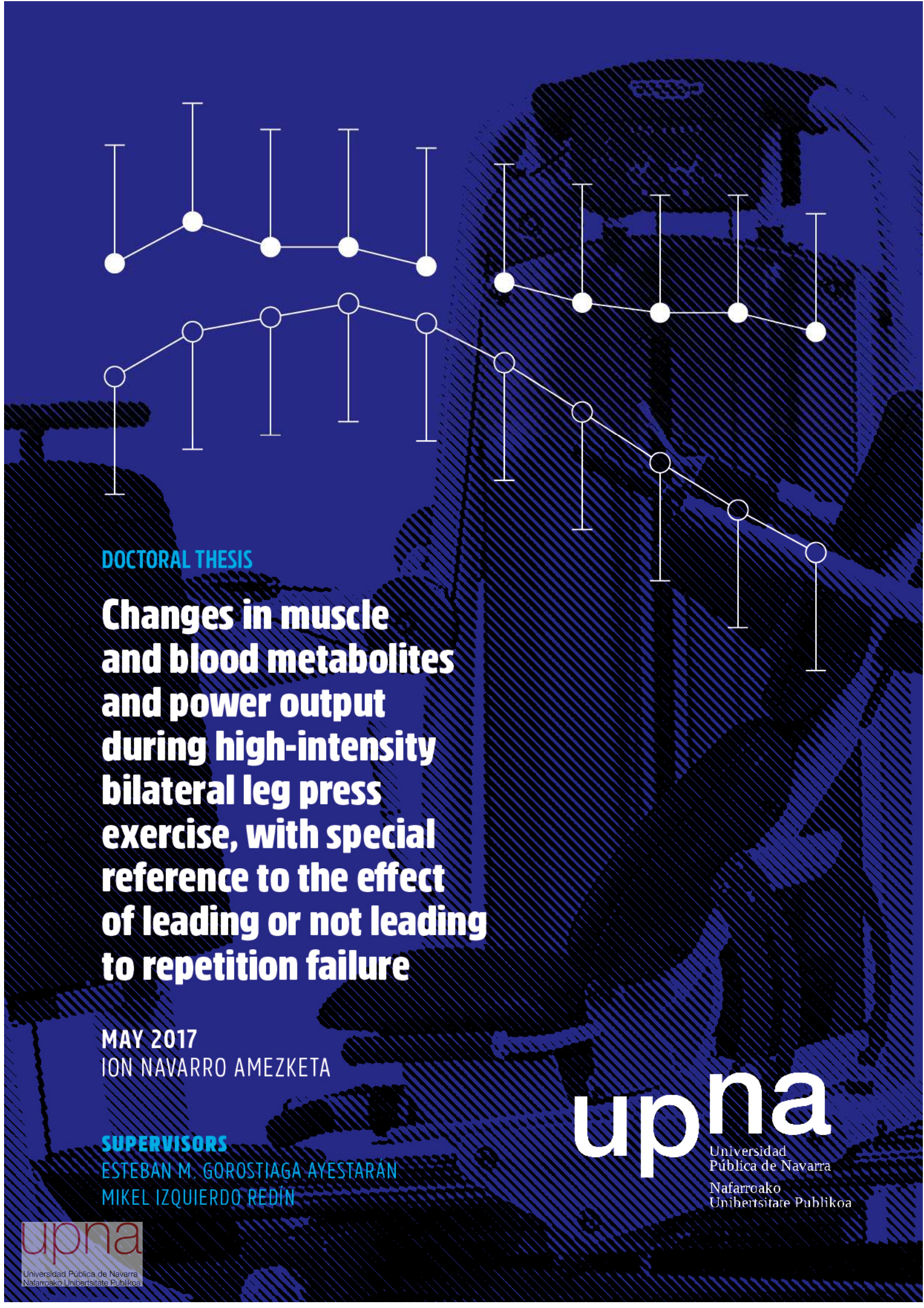

DOCTORAL THESIS

Changes in muscle and blood metabolites and power output during high-intensity bilateral leg press exercise, with special reference to the effect of leading or not leading to repetition failure

MAY 2017

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Declaration

I, Ion Navarro Amezketa, do hereby declare that the research presented in this dissertation is based on 3 articles (chapters 2 to 4) that have been published in international peer-reviewed journals. To meet the stylistic requirements of a thesis, the formats of the papers have been adjusted accordingly throughout. These edits did not substantially change the content of the published articles.

The role which I fulfilled within each of the publications is presented at the end of each study.

Me gustaría mostrar mis más sinceros agradecimientos a todas las personas que me han ayudado y que han estado a mi lado durante el largo periodo de tiempo transcurrido desde que comenzó esta pequeña aventura

Mila esker guztioi!!

Financial support, List of Publications and Conference Papers

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List of Publications

Gorostiaga EM, Llodio I, Ibanez J, Granados C, **Navarro-Amezqueta I**, Ruesta M, Bonnabau H, & Izquierdo M (2009). Differences in physical fitness among indoor and outdoor elite male soccer players. *Eur J Appl Physiol* 106, 483-491.

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Gonzalez-Izal M, Malanda A, **Navarro-Amezqueta I**, Gorostiaga EM, Mallor F, Ibanez J, & Izquierdo M (2010a). EMG spectral indices and muscle power fatigue during dynamic contractions. *J Electromyogr Kinesiol* **20**, 233-240.

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Conference Papers

Aguado-Jimenez R, Izquierdo M, **Navarro-Amezqueta I**, Martínez C, González M, Ruesta M, Gorostiaga EM "Effect of fire-fighting equipment on cardiorespiratory, thermal and neuromuscular responses". European College of Sport Science Congress, July 2006.

Roberto Aguado, Miriam González-Izal, **Navarro-Amezqueta I**, Esteban Gorostiaga, Mikel Izquierdo. "Pattern and level of muscle activation cycling in a new cycloergometer". European College of Sport Science Congress, July 2007.

Navarro-Amezqueta I, Granados C, González-Izal M, Izquierdo M, Vicente-Rodríguez G, Malanda A, Ibáñez J, Calbet JA, Gorostiaga E. "Muscle fiber composition and neuromuscular and metabolic responses Turing high-intensity strength exercise". XXX FIMS World congress of Sports medicine.

González-Izal, M, Malnda A, **Navarro-Amezqueta I**, Gorostiaga EM, Mallor F, Ibañez J, Izquierdo M. "Changes in muscle power fatigue and sEMG parameters during dynamic fatiguing contractions". European College of Sport Science Congress, July 2009.

Izquierdo M1, Ibañez J, Calbet JAL, González-Izal M, **Navarro-Amézqueta I**, Malanda A, Idoate F, González-Badillo JJ, Häkkinen K, Kraemer WJ, Gorostiaga EM. "Neuromuscular and metabolic fatigue with the same absolute and relative inensity alter strength training". European College of Sport Science Congress, July 2009.

Navarro-Amézqueta I, Izquierdo M, González-Izal M, Granados C, Malanda A, Ibáñez J, Vicente-Rodríguez G, Calbet JAL, Gorostiaga EM. "Relationship between muscle fiber distribution, neuromuscular and metabolic responses Turing leg press exercise".European College of Sport Science Congress, July 2009.

Summary (English)

Resumen (Castellano)

Summary

The current PhD dissertation revolves around the influence of the number of repetitions per bout (leading to failure or not) on changes in metabolic and mechanical variables during high-intensity leg press resistance exercise, to attempt to better understand the mechanical and physiological aspects underlying resistance training stimuli that affect adaptations. This doctoral thesis is based on 3 scientific studies that have been published in scientific journals. The first study (Chapter 2) was designed to examine metabolic responses during a single leg press exercise set of 10 repetitions leading to failure (10RM). Study 2 (Chapter 3) was aimed at examining the influence of the number of repetitions per set on changes in muscle metabolites and power output during widely used high-intensity intermittent leg press exercise. The last study (Chapter 4) is a descriptive study to investigate the changes in blood metabolites in relation to muscular metabolites and mechanical power output changes.

Study 1 (Chapter 2)

Bilateral leg press exercise is a multi-joint (hip, knee, and ankle) exercise and is one of the most common resistance training type of exercise used to enhance performance in sports and in knee rehabilitation as it produces greater neural activation than the majority of weight-bearing knee extension exercises. It is a matter of some surprise, however, that literature contains no data that looks into the substrate utilization and the metabolic demand during a brief high-intensity effort carried out with this exercise type. Additionally, bilateral leg press exercise is an interesting muscle contraction model to estimate anaerobic energy production and mechanical efficiency during maximal exercise because it largely differs from the models used up until now. The aim of this first study was to examine the changes in muscle metabolite concentration and power output variations throughout the first and second half of a set of 10 repetitions leading to failure (10RM) of bilateral leg press exercise. Each subject participated in two experiments performed on separate days, in which the number of repetitions was manipulated (leading to repetition failure or performing half of the maximal number of repetitions) and the absolute load was kept equal. The findings of the research provide evidence that performing 5 or 10 repetitions of leg press exercise with the maximum possible load to achieve 10 repetitions provides two main different types of exercise in terms of energy status and muscle metabolism. In addition, the higher energy cost per repetition during the second 5 repetitions is suggestive of decreased mechanical efficiency. The paper relates to previously published works that have delved into the physiological and metabolic responses to maximal running and cycling. The results of this study may enhance the understanding of factors

responsible for muscle power production and fatigue, and thus give an indication of the regulation of the metabolic pathways and of how the anaerobic mechanisms are interrelated during dynamic resistance exercise in man.

Study 2 (Chapter 3)

Although resistance exercise is an essential component of effective intervention programs to improve health and strength performance, the mechanical and physiological aspects underlying resistance training stimuli that affect adaptations are not fully understood. Indeed, the role played by the actual number of repetitions performed in each set with regards to the maximum number that can be completed has been of the utmost interest to coaches and sport scientists to comprehend the physiological mechanisms underlying training-induced gains in strength and power. This investigation examined the impact of the number of repetitions per set on changes in muscle metabolites and power output during multiple sets of high-intensity bilateral leg press exercise whilst simultaneously examining the power output and fatigue developed over the exercise. Each subject participated in two experiments on separate days, in which, to eliminate possible effects of confounding factors, initial load and total number of repetitions (50 repetitions) were controlled by equating their values between both exercise sessions. The results appear to indicate that the actual number of repetitions conducted in each training set with regard to the maximum number that can be completed greatly influences power output patterns, energy balance and cellular homeostasis during leg press exercise. Therefore, this may explain the markedly different metabolic and morphological adaptations reached in response to different resistance training approaches.

Study 3 (Chapter 4)

The muscle biopsy is a technique commonly used under research laboratory conditions. Nevertheless, its regular use in the practice of resistance exercise to monitor training adaptations is unacceptable. Alternatively, it has been suggested that some blood metabolites may be used as biochemical markers of metabolic stress, reflecting changes in the functional state of the muscle contractile machinery. Whilst several studies have consistently found a strong correlation between blood and muscle lactate in a range of exercise models such as cycling and running, there is hardly any information about blood and muscle lactate correspondence during maximal intermittent dynamic resistance exercise. Moreover, although blood ammonia has been postulated as an indirect marker of muscular ATP store changes throughout exercise, the link between blood ammonia and

muscle ATP levels in the course of consecutive bouts of high-intensity dynamic resistance exercise is unknown in humans. Consequently, this last study was designed to determine in what way changes in blood metabolites and changes in muscular metabolites during repeated high-intensity sets of bilateral leg press exercise are related. In addition, interrelation between blood metabolites and power output fatigue profiles was weighed. The findings showed a high positive correlation between muscle and blood lactate, along with a negative correlation between blood ammonia concentration and muscle ATP content. Additionally, negative linear correlations were observed between peak power output and blood lactate and ammonia. Based on all this, these data suggest that the muscle energy status during intermittent leg press exercise can be estimated from different blood metabolic markers (lactate and ammonia) and changes in mechanical power output.

Author's note: Several years have elapsed since the articles that compose this PhD dissertation were published. Hence, some of the studies cited in the introduction of this work are presented as novel results, even though they were divulged to the scientific community after the publication of the articles that compose this PhD thesis. As a result, a kind of temporal paradox occurs in this doctoral thesis, difficult to overcome due to the lengthy period of time elapsed between the publication of the first papers and this last dissertation work.

Resumen

La tesis doctoral que se presenta a continuación tiene como objeto de estudio analizar la influencia que ejerce el número de repeticiones que se realiza en cada serie (repeticiones realizadas hasta el agotamiento o no) sobre los cambios en variables metabólicas y mecánicas durante ejercicio de fuerza muscular de alta intensidad realizado en prensa de pierna. Tiene como objetivo principal poder comprender mejor los aspectos mecánicos y fisiológicos que subyacen a los estímulos producidos por el entrenamiento de resistencia los cuales afectan a las adaptaciones que se producen.

Este trabajo doctoral está basado en tres artículos científicos publicados en revistas científicas. El primer estudio (capítulo 2) fue diseñado con la intención de analizar las respuestas metabólicas que se producen durante una única serie de 10 repeticiones realizadas hasta el fallo muscular (10RM), en ejercicio de prensa de pierna. El segundo estudio (capítulo 3) fue realizado con el objetivo de estudiar la influencia que tiene el número de repeticiones por serie sobre los cambios en metabolitos musculares y en la producción de potencia durante el ejercicio intermitente de alta intensidad realizado en prensa de pierna. La última publicación (capítulo 4) es un estudio descriptivo diseñado para investigar los cambios producidos en diferentes metabolitos sanguíneos en relación a los cambios en metabolitos musculares y las producciones de potencia mecánica.

Estudio 1 (Capítulo 2)

El ejercicio de prensa de pierna bilateral tiene naturaleza multiarticular (cadera, rodilla y tobillo) y es uno de los ejercicios más comúnmente utilizados en el entrenamiento de fuerza muscular para mejorar el rendimiento deportivo y en la rehabilitación de rodilla, debido a que produce mayor activación neural que la mayoría de ejercicios de extensión de rodilla realizados con cargas. Sin embargo, es bastante sorprendente que en la literatura científica no existan datos que examinen la utilización de substratos y la demanda metabólica durante una actividad breve y extenuante realizada con este tipo de ejercicio. Además, el ejercicio de prensa de pierna bilateral es un modelo de contracción muscular interesante para estimar la producción de energía anaeróbica y la eficiencia mecánica durante el ejercicio realizado a máxima intensidad debido a que es un modelo muy diferente a los utilizados hasta la actualidad. El objetivo de este primer estudio fue analizar los cambios en las concentraciones de metabolitos musculares y los cambios en la potencia muscular a lo largo de las primeras y las últimas cinco repeticiones de una serie de 10 repeticiones realizadas hasta el agotamiento (10RM) en ejercicio bilateral de prensa de pierna. Cada sujeto participó en dos experimentos realizados en días diferentes, en los cuales se manipuló el número de repeticiones

(realizando hasta el agotamiento o realizando la mitad del número máximo de repeticiones posibles), mientras que la carga absoluta no se modificó. Los resultados de esta investigación evidencian que el hecho de realizar 5 o 10 repeticiones de prensa de pierna (con una carga máxima que permite realizar 10 repeticiones) produce dos tipos de ejercicio diferentes, en términos de estado energético y metabolismo muscular. Además, el mayor coste energético por repetición observado durante la segunda parte de las 10 repeticiones sugiere que se produce un descenso de la eficiencia mecánica. Este trabajo analiza los estudios publicados anteriormente sobre las respuestas fisiológicas y metabólicas durante ejercicios máximos en carrera a pie y bicicleta. Los resultados de este estudio pueden favorecer la comprensión de los factores responsables de la producción de potencia muscular y de la fatiga. Por lo tanto, da indicaciones sobre la regulación de las vías metabólicas y sobre la forma en que interaccionan los mecanismos anaeróbicos durante el ejercicio de fuerza dinámica en humanos.

