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A Low-Cost MR Compatible Ergometer To Assess Post-Exercise Phosphocreatine Recovery Kinetics

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Abstract

Object—To develop a low-cost pedal ergometer compatible with ultra high (7 T) field MR systems to reliably quantify metabolic parameters in human lower leg muscle using phosphorus magnetic resonance spectroscopy.

Materials and Methods—We constructed an MR compatible ergometer using commercially available materials and elastic bands that provide resistance to movement. We recruited 10 healthy subjects (eight men and two women, mean age $\pm$ standard deviation: 32.8 ± 6.0 years, BMI: 24.1 ± 3.9 kg/m²). All subjects were scanned on a 7 T whole-body magnet. Each subject was scanned on two visits and performed a 90 s plantar flexion exercise at 40 % maximum voluntary contraction during each scan. During the first visit, each subject performed the exercise twice in order for us to estimate the intra-exam repeatability, and once during the second visit in order to estimate the inter-exam repeatability of the time constant of phosphocreatine recovery kinetics. We assessed the intra- and inter-exam repeatability in terms of the within-subject coefficient of variation (CV).

Results—We acquired reliable measurements of PCr recovery kinetics with an intra- and inter-exam CV of 7.9% and 5.7%, respectively.

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Authors' Contribution

Protocol/Project development: Parasoglou, Brown, Walczyk, Regatte. Data collection or management: Naimon, Parasoglou, Khegai, Alon, Che. Data analysis: Parasoglou, Naimon, Babb.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Conclusion—We constructed a low-cost pedal ergometer compatible with ultra high (7 T) field MR systems, which allowed us to reliably quantify PCr recovery kinetics in lower leg muscle using ^{31}P -MRS.

Keywords

Phosphorus; human skeletal muscle; exercise; magnetic resonance spectroscopy

Introduction

Phosphorus magnetic resonance spectroscopy (^{31}P -MRS) is a unique tool that allows direct measurement of important high-energy phosphates in human tissue, such as adenosine triphosphate (ATP), phosphocreatine (PCr) and inorganic phosphate (Pi). ^{31}P -MRS has been used extensively to study skeletal muscle metabolism during rest-exercise-recovery protocols [1–6]. During exercise, ATP is consumed and maintained at a constant level through PCr hydrolysis. This leads to reduction in PCr and increase in Pi, which can be directly observed in ^{31}P -MRS. During recovery, ATP, and thus PCr, are resynthesized through oxidative phosphorylation, which takes place in the mitochondria [6–9]. The recovery of PCr is considered a valid index of mitochondrial oxidative phosphorylation [8, 10–12], and has been used to study mitochondrial impairment in diseases with high socio-economic impact such as mitochondrial myopathy [13], diabetes [14], peripheral arterial disease [15, 16], and heart failure [17]. Mitochondrial ATP synthetic function in human skeletal muscle is best estimated through dynamic ^{31}P -MRS recording of post-exercise PCr recovery [3, 5]. These data are more easily interpreted following low intensity exercise (i.e. below the lactate threshold), when contribution of glycolytic ATP synthesis is considered negligible. In low intensity exercise regimes, PCr recovery follows monoexponential kinetics [5, 18], which implies a linear steady-state relationship of oxidative ATP synthesis to PCr depletion, and vice versa [19–21]. ^{31}P -MRS data interpretation during voluntary exercise (as opposed to during recovery of PCr following exercise) is significantly more involved, and requires several assumptions about motor unit recruitment with the particular exercise protocol and workload used [22].

While high intensity exercise paradigms such as uncontrolled maximum intensity bouts are attractive in terms of their simplicity [23], patients with lower leg complications may not sustain high muscle stress. Therefore, studies oftentimes rely on submaximal exercise paradigms, during which muscles are stressed at a pre-defined level inside the bore of the magnet using MR compatible ergometers. Different types of ergometers have been developed over the years, which either use pneumatic cylinders [24–28], elastic bands [29, 30], or weighted pulleys to allow variable and adjustable resistance to exercise [30, 31]. Several commercially available ergometers offer different exercise modules, though these can be cost prohibitive for many research labs [32].

