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ENERGY CONVERSION IN SKELETAL MUSCLES: OLD AND RECENT VIEWS

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ABSTRACT

The basic principle of the approach to human physiology used by Santorio Santorio was the conservation of mass: perspiratio insensibilis *allowed a perfect balance between input and output:* si cibus et potus unius diei sit ponderis octo librarum, transpiratio insensibilis ascendere solet ad quinque libras circiter. *Muscle contractile activity* (corporis motus violentus)*, however, disturbs the balance between visible and invisible evacuations. At the time of Santorio, the pneuma hypothesis of muscle contraction was still generally accepted and a long series of studies were necessary before the energy conservation principle could be applied to muscle contraction. The idea that in accordance with the energy conservation principle, chemical energy is transformed into work (mechanical energy) and heat (thermal energy) during muscle contraction was accepted only in the first half of the nineteenth century. It took approximately one hundred years before ATP was identified as the unique source of chemical energy for muscle contraction and the different ATP regeneration pathways were discovered. Today a wealth of tools is available to study the energy conversion in muscle contraction in single muscle fibres in vitro as well as in muscle in situ or in the whole organism during exercise. Myosin has been identified as the molecular motor performing the chemo-mechanical energy conversion and a number of specialized variants of myosin, employed for postural tasks or for power generation, have been discovered and analysed in detail.*

Keywords: muscle energetics, thermodynamics, biochemistry, physical activity

PRETVORBA ENERGIJE V SKELETNIH MIŠICAH: STARI IN NOVI POGLEDI

IZVLEČEK

Ohranitev mase je bilo osnovno načelo, s pomočjo katerega je Santorio Santorio pristopal k obravnavi fiziologije človeka: perspiratio insensibilis *je omogočalo popolno ravnovesje med vnosom/vhodom in iznosom/izhodom:* si cibus et potus unius diei sit ponderis octo librarum, transpiratio insensibilis ascendere solet ad quinque libras circiter. *Za mišično aktivnost oz. krčenje* (corporis motus violentus) *pa je bilo tako rekoč mišljeno, da moti ravnovesje med vidno in nevidno evakuacijo. Dihanje mišic med njihovim krčenjem je bila v Santorievem času še vedno splošno sprejeta domneva in bilo je potrebnih še veliko število študij, preden se je načelo ohranitve energije preneslo tudi na mišično krčenje. Ideja, da se kemična energija med mišičnim krčenjem pretvori v delo (mehansko energijo) in toploto (toplotno energijo) po načelu ohranitve energije, je bila sprejeta šele v prvi polovici 19. stoletja. Vendar pa je bilo potrebnih približno sto let, preden se je ugotovilo, da je ATP edini/edinstveni vir kemične energije, ki omogoča mišično krčenje, ter odkrilo različne poti obnove ATP. Danes je na voljo cela vrsta orodij oz. instrumentov, s pomočjo katerih lahko preučujemo pretvarjanje energije med mišičnim krčenjem, tako na nivoju posameznih mišičnih vlaken in vitro kakor tudi na ravni mišice in situ ali pa na ravni celotnega organizma med gibanjem/vadbo. Za miozinske niti je bilo ugotovljeno, da so molekularni motor, ki opravlja kemo-mehansko pretvorbo energije. Odkrite in podrobno preučene pa so bile tudi številne posebne/specializirane različice miozina, ki se vključujejo v naloge ohranjanja ravnotežja ali kadar so potrebe po generiranju moči/silovitosti velike.*