Estudio 2 (Capítulo 3)

Si bien el entrenamiento de resistencia es un componente esencial de un programa efectivo para la mejora de la salud y el rendimiento físico, los aspectos mecánicos y fisiológicos que subyacen al estímulo que produce las adaptaciones al entrenamiento de fuerza no se comprenden completamente. Por ello, el papel real que juega el número de repeticiones realizadas en cada serie en relación al número máximo de repeticiones realizables ha sido de gran interés para entrenadores y científicos del deporte. Esta investigación estudió el impacto que produce el número de repeticiones realizadas por cada serie sobre los cambios en diferentes metabolitos musculares y la producción de potencia muscular durante múltiples series de ejercicio bilateral de prensa de pierna. De forma simultánea se examinó la producción de potencia y la evolución de la fatiga a lo largo del ejercicio. Cada sujeto participó en dos experimentos realizados en días diferentes, en los cuales, con el objetivo de eliminar la posible confusión producida por los efectos de otros factores, la carga inicial y el número total de repeticiones (50 repeticiones) fueron controlados, igualando los valores entre las dos sesiones de ejercicio. Los resultados parecen indicar que el número de repeticiones realizado en cada serie, en relación al número máximo realizable, ejerce una gran influencia sobre los patrones de producción de potencia, el equilibrio energético y la homeostasis celular durante el ejercicio de prensa de pierna. Por lo tanto, esto podría explicar las adaptaciones metabólicas y morfológicas tan diferentes que se producen ante diferentes tipos de entrenamiento de fuerza.

Estudio 3 (Capítulo 4)

La biopsia muscular es una técnica ampliamente utilizada en condiciones de laboratorio. Sin embargo, su uso regular durante la realización de entrenamiento de fuerza con el objetivo de monitorizar las adaptaciones producidas por el entrenamiento es inaceptable. Como alternativa se ha sugerido que algunos metabolitos sanguíneos pueden ser utilizados como marcadores bioquímicos del estrés metabólico, reflejando cambios producidos en el estado funcional de la maquinaria muscular contráctil. Si bien varios estudios han encontrado una fuerte correlación entre el lactato sanguíneo y el muscular en diferentes modelos de ejercicio tales como el ciclismo y la carrera a pie, apenas hay información sobre la correspondencia entre el lactato sanguíneo y el muscular durante el ejercicio dinámico de fuerza, intermitente, realizado a máxima intensidad. Además, a pesar de que el amonio sanguíneo ha sido postulado como un marcador indirecto de los cambios que se producen en los depósitos de ATP muscular a lo largo del ejercicio, el vínculo entre estas dos variables metabólicas a lo largo de series consecutivas de ejercicio dinámico de fuerza de alta intensidad es desconocido en humanos. Por ello, este último estudio fue diseñado para determinar en qué medida están relacionados los cambios producidos en los metabolitos sanguíneos y en los metabolitos musculares durante ejercicio físico de este tipo. Además, se midió la relación entre los metabolitos sanguíneos y los perfiles de fatiga producidos por la potencia muscular. Los resultados mostraron una alta correlación positiva entre el lactato muscular y el sanguíneo, además de una correlación negativa entre la concentración de amonio sanguíneo y las concentraciones de ATP muscular. Adicionalmente, se observaron correlaciones lineales negativas entre el pico de potencia producida y el lactato y amonio sanguíneo. Basándonos en los diferentes aspectos analizados, los datos sugieren que el estado energético del músculo durante el ejercicio intermitente de prensa de pierna puede ser estimado mediante diferentes marcadores metabólicos (lactato y amonio) y mediante los cambios en la producción de potencia muscular.

Nota del autor: Han transcurrido varios años desde que se publicaron los artículos que componen esta tesis doctoral. Por ello, algunos de los estudios citados en la introducción de este trabajo son presentados como resultados novedosos, a pesar de que fueron dados a conocer a la comunidad científica después de la publicación de los artículos que componen la actual tesis doctoral. Por ello, se produce una especie de paradoja temporal en esta tesis doctoral, difícil de superar debido al largo periodo de tiempo que ha transcurrido desde que se publicaron los primeros artículos hasta la realización de este trabajo final.

CHAPTER 1

Updated Introduction

A brief of history

It is thought that 600 before the common era an Indian physician named Susruta was the first to include exercise in prescriptions to maintain equilibrium as well as to prevent and treat diseases (Tipton, 2008). He endorsed moderate exercise because it improved the growth of limbs, enhanced muscle mass, strength, endurance, tone, and development and increased the resistance against fatigue. Susruta thought that to be effective, exercise had to be daily and moderate in intensity and never exceed the half-maximum limit for exhaustion. He strongly opposed excessive exercises, which was interpreted to mean continuous heavy or maximal exercise, as it would cause multiple diseases (Tipton, 2014).

Hundreds of years later, a US army physician, Dr. Thomas L. DeLorme, experimented with a new rehabilitation technique consisting in multiple sets of resistance exercises in which patients performed sets of 10 repetitions up to failure (Odd *et al.*, 2012). In the latter years of the Second World War, the number of US servicemen who had sustained orthopedic injuries was overwhelming the nation's military hospitals. The overflowing of patients was exacerbated by rehabilitation protocols that required lengthy recovery times. The urgent need was to find a faster method to rehabilitate patients who sometimes spent 6-9 months in postoperative therapy with poor results. The physical therapy protocols generally consisted of rest, heat, and various exercises using a high number of repetitions with little to no resistance. In 1945 DeLorme, basing himself on body-building training, started a weight-training involved therapy, in which the number of repetitions was kept relatively high (10 repetitions) and the load was the maximum weight that could be handled for 7 sets of 10 repetitions. In the discussion of his early experimental work in the Journal of Bone and Joint Surgery, DeLorme pointed out that "Rather than attempt to develop endurance in an atrophied, weakened muscle, it seems more logical to restore muscle strength to normal, and then to build endurance" (DeLorme, 1945). In addition, he defined the 1RM and 10RM concepts so widely used today. A few years later, DeLorme refined the system to include 3 progressively heavier sets of 10 repetitions, and he referred to the program as "Progressive Resistance Exercise" (Delorme & Watkins, 1948). DeLorme's academic publications and books on progressive resistance exercise helped legitimize strength training and played a key role in laying the foundation for the science not only of resistance exercise (Odd *et al.*, 2012) in rehabilitation but also in sport training.

Actually, since first proposed as a means of accelerating rehabilitation, the use of training leading repetition failure is currently a pivotal issue of modern resistance training methods. Moreover, the theory that repetitions leading to failure (failure training) will elicit superior muscular strength

gains, compared with repetitions that do not lead to failure (nonfailure training) is commonly associated with Arthur Jones, the founder of Nautilus exercise machines (Smith & Bruce-Low, 2004). Jones advocated that those interested in improving their muscular size, strength, power and/or endurance should perform one set of each exercise to muscular failure (volitional fatigue), train each muscle group no more than once (or, in some cases, twice) per week, perform each exercise in a slow, controlled manner and perform a moderate number of repetitions (for most people, ~8-12). In his writing of over 30 years, Arthur Jones has influenced a number of highly successful athletes (notably, bodybuilders) to use failure training in their programs. Much of what has been written concerning the benefits of training to failure may have originated from a marketing scheme connected with the sale of Nautilus equipment in the 1970s.

Nowadays, resistance training is an essential component of effective intervention programs designed to improve health and strength performance (Kraemer *et al.*, 2009). Unfortunately, the question of what exactly optimal training approximation is for enhancing neuromuscular performance remains unresolved. A description of the metabolic response and degree of fatigue associated to the resistance training stimulus leads to a better understanding of the factors that limit neuromuscular performance and enables us to optimize the manipulation of acute resistance exercise variables to improve the design of resistance training programs. There is a large body of literature which has directly examined the ability of skeletal muscle to provide anaerobic ATP from measurements of the substrates, intermediates, and products of the different metabolic pathways in skeletal muscle throughout maximal efforts carried out by different exercise models such as cycling, sprint running and isometric force. In contrast, there is a paucity of data regarding the specifics of muscle metabolism during a high intensity resistance-type exercise and, therefore, data from other models of high-intensity exercise which approximate the intensity, work to rest interval, and total exercise duration will be examined. Despite the fact that intramuscular energy stores, i.e. adenosine triphosphate, creatine phosphate, glycogen and triglyceride are the major energy fuels during high-intensity exercise, the relative contribution from these sources and the metabolic response depends on mode, intensity and duration of exercise (Essén-Gustavsson & Tesch, 1990). In addition, human skeletal muscle fibers have been reported to have fiber-type-dependent differences in the usage of high-energy phosphates and glycogen degradation, with greater PCr reduction and lactate production in fast-twitch fibers than in slow-twitch fibers (Soderlund & Hultman, 1991; Karatzaferi *et al.*, 2001). The few available data regarding the muscular metabolic changes during resistance exercise will be examined at the end of this section.

Brief overview on the energy metabolism in the contracting muscle

The ability of humans to exercise depends on the conversion of chemical energy to mechanical energy in skeletal muscle. The immediate chemical energy source for muscular contraction, as for most other energy-consuming processes in the cell, is the hydrolysis of ATP to ADP, inorganic phosphate (P_i) and H^+. The concentration of ATP within the muscle cell is, however, limited and for continuous exercise ATP must be resynthesized at essentially the same rate as it is utilized. The ATP turnover rate increases almost 300 times when changing from rest to maximal contraction and the transition can occur within a fraction of a second. The power output of exercise, or the rate of the muscle contraction, determines the demand for ATP. Hence, in biochemical terms, the enzymes which consume ATP during exercise define the challenge facing the pathways that produce ATP (Hargreaves & Spriet, 2006).

When oxidative phosphorylation cannot provide all of the required chemical energy, the ATP resynthesis derived from substrate phosphorylation, is required. The main sources of anaerobic ATP are the degradation of phosphocreatine (PCr) and the production of lactate within the glycolytic pathway (Hargreaves & Spriet, 2006). As ADP is a substrate for the near-equilibrium enzyme CPK, rises in the ADP concentration alter the equilibrium of this reaction and stimulate CPK to convert PCr to ATP. This is a form of substrate control that directly links the rate of ATP use to the provision of anaerobic ATP in the cytoplasm of muscle cells. Increments in free ADP, AMP and P_i also activate glycogen phosphorylase to degrade glycogen and activate the glycolytic enzyme PFK, resulting in a growth of the ATP production and the formation of lactate, which is in part released into the bloodstream. These systems provide the means to engage in exercise requiring high power outputs and bursts of activity. Increased glycolytic rate is linked to an increased cytosolic formation of $NADH^+$; i.e. changes in the redox state of muscle (Ren *et al.*, 1988). The inputs required for oxidative phosphorylation in the electron transport chain (ETC) are oxygen (O_2), free adenosine diphosphate (ADP) and inorganic phosphate (P_i), and the reducing equivalents NADH and $FADH_2$. Owing to the near-equilibrium nature of the ETC and oxidative phosphorylation, the substrates and products of the previous reaction determine the rate of ATP production by this metabolic pathway. Most exercise involves a combination of fat and CHO metabolism to provide the required ATP through aerobic metabolism.

As pointed out above, if cellular homeostasis (a constant concentration of ATP) is to be maintained within an acceptable range during exercise, the ATP synthesis must match ATP utilization. Meanwhile, during intense exercise the rate of ATP utilization in skeletal muscle is higher than the

rate of ATP generation, leading to an accumulation of ADP and AMP, thereby causing a reduction in skeletal muscle ATP content. Atkinson (1977) termed the energy potential of the adenine nucleotides as the energy charge [EC = (ATP + 0.5 ADP)/(ATP + ADP + AMP)] of the contracting cell. EC is a measure of the extent to which the total adenine nucleotide pool of the cell is phosphorylated. As a consequence, the EC is a good indicator of the energy status of the cell (i.e. its capacity to do work). To avoid a large accumulation of ADP within the cell, AMP is deaminated to IMP and ammonia via the enzyme AMP deaminase (Lowenstein, 1990). Deamination of AMP will remove AMP from the adenylate kinase reaction and restore the ATP/ADP ratio and EC. Indeed, the maintenance of a high ATP/ADP ratio has been suggested to be the main function of AMP deamination (Lowenstein, 1972). By preventing excessive accumulation of ADP and AMP, it enables the adenylate kinase reactions to continue, resulting in an increase in the energy charge and continuation contracting. The majority of the IMP accumulated in muscle is reaminated back to AMP after exercise. Nevertheless some IMP is dephosphorylated to inosine, hypoxanthine, and uric acid (Hellsten *et al.*, 1998). The net result is a wasteful loss of purines from muscle and decreased muscle ATP stores that would be replenished by either the purine nucleotide cycle or the novo synthesis, which is a slow, time-consuming process (hours or days) and consumes energy (Hellsten *et al.*, 1998). Some of the ammonia produced in the muscle can efflux from the muscle, enter the bloodstream, and is likely to reflect changes both in the ATP stores and the degree of energy deficiency in skeletal muscle (Sahlin & Broberg, 1990; Harris *et al.*, 1991). Hence, it has been suggested that changes in blood lactate and ammonia can be markers of metabolic stress, reflecting changes in anaerobic glycogenolysis and in the muscular nucleotide pool, respectively (Karlsson *et al.*, 1970; Cheetham *et al.*, 1986; Harris *et al.*, 1991).

Energy metabolism during short-term maximal exercise

When the exercise is pushed to the extreme, as e.g. during a 30-second all-out sprint, the initial power output is at least 3 to 4 times that which can be handled by aerobic means (e.g. 300-400% $\dot{V}O_{2max}$) (Parolin *et al.*, 1999). In this situation, ATP degradation and the accumulation of ADP, AMP, H^+ and P_i are maximal, and PCr use and muscle glycogen degradation to lactate are also maximal (McCartney *et al.*, 1986; Spriet *et al.*, 1989). Simultaneously, the aerobic system is activated but the time is short, so much so that only ~70% of $\dot{V}O_{2max}$ is attained after 30s and merely ~20% of the needed energy are able to be supplied aerobically (Kavanagh & Jacobs, 1988; Bogdanis *et al.*, 1996). The anaerobic systems provide the remaining 80% of the necessary ATP. These high anaerobic ATP regeneration rates lead to a 60-80% fall in phosphocreatine (PCr), a ~30-

45% drop in ATP and a several fold increase of glycolytic intermediates and lactate, as glycolysis yields 65-70% of the anaerobic energy during a 30s sprint (Cheetham *et al.*, 1986; McCartney *et al.*, 1986; Nevill *et al.*, 1989). Studies have found that the PCr and glycolytic systems provide approximately equal amounts of ATP in the first 10 s (Boobis *et al.*, 1981; Jacobs *et al.*, 1983; Jones *et al.*, 1985; Gaitanos *et al.*, 1993; Parolin *et al.*, 1999). Unfortunately, the PCr store is nearly depleted after 10s and can contribute little additional energy. As a result, sustained sprinting relies on the aerobic system and the significant, but diminished, energy provision from the glycogenolytic pathway in the final 20 s of an all-out 30 s sprint (Jacobs *et al.*, 1983; Jones *et al.*, 1985; Bogdanis *et al.*, 1998).

The rapid changes in metabolism and muscle function give rise to an inability to sustain force or the desired exercise intensity (Edwards, 1981). Such fatigue-induced decrements in performance are observed during a supramaximal sprint, where a characteristic fatigue profile is generated. Peak power output is achieved within the initial 3-6 seconds and then declining by ~50-60% by the end of 30 seconds (McCartney *et al.*; 1986; Boobis, 1987; Bogdanis *et al.*, 1998). The decline in force during maximal, short-term contractions has been associated with several metabolic changes in the exercising muscle such as a decrease in muscle phosphocreatine, a corresponding rise in inorganic phosphate (P_i) and its diprotonated form, $H_2PO_4^-$, and a marked fall in muscle pH (Sjoholm *et al.*, 1983; Katz *et al.*, 1986). In spite of the fact that the accumulation of these metabolic by-products in the muscle cell may directly impair the activation of the contractile mechanism, other experiments have indicated that the decline in force may be related to the inability to regenerate ATP at required rates (Sahlin & Ren, 1989; Soderlund *et al.*, 1992). Accordingly, the rates at which anaerobic pathways provide ATP to the exercising muscle cell are critical to the development and maintenance of high power outputs. To assess the rates, serial sampling of muscle is required at frequent intervals. The average rate of ATP regeneration from anaerobic sources during a 6 s sprint on a cycle ergometer is as high as 10-15 mmol · kg^{-1} dm · s^{-1} (Jacobs *et al.*, 1983; Gaitanos *et al.*, 1993; Parolin *et al.*, 1999) and the mean value for a 30 s exercise is ~ 8.5 mmol · kg^{-1} dm · s^{-1} (Bogdanis *et al.*, 1995). The maximal turnover rate of ATP production by means of PCr degradation and anaerobic glycolysis are reported to be approximately 7-9 mmol · kg^{-1} dm · s^{-1} and 5-9 mmol · kg^{-1} dm · s^{-1}, respectively (Jones *et al.*, 1985; Gaitanos *et al.*, 1993; Parolin *et al.*, 1999).
Interestingly, throughout all of the present research, the decline in anaerobic ATP production was coupled with a drop in force generation and power output. It is tempting to postulate therefore, that the development of fatigue was attributable to the decline in ATP provision. Alternatively, however,

the decline in force generation if induced by other mechanisms could be the primary cause for the observed decrease in ATP utilization (Hultman & Greenhaff, 1991).