Herein, we describe the construction and functional abilities of an in-house designed and built MR compatible pedal ergometer, using low-cost, commonly available components. We put an upper limit to the total materials cost at \$500 USD. Our ergometer allows subjects to perform submaximal plantar flexion exercises, while we continuously monitor patient

compliance and power output. It is easy to use and can be set up in a magnet bore in less than ten minutes, and dissembled in the same amount of time. We tested the ergometer in a 7 Tesla whole-body magnet and used it to evaluate repeatability and reliability of dynamic ^{31}P -MRS measurements.

Materials and Methods

Ergometer Description

We built the ergometer using non-ferromagnetic materials, and it was designed to fit in conventional whole-body scanners up to 7 T field strength. The ergometer base consists of a custom-made wooden plank ($101 \times 34 \times 2$ cm) with two length-wise support pieces on the bottom ($69 \times 3 \times 2$ cm each) and a small one ($6 \times 25 \times 3$ cm) spanning the width at the front (Fig. 1a). At one end of the frame is a custom-machined plastic footplate resting on a plastic stand. The pedal moves on the stand between 5° and 35° to allow dynamic plantar flexion exercise. The pedal consists of an outside layer and an inside layer that are compressed together when force is applied to the pedal. The pedal, as well as the stand that supports it, are made of high-density polyethylene (HDPE), which offers high mechanical strength (Fig. 1e). The force is continuously monitored with a transducer (Tekscan, Inc. South Boston, MA) that is secured between the layers. Stainless steel springs between the two layers of the pedal plate keep the force sensor unloaded during rest. The angle of rotation of the pedal is also continuously monitored using a position sensor (Tekscan, Inc. South Boston, MA) as shown in Figure 1a. Analog force and angle signals are digitized outside the magnet on an Arduino Uno Rev 3 board (an open source platform) and inputted to Matlab for real-time processing and display. A multi-line cable connects the Arduino to the sensors through a waveguide to carry DC power to the sensors and force and angle outputs back to the Arduino. Elastic bands are anchored at the bottom of the footplate and the base of a polyvinyl chloride (PVC) structure with freely rotatable pulleys, using plastic hooks (Fig. 1c). They provide resistance from 0 to 400 N (defined by the number of bands used) to counteract movement, which corresponds to a torque from 0 to 60 N m on our setup. The resistance force generated by the elastic bands was estimated by stretching the bands to known lengths by means of known weights (see Appendix). The elastic bands are also attached to a custom-machined plastic holder towards the edge of the wooden baseboard (Fig. 1b). The holder can be used to attach a plastic stopper that provides infinite resistance in order to define maximum voluntary contraction (MVC) for each subject before the beginning of the low intensity exercise (Fig. 1d; 3D model Supporting Material). To limit subject movement and restrict muscle recruitment to the calf during plantar flexion, subjects are secured to the ergometer using an adjustable full-body harness (X-1 Bowhunter, Hunter Safety System Inc, Danville AI) that is attached to the base of the pedal stand (Fig. 1b). To restrict motion while maintaining subject comfort, we mounted a cross-country ski boot to the ergometer (Table 1). The boot's inflexible sole limited toe flexion and provided a base that was easily mounted to the ergometer.

Subjects and MRS Acquisition

The study was fully Health Insurance Portability and Accountability Act (HIPAA)–compliant and approved by the NYU Institutional Review Board. We obtained written

informed consent from all participants in this study. The methods were carried out in accordance with Food and Drugs Administration (FDA) guidelines. We recruited 10 healthy subjects (eight men and two women, mean age $\pm$ standard deviation: 32.8 ± 6.0 years, BMI 24.1 ± 3.9 kg/m², mean $\pm$ standard deviation). All subjects were scanned on a 7 T whole-body magnet (Siemens Medical Solutions, Erlangen, Germany) with a 12 cm diameter dual-tuned (³¹P/¹H) loop coil (Quality Electrodynamics, Mayfield, OH). Participants were familiarized with the exercise procedure prior to the MRI scan. We determined their maximum voluntary isometric plantarflexor contraction (MVC) on the ergometer with the stopper in place (Fig. 1d). We confirmed proper exercise technique, including minimal involvement of accessory muscles. We chose the number of rubber bands that corresponded to 40% of the MVC for each subject.

