Maximum rate of oxygen uptake by human skeletal muscle in relation to maximal activities of enzymes in the Krebs cycle

Eva Blomstrand, Göran Rådegran and Bengt Saltin

Copenhagen Muscle Research Centre, Rigshospitalet, Section 7652, Tagensvej 20, DK-2200 Copenhagen N, Denmark

1. Ten subjects performed incremental exercise up to their maximum work rate with the knee extensors of one leg. Measurements of leg blood flow and femoral arteriovenous differences of oxygen were made in order to be able to calculate oxygen uptake of the leg.
2. The volume of the quadriceps muscle was determined from twenty-one to twenty-five computer tomography section images taken from the patella to the anterior inferior iliac spine of each subject.
3. The maximal activities of three enzymes in the Krebs cycle, citrate synthase, oxoglutarate dehydrogenase and succinate dehydrogenase, were measured in biopsy samples taken from the vastus lateralis muscle.
4. The average rate of oxygen uptake over the quadriceps muscle at maximal work, 353 ml min^{-1} kg^{-1}, corresponded to a Krebs cycle rate of 4·6 μmol min^{-1} g^{-1}. This was similar to the maximal activity of oxoglutarate dehydrogenase (5·1 μmol min^{-1} g^{-1}), whereas the activities of succinate dehydrogenase and citrate synthase averaged 7·2 and 48·0 μmol min^{-1} g^{-1}, respectively.
5. It is suggested that of these enzymes, only the maximum activity of oxoglutarate dehydrogenase can provide a quantitative measure of the capacity of oxidative metabolism, and it appears that the enzyme is fully activated during one-legged knee extension exercise at the maximal work rate.

During whole-body exercise, e.g. running or cycling, there is evidence to support the view that the maximal oxygen uptake is primarily limited by the cardiac output rather than the capacity of the skeletal muscle to extract and utilize oxygen (Saltin & Rowell, 1980; Blomqvist & Saltin, 1983). On the other hand, when exercise is performed with one muscle or muscle group, oxygen uptake should not be limited by the central circulation (Andersen & Saltin, 1985). In this situation the amount of oxygen consumed by the muscle is probably determined by peripheral factors, e.g. peak muscle perfusion, oxygen diffusion or mitochondrial respiratory capacity. The question of whether oxidative enzymes play a role in determining maximal oxygen uptake is still debated, since the activities of many oxidative enzymes are considerably higher than the estimated or calculated maximal rate of oxygen consumption in skeletal muscle. However, it has been shown that in some cases the maximum *in vitro* activity of certain enzymes is similar to the maximum flux through the pathway so that the activities of such enzymes may limit oxygen consumption and, moreover, provide a simple means of obtaining quantitative information about the maximal flux through a given pathway (Newsholme, Crabtree & Zammit, 1980). Based on studies of muscles from different species, the maximal activity of oxoglutarate dehydrogenase appears to provide quantitative information on the maximal capacity of oxidative metabolism (Read, Crabtree & Smith, 1977; Newsholme & Leech, 1983). This is based on the calculation of maximum flux through the Krebs cycle from oxygen consumption data found in the literature, which is then compared with enzyme activity measurements in extracts of muscles from the same species.

In a previous study, a weak relationship was found between the maximal activity of oxoglutarate dehydrogenase in muscle samples from the vastus lateralis and the maximal oxygen uptake during running (Blomstrand, Ekblom & Newsholme, 1986). However, in that study it was not possible to calculate the oxygen uptake by the exercising muscles, as the active muscle mass is not known. Furthermore, since the oxygen delivery to the legs is limited by the capacity of the central circulation (see above), the value of oxygen uptake will not be maximal.

The model employed in the present study is the one-legged knee extension exercise, during which power output is produced mainly by the quadriceps femoris muscle (Andersen, Adams, Sjøgaard, Thorboe & Saltin, 1985). This provides an opportunity to measure the peak oxygen

uptake by a single muscle group and then compare the calculated flux through the Krebs cycle with enzyme activities measured in extracts of biopsy samples taken from the same muscle. This has not been done previously in experimental animals or humans. The maximal activities of three enzymes in the Krebs cycle were measured in extracts of biopsy samples taken from the vastus lateralis: oxoglutarate dehydrogenase, citrate synthase and succinate dehydrogenase. The latter two enzymes are often used as markers of oxidative capacity in human skeletal muscle (see Saltin & Gollnick, 1983). In addition, the maximal activities of hexokinase and phosphofructokinase were measured.