Energy metabolism during intermittent short-term maximal exercise

There is scarce data on the relative energy system contribution during exercise involving consecutive, all-out short duration sprints. As seen above, during brief periods of maximal work, ATP provision is sustained through the integration of various metabolic processes. Nonetheless, as work bouts are repeated, the metabolic response to subsequent work bouts will be affected by the previous exercise and the length of the intervening rest periods. With repetitive bouts of high-intensity exercise, the contribution of PCr and anaerobic glycogenolysis to the ATP turnover diminishes, and though there is an growth in the aerobic contribution to exercise, reduced power output and total work production result (McCartney *et al.*, 1986; Spriet *et al.*, 1989; Bogdanis *et al.*, 1996). Bogdanis et al (1995, 1996) observed a close relationship between PCr availability and power output during consecutive intense exercise bouts, suggesting that the ability to reproduce high power outputs is directly related to the resynthesis of PCr during rest periods. In a classic paper by McCartney et al. (1986), research was conducted on muscle power and the subsequent metabolic changes in muscle during four 30-s bouts of maximal isokinetic cycling, with 4-min recovery intervals. Glycogenolysis was detected to be almost inhibited in the third and fourth exercise periods, an indication that substantial utilization of aerobic metabolic pathways must have occurred. The enhanced supply of oxidative metabolism to repeated all-out exercise was quantified in a later study using a protocol of two 30-s sprints separated by 4-min intervals of passive rest (Bogdanis *et al.*, 1996). Aerobic energy contribution during the first 30 s sprint was roughly 29% and this augmented to 43% during the second 30 s sprint performed 4 minutes afterwards. In contrast, a reduction in anaerobic energy production of approximately 41% was reported in the course of the second sprint, but the decline in total work production was solely 18%. This discrepancy in anaerobic energy production and total work was partly explained by the 15% increase in oxygen uptake ($\dot{V}O_2$) in the second sprint. Furthermore, aerobic energy contribution was further increased to 65% of the energy during the last 20 s of the second sprint, at which point 85% of $\dot{V}O_{2max}$ was attained.

In addition to the reduction in PCr, intramuscular contents of the total adenine nucleotides (TAN), are reduced during high-intensity exercise, and there is purine efflux from active skeletal muscle (Stathis *et al.*, 1994; Hargreaves *et al.*, 1998). As a consequence, muscular fatigue that develops

during this kind of exercise could be attributed to signs of energy deficiency, i.e. higher concentrations of IMP, hypoxanthine and uric acid (M Hargreaves et al., 1998; C. G. Stathis et al., 1999). Elevated blood ammonia during RT may indicate an imbalance between the rate of ATP utilization and resynthesis within the contracting muscle and it has been thought to play a key role in fatigue (Izquierdo *et al.*, 2009; Sánchez-Medina & González-Badillo, 2011).

Nevertheless, it is plausible that the fatigue experienced in these types of exercises is mediated centrally. In such a situation reductions in external power output and muscle metabolism may be brought about by a common cause rather than being explained on a cause-and-effect basis (McCartney et al, 1986). In summary, in spite of the complexity of the physiological processes that regulate this type of activity, research clearly shows that intermittent short-term maximal exercise places considerable demands on both substrate and aerobic phosphorylation pathways.

Energy metabolism during intermittent short-term maximal resistance exercise

Compared with cycling and running, limited research has been completed to evaluate muscle energy metabolism during resistance-type exercises (MacDougall *et al.*, 1977; Tesch *et al.*, 1986, 1998; Dudley, 1988; Dudley *et al.*, 1991; Robergs *et al.*, 1991; MacDougall *et al.*, 1999). Early research indicated that short-duration weight-resistance exercise predominantly taxes the muscle's stores of ATP and creatine phosphate, with negligible glycogenolysis and a minor glycolytic energy contribution (MacDougall *et al.*, 1977). Several years later, however, they pointed out that muscle glycogen content can decrease by 25% in the biceps brachii after three sets of 10 weight-resistance repetitions to muscular failure (MacDougall *et al.*, 1988). More than a decade later, the same laboratory (MacDougall *et al.*, 1999) published a study examining PCr and glycogen degradation and lactate accumulation in response to high-intensity resistance exercise carried out by bodybuilders. Muscle biopsies were obtained from the biceps brachii prior to and after one set of 12 repetitions to muscular failure at 80% of the 1RM (averaged 37 seconds and 12 repetitions). It was reported that PCr decreased by 62% without significant changes in ATP concentration. In addition, glycogen diminished around 12% and muscle lactate grew 12 fold. Furthermore, other investigations pointed out that resistance-exercise of the legs reduced muscle glycogen content by 26-38% and reflected that muscle lactate glycolytic intermediates rose, indicating the flux of glycogen derives into the glycolytic pathway (Tesch *et al.*, 1986; Robergs *et al.*, 1991). Additionally, these investigators reported a greater rate of glycogen degradation in type II fibers than in type I fibers. Rogers et al (Robergs *et al.*, 1991) found that during 6 high-intensity (70% of 1RM) leg extension sets, with 2 min rest periods, skeletal muscle glycogenolysis took place at

comparable rates with those reported during maximal isokinetic cycle ergometry (McCartney *et al.*, 1986; Spriet *et al.*, 1989). Nevertheless, a near linear drop in muscle glycogen in the course of six sets of a leg extension exercise differs from the reported fall in glycogenolysis during maximal intermittent cycling (McCartney *et al.*, 1986; Spriet *et al.*, 1989), where skeletal muscle glycogenolysis was high during the initial bouts but then declined after the third bout of intense exercise. What is more, several studies (Tesch *et al.*, 1986, 1998; Essén-Gustavsson & Tesch, 1990; Robergs *et al.*, 1991) demonstrated that muscle glycogen loss is related to load, mainly due to fast fiber usage. They infer from single fiber glycogen utilization patterns that fast—twitch fibre type use depends on exercise intensity-holds for resistance exercise, as previously shown for cycling and running (Tesch *et al.*, 1998). Schott *et al. (1995)* compared intermittent and sustained isometric contractions, with the two protocols involving the same load and duration of loading. The sustained contractions led to higher metabolite accumulation and resulted in significantly greater gains (about 80%) in isometric strength.

Resistance training manipulable variables

During the past 30 years, the body of literature on the topic of resistance exercise has reached enormous proportions. When designing resistance exercise programs, many training variables must be taken into account for optimal results to occur (Kraemer & Ratamess, 2004). In a position stand, written for the American College of Sports Medicine, training variables such as the type of muscle action, load, volume, exercise selection, exercise order, rest periods, and frequency were discussed and recommendations were made based on different goals (Kraemer *et al.*, 2009). How these variables are structured over time determines specific muscular adaptations that are associated with measurable characteristics, such as power, absolute strength, hypertrophy, and localized muscular endurance. However, this position stand did not include specific discussion or recommendations on the application of training to failure, that is, if resistance exercise sets should be performed leading to muscular failure or not. Failure typically occurs initially during the concentric phase of a repetition when the muscles cannot produce sufficient torque to lift a given load beyond a critical joint angle or sticking region (Kompf & Arandjelovic, 2016). Although intentionally reaching failure during resistance exercise sets is a common practice in recreational and sports conditioning settings (Willardson *et al.*, 2010), it has been studied less frequently versus other prescriptive variables (e.g., intensity and volume, number of sets, repetition ranges).

Coaches and sport scientists in the field of strength training have attempted to identify proper handling of training variables to determine the training stimulus that maximizes performance

enhancement, although the optimal combination of such training variables is still under debate (González-Badillo *et al.*, 2005; Izquierdo *et al.*, 2006*a*). Even though training leading to repetition failure or not leading to failure has been of interest in the past two decades (Drinkwater et al., 2005; Izquierdo et al., 2006; Rooney et al., 1994) it has still received scarce research attention. Based on the current literature and to the best of our knowledge, remains unclear how this training variable determines the heavy-resistance exercise produced acute metabolic responses that can differ depending on the magnitude of the muscular stress of the exercise protocol utilized. However, the neuromuscular adaptations must differ depending on the relative velocity of each repetition, that is, maximal versus submaximal intended concentric velocity (Pareja-Blanco *et al.*, 2014).

Muscular failure defined

Muscular failure can be defined as the inability to move a specific load beyond a critical joint angle (i.e., sticking point; Drinkwater et al., 2005) or as incapacity to complete a repetition in a full range of motion due to fatigue (Izquierdo *et al.*, 2006*a*). Eventually, fatigue of motor units increases to the extent that force production is not sufficient to lift a given load beyond a critical joint angle or sticking region; this is typically considered the point at which failure has been initially reached (Willardson et al., 2010). However, when failure is initially encountered during the concentric phase, the muscles are still not maximally fatigued. Lifters can typically sustain adequate force to perform additional repetitions through the use of partner-assisted repetitions or descending imposed external load (Willardson *et al.*, 2010). Thus, a common technique among resistance trainers, upon reaching concentric failure, is to immediately reduce the resistance and carry on with the repetitions. Due to the fact that the muscles are not exhausted, sufficient force can be generated to execute additional repetitions with less resistance (Drinkwater *et al.*, 2005).

Rationale for training to muscle failure

The primary role of training leading to repetition failure has been tied to the increase of the motor unit (MU) activation capacity and high stress levels to the tissues, which would increase protein synthesis in order to repair damaged muscle during the training process (Sale, 1987; Rooney *et al.*, 1994; Schott *et al.*, 1995; Drinkwater *et al.*, 2005; Akima & Saito, 2013). Indeed, resistance exercise performed to failure elevates muscle protein synthesis regardless of volume or percentage of 1RM load (Burd *et al.*, 2010; Mitchell *et al.*, 2012). In this kind of high-intensity resistance training (HI-RT), fatiguing protocols yield greater motor units activation than do nonfatiguing protocols and can enhances the magnitude of the strength training response (Rooney *et al.*, 1994;

Drinkwater *et al.*, 2005) and, consequently, leads to greater neural adaptations (Drinkwater *et al.*, 2005). During a HI-RT session, MUs recruitment pattern follows the size principle, in which the low threshold MUs are recruited first, followed by high threshold MUs (Henneman, 1957). It has been speculated that even higher excitability threshold MUs, composed predominantly of type IIx muscle fibers, are recruited when repetitions are performed to failure, possibly due to fatigue in MUs (Willardson, 2007). In fact, HI-RT to failure might promote increased electromyography (EMG) activity, which indicates increased recruitment of high threshold MUs (Akima & Saito, 2013). In this regard, it is believed that recruiting as many MUs as possible delivers maximal gains in muscle hypertrophy and strength on the target muscles (Wernbom *et al.*, 2007). Thus, collectively, the evidence appears to suggest that repetition failure is an essential feature of resistance training regimen when muscle strength and hypertrophy are targeted.

Acute mechanical and metabolic responses to Failure vs Non Failure exercise protocols

Some studies have already looked into the acute mechanical and neuromuscular responses produced by both leading to repetition failure or not leading exercise protocols, which can aid us to understand the training induced-adaptations which arise by resorting to failure versus nonfailure training programs. Lawton et al. (2006) demonstrated that a traditional full-RM set was detrimental to acute repetition power output. In this study, acute repetition power output was examined during 4 bench press sessions that consisted of the following: (a) continuous: full 6RM set to failure, (b) singles: 6 repetitions with the 6RM load and 20-second rest between single repetitions, (c) doubles: 3 sets of 2 repetitions with a 50-second rest between clusters, and (d) triples: 2 sets of 3 repetitions with 100-second rest between clusters. The results demonstrated significantly greater power output for each repetition of the singles, doubles, and triples conditions versus the continuous condition; this was clearly obvious for the last 3 repetitions. Throughout typical resistance exercise in isoinertial conditions, and assuming every repetition is carried out with maximal voluntary effort, velocity unintentionally declines as fatigue develops (Izquierdo *et al.*, 2006*b*). However, few cases of research analyzing the response to different resistance training schemes have described variations in repetition velocity or power (Izquierdo *et al.*, 2006*b*, 2009). Sánchez-Medina and González-Badillo (2011) demonstrated that the number of repetitions actually performed in relation to the maximum number that can be completed was tied to the neuromuscular fatigue degree and the loss of repetition velocity. They proved that by conducting training sets consisting of eight or more repetitions per set leading to failure, caused ammonia to substantially rise above resting values, which could indicate an accelerated purine nucleotide degradation and a corresponding muscle

energetic disturbance. Unlike lactate, ammonia displayed a curvilinear response to loss of velocity, only increasing above resting levels when the number of repetitions was at least two repetitions over 50% of maximum predicted number. Izquierdo et al. (2006b) demonstrated that maintenance of a high repetition velocity is best accomplished by ending a set well ahead of reaching to failure and stated that significant reductions in repetition velocity occurred at 48% of the overall repetitions for the back squat, irrespective of the intensity. The maintenance of a high repetition velocity is another key variable that may determine increases in muscular power. These results suggest that if the objective is to maintain a high repetition velocity or to lose a low percentage of the initial speed, with the intent of increasing muscular power, the intention should be to end a set well ahead of reaching failure.

In another series of studies performed by the same research group (Gonzalez-Badillo *et al.*, 2016; Pareja-Blanco *et al.*, 2016*a*), the authors compared the time course of recovery following two resistance exercise protocols differing in the number of repetitions per set with in relation to the maximum possible (to failure) number in the bench press and squat exercises in a single training session. This study showed that the mechanical and neuroendocrine response and muscular damage is notably different when manipulating the number of repetition per set (exercising up to failure vs performing a half-maximum number of repetitions per set). Leading to failure protocol resulted in a significantly decreased ability to apply force rapidly with the lower limbs (CMJ height) during the initial 48h-post exercise at least, greater increments in hormonal response (cortisol and prolactin) and more extensive muscle damage (CK) 48 h-post exercise, compared with the protocol comprised of half of the maximal number of repetitions per set. These findings may suggest that the muscular ability to rapidly develop force with the lower limbs may be considerably compromised up to 48h after resistance exercise to repetition failure.

Controversy between leading to failure versus not to failure training approaches findings

Despite previously described rationale, it is unclear whether HI-RT to failure is more effective than not failure for strength, power and/or muscle hypertrophy adaptations. Few studies directly compared the resistance-type training program to repetition failure and no repetition failure on neuromuscular and morphological adaptations (Table 1). Besides, these studies displayed conflicting results. These inconsistent findings could be explained by virtue of the differences among the studies depending on the type of muscle action, the muscular characteristic being trained, the length of the study period, the training status of the subjects, and the total volume of training performed.

