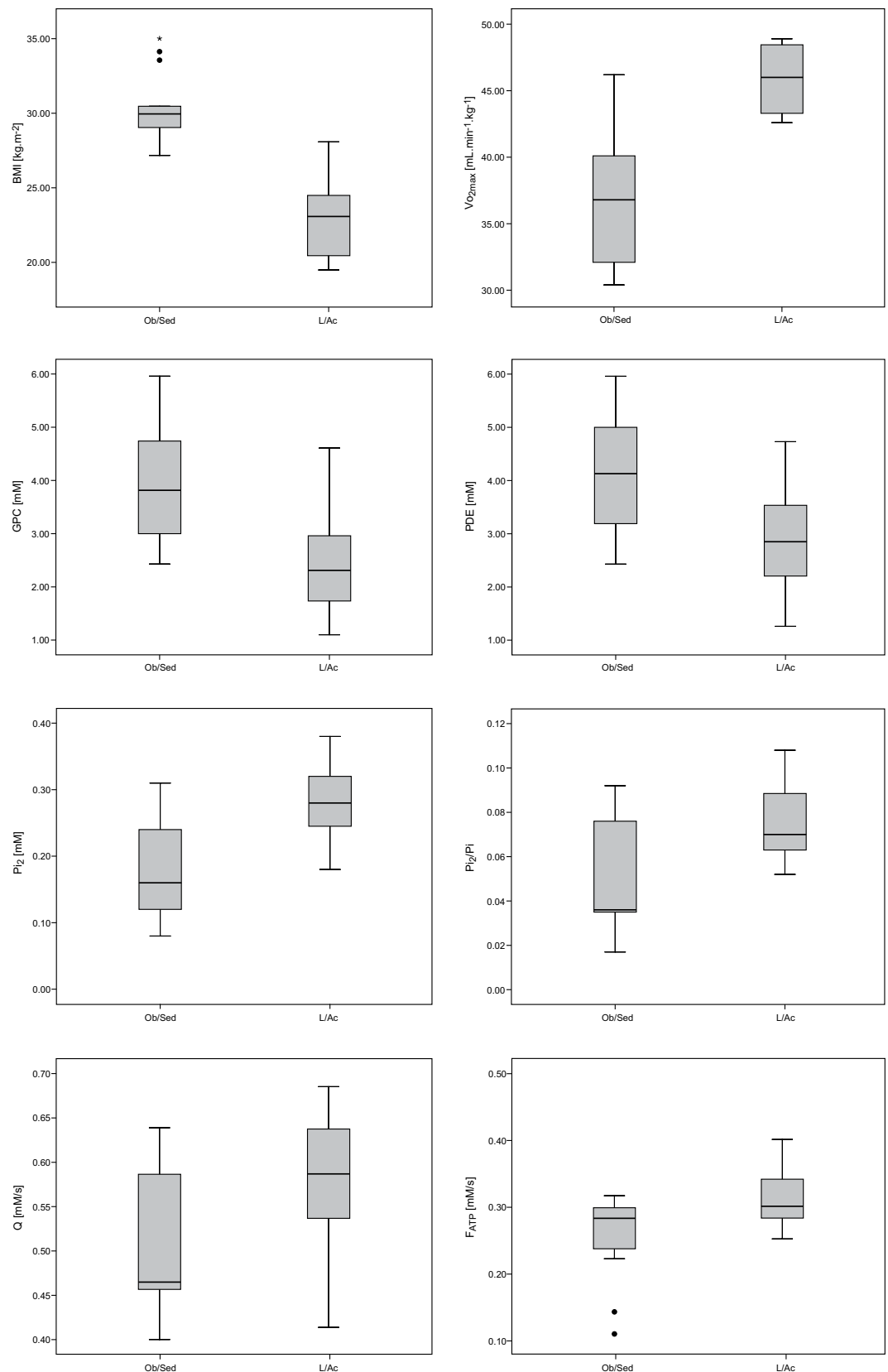


Figure 2. Box plots depicting the significantly different physiological and metabolic parameters between the two groups. The solid lines represent the median, boxes represent lower and upper quartiles, and whiskers the minimum and maximum. Outliers and extreme outliers are denoted by circles and stars, respectively. The outliers were also taken into account for all statistical tests.