For the MRI scan, subjects laid supine on the MRI bed with the coil fixed underneath the medial head of the right gastrocnemius muscle. The right foot was placed into a boot on top of the pedal, which minimized the use of stabilizer muscles and prevented excess energy dissipation.

We acquired ³¹P-MRS data using an unlocalized pulse-acquire product sequence with a repetition time of 6 s. We acquired 2048 points of the free induction decay, with a spectral width of 5000 Hz. PCr time courses were normalized to the value at rest. To calculate PCr and pH, data were quantified using the AMARES time domain fit routine, as incorporated in the jMRUI software package [33, 34]. No data filtering or zero-filling was performed prior to spectra fitting. We fitted the PCr recovery data to a single exponential recovery function, using a least squares minimization algorithm, and estimated the time constant of PCr resynthesis (τ_{PCr}). We also estimated the end-of-exercise PCr level (PCr_{min}) and the pH during rest and at the end of exercise, as well as the work output (W) generated by each participant during each scan (for calculation of work see Appendix).

Inter and Intra-Subject Reliability Measurement Protocol

Subjects were scanned with the following procedure:

- Two scanning exams: Each subject was scanned on two days in the same week and we will refer to scans of the same subject on different days as **exams**.
- Two scans in exam one: Each subject was scanned during two successive acquisitions with a brief (~ 10 min long) pause between experiments. During the pause, the subject was removed from the magnet and required to remove fastenings and leave the MR bed. We will refer to scans of the same subject on the same day as **sessions**.
- One scan in exam two: Each subject returned within the week to perform one additional data acquisition.
- Each scan consisted of 30 s of rest followed by 90 s of exercise and 4 min of rest for PCr recovery.
- The 90 s exercise was composed of pushing the pedal at approximately 40% maximum voluntary contraction at the beat of a metronome at 40 bpm.

Statistical Analysis

We will refer to the variance among the results from the two sessions on day 1 for the same subject as the intra-exam component of the intra-subject variance, $\sigma^2(\text{Intra})$, and to the variance among results derived from exams on different days for the same subject as the inter-exam component of the intra-subject variance, $\sigma^2(\text{Inter})$. We will refer to the variance among results for different subjects as the inter-subject component of the overall variance and denoted $\sigma^2(\text{Subject})$.

We used restricted maximum estimation (REML) of variance components in a random effects model to estimate these individual components of the overall variance for each measure (i.e. τ_{PCr} , PCr_{min} , and W). The model included subject ID, exam nested within subject and session nested within exam as random classification factors. With this model, the error variance corresponds to $\sigma^2(\text{Intra})$, the variance associated with exams nested within subjects corresponds to $\sigma^2(\text{Inter})$ and the variance associated with subject corresponds to $\sigma^2(\text{Subject})$. The estimated variance components were used to compute the within-subjects coefficient of variation (CV) and intra-class correlation coefficients (ICC) associated with the intra-exam and inter-exam components of the intra-subject variance. For the intra-exam variation, two ICC values were computed as $\text{ICC1} = \sigma^2(\text{Inter}) / [\sigma^2(\text{Inter}) + \sigma^2(\text{Intra})]$ and $\text{ICC2} = \sigma^2(\text{Inter}) / [\sigma^2(\text{Inter}) + \sigma^2(\text{Subject})]$, this accounts for the nesting of the intra-exam variation within both exams and subjects. For the inter-exam variation, a single ICC value was computed as $\text{ICC} = \sigma^2(\text{Subject}) / [\sigma^2(\text{Subject}) + \sigma^2(\text{Inter})]$. The CV values for each component were computed as the square root of the relevant variance estimate expressed as a percentage of the overall mean.

Results

Typical ^{31}P -MRS spectra from a male participant during rest and at the end of exercise can be seen in Figure 2a. The time course of PCr signal depletion and subsequent recovery of the same volunteer can be seen in Figure 2b. Continuous measurement and recording of angle displacement (Fig. 2c) allows subject compliance to be monitored with the exercise protocol. Figure 3 shows examples of PCr timecourses in a female participant in each of the three scans, during which she performed 90 s of plantar flexion at 40 bpm. The reliability results are summarized in Tables 2 and 3.