Study	Group	Subjects	Exercise prescription	Controlled variables	Frequency (days/ week)	Training program Duration	Measured variables
Rooney et al. (1994)	Failure	N = 13 Untrained males and females	BC: 1 x 6–10 reps at 6RM	Volume Intensity Frequency Exercise type	3	6	1RM BC Maximal isometric strength
	Non-failure	N = 14 Untrained males and females	BC: 6–10 x 1 rep at 6RM, 30 s rest between sets				
Kramer et al. (1997)	Failure	N = 16 Trained males	SQ, PP, BP, MTP, LC, BR: 1 x 8–12RM		3	14	1RM SQ
	Non-failure	N = 14 Trained males	SQ, PP, BP, MTP, LC, BR: 3 x 10 reps at 90–100 % of 10RM, 2 min rest between sets				
Sanborn et al. (2000)	Failure	N = 9 Untrained females	SQ, SQ, BP, SP, MTP, SS, SLDL, UR: 1 x 8–12RM		3	8	1RM SQ CMJ
	Non-failure	N = 8 Untrained females	SQ, SQ, BP, SP, MTP, SS, SLDL, UR: 3–5 x 2–10 reps at 80–100 % of 2–10RM				
Folland et al. (2002)	Failure	N = 12 Untrained males and females	LE: 4 x 10 reps at 75 % of 1RM, 30 s rest between sets	Volume Intensity Frequency Exercise type	3	9	1RM LE
	Non-failure	N = 11 Untrained males and females	LE: 40 x 1 rep at 75 % of 1RM, 30 s rest between sets				
Drinkwater et al. (2005)	Failure	n= 15 Trained males	BP: 4 x 6 reps at 80–105 % of 6RM, 3 min 50 s rest between sets	Volume Intensity Frequency Exercise type	3	6	6RM BP 40-kg bench throw power
	Non-failure	N = 11 Trained males	BP: 8 x 3 reps at 80–105 % of 6RM, 1 min 40 s rest between sets				

Izquierdo et al. (2006a)	Failure	N = 14 Trained males	BP: 3 x 6–10RM, 2 min rest between sets SQ: 3 x 6–10 reps at 80 % of 6–10RM, 2 min rest between sets	Volume Intensity Frequency Exercise type	2	11	1RM BP and SQ CMJ, CMJ with 30% body mass extra load Leg and arm power output with 60%1RM load BP and SQ endurance test (maximal nº reps until failure with 75%1RM) Resting Hormone concentrations
	Non-failure	N = 13 Trained males	BP: 6 x 3–5 reps at 6–10RM, 2 min rest between sets SQ: 6 x 3–5 reps at 80 % of 6–10RM, 2 min rest between sets				
Willardson et al. (2008)	Failure	N = 10 Trained males	SQ, LC, LE: 3 x 13-15 at 60-115% 15RM, 1 min rest between sets and 2 min between exercises	Volume Intensity	1	6	Muscular endurance test. 3 sets each for SQ, LC, LE Blood lactate Heart Rate
	Non-failure	N = 10 Trained males	SQ, LC, LE: 4 x 10-12 at 60-115% 15RM, 1 min rest between sets and 2 min between exercises				
Izquierdo-Gabarren et al. (2010)	Failure	N = 14 Trained males	BP, SCR, LPD, PC: 3–4 x 4–10 reps at 75–92 % of 1RM, 2 min rest between sets	Intensity Frequency	2	8	1RM BP Upper-body power-load relationship (15-100% 1RM) BP power with the same absolute load (70% pre-training 1RM) Rowing ergometer performance tests
	Non-failure	N = 15 Trained males	BP, SCR, LPD, PC: 3–4 x 2–5 reps at 75–92 % of 1RM, 2 min rest between sets				
Sampson and Groeller (2015)	Failure	N = 10 Untrained males	BC: 4 x 6 reps at 85 % of 1RM, 3 min rest between sets	Intensity Frequency Exercise type	3	12	1RM BC Maximal Voluntary contraction CSA EMGRMS
	Non-failure	N = 10 Untrained males	BC: 4 x 4 reps at 85 % of 1RM, 3 min rest between sets				
Pareja-Blanco et al. (2016)	Close to Failure	N = 10 Trained males	FSQ: 3 x 3-9 reps at 70- 85% 1RM, 4 min rest between sets	Intensity Frequency Exercise type	2	8	CSA 1RM, full load-velocity profile CMJ, 20m sprint Muscle fibre composition
	Non-failure	N = 12 Trained males	FSQ: 3 x 2-5 reps at 70- 85% 1RM, 4 min rest between sets				

Table 1. SQ quarter squat, BC bicep curl, BP bench press, BR bent-over row, FSQ full squat, (F) higher volume completed by the failure group, LC leg curl, LE leg extension, LPD lateral pull-down, MTP mid-thigh pull, (NF) higher volume completed by the non-failure group, NR not reported, PC power clean, PP push press, rep(s) repetition(s), RM repetition maximum, SCR seated cable row, SLDL straight-legged deadlift, SP shoulder press, CMJ countermovement jump, CSA cross sectional area, EMG electromyography,

The following sections will provide an overview of the results obtained in the most relevant studies that made a comparison between resistance training leading to repetition failure and no repetition failure, within the framework of different training objectives and performance adaptations.

Absolute strength. Of the ten studies analyzed, muscular strength gains were measured in nine of them. Two of them demonstrated greater increases by the RF group (Rooney *et al.*, 1994; Drinkwater *et al.*, 2005), another three studies indicated higher gains by the NF group (Kraemer *et al.*, 1997; Sanborn *et al.*, 2000; Izquierdo-Gabarren *et al.*, 2010) and the remaining four studies did not observe significant differences among training protocols (Folland *et al.*, 2002; Izquierdo *et al.*, 2006*a*; Sampson & Groeller, 2015; Pareja-Blanco *et al.*, 2016*b*). In a recent systematic review with a meta-analysis (Davies *et al.*, 2016) the effect of failure versus non-failure resistance training on muscular strength gains was investigated. The authors showed that despite increasing muscular strength in both practices, non-failure training was found to be slightly more effective. However, the effectiveness of non-failure training was influenced by training volume, training status and exercise type. When the volume and intensity variables were equated, training not leading to repetition failure led to similar (Folland *et al.*, 2002; Izquierdo *et al.*, 2006*a*; Pareja-Blanco *et al.*, 2016*b*) or higher improvements in maximum strength by repetition failure training (Drinkwater *et al.*, 2005).

It has been suggested (Folland *et al.*, 2002; Izquierdo *et al.*, 2006*a*) that high fatigue is not an essential or primary stimulus for gains in strength due to the fact that low fatigue strength training designed to minimize metabolite accumulation produced noteworthy increases in strength that were slightly higher compared with training designed to maximize fatigue and metabolite accumulation. This means that substantial and comparable strength gains can be achieved with training that involves a low level of discomfort and physical effort. Two of the studies (Rooney *et al.*, 1994; Drinkwater *et al.*, 2005) reported that RT to failure induced greater strength rises as opposed to training not leading to failure. Nonetheless, in these two pieces of research repetition velocity during training was not monitored, nor was it intended to be maximal. Training at maximal voluntary velocity has recently been described to be of paramount importance for maximizing strength gains and athletic performance (jumping ability) (Izquierdo *et al.*, 2006*a*; Pareja-Blanco *et al.*, 2014; Gonzalez-Badillo *et al.*, 2014).

In short, accumulation of intramuscular metabolites or elevated endogenous circulating hormones, physiological responses associated with resistance exercise to failure, may prove not to be the most effective approach to elicit major changes in skeletal muscle structure or function (Wilkinson et al., 2006; West et al., 2009). This suggests that there are multiple signaling pathways that may promote muscular hypertrophy and strength in the absence of repetition failure (Goldberg, 1967; Spangenburg et al., 2008).

Muscle hypertrophy. Of the ten studies analyzed, muscular strength gains were solely directly measured in two of them (Sampson & Groeller, 2015; Pareja-Blanco *et al.*, 2016*b*), with contradictory findings. Despite the relative absence of fatiguing bouts of resistance exercise to failure and a considerable reduction in the total exercise volume, Sampson and Groeller (2015) detected no significant difference in agonist elbow flexors cross sectional area (CSA) among the experimental groups after a 12-week training period. On the other hand, Pareja-Blanco et al (2016), noticed that training close to muscle failure elicited a greater hypertrophy compared with the leading not to failure group, in spite of both groups securing increased mean fiber CSA. The greater mechanical and metabolic stress (Sánchez-Medina & González-Badillo, 2011), hormonal response (Pareja-Blanco *et al.*, 2016*a*), and muscle damage (Pareja-Blanco *et al.*, 2016*a*) associated to typical HI-RT to failure protocols might explain the more extensive hypertrophy response observed in the latter study.

Power output. Of the ten studies analyzed, muscle power output changes were measured in four of them. In elite athletes, Izquierdo et al. (2006a) found that after the initial 11 weeks, increases in power were similar in both groups for the bench press (20% RF, 23% NRF) and back squat (26% RF; 29% NRF). Nevertheless, during the final 5-week peaking period, the NRF group continued to increase muscular power, despite both groups performing the same ballistic style nonfailure training program. Drinkwater et al. (2005) found that using a training program that equated training intensity (i.e. percentage of 6RM), training volume (i.e., total number of repetitions), and duration of training time (13 minutes 20 seconds), the group that trained leading to repetition failure experienced substantially larger augments in bench throw power as opposed to the group allowed to fulfil all repetitions during the training-program. The authors speculated that the RF group experienced greater increases in strength and power by maximizing the number of active motor units leading to greater neural adaptations. Pareja et al. (2016) demonstrated greater enhancement in the vertical jump

height in the group trained with low magnitude of velocity loss within each set (20%), although the group trained close to failure performed 40% more repetitions with the same training intensity. Izquierdo-Gabarren et al. (2010), observed in elite athletes larger gains in maximal power output in the non repetition failure group compared with the training to volitional fatigue group, in response to the combined endurance and resistance training intervention. Taken together, most of the studies indicate that non failure training is more effective than training to failure for increasing power.

Localized muscular endurance. Of the ten studies analyzed, localized muscular endurance changes were measured in two of them. The aforementioned study by Izquierdo et al. (2006a) demonstrated that a repetition failure approach resulted in greater gains in localized muscular endurance (i.e., maximal bench press repetitions with 75% of 1RM) after 11 weeks. However, Willardson et al. (2008) demonstrated similar results in comparing failure (F) versus nonfailure (NF) training approaches with equated intensity and volume on lower-body muscular endurance in trained men. It should be noted that in the Izquierdo et al. (2006) study, despite significant greater gains in bench press repetitions with the failure approach, back squat repetitions were similar with both the failure and nonfailure approaches, similar to the Willardson et al. (2008) study.

These results imply that when intensity and volume are equated, failure or nonfailure training approaches will yield similar gains in lower body muscular endurance. Accordingly, it appears that a repetition failure approach might be superior for upper body endurance. Conversely, when training for lower-body endurance, the total volume (load 3 sets 3 repetitions) might be more important versus whether or not sets are performed to failure.

Concurrent strength and endurance gains. Izquierdo-Gabarren et al. (2010) found that throughout an 8-week periodized cycle of combined strength and endurance training that incorporated three to five sets in four global and multi-joint exercises (prone bench pull, seated cable row, pulldown, power clean), notable increases were generated in strength, muscle power and rowing performance in highly trained rowers. In contrast, both muscle strength and rowing performance could be compromised if a given threshold volume is surpassed or drastically reduced during a short-term training program. It is crucially important when both strength and aerobic endurance need to be concurrently enhanced. These findings suggest that a concurrent strength and endurance training program using a moderate number of repetitions for not to repetition failure

training provides a favorable environment for achieving greater enhancements in strength, muscle power and specific performance when compared to higher training volumes of repetition to failure.

Muscle fiber composition. Pareja-Blanco et al (2016), observed that training with a higher magnitude of velocity loss (close to muscle failure) gave rise to a greater muscle hypertrophy, but induced a substantial reduction in the expression of the fastest myosin isoform (MHC-IIx), as opposed to the non failure training group, in which it was preserved. It was concluded that if only low or moderate repetition velocity losses (~20%) are experienced, higher forces at fast velocities will be achieved during training, while minimizing fatigue and favoring rapid force production adaptations required by many sports and athletic disciplines.

Approach to the topic and purposes

Regular resistance exercise is an essential component of effective intervention programs designed to improve strength in athletes and adults with chronic diseases and disabilities (Kraemer *et al.*, 2009; Colberg *et al.*, 2010). The response to the resistance training program depends ultimately on pronounced metabolic and morphological adaptations of multiple cellular functions which depend to a great extent on changes of a complex signaling network that is involved in the course of each training session in response to contractile activity (Winder & Hardie, 1999; Hancock *et al.*, 2006).

Whilst changes in muscle metabolites and power output during exhausting and non exhausting heavy intermittent cycling (Saltin & Essén, 1971; McCartney *et al.*, 1986), running (Cheetham *et al.*, 1986) or isometric knee extension (Edwards *et al.*, 1972) exercises are well characterized, little is known on substrate utilization and metabolic demand during one or consecutive sets of exhausting compared with non exhausting high-intensity dynamic resistance exercise. The adaptive response to strength training may diverge when training leads to failure is compared to when it does not lead to failure, as different degrees of fatigue and muscle metabolite accumulation are elicited by the training (Spriet *et al.*, 1989).

Analyzing different conditions pertaining to changes in power output may provide some clues as to the understanding of the mechanisms by which the process of muscle contraction attempts to maintain an adequate function during dynamic resistance exercise (Sahlin & Broberg, 1990). This kind of examination may enhance the understanding of factors that limit fatigue during leg press exercise, and thus give an indication of the regulation of the metabolic pathways and of how the anaerobic mechanisms are interrelated during dynamic resistance exercise in humans.

Bilateral leg press is an interesting muscle contraction model to examine changes in power output, muscle and blood metabolites as well as to estimate anaerobic energy production and mechanical efficiency during maximal exercise because it largely differs from the models used up till now. Thus, in the course of one or several sets of 10 repetitions of bilateral leg press exercise the duration of the muscle contraction is between 1,500 to 2,000 ms, the contraction frequency is ~0.30 Hz, force and intramuscular pressures are high, the blood flow to the exercising muscle and the

lactate released from muscle to the blood stream is greatly restricted, power output declines over time and the external mechanical work produced during each muscle contraction remains constant. This leg press exercise model is different from the model of maximal cycling or treadmill running (contraction duration of ~80–200 ms; contraction frequency of ~2 Hz; lower force and intramuscular pressures, low blood flow restriction, and power output and external mechanical work declining with time) or the classical intermediate constant-load one-legged knee extensor model (Bangsbo *et al.*, 2001), in which the contraction duration is ~500 ms, the contraction frequency is ~1 Hz and power output and external mechanical work does not vary during exercise. Consequently, whether power output, muscle and blood metabolites and mechanical efficiency vary over time during the course of short-lasting maximal dynamic exercise such as the leg press model remains an unresolved matter.

Taking into account the above considerations, the purposes of the studies that compose this doctoral thesis were:

1) To examine changes in muscle metabolism and power output during the initial and final 5 repetitions of a set of 10 repetitions to failure of bilateral leg press exercise, identifying the metabolic factors that could contribute to the decline in muscle power production during leg press exercise, and estimating the changes over time in anaerobic energy production and mechanical efficiency. (**Study 1**). Literature on anaerobic energy production and mechanical efficiency (expressed as external work or power done per a given ATP produced) (Awan & Goldspink, 1970; Chasiotis *et al.*, 1987) that occurs over time during short-lasting maximal exercise is scarce and controversial (Bangsbo *et al.*, 2001). A limited number of research studies that employ cycle exercise (Boobis, 1987; Medbø & Tabata, 1993), static knee-extension (Spriet *et al.*, 1987; Bergström & Hultman, 1988) or dynamic one-legged knee-extensor (Bangsbo *et al.*, 1990, 2001; Gonzalez-Alonso *et al.*, 2000; Krustrup *et al.*, 2003) exercise at a constant work rate (Bangsbo *et al.*, 1990) have estimated that ATP utilization per work unit either decreased (Spriet *et al.*, 1987; Bergström & Hultman, 1988; Sahlin & Ren, 1989; Bangsbo *et al.*, 1990) or increased over time (Bangsbo *et al.*, 2001) throughout exercise. This may be partly due to the variety of experimental conditions, the mode of exercise chosen to determine mechanical efficiency and the difficulties

encountered whilst quantifying anaerobic energy production based on the decrease in muscle adenosine triphosphate and PCr, as well as the accumulation of metabolites such as lactate. Bilateral leg press is an interesting muscle contraction model to estimate anaerobic energy production and mechanical efficiency in the course of maximal exercise owing to the fact that it largely differs from the models used until now.

One hypothesis was that the decline in power production while exercising were related to changes in muscle metabolites. It was also hypothesized that changes in mechanical efficiency would occur over time during a set of exhaustive leg press exercise.