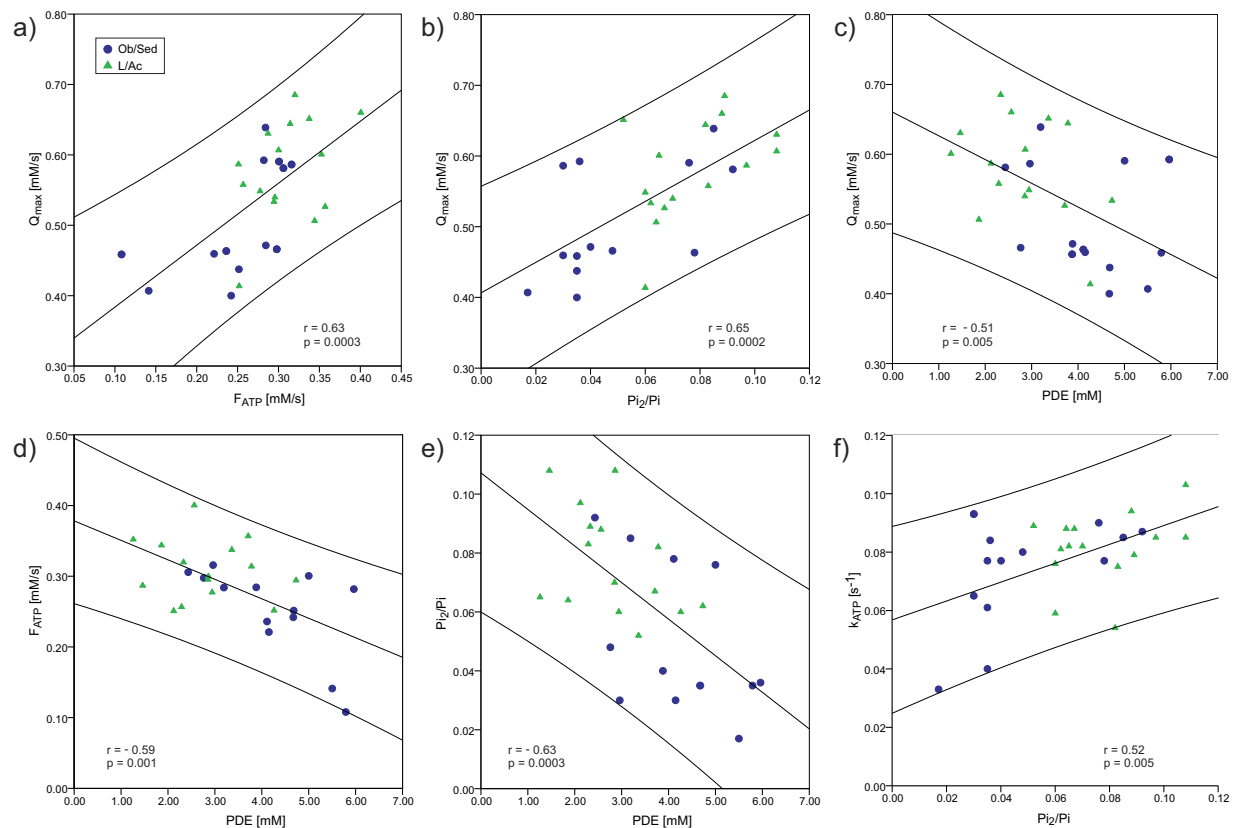


Figure 3. Plots of correlations between myocellular energy metabolism parameters measured by dynamic and static ^{31}P -MRS in Ob/Sed (●) and L/Ac (▲) individuals: (a) mitochondrial capacity (Q_{\max}) with the Pi -to-ATP forward metabolic flux at rest (F_{ATP}); (b) Q_{\max} with the ratio of alkaline Pi to main Pi (Pi_2/Pi); and (c) Q_{\max} with the concentration of phosphodiesterases ([PDE]). Further correlations of the ^{31}P -MRS parameters measured at rest: (d) F_{ATP} with [PDE]; (e) Pi_2/Pi with [PDE]; and (f) Pi -to-ATP exchange rate constant (k_{ATP}) with Pi_2/Pi . 95% confidence intervals are also depicted.

Independent variables per Q_{\max}	F value	p value
Pi_2^*	22.035	0.0001
F_{ATP}^*	14.653	0.0001
Pi_2/Pi	<0.05	0.217
BMI	<0.05	0.536
k_{ATP}	<0.05	0.537
GPC	<0.05	0.689
PDE	<0.05	0.940
age	<0.05	0.961

Table 2. Results of multivariable stepwise regression of Q_{\max} (dependent variable) and physiological and metabolic variables measured at rest (independent variable). *Variables accepted into the model as predictors; all other variables not accepted into the model.