Ključne besede: energetika mišic, termodinamika, biokemija, telesna aktivnost

Santorius and the old views on muscle energetics

The present view on skeletal muscle energetics is the end point of a long path which started centuries ago. Along this path, the work of Santorius marked one of the first steps with two extremely important contributions: the first contribution was the careful application of a quantitative approach in the study of biological processes, while the second contribution was the proposal of a mass balance in the human body – or in other words, of a mass conservation principle. The amount of food and drink must precisely correspond to the amount of weight loss measured on a balance "stadera or weighing chair" made to the appropriate size for the human body. While the amount of food and drink could be precisely weighed, the weight loss could be divided into fractions: the amount lost in measurable excretions (urine, perspiration, faeces …) and the amount of weight lost in a form not measurable was referred to as *"transpiratio insensibilis"*. According to the fifth aphorism: *"Insensible Perspiration is either made by the Pores of the Body, which is all over perspirable, and cover'd with a Skin like a Net; or it is performed by Respiration through the Mouth, which usually, in the Space of one Day, amounts to about the Quantity of half a Pound, as may plainly be made* *appear by breathing upon a Glass"*. Thus, a basic equation was proposed by Santorius in the sixth aphorism: "*si cibus et potus unius diei sit ponderis octo librarum, transpiratio insensibilis ascendere solet ad quinque libras circiter"* i.e. every day 8 pounds of food and drink enter the body and 8 pounds leave the body: 5 as detectable or measurable excretion and 3 as undetectable excretion (*transpiratio insensibilis*). The lack of balance between the two components was considered to be either an indication of the existence of or the cause of pathological conditions (Santorius, 1614).

In the above depicted frame, muscle contractile activity was considered a possible cause of balance alterations and to some extent it was considered advisable to avoid any heavy physical activity *motus violentus*. The suggestion to keep physical activity within moderate limits was reminiscent of the view presented just some decades previously by Girolamo Mercuriale (1530–1606), who was also professor at the University of Padova (1575–1587) in his book *De Arte Gymnastica* (Mercuriale, 1569).

At the time of Santorius, there was no idea of a possible connection between energy derived from food intake and energy released during muscle contraction. The theory of muscle contraction widely accepted at the University of Padova by Vesalius and Harvey, not to mention Galileus, and shared by Santorius himself was based on the the hypothesis that a biological fluid indicated as *pneuma* could reach the muscles running along the nerves, inflate the muscles and cause them to shorten. Such a theory did not give much consideration to the quantitative application of the mass conservation principle put forward by Santorius and was destined to be challenged over the subsequent decades. Swammerdam (1737) showed that muscle volume remains constant during a contraction, Stensen (1667) provided evidence in favour of fibre shortening as the first process in muscle contraction and a complete presentation of the *iatromechanical* approach to muscle function was reported by Borelli (1680) in his book *De Motu Animalium*.

Historical development of muscle energetics

Scientific development in the 18th century brought two great advancements in the understanding of muscle contraction: the development of chemistry lead to the discovery that oxygen is used for muscle contraction, thus chemical processes give power to muscles (Lavoisier, 1777). In parallel to this, the demonstration that electrical phenomena take place in muscles established the basis for the foundation of electrophysiology (Galvani, 1791).

It was only around the first half of the 19th century that the principle of energy conversation came into consideration in muscle physiology in close relation to the development of thermodynamics. This achievement can be ascribed to the German school of muscle physiology: mechanical recordings and heat production measurements were carried out during contraction and related to the catabolism of some not yet defined chemical compounds (Schwann, 1835; Helmoltz, 1847; Heidenhain, 1864)

In more recent years, the modern view of the energetics of muscle contraction emerged as the result of work carried out in the first half of the 20th century: Hill and his colleagues provided a complete analysis of the mechanical output and heat production in muscle contraction (summarized by AV Hill in his book published in 1970), while Meyeroff and Lohmann (1930, 1931) studied the metabolic reactions which supply energy for muscle contraction and this lead to the discovery of ATP. In the emerging picture all chemical energy from energy substrates is spent to produce ATP, which is in balance with CP. ATP hydrolysis releases energy which is converted into work and heat.

Modern views of muscle energetics

According to the currently accepted view, the interaction between myosin and actin or the cross bridge cycle is the specific process that powers muscle contraction. After the action potential triggered by electrical stimulation or acetyl choline release at the neuromuscular junction, force and work are produced in the muscle as the result of the activity of many individual cross bridges, each of which is formed by the attachment of a single myosin head to actin molecules on the thin filament. The acto-myosin interactions split ATP in a cyclical process, as a first approximation one molecule of ATP is split in each cycle of attachment and detachment (for a review on cross bridge mechanism, see Barclay et al., 2010). Thus, most of the ATP consumption associated with muscle contraction is accounted for by the interactions of myosin molecules on actin filaments which generate force and displacement, with a lower but not negligeable component related to ions pumping across membranes, in particular Na^+ and K^+ through the sarcolemma (Na/K pump) and Ca^{2+} through sarcoplasmic reticulum membranes (SERCA pump). The ATP consumed for ion pumping is approximately 30–40% of the total ATP hydrolysed during contraction (for a review, see Barclay et al., 2007).