2) To investigate the influence of the number of repetitions per set (leading to failure or not) on changes in muscle and blood metabolites and power output during high-intensity bilateral leg press exercise performed with the same initial load (~83% 1RM) in young trained men, while simultaneously examining the power output and fatigue developed throughout the exercise (**Study 2**). A secondary purpose was to examine the relationship between the metabolic status of muscle and changes in power output. These two exercise sessions are traditionally used for reaching specific training goals. Thus, the ‘‘leading to failure’’ exercise is characterized by a progressive decrease in load and power throughout the repeated sets and is primarily used for increasing muscular strength and hypertrophy (Kraemer *et al.*, 1987; Burd *et al.*, 2012). The ‘‘not leading to failure’’ exercise is characterized by the maintenance of load and average power throughout the sets and is used primarily for optimizing muscle power development (Izquierdo *et al.*, 2006*a*). To the best of the author’s knowledge, no study has analyzed the changes in muscle and blood metabolites during high-intensity dynamic exercise characterized by the maintenance of power output throughout the sets.

3) To investigate how muscle lactate and ATP levels are affected in relation to, blood lactate and ammonia concentrations respectively during repeated sets of bilateral leg press exercise, and to analyze changes in mechanical power output throughout exercise and blood metabolites (**Study 3**). We hypothesized that

blood lactate and ammonia concentrations would reflect muscle lactate and ATP levels, respectively, and would predict changes in power output production. Two resistance leg press exercise protocols (leading or not to failure) were chosen as this was a useful model to examine the relationships between power output, muscle and blood metabolites because of the different degree of fatigue induced and rate of utilization of various muscle substrates.

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List of abbreviations

Abbreviation	Meaning
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
AMPK	AMP-activated protein kinase
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BC	Biceps Curl
BP	Bench press
BR	Bent-over row
Ca^{2+}	Calcium ions
CHO	Carbohydrate
CMJ	Counter movement jump
Cr	Creatine
CSA	Cross sectional area
CV	Coefficient of variation
dm	Dry matter
Δ	Delta: change
EC	Energy charge
e.g.	Exempli gratia
EMG	Electromyography
ETC	Electron transport chain
$FADH_2$	Flavin adenine dinucleotide (reduced form)
FSQ	Full squat
Fig	Figure
F6P	Fructose 6-phosphate
G1P	Glucose 1-phosphate
G6P	Glucose 6-phosphate
H_2PO^{4-}	Diprotonated inorganic phosphate
H^+	Hydrogen ion or proton
HI-RT	High-intensity resistance training
HMP	Hexose monophophosphates
HPLC	High-performance liquid chromatography
Hz	Hertz
IMP	Inosine 5-monophosphate
J	Joule

K^+	Potassium ion
$[La^-]$	Lactate concentration
LC	Leg curl
LE	Leg extension
LSD	Fisher's least significant difference
ms	millisecond
MU	Motor unit
µmol	micromole
mmol	millimole
N	Newton
NADH	Nicotinamide adenine dinucleotide
NH_3	Ammonia
NRF	Non repetition failure
PCr	Phosphocreatine or creatine phosphate
P_i	Inorganic phosphate
PFK	Phosphofructokinase
PHOS	Glycogen phosphorylase
r	Pearson's product-moment correlation coefficient
R^2	Coefficient of determination
rep or REP	Repetition
RF	Repetition to failure
RM	Repetition maximum
RNF	Repetitions not to failure
RT	Resistance training
SD	Standard deviation
SPSS	Statistical Package for the Social Sciences
SQ	Quarter Squat
ST	Slow-twitch fibers
TAN	Total adenine nucleotide
US	United States of America
$\dot{V}O_2$	Oxygen uptake
$\dot{V}O_{2max}$	Maximal oxygen uptake
W	Watt
wm	Wet matter
wt	Weight
^{31}P NMR	Phosphorus-31 nuclear magnetic resonance

CHAPTER 2

Anaerobic energy expenditure and mechanical efficiency during exhaustive leg press exercise

2010

Gorostiaga, E. M., Navarro-Amézqueta, I., Cusso, R., Hellsten, Y., Calbet, J. A., Guerrero, M., Granados, C., González-Izal, M., Ibañez, J. & Izquierdo, M

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Abstract

Information about anaerobic energy production and mechanical efficiency that occurs over time during short-lasting maximal exercise is scarce and controversial. Bilateral leg press is an interesting muscle contraction model to estimate anaerobic energy production and mechanical efficiency during maximal exercise because it largely differs from the models used until now. This study examined the changes in muscle metabolite concentration and power output production during the first and the second half of a set of 10 repetitions to failure (10RM) of bilateral leg press exercise. On two separate days, muscle biopsies were obtained from vastus lateralis prior and immediately after a set of 5 or a set of 10 repetitions. During the second set of 5 repetitions, mean power production decreased by 19% and the average ATP utilization accounted for by phosphagen decreased from 54% to 19%, whereas ATP utilization from anaerobic glycolysis increased from 46 to 81%. Changes in contraction time and power output were correlated to the changes in muscle Phosphocreatine (PCr; $r=-0.76$; $P<0.01$) and lactate ($r=-0.91$; $P<0.01$), respectively, and were accompanied by parallel decreases ($P<0.01$-0.05) in muscle energy charge (0.6%), muscle ATP/ADP (8%) and ATP/AMP (19%) ratios, as well as by increases in ADP content (7%). The estimated average rate of ATP utilization from anaerobic sources during the final 5 repetitions fell to 83% whereas total anaerobic ATP production increased by 9% due to a 30% longer average duration of exercise (18.4 ± 4.0 vs 14.2 ± 2.1 s). These data indicate that during a set of 10RM of bilateral leg press exercise there is a decrease in power output which is associated with a decrease in the contribution of PCr and/or an increase in muscle lactate. The higher energy cost per repetition during the second 5 repetitions is suggestive of decreased mechanical efficiency.

Introduction

Whereas the physiological and metabolic responses to maximal running, cycling and knee extension exercise using the leg extension ergometer have been well documented, to our knowledge, careful investigations examining substrate utilization across a single set of bilateral leg press exercise have not been performed. Bilateral leg press exercise is a multi-joint (hip, knee, and ankle) exercise and is one of the most common resistance training type of exercise used to enhance performance in sports and in knee rehabilitation as it produces greatest neural activation than the majority of weight-bearing knee extension exercises (Bolgla *et al.*, 2008).

Information about anaerobic energy production and mechanical efficiency (expressed as external work or power done per a given ATP produced (Chasiotis *et al.*, 1987; Awan & Goldspink, 1970) that occurs over time during short-lasting maximal exercise is scarce and controversial (Bangsbo *et al.*, 2001). A limited number of studies that employ cycle exercise (Boobis, 1987; Medbo & Tabata, 1993), static knee-extension (Bergstrom & Hultman, 1988; Sahlin & Ren, 1989; Spriet *et al.*, 1987) or dynamic one-legged knee-extensor (Bangsbo *et al.*, 1990; Bangsbo *et al.*, 2001; Gonzalez-Alonso *et al.*, 2000; Krustrup *et al.*, 2003) exercise at a constant work rate (Bangsbo *et al.*, 1990) have estimated that ATP utilization per work unit either decreased (Bangsbo *et al.*, 1990; Bergstrom & Hultman, 1988; Sahlin & Ren, 1989; Spriet *et al.*, 1987) or increased over time (Bangsbo *et al.*, 2001) during exercise. This may be partly due to the variety of experimental conditions, the mode of exercise chosen to estimate mechanical efficiency and the difficulties of quantifying anaerobic energy production based on the decrease in muscle adenosine triphosphate and PCr, as well as the accumulation of metabolites such as lactate.

Bilateral leg press is an interesting muscle contraction model to estimate anaerobic energy production and mechanical efficiency during maximal exercise because it largely differs from the models used until now. Thus, during a set of 10 repetitions of bilateral leg press exercise the duration of the muscle contraction is between 1500 to 2000 ms, the contraction frequency is ~ 0.30 Hz, force and intramuscular pressures are high, the blood flow to the exercising muscle and the lactate released from muscle to the blood stream is highly restricted, power output declines over time and the external mechanical work produced during each muscle contraction remains constant. This leg press exercise model is different from the model of maximal cycling or treadmill running (contraction duration of ~ 80 – 200 ms; contraction

frequency of ~ 2 Hz; lower force and intramuscular pressures, low blood flow restriction, and power output and external mechanical work declining with time) or the classical intermediate constant-load one-legged knee extensor model (Bangsbo *et al.*, 2001) in which the contraction duration is ~ 500 ms, the contraction frequency is ~ 1 Hz and power output and external mechanical work does not change during exercise. It is therefore still uncertain whether mechanical efficiency changes over time during short-lasting maximal dynamic exercise such as the leg press model.

The purposes of the present study were: 1) to examine changes in muscle metabolism and power output during the initial and final 5 repetitions of a set of 10 repetitions to failure of bilateral leg press exercise, 2) to identify the metabolic factors that could contribute to the decline in muscle power production during leg press exercise, and 3) to estimate the changes over time in anaerobic energy production and mechanical efficiency. One hypothesis was that the decline in power production during exercise would be related to changes in muscle metabolites. It was also hypothesised that changes in mechanical efficiency would occur over time during a set of exhaustive leg press exercise.

Material and methods

Subjects. Six healthy male volunteers participated in this study. Their mean (± SD) age, height, body mass, estimated maximal oxygen uptake ($\dot{V}O_{2max}$) in cycloergometer and maximal strength (1RM) during leg press exercise were 34 ± 6 years, 179 ± 5 cm, 74.5 ± 7.2 kg, 57.1 ± 4.9 $ml \cdot kg^{-1} \cdot min^{-1}$ and 199 ± 43 kg, respectively. All were recreational athletes, mainly in endurance events, but none trained for competition. The subjects were thoroughly informed of the purpose, nature, practical details and possible risks associated with the experiment, as well as the right to terminate participation at will, before they gave their voluntary written consent to participate. A medical examination was also completed by a physician. The present study is part of a project which has been approved by the Institutional Review Committee of the Instituto Navarro del Deporte, according to the Declaration of Helsinki.

Experimental design. This study was designed to examine metabolic responses elicited during one set of 10 repetitions to failure (10RM) of bilateral leg press exercise. Failure was indicated by the inability to complete the next repetition after 10 repetitions. Each subject participated in two experiments performed on separate days. In one experimental day the subjects performed a set of 5 repetitions of leg press exercise and on the other day a set of 10 repetitions were performed. Both experiments were performed with the same absolute load and averaged 168 ± 39 Kg (~ 85% of the individual 1RM). On both days percutaneous muscle biopsy samples were obtained from the vastus lateralis prior and immediately after the last repetition. All subjects participated in the two experiments in random order. The two randomly assigned main tests were performed at the same time of the day and were separated by one to two months. Subjects were requested to repeat their pre-recorded normal diet and refrain from any form of intense physical exercise for 48 h prior to each test.

Preliminary tests. Several pre-test sessions were done during the 3 weeks preceding the experiments. First, the subjects were familiarized with the experimental testing procedures about 2 week beforehand. Second, two weeks before the first experiment subjects participated in a control testing day where resistance-load verifications for one repetition maximum strength (1RM: the heaviest load that could be correctly pressed only once using the correct technique) and for 10 repetition maximum strength (10RM: the individual maximum load that produced 10 repetitions to fatigue) were determined

in the leg press exercise machine. After measuring 1RM, we estimated the absolute load that should produce 10 repetitions to fatigue (10RM). Then, after at least 10 min rest, the subjects performed a maximal repetitive set until failure with the load that theoretically should produce 10 repetitions to fatigue (~85% of 1RM). If the number of repetitions until failure was equal to 10, the load was defined as a 10RM and used during the experimental main tests. If the number of repetitions until failure was different from 10, several trials of a maximal repetitive set until failure were performed on different days with lower or higher loads during subsequent test sessions, in order to determine the load leading to failure in exactly 10 repetitions. Third, on a separate day the maximum oxygen uptake ($\dot{V}O_{2max}$) of each subject was estimated (Storer *et al.*, 1990) by using a continuous incremental test until exhaustion on a friction-loaded cycle ergometer (Monark Ergomedic 818E, Varberg, Sweden). The first work load (60W) was high enough to ensure that exhaustion would occur within 8-14 min, the load being increased by 30 W at the end of every min. Heart rate was monitored throughout the test continuously (15s) with a cardiotachometer (Sportester Polar, Kempele, Finland). Average power output at exhaustion was 347 ± 27 W.

Main tests. On the morning of the experiment, the subject arrived after a light breakfast and 2 h fast period. On arrival at the laboratory subjects rested on bed for 20 minutes in order that small incisions could be made under local anaesthesia (1% lidocaine) through the skin and fascia over the vastus lateralis muscle of one leg. The subjects then completed a period of warm-up consisting of a set of 5 repetitions at 50%, three to four repetitions at 75% and 1 repetition at 90% of maximal bilateral leg press strength (1RM). Three to four subsequent attempts were made to determine the 1RM. The resting period between maximal attempts was always 2 min. After 10 min rest, a muscle biopsy (resting biopsy) was taken from muscle vastus lateralis and arterialized blood sample was drawn from the earlobe previously hyperemized with a warming ointment (Finalgon, Boehringer Ingelheim, Germany). Then an intense bilateral leg press exercise, 5 or 10 repetitions with the maximum load possible to achieve 10 repetitions (10RM), was performed. In each repetition the subject was instructed to move the weight as fast as possible. The time for each repetition and the concentric and eccentric components were recorded. There was about 1-s pause between repetitions to ensure that the stretch-shortening cycle did not enhance performance of the subsequent concentric action. The power output of each repetition was monitored continuously and

measured during the concentric phase of leg press action. Immediately after the last repetition (within 5-10 s) an additional muscle biopsy and arterialized blood samples were taken. All subjects were highly motivated and strong verbal encouragement was given to all subjects to motivate them to perform each repetition maximally and as rapidly as possible.

Equipment. The study was performed on a horizontal bilateral leg extension variable resistance machine (i.e. leg press action in a sitting position) (Technogym, Gambettola, Italy). The sitting was individually adjusted to minimize displacement between the lower back and backrest during muscular force exertion and, therefore, to avoid posture change. Each subject was instructed to fix their feet in the same position on the force platform to ensure a constant foot position. The exercise machine incorporated four force transducers on a foot platform located below the subject's feet. The strain gauges recorded the applied force (N) within an accuracy of 1 Newton at 1000 Hz. The force platform and leg press plate all remained stationary throughout the lift, while the body moved away from the feet. In addition, a rotational encoder (Computer Optical Products Inc, California, USA) was attached to the weight plates to record the position and direction of the displacement within an accuracy of 0.2 mm at 1000 Hz. Customized software was used to calculate power (immediate product of displacement velocity and applied force) and work output (vertical displacement of the weight plates multiplied by applied force) per repetition. After the end of the exercise, results were integrated over 1-ms intervals. The maximum 10-ms integral of applied force and displacement velocity during each repetition is referred to as "peak power output". The average 10-ms integral of applied force and displacement velocity over the total concentric contraction time of each repetition is referred to as "mean power output".

Muscle samples. Muscle biopsies, as described by Bergstrom (Bergstrom, 1975), were taken from the vastus lateralis muscle of the left leg before and immediately after 5 and 10 repetitions of the leg press exercise. The muscle sample was immediately frozen (in 5–10 s) in liquid nitrogen and stored at -80º C for subsequent metabolite assay, after being freed from visible fat and connective tissue.

Analysis. Muscle Phosphocreatine (PCr), creatine (Cr), lactate, glucose 1-phosphate (G-1-P), glucose 6-phosphate (G-6-P), fructose 6-phosphate (F-6-P) and free glucose were

analysed by fluorometic analysis (Lowry & Passonneau, 1971). Skeletal muscle adenine nucleotides and inosine monophosphate (IMP) were analysed by high-performance liquid chromatography (HPLC) using a modified version (Norman *et al.*, 1991) of the method originally described by Dellevold et al. (Sellevold *et al.*, 1986). All muscle metabolite concentrations are expressed as mmol·Kg^{-1} wet muscle.