METHODS

Subjects

Eight male and two female subjects volunteered to participate in this study after being fully informed about the risks involved. Their mean age was 26 ± 1 years, their height 177 ± 2 cm and their weight 79 ± 4 kg. The engagement of the subjects in exercise training ranged from daily activities to regular endurance training. The study was approved by the Ethical Committees of the municipalities of Copenhagen and Fredriksberg.

Preliminary tests

The subjects performed one-legged dynamic knee extension exercise (Andersen & Saltin, 1985) with sixty contractions per minute. Two or three 30–45 min exercise sessions were performed to accustomize the subjects to the exercise model, i.e. to enable them to fully relax the hamstrings and perform the exercise exclusively with the quadriceps muscle. The maximal work rate of the subjects was determined at the end of each session.

Experimental design

Catheters were inserted into the femoral artery and vein, the latter 4 cm distal to the inguinal ligamentum. A thermistor was placed in this catheter approximately 8 cm proximal to the tip, which also had side holes for infusing saline or withdrawing blood. The arterial catheter was advanced 10 cm upstream and connected to a blood pressure transducer and monitor (Patient Data Monitor 565A, Medicoline, Valby, Denmark). After the catheters were positioned, the subjects rested for approximately 30 min in the supine position and then moved over to the knee extension chair. ECG chest electrodes were attached to the subject to monitor heart rate. The subjects performed exercise at two submaximal work rates: 10 min at 30 W and 5–6 min at a work rate corresponding to approximately 75–80% of their maximal work rate. After 30–40 min of rest, the subjects performed incremental exercise, starting at 20 or 30 W for 3 min, followed by 5 W elevation every 30 s up to their maximum work rate, which was maintained for at least 1 min. The maximum work rate was defined as the highest rate at which the subjects were still able to maintain the pace. Furthermore, the recording of the force curves (see below) confirmed that the work was performed exclusively by the quadriceps muscle. Measurements of blood flow in the femoral vein (using the thermodilution technique described by Andersen & Saltin, 1985) were made at rest and repeatedly during exercise. To diminish mixing with blood from the lower leg, a cuff was placed just below the knee of the working leg. The cuff was kept inflated (280 mmHg) during the measurements of blood flow and blood sampling. Blood samples were drawn simultaneously from the arterial and venous catheters at rest, and during submaximal and maximal exercise for measurements of haemoglobin concentration and oxygen saturation. The oxygen content of arterial and venous blood was calculated from these measurements. The knee extensor force was recorded from a strain-gauge ring connected to a Peekel bridge. The signal was recorded along with the heart rate and blood pressure on a Gould recorder (Model TA 200, Gould Inc., Ohio, USA). Pulmonary oxygen uptake was measured at rest, during the last 2–3 min of the two submaximal exercise sessions, and during the last minute of the maximal exercise using an on-line system (MedGraphics, Spiropharma A/S, Klampenborg, Denmark). Values of leg blood flow and oxygen uptake, heart rate, mean arterial pressure and pulmonary oxygen uptake are given in Table 1.

Measurements of the quadriceps muscle mass

Computer tomography (CT) scans were performed using a Prospeed VX (General Electric CGR, Paris, France). Consecutive axial images were taken from the patella to the anterior inferior iliac spine with the genital region covered by a protective lead capsule. Twenty-one to twenty-five images were obtained from each subject. The slice thickness was 10 mm and the distance between the images was 20 mm. The spatial resolution was 0·59–0·87 mm. The muscle volume was calculated as follows: the mean area of two neighbouring sections was multiplied by the section distance (20 mm) and the muscle volume was calculated as the sum of these calculations over the whole muscle length. Assuming a muscle tissue density of 1·04 kg l^{-1}, which has been determined in young healthy individuals (Nadeshdin, 1932), the muscle mass was calculated and found to be 2·40 ± 0·17 kg (range 1·36–3·40 kg).

Enzyme activities

Two to three days after the experiment, muscle biopsy samples were taken at rest from the lateral part of the quadriceps muscle, vastus lateralis, and in five subjects also from the rectus femoris muscle, using the needle biopsy technique (Bergström, 1962). The samples were immediately frozen in liquid nitrogen and stored at −80 °C. The muscle samples were weighed and homogenized in ten volumes of ice-cooled extraction medium using a ground-glass homogenizer. The extraction medium contained 50 mM Tris hydrochloride, 5 mM $MgCl_2$ and 1 mM EDTA at pH 8·2.