The findings of this study support our previous report on correlations between dynamic and ST parameters in this Ob/Sed group²² and provide additional information through analysis of Pi_2/Pi and GPC, and moreover, by comparison to a lean active group of individuals. As to the technical limitations of our study, we should note that although care was taken to perfectly reposition the subject in the second MR system, when applicable, some small mislocalizations could not be fully excluded. The effect of individual anatomy must be also considered, as the localization through the sensitivity of the surface coil used in this study might cover different portions of the quadriceps muscles between subjects. Localization techniques, recently proposed for dynamic examinations of the lower leg muscles, e.g., frequency selective ^{31}P -MRI^{38–40}, semi-LASER for single voxel localization⁴¹ or depth-resolved surface coil MRS⁴², could be used in future studies to measure muscle-specific metabolism. However, the muscles of the quadriceps covered by the sensitivity volume of the used surface coil are all active

during knee-extension⁴³, and, therefore, the inter-subject variability of the covered muscle volumes should have had only a minor effect on our results.

In conclusion, overweight-to-obese sedentary pre-diabetics exhibit increased concentrations of glycerophosphocholine, a lower amount of alkaline Pi, a slower Pi-to-ATP exchange rate, and decreased mitochondrial capacity compared to lean active individuals. Associations found between the parameters of myocellular metabolism measured at rest and during exercise suggest that highly spectrally resolved static ³¹P-MRS and saturation transfer measurements at rest could provide markers of muscle mitochondrial metabolism.

Methods

Fifteen young, overweight-to-obese, sedentary individuals (10/5 male/female; age 34.6 ± 7.1 years) with a body mass index (BMI) $\geq 27.0 \text{ kg.m}^{-2}$, a sedentary lifestyle without regular physical activity, no pharmacotherapy, and no medical history of type 2 diabetes were recruited for this study and classified as the overweight-to-obese/sedentary (Ob/Sed) group. Thirteen of these volunteers had already participated in our previous study on the interrelations between mitochondrial capacity and Pi-to-ATP exchange rates in this particular type of population²². Fifteen young, lean, physically active participants (10/5 male/female; age 29.3 ± 5.5 years) were recruited for the current study as the control lean/active (L/Ac) group.

Written, informed consent was obtained from each participant in the study after an explanation of the purpose, nature and potential risks of the study. The examination protocol was approved by the appropriate institutional ethical boards of the Medical University of Vienna and of the University Hospital Bratislava, Comenius University Bratislava, and the study was carried out in accordance with the approved guidelines.

Physiological tests. Within a week before the MR examination, the participants underwent a physical examination and physiological testing. BMI was measured by an analog weight scale and standard measuring tape. Bioelectric impedance, measured using an Omron BF511 (Omron Healthcare, Matsusaka, Japan), was used to evaluate total adiposity (%Fat) and to estimate the lean body mass (LBM). The maximal aerobic capacity (i.e., whole-body oxygen uptake [$\text{VO}_{2\text{max}}$]) was measured during an incremental exercise test performed on a Lode Corival cycle ergometer (Lode, Groningen, The Netherlands). Continuous measurement of the gas exchange rate was obtained with the Ergostik (Geratherm Respiratory, Bad Kissingen, Germany), and the maximal oxygen consumption rate was expressed relative to LBM. The ergometry was performed at least three days prior to the MR examinations. The activity level was evaluated based on two working days and a weekend of accelerometer recordings and expressed as the number of steps per 24 hours.

³¹P-MRS. Each participant underwent the entire MR examination protocol in one day, starting two hours after a standardized meal. The dynamic ³¹P-MRS exercise-recovery experiment was performed on either a 7 T MR system (Magnetom, Siemens Healthcare, Erlangen, Germany) or a 3 T MR system (TIM Trio) from the same manufacturer, due to initial compatibility problems of our ergometer (Quadspect, Ergospect, Innsbruck, Austria) with the 7 T. Dual-tuned (³¹P-¹H) circular surface coils (10 cm diameter, Rapid Biomedical, Rimpfing, Germany), with similar sensitivity volumes²² were used on both MR systems. The use of two MR systems, equipped with the same ergometer and surface coils with a similar sensitivity volume, has recently been shown to have no effect on the metabolic data derived from dynamic ³¹P-MRS⁴⁴.

Static ³¹P-MRS experiments were performed exclusively at 7 T, as the increased spectral resolution is necessary for separation of the Pi₂, as well as the GPE and GPC signals²⁷, and the increase in signal-to-noise ratio allows significant reduction in measurement time of the ST experiment, compared to 3 T⁴⁵. The subjects were investigated while lying inside the MR scanner with the surface coil fixed to the quadriceps femoris muscle (Fig. 1a) and the coil positions were marked to allow precise repositioning in the other MR system, if applicable. When the dynamic measurements were performed at 3 T (i.e., in case of first 10 Ob/Sed subjects), the order of examinations was randomized to allow simultaneous examinations of two subjects; otherwise the measurements at rest were always performed prior to the exercise-recovery experiment.