Calculations. Total average ATP production (mmol·Kg^{-1} wet muscle) from anaerobic sources, assuming a closed system, was estimated as previously described by Chasiotis et al. (Chasiotis *et al.*, 1987), from changes in average metabolite values obtained before and immediately after exercise using the following formula:

$$\text{ATP production} = 1.5 \cdot \Delta[\text{La}] - \Delta[\text{PCr}] + 2\Delta[\text{ATP}] - \Delta[\text{ADP}]$$

where Δ refers to exercise-induced change in each metabolite. The relative contributions to ATP utilization from ATP and PCr degradation were calculated from concentration changes during the period, whereas the amount of ATP re-synthesised through glycolysis was calculated from the lactate formed, assuming 1.5 mmol ATP·$mmol^{-1}$. Although it is recognized that some lactate may have left the muscle during exercise, this calculation ignores lactate released to the blood during exercise and assumes that blood flow in the active muscles is arrested during the ~ 30 s of the exercise (Bogdanis *et al.*, 1995; Cheetham *et al.*, 1986; Medbo & Tabata, 1993) and that the majority of carbohydrate substrate originates from muscle glycogen. This creates a complete anaerobic situation (Hultman & Sjoholm, 1983). It also assumes that decreases in muscle ATP concentration are accounted for by increases in inosine 5'-monophosphate (IMP).

The average anaerobic ATP utilization rate (mmol·Kg^{-1} wet muscle·s^{-1}) was obtained by dividing the average anaerobic ATP production by the total duration of the exercise.

The minimum average anaerobic glycogenolytic and glycolytic rates during each experiment were calculated from accumulation of glycolytic metabolites using the formulas described by Hultman and Sjoholm (Hultman & Sjoholm, 1983), as follows:

$$\text{glycogenolysis} = \Delta\text{G1P} + \Delta\text{G6P} + \Delta\text{F6P} + 0.5\ (\Delta\text{lactate} + \Delta 0.1 \cdot \text{lactate})$$

$$\text{glycolysis} = 0.5\ (\Delta\text{lactate} + \Delta 0.1 \cdot \text{lactate})$$

The values were divided by time to give millimole glycosyl units per kilogram per second (mmol glucosyl · units Kg^{-1} wet muscle·s^{-1}). Increases in intracellular glucose have not been included in the calculation of glycogenolysis because the free glucose in muscle was probably released by the action of the debranching enzyme and not the action of phosphorylase (Gaitanos *et al.*, 1993).

The concentration of inorganic phosphate (P_i) in the muscle after each exercise was calculated from changes in ATP, ADP, PCr and hexose monophosphates (HMP) using the formula (Chasiotis *et al.*, 1987):

$$[P_i] = \text{resting value} - \Delta[PCr] + 2\,\Delta[ATP] - \Delta[ADP] - \Delta[HMP]$$

A resting value of 2.9 mmol·l^{-1} was used (Chasiotis *et al.*, 1987).

Muscle pH was estimated from muscle lactate content by using the Sahlin et al. (Sahlin *et al.*, 1975) equation.

Cellular energy charge, a measure of the extent to which the total adenine nucleotide pool of the cell (ATP, ADP and AMP) is phosphorylated, was estimated by using the following equation (Atkinson, 1977):

$$\text{Energy charge} = ([ATP] + 0.5\,[ADP]) / ([ATP] + [ADP] + [AMP])$$

Blood samples. Capillary blood lactate and ammonia samples were obtained from a hyperemic earlobe. After cleaning and puncturing, a 5-µl sample of whole blood was automatically aspirated into a single use enzyme-coated electrode test strip. Lactate concentration was determined via amperometric measurement and the result displayed in 60 s (Lactate Pro LT-1710; Arkray KDK Corporation, Shiga, Japan). In terms of reliability, the manufacturers report a coefficient of variation (CV) of 3.2% and 2.6% with lactate standards of 2 and 11 mmol·l^{-1}, respectively. The Lactate Pro was checked for accuracy according to manufacturer's instructions before every test. A single 20 µl of whole blood sample was also taken from hyperemic earlobe with an Eppendorf varipipette and immediately analyzed for ammonia concentration with an ammonia checker (BAC) II (model AA-4120, Kyoto Daiichi, Kayaku Co., Ltd. Japan, Menarini Diagnostic, Italy). This analyzer uses a reflectometer to optically measure the reflection

intensity (45º) of reagent colour reaction in biochromatic mode and was calibrated before and after every test with a known control (58.7 µmol·l^{-1}).

Statistical analysis. Standard statistical methods were used for the calculation of mean and standard deviation (SD). Student's paired t-test was used for comparisons of analytic values during the two different experimental conditions in this study, whereas one-way analysis of variance for repeated measures was used to examine the differences in performance indexes and metabolite concentrations over time. When a significant F-value was achieved ($P<0.05$), the means were compared using a LSD post-hoc test. For the purposes of comparison the power output for the second 5 repetitions were compared with the first 5 repetitions. Pearson product-moment correlation test was performed to examine the relationship between variables. Statistical significance was accepted at the $P<0.05$ level.

Results

Power and force production. The peak power output profiles during each repetition in the two experimental conditions (5 or 10 repetitions) are shown in Figure 1. Average peak power production during the first 5 repetitions was similar in both experimental periods. The highest average peak power for a leg extension was recorded within the 2-3 initial repetitions of the exercise and amounted to 897 ± 194 W. The average peak power was well maintained during the first 5 repetitions of the exercise, however after that peak power declined progressively during exercise ($P<0.05$), reaching a value of 64 ± 17% of the highest value after 10 repetitions. Average peak power decreased 19 ± 12% ($P<0.05$) from the first (821 ± 207 W) to the second (667 ± 206 W; Fig. 1) 5 repetitions of the exercise. The magnitude of the decline in average mean power production between the first and the second 5 repetitions was 23 ± 10% (from 349 ± 68 to 268 ± 58 W: $P<0.05$).

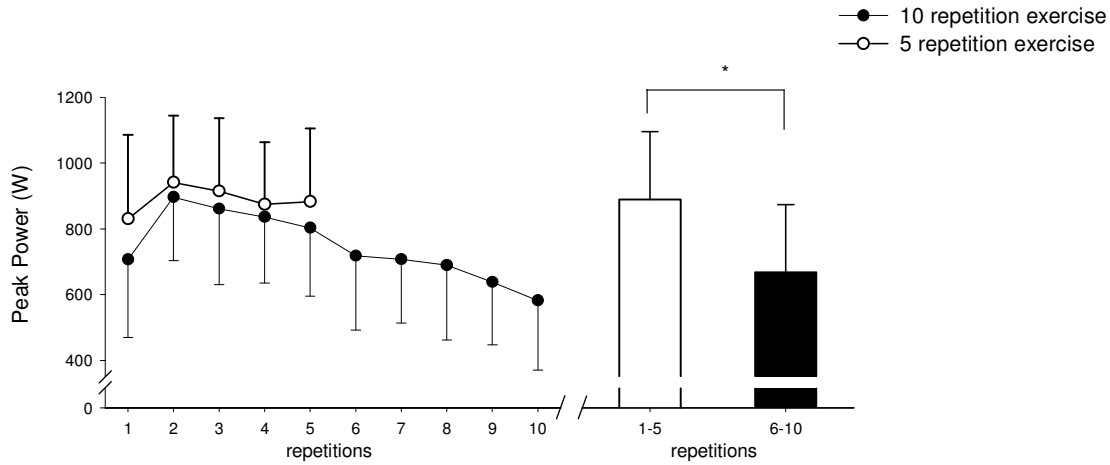

Figure 1. Peak power output profiles (average for n=6 subjects) for each exercise during the two experimental conditions: when exercise was 5 repetitions (open circles) and when exercise was 10 repetitions (filled circles). Boxes represent mean of the peak power output for the first and the second half of a set of 10 repetitions. *significant difference ($P<0.01$) between the first and the second 5 repetitions. Values are means ± SD.

The total duration of exercise was 32.6 ± 4.4 s: i.e. 14.2 ± 2.1 s for the first 5 repetitions and 18.4 ± 4.0 s for the second 5 repetitions ($P < 0.05$). During the first 5 repetitions average contraction time per repetition was 1.59 ± 0.3 s and average relaxation time per repetition was 1.25 ± 0.26 s, whereas during the second 5 repetitions the corresponding contraction and relaxation times were 20-25% longer (1.96 ± 0.35 s and 1.71 ± 0.56 s, respectively) compared with the first 5 (Fig. 2). For each second of

exercise the contraction/relaxation cycle was similar for the first and the second 5 repetitions (~ 0.55). The total amount of work performed was slightly (3%) but significantly less ($P<0.05$) during the second 5 repetitions (433 ± 92 J) than for the first 5 repetitions (453 ± 87 J). This drop was due to a slight decline ($P<0.05$) in the load displacement from 35.5 ± 5.6 cm for the first 5 repetitions to 34.3 ± 6.3 cm for the second 5 repetitions.

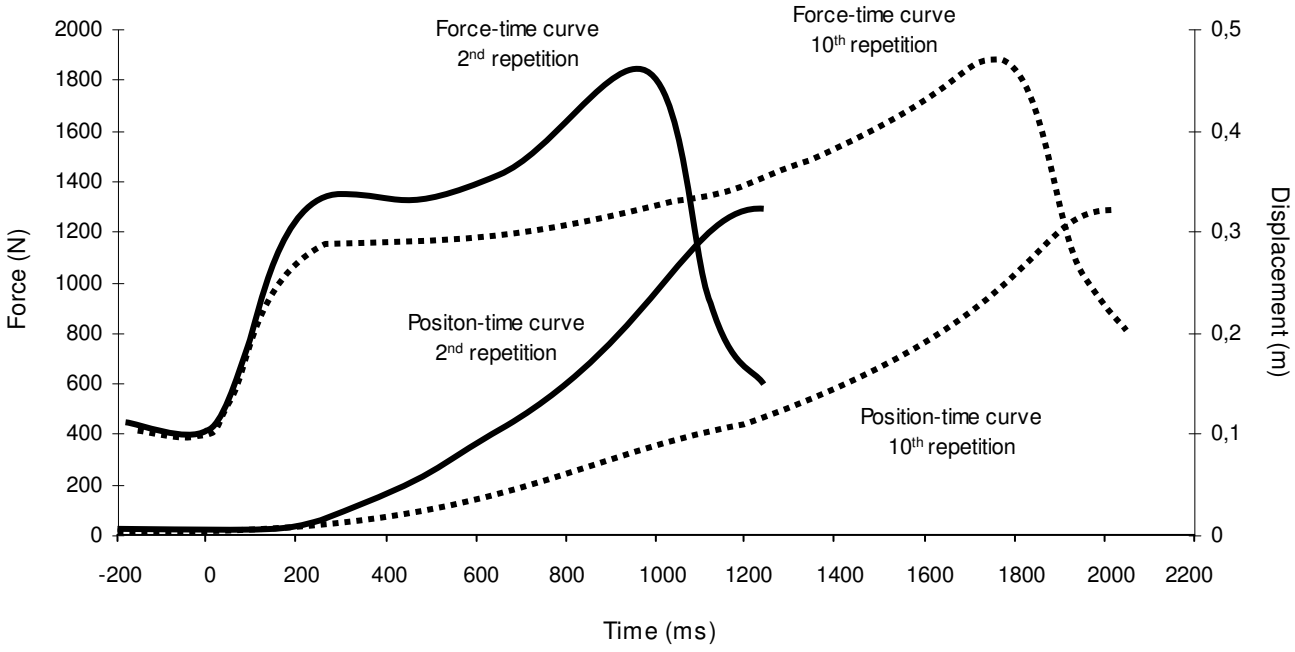

Figure 2. Curves of the position-time of the encoder attached to the weight plates, and applied force-time on the force platform by one representative subject, during the second (solid line) and the tenth (dash line) repetition of a set of 10 repetitions. This profile was similar for all subjects.

Muscle metabolites. Tables 1 to 3 show muscle metabolite concentrations at rest and after exercise. Resting metabolite concentrations, averaged from both experimental days, were within the normal range for human skeletal muscle. Concentrations of ATP, AMP and IMP were unchanged during exercise (Table 1). The ADP concentration increased ($P<0.05$) during the first 5 repetitions, and remained high at the end of exercise. PCr concentration decreased to 58 % and 38% of the resting value after the first and the second 5 repetitions, respectively. Changes in calculated P_i generally followed those of PCr. Compared with rest values the energy charge decreased ($P<0.05$) after 5 and 10 repetitions. The calculated ATP/ADP ratios decreased by 9% ($P<0.05$) after 5 repetitions and remained at this level until the end of the exercise (Table 2). The

ATP/AMP ratio did not change after 5 repetitions but decreased by 19% (P<0.05) at the end of the exercise. No change occurred in either the ADP/AMP and ATP/IMP ratios throughout exercise.

Table 1. Effects of leg press exercise on adenine nucleotides, IMP, PCr, Cr, P_i and energy charge at rest and during exercise.

	Pre exercise	Mid exercise (5 reps)	Post exercise (10 reps)
ATP	6.52 ± 0.38	6.19 ± 0.59	6.42 ± 0.57
ADP	0.85 ± 0.03	0.89 ± 0.08 [a]	0.91 ± 0.10
AMP	0.08 ± 0.04	0.08 ± 0.03	0.09 ± 0.03
IMP	0.01 ± 0.00	0.01 ± 0.00	0.08 ± 0.11
TAN	7.44 ± 0.40	7.15 ± 0.66	7.41 ± 0.67
PCr	20.24 ± 6.31	11.68 ± 7.82	7.74 ± 5.53 [ab]
Cr	8.66 ± 3.92	16.97 ± 6.33	25.45 ± 3.8 [a]
PCr + Cr	28.9 ± 3.94	30.56 ± 6.19	34. 55 ± 6.23
Energy charge	0.933 ± 0.01	0.927 ± 0.01 [a]	0.927 ± 0.004 [a]
P_i	2.9	17.63 ± 15.74	24.8 ± 15.8 [a]

Values are means ± SD in mmol·kg^{-1} wet muscle, except [P_i] (mmol·l^{-1} intracellular water) and energy charge; n=6, except at post exercise, where n=4-5. TAN, total adenine nucleotides (ATP + ADP + AMP); IMP, Inosine 5'-monophosphate; Cr, Creatine. For calculations of P_i and energy charge see METHODS. [a] significant difference (P<0.05) with pre exercise value. [b] significant difference (P<0.05) with middle exercise value.

After the first 5 repetitions there was a 3.5-fold increase in the concentration of muscle lactate (P<0.05), whereas a more marked increase (~ 9 fold) was noticed during the second 5 repetitions (P<0.05) (Table 3). The muscle concentration of F-6-P increased significantly (P<0.05) from rest up to the tenth repetition. In contrast, the muscle concentrations of free glucose, G-1-P and G-6-P did not increase above resting values during exercise. By using the Sahlin et al. (1975) equation to relate changes in muscle lactate concentration to changes in average muscle pH it can be calculated that in the present study the first 5 repetitions would have resulted in a fall (P<0.05) in intramuscular pH from 7.02 ± 0.02 (rest) to 6.93 ± 0.05 and a further decrease (P<0.05) to 6.79 ± 0.14 after the second 5 repetitions.

Table 2. Effects of leg press exercise on nucleotide metabolite ratios at rest and during exercise.

	Pre exercise	Middle exercise (5 reps)	Post exercise (10 reps)
ATP/ADP	7,7 ± 0,3	7,0 ± 0,3 [a]	7,1 ± 0,2 [a]
ATP/AMP	95,9 ± 27,2	88,4 ± 24,8	77,8 ± 19,7 [a b]
ADP/AMP	12,4 ± 3,4	12,6 ± 3,3	10,9 ± 2,7
ATP/IMP	1051.3 ± 50.5	1055.3 ± 23.2	648.4 ± 553.8

Values are means ± SD. [a] significant difference (P<0.05) with pre exercise value. [b] significant difference (P<0.05) with middle exercise value.

During exercise the energy was mainly derived from utilization of high-energy phosphates and glycolysis. Table 4 shows the summary of measurements and estimates of ATP production from anaerobic sources during exercise made from the average values of the Tables 1 and 2. Assuming a closed system, the minimum anaerobic ATP production over the whole exercise (10 repetitions) calculated from changes in muscle lactate, phosphocreatine, and ATP concentrations was 35.7 mmol·Kg^{-1} wet muscle while glycogenolysis accounted for 64% and phosphagens contributed to 36% of the anaerobic ATP produced. When the whole exercise was divided into two parts, the minimum anaerobic ATP production was 9% higher during the second 5 repetitions (18.6 mmol·Kg^{-1} wet muscle) than during the first 5 (17.1 mmol·Kg^{-1} wet muscle). The fraction of ATP resynthesis derived from phosphagens decreased from the first 5 repetitions (54%) to the second 5 (19%) whereas the fraction of ATP production derived from glycogenolysis increased from the first 5 repetitions (46%) to the second 5 (81%).