Mechanical Power Output

The power output during ^{31}P -MRS was estimated from the known resistance of the elastic bands, the angular displacement of the pedal, and the frequency of muscle contractions. The power output for the participants had a mean of 4.57 W, with $\sigma^2(\text{Intra}) = 0.04$, $\sigma^2(\text{Inter}) = 0.03$, and $\sigma^2(\text{Subject}) = 1.13$. Intra-Exam CV was 4.4% ($\text{ICC1} = 0.40$, $\text{ICC2} = 0.97$), and Inter-Exam CV was 3.6% ($\text{ICC} = 0.98$).

End of exercise pH and PCr levels

Mean PCr_{min} was 70.49%, with $\sigma^2(\text{Intra}) = 5.14$, $\sigma^2(\text{Inter}) = 14.50$, and $\sigma^2(\text{Subject}) = 63.85$. The Intra-Exam CV was 3.2% ($\text{ICC1} = 0.74$, $\text{ICC2} = 0.93$), and the Inter-Exam CV was 5.4% ($\text{ICC} = 0.81$).

We did not observe significant acidification during exercise in any of the subjects. The mean end of exercise pH was 7.05 with $\sigma^2(\text{Intra}) = 0.00069$, $\sigma^2(\text{Inter}) = 0.00072$, and $\sigma^2(\text{Subject}) = 0.0027$. The Intra-Exam CV was 0.4% (ICC1 = 0.51, ICC2 = 0.80), and the Inter-Exam CV was 0.4% (ICC = 0.79).

PCr Recovery Time Constant

The calculation of τ_{PCr} revealed a high median coefficient of determination ($r^2 = 0.959$, min/max = 0.898 – 0.999). Mean k_{PCr} was 26.40 s, with $\sigma^2(\text{Intra}) = 4.31$, $\sigma^2(\text{Inter}) = 2.29$, and $\sigma^2(\text{Subject}) = 40.16$. The Intra-Exam CV was 7.9% (ICC1 = 0.35, ICC2 = 0.90), and the Inter-Exam CV was 5.7% (ICC = 0.95).

Discussion

In this work, we designed and constructed a robust low-cost MR compatible ergometer that was used to stress the muscles of the lower leg at a pre-defined level inside the bore of a modern ultrahigh (7 T) field magnet. The main advantage of the device is that it is easy to construct it at a very low cost (under \$500 USD). In addition, it can be placed on the magnet bed within a reasonable amount of time (approximately 10 min). The reported amount includes only the cost of the materials used to build the device. In the supporting material, we have included 3D models of the device on a free software platform (Google SketchUp) and a basic signal acquisition code for the Arduino board (in Matlab), which can enable other research groups with basic machining and programming skills to replicate the device. Importantly, one of the most expensive parts for building such a device can be the digitizer. We are showing how an inexpensive board (i.e. Arduino) with freely available drivers can replace the need for expensive digitizers.

Our ergometer produced well-controlled PCr depletion with excellent patient compliance and highly reliable measurements of PCr recovery kinetics with an intra- and inter-exam coefficient of variation of 7.9% and 5.7%, respectively. These values are comparable with those reported in the literature [23, 35–39]. The power output showed high reliability with intra- and inter-exam coefficient of variation of 4.4% and 3.6%, respectively. No significant acidosis (pH <6.88) was observed in any of the participants, which justifies fitting PCr recovery data to a monoexponential function [40].

In our study, the intra exam variation is greater than extra inter exam variation. Subjects learning to perform the task in a more uniform manner over time could result in the data showing increased reliability over time. Since the intra-exam variation was assessed only in Day 1 of the study, this could result in the estimate of intra-exam variation being greater than the estimate of inter-exam variation since both components of variation would decline over time.

Our ergometer uses elastic cords that generate forces up to 400 N, which correspond to a maximum torque of 60 N m on our setup. Healthy subjects between the ages of 30 and 39 years typically generate maximum torque of 110 N m during plantar flexion when both the soleus and the gastrocnemii muscle groups are recruited [41], and that of about 70 N m when only the gastrocnemii muscles are recruited [42]. Therefore, the device can be used for low-

grade exercise (of 40% MVC) in a relative large range of subjects. In studies where higher torque is needed, the number of cords can be increased, or different elastic cords that provide higher resistance could be used.