Force development in isometric contractions has a cost in ATP, a close proportion existing in number of ATP hydrolysed per second and the amount of force developed. When shortening occurs, ATP is further hydrolysed (Figure 1) and the degree of conversion of the chemical energy released from ATP hydrolysis in mechanical work is quantified by the efficiency. Efficiency can be determined at the level of the cross bridge cycle and at myofibrillar level and can reach values close to 50% (Smith et al., 2005). When the efficiency is determined during a contraction of an intact fibre, the fraction of ATP, and thus of energy, used for ionic pumps must be also considered. The value of efficiency in intact muscles or muscle fibres is therefore lower. Furthermore, efficiency can be also determined in muscles taking into account the regeneration process of ATP. Three distinct processes support ATP regeneration: CreatinPhosphate to ATP conversion, anaerobic glycolysis and mitochondrial oxidative phosphorylation.

It is important to underline that distinct muscle fiber types have different energetic properties. The difference is very evident in the cost of tension generation (ATP per unit force per unit time) which is several times lower in slow compared to fast fibers (Stienen et al., 1996; He et al., 2000), see Figure 2. This makes slow fibres more convenient than fast fibres in being recruited for long lasting force generation tasks such as those related with posture. When external work is performed, efficiency is not very different between fast and slow fibres (Reggiani et al., 1997; He et al., 2000). This implies that the higher power developed by fast fibres can be exploited without energetic disadvantage.

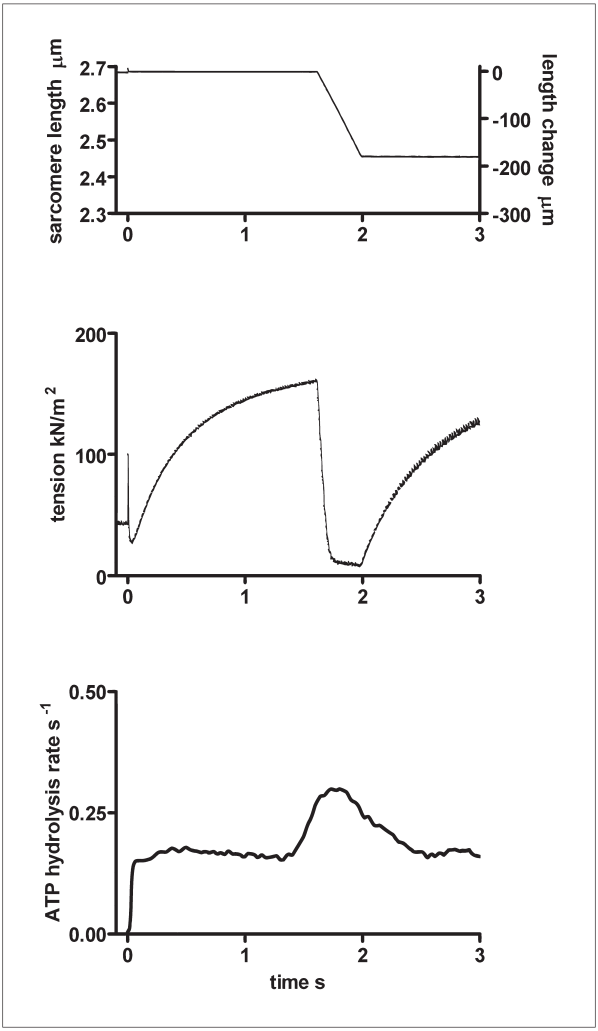

Figure 1: ATP consumption during contraction of a slow fibre of human muscle. Measurements are carried out on a muscle fibre segment, dissected from a needle biopsy, permeabilized with Triton, activated with calcium at 20 °C. Contraction starts in an isometric condition and then shortening is allowed from sarcomere length of 2.7 µm to sarcomere length of 2.5 µm, as can be seen in the uppermost panel. Tension decreases during the shortening phase and reaches the level determined by the force-velocity relation (see middle panel). ATP consumption is determined by the binding of inorganic phosphate to a fluorescent protein binding protein as described by He et al. (2000) and is shown in the lowest panel. Note the increase in ATP hydrolysis rate when shortening occurs and external work is performed, in comparison to the isometric condition.