The previous estimate does not take into account the amount of lactate released from the muscle during the exercise. However, some lactate efflux from the active muscle occurred during each bout of exercise, since blood lactate concentrations immediately after the end of the fifth (2.4 ± 0.6 mmol·l^{-1}) and the tenth (4.5 ± 1.0 mmol·l^{-1}) repetition were elevated compared with resting values (1.0 ± 0.1 mmol·l^{-1}). Assuming that: 1) lactate was uniformly distributed in total extracellular water space (22.4 l: about 30% of the whole body mass) (Karlsson & Saltin, 1970), 2) no lactate removal occurred from this pool during exercise, 3) the biopsy represents the entire mass of all the muscle groups involved during exercise (Karlsson & Saltin, 1970), and 4) 10 Kg of muscle are involved in leg press exercise (Essen *et al.*, 1977), it may be

calculated that a lactate concentration of approximately 3.02 mmol·Kg^{-1} wet muscle (22.4 · (2.4-1.0) · 10^{-1}) and 4.66 mmol·Kg^{-1} wet muscle (22.4 · (4.5-2.4) ·10^{-1}) escaped the muscle during the first and last 5 repetitions, respectively. Therefore, a lactate concentration of 7.68 mmol·Kg^{-1} wet muscle (3.02 + 4.66) escaped the muscle during the entire 10 repetitions. Since average muscle lactate concentration increased by 5.2 (first 5 repetitions) and 10.1 mmol·Kg^{-1} wet muscle (second 5 repetitions) during these periods, lactate efflux represented approximately 37% (first 5 repetitions), 32% (second 5 repetitions) and 33% (whole exercise) of the total lactate produced. These estimates of lactate released from muscle during the present exercise (~ 30% of total lactate production) are consistent with those measured during one-legged, dynamic knee-extensor exercise (Bangsbo *et al.*, 1990) and during 80-s electrical stimulations of the quadriceps muscle (Hultman & Spriet, 1986). Assuming that 1.5 mol of ATP are produced for every mol of lactate accumulated, the addition of the above estimate of lactate escaped from the muscle should give a total estimated anaerobic ATP production of 21.6 mmol·Kg^{-1} wet muscle during the first 5 repetitions, 19% higher anaerobic ATP production during the last 5 repetitions (25.6 mmol·Kg^{-1} wet muscle), and a total production of 47.2 mmol·Kg^{-1} wet muscle during the entire exercise (Table 4). In this case phosphagens contributed to 46%, 15% and 29% of the total estimated anaerobic ATP produced during the first 5, second 5 and 10 repetitions respectively, while the corresponding contribution from glycogenolysis accounted for 54%, 85% and 71% respectively.

Table 3. Effects of leg press exercise on glycolytic intermediates at rest and during exercise.

	Pre exercise	Middle exercise (5 reps)	Post exercise (10 reps)
Glucose	0.47 ± 0.17	0.52 ± 0.38	0.37 ± 0.27
G-1-P	0.01 ± 0.01	0.03 ± 0.03	0.06 ± 0.05
G-6-P	0.07 ± 0.05	0.62 ± 0.74	1.43 ± 1.05
F-6-P	0.02 ± 0.01	0.08 ± 0.07	0.09 ± 0.05 [a]
Lactate	1.86 ± 0.95	7.1 ± 2.54 [a]	17.2 ± 3.5 [ab]
pH	7.02 ± 0.02	6.93 ± 0.05 [a]	6.79 ± 0.14 [ab]

Values are means ± SD in mmol·kg^{-1} wet muscle; n=6, except at post exercise, where n=4-5. G-1-P, Glucose 1-Phosphate; G-6-P, Glucose 6-phosphate; F-6-P, Fructose 6-phosphate. For calculations of pH, see METHODS.

The mean rate of ATP utilization decreased during exercise because during the second 5 repetitions (1.40 mmol·kg^{-1}·s^{-1}) the values were 8% lower than during the first 5 repetitions (1.53 mmol·kg^{-1}·s^{-1}) (Table 5). The ATP utilization per repetition in relation to units of mean power (µmol·watt^{-1}·rep^{-1}) and units of work (µmol·J^{-1}·rep^{-1}), which is a measure of the energy cost is also reported in Table 5. The ATP utilization per repetition per unit of power was 35% higher during the second 5 repetitions than during the first 5. The ATP utilization per repetition per unit of work was 21% higher in the second 5 repetitions than in the first 5.

Table 4. Summary of measurements and estimations of ATP production from anaerobic metabolism.

	1 to 5 rep	6 to 10 rep	1 to 10 rep
	Assuming a closed system		
Phosphagens	9.2	3.5	12.6
Glycogenolysis	7.9	15.1	23.0
ATP Turnover	17.1	18.6	35.7
	Assuming an open system		
Estimated lactate release	4.5	7.1	11.6
ATP turnover	21.6	25.6	47.2

Mean ATP turnover estimations in mmol·kg^{-1} wet wt. Calculation for released lactate data is derived from product of measured increase in blood lactate and the estimated magnitude of extracellular water space (22.38 l; (Karlsson & Saltin, 1970). Active mass (10 kg) was calculated using data of Essen et al. (1977).

The minimum anaerobic glycogenolytic and glycolytic rates during leg press exercise were estimated. The results of these calculations show that during the first 5 repetitions the glycogenolytic rate exceeded the glycolytic rate by 25%. The glycogenolytic rate of 0.25 mmol·Kg^{-1} wet wt·s^{-1} calculated during the first 5 repetitions increased to 0.35 mmol·Kg^{-1} wet wt·s^{-1} during the second 5, however this 40% increase was less than the 50% increase in glycolytic rate (from 0.20 mmol·Kg^{-1} wet wt·s^{-1} to 0.30 mmol·Kg^{-1} wet wt·s^{-1}). During the second 5 repetitions, however, the glycogenolytic rate still exceeded the glycolytic rate by 17%.

Blood metabolite. Blood lactate increased ($P < 0.01$) from 1.0 ± 0.1 mmol·l^{-1} at rest to 2.4 ± 0.6 mmol·l^{-1} and 4.4 ± 1.0 mmol·l^{-1} after 5 repetitions and 10 repetitions, respectively. Average blood ammonia concentrations were not different from resting levels (19.6 ± 6.6 µmol·l^{-1}) immediately after 5 (21.4 ± 7.4 µmol·l^{-1}) and 10 repetitions (28.3 ± 12.6 µmol·l^{-1}).

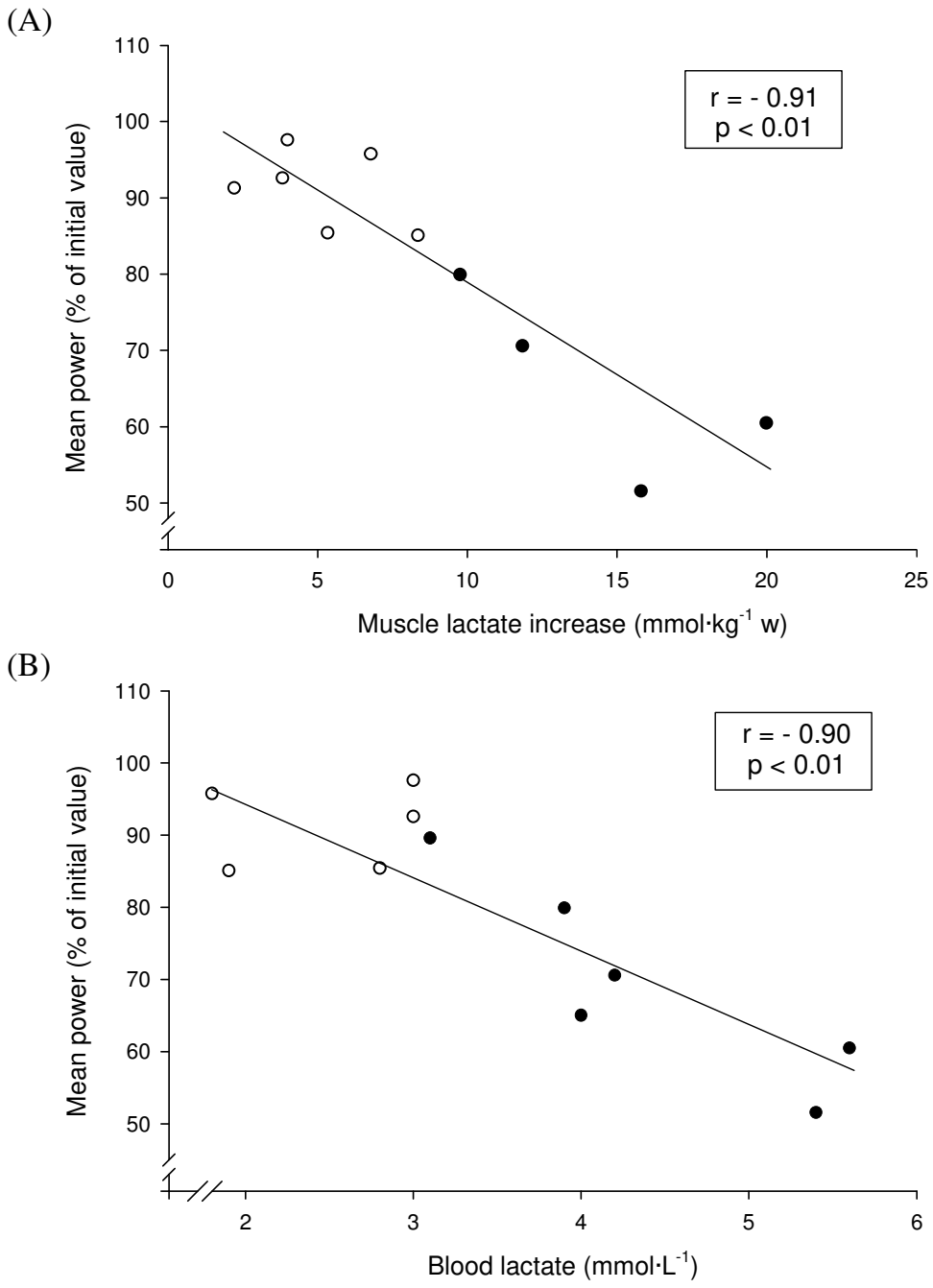

Figure 3. Individual relationships between the relative mean power output changes (expressed in percent of initial value) between the first and the last two repetitions of both experimental periods, and the muscle lactate concentration increases (3A) as well as with the final blood lactate concentration values (3B), during a set of 5 (open circles) and a set of 10 (filled circles) repetitions.

Correlations. There was a negative correlation between individual contraction-induced relative changes (expressed as percent of initial values) in mean power output between the first (initial values) and the last (final values) two repetitions in both experimental periods, and individual changes in muscle ($r = - 0.91$, $P<0.01$) (Fig. 3A) as well as blood ($r = -0.90$, $P<0.01$) (Fig. 3B) lactate concentrations. Similarly, inverse relationships ($r = - 0.75$, $P<0.01$) were observed between the individual changes in PCr and the individual relative changes in the average duration of the concentric phase of leg press actions, expressed in percent of initial values (Fig. 4). A positive correlation ($r = 0.89$, $P<0.01$) was found between individual changes in muscle and blood lactate concentrations.

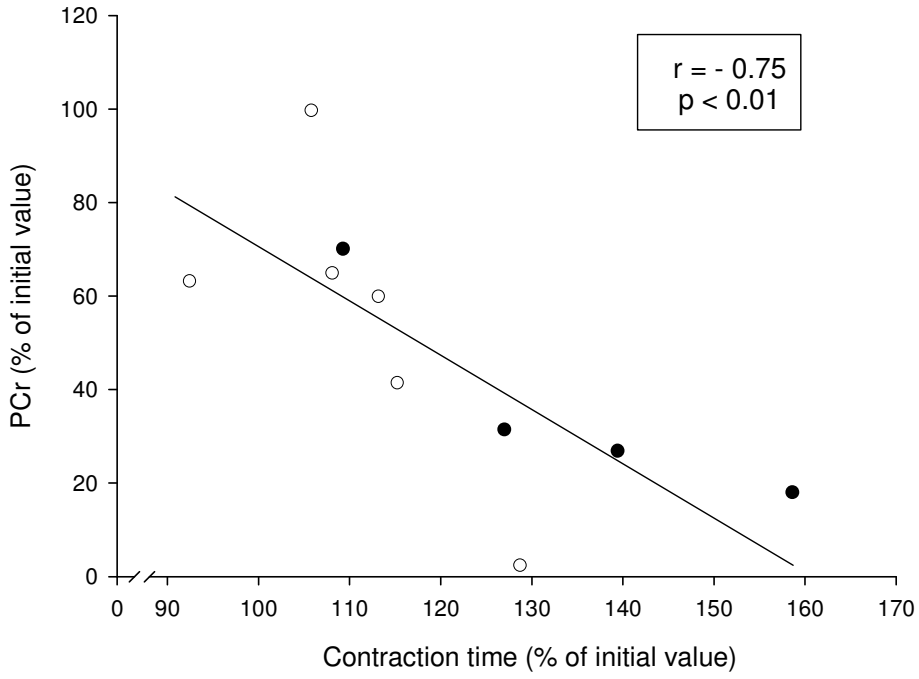

Figure 4. Individual relationship between PCr changes (expressed in percent of initial value) and the changes in the relative average duration of the concentric phase of leg press exercise (expressed in percent of initial value) during a set of 5 (open circles) and a set of 10 (filled circles) repetitions.

Discussion

The primary aims of the present study were to determine the changes in muscle metabolite concentration and power output production during the first and the second 5 repetitions of a 10 repetition to failure of bilateral leg press exercise, and secondly to relate these changes to decreases in power. The main findings were: 1) the major reduction in PCr concentration mainly occurred within the first 5 repetitions of exercise, whereas muscle lactate accumulation was more substantial during the second 5 repetitions, and 2) a significant drop in power output was observed from the first to the second 5 repetitions, correlating with the reduction in phosphagens and the accumulation of muscle lactate, and accompanied by a parallel decrease in muscle energy charge as well as increases in ADP content. The present data shows that during the first 5 repetitions energy is primarily derived from PCr with a smaller contribution of glycolysis, while during the second 5 repetitions glycolysis was the major energy source. Finally, our study shows that the rate of ATP turnover per work unit increases as the exercise progresses, implying that mechanical efficiency is reduced during the second half of the exercise.

Muscle metabolites. The bilateral leg press exercise produced similar fatigue profile and changes in muscle PCr, lactate and glycolytic intermediates over ~ 35 seconds as that reported after 30 second cycling sprints (Boobis, 1987; Jones *et al.*, 1985; Medbo & Tabata, 1993; Parolin *et al.*, 1999), maximal running sprints on a non-motorised treadmill (Boobis, 1987; Cheetham *et al.*, 1986) or during isometric, isokinetic (Jones *et al.*, 1985) or electrical stimulation (Bergstrom & Hultman, 1988; Hultman & Sjoholm, 1983) muscle contractions. The calculated ATP production from glycolysis (64%) and from phosphagens (36%) during the whole leg press exercise agrees with the values of 60 to 70% (glycolysis) and from 30 to 40% (phosphagens) reported in maximal sprint runs in a non-motorised treadmill (Cheetham *et al.*, 1986), maximal cycling (Bogdanis *et al.*, 1995; Boobis, 1987) or isometric contraction (Hultman & Greenhaff, 1991) exercises lasting around 30 s, assuming a metabolically closed compartment. Our finding of the increase in anaerobic glycolysis between the second and the first 5 repetitions agrees with the increased anaerobic glycolysis observed by Hultman and Sjoholm (Hultman & Sjoholm, 1983) during the final part of 30 s of electrical stimulation of knee extension with occluded circulation. However, other authors have found that during the last part of a 20-30 s all-out sprint cycling exercise (Bogdanis *et*

al., 1998; Boobis, 1987; Jones *et al.*, 1985; Parolin *et al.*, 1999) or one-legged knee-extensor exercise at a constant work rate (Bangsbo *et al.*, 2001), there is a decrease in anaerobic glycolysis compared with the initial part of the exercise. This difference is likely due to differences in exercise mode (cycling, running, electrical stimulation), type of contraction (isometric-isotonic, intermittent-sustained, constant-changing work rate), frequency, intensity, duration of muscle contraction, type of muscle and species, and different amounts of lactate efflux from the active muscles.