One of the limitations of our ergometer is that it does not allow the user to change the level of resistance from the control room. This requires changing the number of elastic bands that resist pedal movement. Such changes can be more easily performed from the control area using pneumatic pistons as previously shown [24–28]. Another limitation of our system was the lack of a sophisticated feedback system for real-time self-adjustment of exercise conditions such as the visual system reported by Tschiesche *et al.* [24]. To the best of our knowledge, such a feedback system would be prohibitively expensive in order to be compatible with ultra high (7 T) field magnets. Despite the lack of a real-time feedback system, all subjects showed excellent compliance and the power output was very reliable in all three scans of the participants. Another limitation in our study is the relatively low temporal resolution of 6s per spectrum, which may not allow to accurately characterize fast resynthesis rates in some healthy subjects. Despite this limitation, we observed an excellent fitting quality with high median coefficient of determination ($r^2 = 0.959$, min/max = 0.898 – 0.999).

Prior to MRI experiments, subjects were trained and their range of motion was restricted in order to emphasize gastrocnemius recruitment during plantar flexion. However, we did not confirm muscle involvement by, for example, performing localized spectroscopy or imaging, during plantar flexion with flexed or straight knee [43, 44]. While the ergometer was tested on healthy subjects, we anticipate that the device will be well tolerated by patients similar to other MR compatible ergometers used in patient studies [27, 30].

In summary, we constructed a low-cost MR compatible pedal ergometer with commonly available components, which allows subjects to perform submaximal plantar flexion exercises. The device allows us to continuously monitor patient compliance and power output, leading to highly reliable measurements of skeletal muscle metabolic parameters assessed through ^{31}P -MRS. This device can be used to design reliable longitudinal studies to assess treatments aimed at improving mitochondrial function in several patient groups.

Conclusion

In this work, we developed a low-cost pedal ergometer compatible with ultra high (7 T) field MR systems that allowed us to reliably quantify metabolic parameters in the lower leg muscles using ^{31}P -MRS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Appendix

Elastic Band Force Calibration

Commercially available polyurethane elastic bands provide resistance to counteract movement during plantar flexion (Table 1). They are anchored at the bottom of the foot-plate and the base of a PVC structure that is mounted on the wooden baseboard using plastic hooks (Fig. 1c). The bands are 30 cm in length when relaxed. We measured the length of 10 identical elastic bands by suspending blocks of lead of 2.5, 3.5, 5, and 6 kg (Fig. 4). When the pedal is at rest, the bands are stretched to 37 cm in length, resulting in an initial loading of 38 N per elastic band. At maximum stretch the bands are 42 cm in length, providing resistance of 53 N per elastic band. Within the 5 cm operating range, the bands have an approximately linear response. Currently, the device allows the use of up to eight rubber bands.

At the time of preparation of this manuscript, we have used the bands for over 8 months. We are performing a monthly recalibration and so far we have not noticed change in their elastic properties. The bands will be replaced when their elastic properties exceed a 5% deviation from the baseline. When we store the device, we keep the bands relaxed by unmounting the hook on one end to avoid unnecessary wearing when the device is not being used.

Mechanical Power Output Calculation

The work calculation relies on data from the angle sensor. Angle sensor calibration was tested both inside and outside the magnet and no RF interference with the recorded signal was observed. The positive mechanical work during plantar flexion is estimated based on information about the angle of the pedal, which is recorded by the angle sensor at any instance of time, and the force generated by the sum of elastic bands that resist motion, using the following formula:

$$W = \begin{cases} d \sum_t F(\alpha) \Delta \alpha & \text{if } \Delta \alpha > 0 \\ 0 & \text{if } \Delta \alpha < 0 \end{cases} \quad [1]$$

Where W is the work (J), d is the distance between the anchoring point of the rubber band and the axis of rotation of the pedal (m), F is the instantaneous force of the elastic bands (N), and α the instantaneous angle measured through the sensor (in rad). Energy is absorbed by the subject during the return of the pedal to rest position, and is not included in the calculation of positive mechanical work.