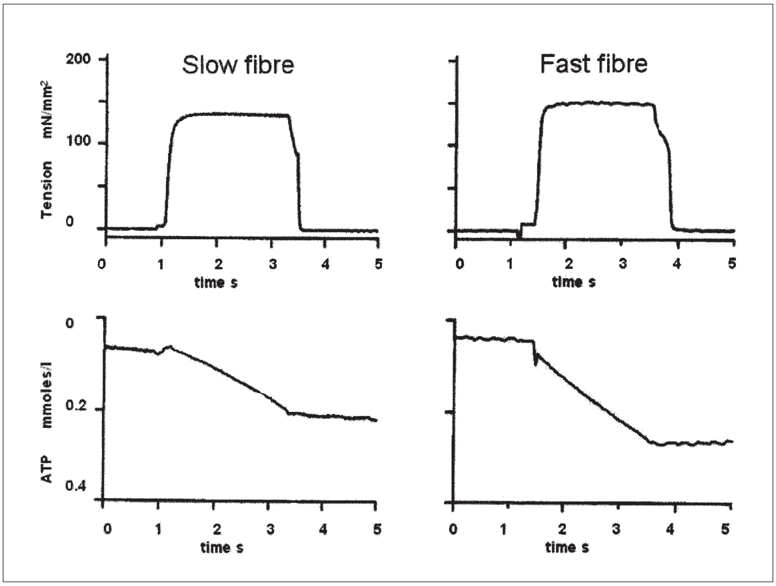

Figure 2: ATP consumption (lower panels) and tension development (upper panels) during an isometric tetanus in a slow fibre (left) and in a fast fibre (right) from human vastus lateralis. Figure re-drawn and modified from Stienen et al. (1996). Fibre segments were dissected via a needle biopsy, permeabilized with Triton, activated with calcium at 20 °C. ATP consumption was determined from NADH oxidation in a spectrophotometer. NADH oxidation was enzymatically coupled with ATP hydrolysis in 1:1 proportion as described in Stienen et al. (1996). Note the greater slope of ATP consumption, i.e. the higher ATP consumption rate, in the fast compared to the slow fibre.

Santorius was deeply interested in developing equipment to measure physiological parameters. Nowadays, an unprecedented wealth of experimental tools enable muscle physiologists to quantitatively determine the energy conversion in vitro as well as in vivo, in single muscle fibers, in whole muscles or in the body. Specific methods allows the determination of the energy consumption rate in muscle fibers, ranging from experiments on permeabilized fibers where the spectrophotometric determination of NADH oxidation (Stienen et al., 1996) or the use of a specific probe for inorganic phosphate permit to monitor the ATP hydrolysis (He et al., 2000), to microchemical determination of high energy phosphate in muscle biopsy samples (Karatzaferi et al., 2001), to enthalpy determination from heat and mechanical measurements (Barclay et al., 1993), to the measurement of oxygen consumption (Homsher, 1987) and to ^{31}P MR spectroscopy in vivo (Blei et al., 1993; Conley et al., 2000). Since all energy required for cellular functions, including contractile activity, is provided by ATP hydrolysis to ADP and Pi, the energy consumed by a muscle fiber can be measured in moles of ATP consumed as well as in energy units (calories or Joules) or in oxygen consumed for ATP regeneration in the mitochondria, if contraction occurs in aerobic conditions. It is generally accepted that the hydrolysis of 1 mole ATP releases between 50 and 60 kJ under physiological conditions.

CONCLUSIONS

In conclusion, looking back over the four centuries which have passed since the time when Santorius was called to the chair of *Medicina Teorica* in Padua, we can recognize and identify the steps of the long path which lead from the mass balance principle to the energy balance principle in muscles. The legacy of Sartorius is, however, still recognizable and can be well appreciated. We owe to him our quantitative approach in physiology: the passion for measuring all parameters in precise quantitative terms and designing the most appropriate tools necessary to meet this goal. We also owe to him the view that a balance must exist, the balance of mass and energy in the reactions which occur within the muscles and the body.

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