Hexokinase, 6-phosphofructokinase and oxoglutarate dehydrogenase were assayed as described previously (Blomstrand, Challiss, Cooney & Newsholme, 1983), citrate synthase as described by Alp, Newsholme & Zammit (1976) and succinate dehydrogenase as described by Cooney, Taegtmeyer & Newsholme (1981) after incubation of the homogenate with succinate for 30 min at 30 °C. All enzyme activities were measured under optimal conditions according to the original references. Measurements were performed in a Beckman DU 640 spectrophotometer at 25 °C. However, to be comparable to the Krebs cycle flux during maximal exercise, when the muscle temperature was 37–38 °C, the enzyme activities at this temperature were calculated from the Arrhenius equation (see below), applying the knowledge that the rate of most enzyme reactions approximately doubles for every 10 °C rise in temperature. The Arrhenius equation is as follows: rate $= A e^{-E/RT}$, where A is a constant, E is the activation energy (J mol^{-1}), R is the gas constant (8·31 J mol^{-1} K^{-1}) and T is the absolute temperature (K).

Statistics

Parametric statistical methods were used to calculate means, standard error of the mean (S.E.M.) and the linear correlation coefficient (r). Values given in the text are means ± S.E.M. unless indicated otherwise.

Table 1. Leg blood flow and oxygen uptake ($\dot{V}_{O_2}$), pulmonary $\dot{V}_{O_2}$, heart rate and mean arterial pressure (MAP) at rest, during submaximal and maximal exercise with the knee extensors

Work rate	Leg blood flow ($l\ min^{-1}$)	Leg $\dot{V}_{O_2}$ ($ml\ min^{-1}$)	Pulmonary $\dot{V}_{O_2}$ ($l\ min^{-1}$)	Heart rate (beats min^{-1})	MAP (mmHg)
Rest	0·24 ± 0·02	18·0 ± 2·4	0·32 ± 0·03	66 ± 3	90 ± 4
30 W	3·71 ± 0·22	448 ± 23	0·86 ± 0·04	98 ± 3	101 ± 4
Maximal, 65 W (45–100 W)	5·91 ± 0·59 (4·07–9·52)	845 ± 100 (574–1521)	1·77 ± 0·19 (1·11–2·33)	136 ± 7 (112–171)	119 ± 2 (114–130)

Data are presented as means ± S.E.M. for ten subjects. For the maximal work rate, the range is given in parentheses.

RESULTS

At maximal work rate, leg blood flow averaged 5·9 ± 0·6 $l\ min^{-1}$ and the leg oxygen uptake 845 ± 100 $ml\ min^{-1}$ or 353 ± 33 $ml\ min^{-1}\ kg^{-1}$ of quadriceps muscle. The flux through the Krebs cycle has been calculated from this rate of oxygen uptake with the assumption that carbohydrate is the only substrate being oxidized during maximal exercise (Åstrand & Rodahl, 1986; Bangsbo *et al.* 1990). According to the stoichiometry of the pathway of glucose oxidation (one glucose molecule produces two molecules of acetyl-coenzyme A for oxidation by the Krebs cycle), the flux through the Krebs cycle is equal to one-third of the oxygen uptake. At a temperature of 37–38 °C, the volume of 1 mol of oxygen is 25·4–25·5 l according to the General Law for gases. The average rate of oxygen uptake therefore corresponds to a Krebs cycle rate of 4·6 ± 0·4 $\mu mol\ min^{-1}\ g^{-1}$ of quadriceps muscle.

There was a significant correlation between leg oxygen uptake at the maximal work rate and the maximal activity of the enzymes in the Krebs cycle measured in the vastus lateralis: r was 0·79, 0·72 and 0·73 for citrate synthase, oxoglutarate dehydrogenase and succinate dehydrogenase, respectively (Fig. 1). However, only the activity of oxoglutarate dehydrogenase (5·1 ± 0·3 $\mu mol\ min^{-1}\ g^{-1}$ at 37–38 °C) was similar to the calculated Krebs cycle flux at the maximal work rate. Figure 2 illustrates the agreement between the calculated flux through the Krebs cycle and the maximal activity of oxoglutarate dehydrogenase at 37–38 °C. The maximal activities of succinate dehydrogenase and citrate synthase averaged 7·2 ± 0·3 and 48·0 ± 1·5 $\mu mol\ min^{-1}\ g^{-1}$ at 37–38 °C, respectively, i.e. 55 and 940% higher than the estimated flux through the Krebs cycle.

For the five subjects in whom biopsy samples were also taken from the rectus femoris muscle, the maximal oxygen uptake averaged 309 ± 28 $ml\ min^{-1}\ kg^{-1}$, corresponding to a Krebs cycle rate of 4·1 ± 0·4 $\mu mol\ min^{-1}\ g^{-1}$. The activity of oxoglutarate dehydrogenase in the rectus femoris muscle was 4·0 ± 0·5 $\mu mol\ min^{-1}\ g^{-1}$ at 37–38 °C and the average activity from the two sets of samples (rectus femoris and vastus lateralis) was 4·3 ± 0·4 $\mu mol\ min^{-1}\ g^{-1}$ at 37–38 °C.