The mechanical power output (P , Watt) for the exercise is the total work over the duration of the exercise (T ,s):

$$\overline{P} = \frac{W}{T} \quad [2]$$

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Figure 1.

(a) Side view of the ergometer with the force sensor underneath the pedal. A stopper is installed to provide infinite resistance for defining MVC for each subject. The angle sensor can be seen in the magnified box. Rotation of the pedal results in rotation of the wiper on the resistance wire, which leads to voltage changes. (b) Harness. (c) Close-up view of the elastic bands on freely rotatable pulleys. The bands are anchored on the pedal and base of the device using plastic hooks. (d) Close-up view of the boot secured on the pedal. (e) The pedal, as well as the stand that supports it, are made of high-density polyethylene (HDPE), which offers high mechanical strength, while the pulley system is made of PVC.

Figure 2.

(a) ^{31}P -MRS spectra from a male participant during rest and at the end of exercise. (b) Time course of PCr signal depletion and subsequent recovery. The PCr recovery rate was 24 s ($r^2 = 0.989$). (c) Angle displacement of the pedal during 90 s plantar flexion exercise at 40 bpm.

Figure 3.

PCr time courses in a female participant in each of the three scans during which she performed 90 s of plantar flexion at 40 bpm. PCr_{min} was 68.92% during Exam 1 Session 1, 66.01% during Exam 1 Session 2, and 65.62% during Exam 2. Power output was 4.47 W, 4.77 W, and 4.68 W, respectively, while τ_{PCr} was estimated at 31.23 s, 30.46 s, and 31.65 s, respectively.

Figure 4.

Mean and standard deviation of length measurements from 10 identical elastic bands by suspending blocks of lead of 2.5, 3.5, 5, and 6 kg. When the pedal is at rest, the bands are stretched to 37 cm in length, resulting in an initial loading of 38 N per elastic band. At maximum stretch the bands are 42 cm in length, providing resistance of 53 N per elastic band. Within the 5 cm operating range, the bands have an approximately linear response.

Table 1

Materials Used for the Construction of the Ergometer

Item	Vendor	Product Name	Model/Material
Angle Sensor	Tekscan	FlexiPot Ring Position Sensor	ZFLEX(PR101)-4PK
Arduino	Arduino Store USA	Arduino UNO	REV3
Band Clips	American Cord and Webbing	Plastic Hardware Black Hook	CH-25
Buckles	McMaster—Carr	Buckles for Webbing	29705T14/29705T87
Cable	AlphaWire	Shielded Cable	2214C SL005
Force Sensor	Tekscan	FlexiForce Sensor	ZFLEX(A401)25-4
Nylon Fabric	McMaster—Carr	Nylon Fabric	87425k77
Resistance Bands	McMaster—Carr	Polyurethane bungee cords	3097T85
Plastic Pedal	McMaster—Carr	HDPE	8619K471
Elastic Band Support	McMaster—Carr	PVC	87025K113
Harness	Hunter Safety System Inc	X-1 Bowhunter	—
Boot	Alpina	Blazer 75 mm Boot	—

Table 2

The mean of each imaging measure over sessions, exams and subjects and the REML estimates of the variance components for each imaging measure.

Measure	Mean	σ^2 (Intra)	σ^2 (Inter)	σ^2 (Subject)
PCr _{min} (%)	70.49	5.14	14.50	63.85
τ_{PCr} (s)	26.40	4.31	2.29	40.16
Power (W)	4.57	0.04	0.03	1.13
pH	7.05	0.00069	0.00072	0.0027

Table 3

The ICC and CV values as measures of intra-exam and inter-exam reliability.

Measure	Intra-Exam			Inter-Exam	
	CV	ICC1	ICC2	CV	ICC
PCr _{min}	3.2%	0.74	0.93	5.4%	0.81
τ _{pcr}	7.9%	0.35	0.90	5.7%	0.95
Power	4.4%	0.40	0.97	3.6%	0.98
pH	0.4%	0.51	0.80	0.4%	0.79