For the assessment of intramyocellular metabolite concentrations and the Pi₂/Pi ratio, a pulse-acquire ³¹P-MR spectrum (acquisition delay = 0.4 ms; repetition time = 15 s; bandwidth = 5 kHz; 16 averages in 4 minutes) was acquired at rest (Fig. 1b) and corrected for longitudinal relaxation times, as measured for ³¹P muscle metabolites at 7 T^{27,46}. The γ -ATP signal was used as an internal concentration reference, assuming a stable ATP concentration of 8.2 mM in the skeletal muscle¹².

The exchange rate between ATP and Pi (i.e., ATP synthesis) was investigated using an ST experiment applying continuous irradiation, and the apparent longitudinal relaxation time (T_1^{app}) was determined via an inversion recovery experiment, as described previously⁴⁵. The total measurement time of the ST experiment was under 9 minutes.

The exercise-recovery protocol involved six minutes of repeated knee extensions against an air pressure, set to 30% of the maximal voluntary contraction force, once every repetition time (i.e., 2 s), followed by six minutes of recovery¹⁵. The volunteers were instructed by an audio signal to time the contraction-relaxation periods, so that the spectra were acquired always in the relaxed state of the muscle.

Analyses and calculations. Due to patient noncompliance ($n_{\text{Ob/Sed}} = 1$) and technical problems ($n_{\text{Ob/Sed}} = 1$), 28 complete datasets and one incomplete dataset (dynamic ³¹P-MRS only), were available for analyses.

All acquired ³¹P-MR spectra were analyzed using jMRUI software with the AMARES time domain fitting algorithm⁴⁷. The resonance lines of PCr, two Pi signals, and two PDEs—glycerophosphocholine (GPC) and glycerophosphoethanolamine (GPE)—were fitted as single Lorentzians, whereas γ - and α -ATP were fitted as doublets and β -ATP as a triplet. The line width of the Pi₂ peak was constrained with respect to the line width of the main Pi peak, and the expected frequency difference between Pi₂ and Pi was set to ~ 0.4 ppm, to ensure a good

fit for the Pi_2 peak^{27,28}. The shift in resonance position between PCr and Pi signals in parts per million (δ) was used to calculate intramyocellular pH⁴⁸, according to the Henderson-Hasselbalch equation: $\text{pH} = 6.75 + \log((\delta - 3.27)/(5.63 - \delta))$. The free cytosolic ADP concentration ($[\text{ADP}]$) was calculated according to the method described by Kemp *et al.*⁴⁹, assuming that 15% of total creatine $[\text{Cr}]$ was not phosphorylated in the resting state⁵⁰.

The chemical exchange rate constant (k_{ATP}) was calculated from the fractional reduction of Pi magnetization upon selective saturation of γ -ATP (Fig. 1c)⁴⁵. The resting unidirectional forward exchange flux was then calculated as $F_{\text{ATP}} = k_{\text{ATP}} \times [\text{Pi}]$.

To calculate the time constant of PCr resynthesis (τ_{PCr}), the PCr signal changes during the recovery period of the dynamic experiment (Fig. 1d) were fitted to a monoexponential function using MATLAB (MathWorks, Natick, MA, USA). The initial PCr recovery rate (V_{PCr}), which roughly represents ATP turnover at the end of exercise, was determined and used to calculate the maximal rate of oxidative phosphorylation (Q_{max}) according to the ADP-based model of Michaelis and Menten⁴⁹.

Data are presented as means \pm standard deviations and compared between the groups by an unpaired Student t-test. The relationships between metabolic parameters were analyzed by linear regression analysis, using Pearson's correlation coefficient, to estimate the strength of the relationship. Multivariate stepwise regression analysis for the dependent variable Q_{max} was performed using the independent variables (i.e., BMI, age, [PDE], [GPC], $[\text{Pi}_2]$, Pi_2/Pi , k_{ATP} and F_{ATP}). A similar multivariate regression analysis was performed for the dependent variables [PDE] and Pi_2/Pi . The results were considered statistically significant at $p < 0.05$.

Data. Parts of the data were presented as abstracts at the following meetings: ISMRM 2012 Melbourne Australia, ISMRM 2015 Toronto Canada. Some ^{31}P -MRS data of the dynamic and ST experiment, from the Ob/Sed group exclusively, were published in the *NMR in Biomedicine* journal (ref. 22).

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Author Contributions

L.V., B.U., J.U. and M.K. designed the study, L.V., M.C. and T.H. acquired and analyzed the data. L.V. and M.K. wrote the manuscript. W.B., I.F., H.T., M.K., N.B., J.U. and S.T. contributed to the discussion and all authors reviewed the final form of the manuscript.

Additional Information

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