A correlation was also found between the maximal rate of oxygen uptake over the leg and the maximal activity of hexokinase ($r = 0·77$, $P < 0·05$), whereas no correlation was found for 6-phosphofructokinase. The enzyme activities are given in Table 2.

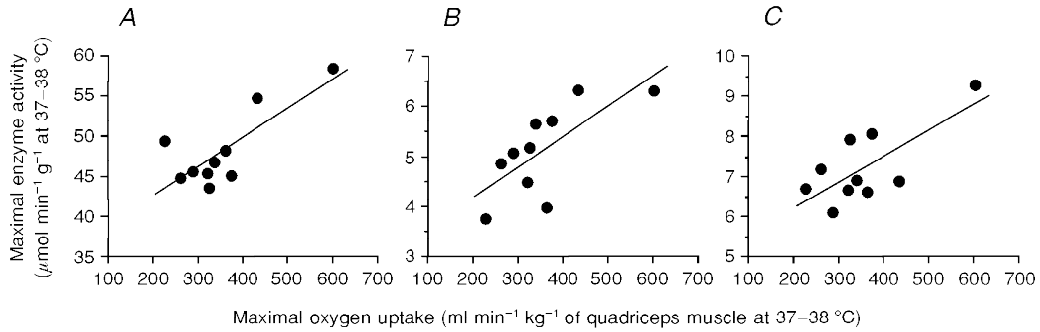

Figure 1. Relationship between leg oxygen uptake during maximal exercise with the knee extensors of one leg and the maximal activities at 37–38 °C of three enzymes in the Krebs cycle

A, citrate synthase, $r = 0·79$, $P < 0·05$. *B*, oxoglutarate dehydrogenase, $r = 0·72$, $P < 0·05$. *C*, succinate dehydrogenase, $r = 0·73$, $P < 0·05$.

Table 2. Maximal enzyme activities in biopsy samples taken from the vastus lateralis and rectus femoris muscles

Enzyme	Enzyme activity (μmol min^{-1} g^{-1} at 25 °C)	
	Vastus lateralis	Rectus femoris
Hexokinase	2·1 ± 0·08 (10)	2·0 ± 0·1 (5)
Phosphofructokinase	33 ± 1·5 (10)	45 ± 2·6 (5)
Citrate synthase	20 ± 0·6 (10)	17 ± 1·6 (5)
Oxoglutarate dehydrogenase	2·2 ± 0·1 (10)	1·7 ± 0·2 (5)
Succinate dehydrogenase	3·1 ± 0·1 (10)	—

Data are presented as means ± S.E.M., with the number of subjects in parentheses.

DISCUSSION

The major finding in the present study is that the average maximal activity of the enzyme oxoglutarate dehydrogenase in extracts of muscle is almost the same as the average flux through the Krebs cycle calculated from peak oxygen uptake by the quadriceps muscle. This indicates that the enzyme is fully activated during maximal exercise with the quadriceps muscle and is one factor limiting the flux through the Krebs cycle and thus the oxygen uptake by muscles. It is to be noted that blood supply to the muscle is extraordinarily high, and thus the oxygen availability is also high when only a small muscle group is performing the intensive exercise (see Table 1; Savard, Kiens & Saltin, 1987). Peak muscle oxygen uptake is also markedly higher than that which can be achieved when exercise such as cycling or running is performed. It is difficult to give a precise number for how much higher the oxygen uptake may be when comparing peak knee extension work with exercise eliciting a whole-body maximal oxygen uptake, since the muscle mass involvement in the latter case is not known. One estimate indicates that there may be a 3- to 4-fold difference (Savard *et al.* 1987). Thus although it may be possible to reach a peak Krebs cycle flux by performing small muscle group exercise in man, this does not mean that such a high flux is reached in more ordinary work when maximal oxygen uptake of the human body is reached.

Weibel and colleagues introduced the concept of symmorphosis, which suggested that structure and function are conjointly linked such that for maximal oxygen uptake, all systems are matched and none is present in excess (Weibel & Taylor, 1981). They based this concept on studies of many different-sized species, measuring various links in the chain of oxygen transport, including the total volume of mithocondria (mitochondrial volume (%) × total muscle mass) and maximal oxygen uptake. In a log–log correlation analysis, they found a very close relationship between these two variables ($r > 0·9$) and concluded that the mitochondrial volume limits the maximal oxygen uptake of animals, including man. The present study does not lend support to such a conclusion in ordinary exercise performed by humans. Indeed, less than 20% of the muscle mass has to be recruited when humans are exercising to utilize the maximal oxidative capacity of the mitochondria, which probably means that when humans perform whole-body exercise, the mitochondria do not respire maximally.

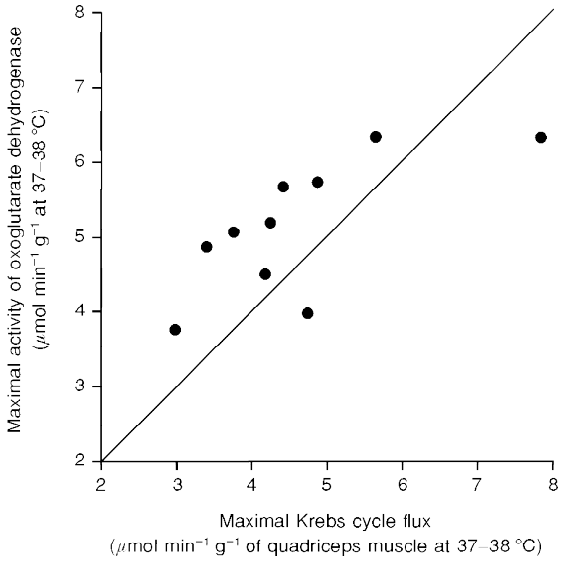

Figure 2

Relationship between the maximal activity of oxoglutarate dehydrogenase at 37–38 °C measured in m. vastus lateralis and the calculated maximal flux in the Krebs cycle during one-legged knee extension exercise. The continuous line denotes the line of identity.

The maximal activity of oxoglutarate dehydrogenase was found to be similar to the maximum flux through the Krebs cycle (Fig. 2) and thus supports the theory that this enzyme can be used as a quantitative measure of the maximum flux through the Krebs cycle and therefore the respiratory chain (Newsholme *et al.* 1980). A similar agreement is found in muscles in a variety of species, e.g. insect flight muscles where the maximal activity of oxoglutarate dehydrogenase and therefore the Krebs cycle rate was found to be five to ten times higher than the maximal rate in human muscle (Newsholme & Leech, 1983).

Only in one previous study have measurements of enzyme activities and oxygen consumption been done in the same tissue – the isolated rat heart (Cooney *et al.* 1981). In that study, a correlation was found between the activity of oxoglutarate dehydrogenase and the maximal flux through the Krebs cycle calculated from the oxygen consumption of the perfused maximally working rat heart. No correlation was found between the activities of citrate synthase or succinate dehydrogenase and the Krebs cycle flux. In the present work, the maximal activities of succinate dehydrogenase and citrate synthase were 55 and 940 % higher, respectively, than the maximal flux through the Krebs cycle and therefore these enzymes cannot be used as accurate indicators of the maximum flux through the cycle. There was, however, a positive correlation between the rate of leg oxygen uptake and the activity of all three enzymes, suggesting a constant relationship between the activities of the Krebs cycle enzymes. Some caution should be observed, however, when interpreting these correlations since the number of subjects is small.

The present results show a correlation also between the maximal activity of hexokinase and the maximal rate of oxygen uptake. This is expected since the activity of hexokinase has been reported to change in parallel with the Krebs cycle enzymes as a result of exercise training and electrical stimulation of skeletal muscle (Baldwin, Winder, Terjung & Holloszy, 1973; Reichmann, Hoppeler, Mathieu-Costello, von Bergen & Pette, 1985). The activity of hexokinase appears to provide a quantitative indication of the maximum rate of glucose utilization (Newsholme *et al.* 1980). In the present work the maximal activities of hexokinase and oxoglutarate dehydrogenase were almost the same (Table 2), indicating that the capacity of muscle to utilize glucose is twice the maximal flux through the Krebs cycle.

The maximal rate of oxygen uptake and thus ATP production found in the present study is considerably higher than the values obtained from *in vitro* preparations of isolated mitochondria from human skeletal muscle (Wibom & Hultman, 1990) and from the perfused rat hindlimb preparation during tetanic contractions (McAllister & Terjung, 1991; Robinson, Ogilvie, Tullson & Terjung, 1994). This might be explained by the difficulties in obtaining optimal conditions during the latter two experimental conditions and may therefore indicate that an *in vivo* situation, with for example intact circulation, proper hormonal response and substrate availability, is needed to reach maximal oxygen uptake with one muscle or muscle group. In addition, the recruitment pattern of muscle fibres *in vivo* is known to be different from that during tetanic contractions, which may also influence the rate of oxygen uptake.

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