At the end of the first 5 repetitions of leg press exercise a significant drop in power output was observed. The decrease in power output was accompanied by a parallel decrease in the concentration of PCr and energy charge as well as by an increase in ADP content in muscle whereas muscle AMP remained unchanged. Increases in the estimated muscle-free ADP content, without any changes in estimated free AMP, have been observed during 15 s sprint isokinetic cycling exercise (Parolin *et al.*, 1999). The decrease in energy charge observed after the first 5 repetitions has been found after maximal exercise (Sahlin *et al.*, 1978), indicates a diminished capacity to do work and seems to fill the purpose of energy conservation (Atkinson, 1977).

No significant changes in ATP and IMP muscle concentrations were observed during exercise despite the fact that at the end of the exercise the demand for ATP was at its highest. The finding of a lack of IMP accumulation or ATP decrease during exercise in the present study is consistent with other studies showing that no changes in muscle IMP, ATP or NH_3 occur until PCr levels have significantly decreased to values below ~7 $mmol \cdot Kg^{-1}$ wet wt (Sahlin *et al.*, 1975; Spriet *et al.*, 1987), muscle lactate reaches 12-18 $mmol \cdot Kg^{-1}$ wet wt (Dudley & Terjung, 1985) and muscle pH values have fallen below 6.6 (Dudley & Terjung, 1985). The absence of changes in ATP and IMP concentrations in parallel with a decrease in power production indicates that fatigue occurred well before the metabolic conditions necessary to elicit major metabolic stress and a higher rate of AMP deamination could be established (Tullson *et al.*, 1995).

The estimated rates of glycogenolysis and glycolysis were based on increases of HMP and lactate. The finding of a significant increase of F-6-P during exercise suggests that the rate of glycogenolysis was higher than the rate of glycolysis. A higher rate of glycogenolysis compared to the glycolysis rate is in agreement with studies on maximal cycling (Boobis, 1987; Jones *et al.*, 1985) and isometric (Hultman & Sjoholm, 1983) exercises lasting 30 s (Hultman & Sjoholm, 1983). Furthermore, the rate of both glycogenolysis (40%) and glycolysis (50%) increased throughout the exercise in

connection with decreases in PCr concentration and ATP/ADP ratio as well as with increases in ADP concentrations. The changes in glycolytic intermediates were consistent with rate-limiting steps in the phosphofructokinase, pyruvate dehydrogenase (Jones *et al.*, 1985) and glycogen phosphorylase (Chasiotis *et al.*, 1983) reactions. In this hypothesis, control of the regulatory glycolytic enzymes in this manner would prevent a continued high rate of muscle glycogen utilization and accumulation of H^+ (Hultman & Spriet, 1986). However, conclusions about the rate-limiting steps in these pathways, made on the basis of changes in concentrations of intermediates, should be considered to be tentative rather than final.

Anaerobic ATP production. In agreement with previous studies that have used cycling, treadmill running or one-legged knee-extensor exercise at a constant work rate (Bangsbo *et al.*, 2001; Bogdanis *et al.*, 1995; Bogdanis *et al.*, 1998; Chasiotis *et al.*, 1987; Cheetham *et al.*, 1986; Gaitanos *et al.*, 1993; Gonzalez-Alonso *et al.*, 2000; Jones *et al.*, 1985; Krustrup *et al.*, 2003; Medbo & Tabata, 1993; Spriet *et al.*, 1987), the anaerobic energy production was estimated from muscle biopsies before and after exercise on the basis of the decrease in muscle ATP and PCr, as well as the accumulation of metabolites such a lactate. Limitations in these estimations, however, exist because it is difficult from measurements on muscle biopsy material to determine the anaerobic energy turnover during whole body exercise such as cycling, treadmill running or knee extension exercise, because the mass and the activity of the muscles involved are unknown (Bangsbo *et al.*, 2001). Furthermore, the metabolic response of the biopsied muscle may not be representative of all of the muscles included in the exercise and the muscle sample may not properly interpret recruitment patterns (Bangsbo *et al.*, 2001). Another problem is that the release of metabolites into the blood from the exercising muscles is frequently not taken into account when energy turnover is calculated, although this may represent a substantial contribution to the total energy production when the exercise lasts more than a few seconds (Bangsbo *et al.*, 2001).

Taking into account the above limitations in the estimations of the anaerobic energy production, when the muscle was regarded as a metabolically closed compartment which does not exchange metabolites with its surroundings, the calculated total anaerobic ATP production was 9% greater during the second 5 repetitions compared with the first 5. However, since the exercise was dynamic and the circulation was not restricted, some lactate diffused into the circulation during the exercise. When

the amount of lactate released from the muscle to the blood during exercise was estimated from the changes in average blood lactate (see RESULTS section) the difference in the total anaerobic energy release between the second and the first 5 repetitions increased from 9% (closed system) to 19% (adding the lactate released from the muscles). In the above calculations of ATP production during contraction the contribution from oxidative metabolism was not included. Although the aerobic metabolism was not actually measured in this study, Dudley et al. (Dudley *et al.*, 1991) have shown that during one set of 10 repetitions leading to failure of bilateral leg press exercise, pulmonary oxygen uptake increased throughout the exercise, reaching 100% higher levels during the second 5 repetitions than the first (~50% versus ~25% of maximal aerobic power reached during treadmill running). Moreover, it has been shown that during unilateral knee extensions oxygen utilization of the contracting muscles is much more rapid than suggested previously and muscular and pulmonary oxygen uptake kinetics are functionally identical (Bangsbo *et al.*, 1990; Krustrup *et al.*, 2009). Taken together, these findings suggest that a significant amount of ATP production during leg press exercise could come from aerobic metabolism and that aerobic ATP production was substantially greater during the second 5 repetitions than the first. The higher aerobic contribution during the second 5 repetitions would make the observed difference in ATP production between the second and the first repetitions even higher than the difference observed when only the anaerobic contribution is considered. Since the total amount of external work sustained by the quadriceps was practically the same for the first and the second 5 repetitions, the significantly higher energy cost per unit of work during the second 5 repetitions implies a reduction in mechanical efficiency, expressed as work carried out per ATP production, in the final part of exercise. This finding is in agreement with previous studies using leg extension model (Bangsbo *et al.*, 2001; Gonzalez-Alonso *et al.*, 2000).

Why does mechanical efficiency decrease in the final part of exercise? Our results could indicate that the efficiency of converting chemical energy into mechanical power is high in the transition from rest to leg press exercise, and then gradually declines in proportion to the source of ATP production. This is in contrast, however, to static electrical stimulation (Bergstrom & Hultman, 1988; Edwards *et al.*, 1975; Spriet *et al.*, 1987), one-legged isometric knee-extensor exercise (Sahlin & Ren, 1989), or sprint cycling exercise (Bogdanis *et al.*, 1998), in which decreases in the energy cost per force

or per unit of work or power produced have been observed when contractions are extended over time.

Several reasons may explain the reduction in mechanical efficiency observed during the second 5 repetitions of leg press exercise. Firstly, the increased proportion of energy derived from glycolysis and oxidative phosphorylation estimated during the second 5 repetitions would increase the heat associated with ATP hydrolysis (Edwards *et al.*, 1975; Gonzalez-Alonso *et al.*, 2000; Krustrup *et al.*, 2003) and, therefore, would decrease the efficiency of conversion of chemical energy into mechanical power. This increase in energy derived from anaerobic glycolysis found during leg press exercise is opposite to the decrease in anaerobic glycolysis observed in the studies in which mechanical efficiency increased throughout exercise (Bergstrom & Hultman, 1988; Bogdanis *et al.*, 1998; Edwards *et al.*, 1975; Sahlin & Ren, 1989; Spriet *et al.*, 1987). Secondly, the decrease in the ATP/ADP ratio observed after the first 5 repetitions can reduce the amount of energy released per mol of ATP hydrolysed, and may limit the rate of energy-requiring processes or even block energy-requiring reactions, forcing more ATP to be hydrolyzed for a given amount of work (Sahlin *et al.*, 1978). Thirdly, it has been shown in mammalian muscle that during isotonic contractions at high speeds of muscle contractions, slow-twitch muscles are less efficient in doing external work than fast muscles (Awan & Goldspink, 1970). Therefore, selective fatigue of fast-twitch fibers and progressively greater recruitment of slow-twitch fibers throughout exercise might explain the higher energy cost per unit of work observed during the final part of leg press exercise. And fourthly, the progressive increase in biceps femoris activity that has been observed during a set of 10 repetitions leading to failure of leg press exercise (Gonzalez-Izal *et al.*, 2010) may detract from the force produced by the quadriceps and decrease mechanical efficiency when approaching exhaustion.

What causes fatigue during leg press exercise. The cause of muscle fatigue during maximal intense exercise remains uncertain, although many biochemical and electrophysiological changes that accompany fatigue have been described. In the present study the fall in power production during exercise was strongly correlated to the fall in phosphagens and the increase in muscle lactate. Associations between decline in muscle tension and proportional changes in muscle phosphagens, as well as changes in muscle lactate or pH have been observed in many studies during repeated isometric contractions in frog stimulated muscle using ^{31}P NMR (Dawson *et al.*, 1978) and during

maximal short-term dynamic exercise in humans (Karlsson & Saltin, 1971). Thus, it seems reasonable to suggest that some biochemical changes, such as a decrease in the contribution of PCr (Bogdanis *et al.*, 1995), an abrupt increase in muscle lactate and by-products of ATP hydrolysis (H^+, P_i, ADP) and the inability of the glyconeogenolytic rate to compensate for the fall in ATP production when the PCr store is depleted (Bogdanis *et al.*, 1995; Hultman & Spriet, 1986; Hultman & Greenhaff, 1991) contributed to fatigue during leg press exercise.

In agreement with our results, decreases in the ATP production rate over time have been observed during isometric contractions (Hultman & Greenhaff, 1991) lasting around 30 s and during the second half of a 30-s maximal sprint-cycling exercise (Parolin *et al.*, 1999). The calculated anaerobic ATP turnover rate of 1.09 $mmol \cdot Kg^{-1}$ wet $muscle \cdot s^{-1}$ observed during this whole exercise (10 repetitions), corresponds well with earlier estimates for human muscle where values ranging from 0.5 to 2.0 $mmol \cdot Kg^{-1}$ wet $wt \cdot s^{-1}$ were reported in cycling (Bogdanis *et al.*, 1995; Boobis, 1987; Jones *et al.*, 1985; Medbo & Tabata, 1993; Parolin *et al.*, 1999) and maximal running sprints (Cheetham *et al.*, 1986) or during isokinetic or electrical stimulation (Hultman & Sjoholm, 1983; Ren *et al.*, 1988) exercises lasting 10-30 s. These values of anaerobic ATP turnover rate are, however, lower than the highest rates for PCr and glycolytic ATP provision reported during all-out 6 s sprints (Gaitanos *et al.*, 1993). The decrease in the energy rate of utilization as the exercise progresses has been related to a decline in the rate of ATP production as a result of PCr hydrolysis (Hultman & Greenhaff, 1991), the accumulation of ADP slowing down cross-bridge ATP utilization (Karatzaferi *et al.*, 2003), phosphorylation of the so-called regulatory light chain of myosin, regulation by intracellular acidosis, accumulation of inorganic phosphate (P_i) or lowered ATP/ADP ratios (Kushmerick, 1985; Westerblad *et al.*, 1998). Our results are consistent with the concept that the decrease in power output during leg press exercise is caused by the reduction in the capacity to generate ATP at a high rate from anaerobic sources (Edwards *et al.*, 1972).

In summary, this data shows that during the first 5 repetitions of a set of 10 repetitions leading to failure of bilateral leg press exercise ATP is resynthesized predominantly from PCr with a smaller contribution of glycolysis, while during the second 5 repetitions this pattern is inverted, i.e. the anaerobic ATP resynthesis is produced mostly by glycolysis. Over the repetitions power output is reduced and the work is produced by slowing the speed of muscle contraction. This reduces the energy

demand but not enough to balance ATP production and utilization, due to the impairment of mechanical efficiency with fatigue. The mismatch between ATP production and demand, reflected by the reduction in the ATP/ADP ratio, combined with the accumulation of by-products of the energy metabolism may lead to a final task failure. It remains to be explained why mechanical efficiency is reduced during leg press fatiguing exercise.

Application statement. Although the leg press exercise is one of the most common exercises performed by trained athletes or knee injured people, there is a significant gap in the body of knowledge pertaining to the metabolic and power output changes during bilateral leg press exercise, as no measurements have been made, and it is inappropriate to suppose that it will be the same as that found with other muscle contraction models. This study reports on the acute muscle metabolic response to a single set of this exercise. The results provide the first evidence that performing 5 or 10 repetitions of leg press exercise with the maximal load possible to achieve 10 repetitions (10RM) gives two main different types of exercise in terms of energy status and muscle metabolism: 1) the first 5 repetitions (duration ~ 14 s) result in modest increases in blood (~ 1.4 $mmol \bullet l^{-1}$) and muscle (~ 5 $mmol \bullet Kg^{-1}$ wet muscle) lactate and in high decreases of the muscle PC (~ 42%) content while muscle generating capacity are maintained, and 2) compared with the first, the second 5 repetitions (duration ~ 18 s) are characterized by markedly higher increases in blood (~ 2.0 $mmol \bullet l^{-1}$) and muscle (~ 10 $mmol \bullet Kg^{-1}$ wet muscle) lactate that may reach average values up to 17 $mmol \cdot l^{-1}$ at the end, and by markedly lower decreases in muscle PC (~ 19%) content leading to a significant decrease in muscle generating capacity and mechanical efficiency. Compared to the final part of leg press exercise, the lower lactate accumulation and the maintenance of muscle generating capacity during the initial part of exercise are opposite to those reported during 20-30 s all-out sprint cycling exercise or one-legged knee-extensor exercise at a constant work rate. This type of fatigue may relate to decreased contribution of PCr and/or abrupt increases in muscle lactate or by-products of ATP hydrolysis. The strong correlations found in the present study between blood and muscle lactate, as well as between changes in power output and changes in muscle lactate, indicate that blood lactate concentration may give valuable information about the changes taking place in muscle lactate and in power output during a set of leg press

exercise. The present description of the metabolic response to a single set of bilateral leg press exercise is of importance in the search for a better understanding of the factors that limit performance in this type of exercise and would help to compose a scientifically-based and well-balanced training program that improves knee extension performance in trained athletes or knee injured people.

Author Contributions

Conceived and designed the experiments: EG YH JALC MGI JI MI. Performed the experiments: EG RC JALC CG JI MI. Analyzed the data: EG INA RC YH JALC MG CG MGI JI MI. Contributed reagents/ materials/analysis tools: EG INA RC YH JALC MG CG MGI JI MI.

Wrote the paper: EG INA RC YH JALC MG CG MGI JI MI.

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CHAPTER 3

Energy metabolism during repeated sets of leg press exercise leading to failure or not

2012

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Abstract

This investigation examined the influence of the number of repetitions per set on power output and muscle metabolism during leg press exercise. Six trained men (age 34 ± 6 yr) randomly performed either 5 sets of 10 repetitions (10REP), or 10 sets of 5 repetitions (5REP) of bilateral leg press exercise, with the same initial load and rest intervals between sets. Muscle biopsies (vastus lateralis) were taken before the first set, and after the first and the final sets. Compared with 5REP, 10REP resulted in a markedly greater decrease ($P<0.05$) of the power output, muscle PCr and ATP content, and markedly higher ($P<0.05$) levels of muscle lactate and IMP. Significant correlations ($P<0.01$) were observed between changes in muscle PCr and muscle lactate ($R^2=0.46$), between changes in muscle PCr and IMP ($R^2=0.44$) as well as between changes in power output and changes in muscle ATP ($R^2=0.59$) and lactate ($R^2=0.64$) levels. Reducing the number of repetitions per set by 50% causes a lower disruption to the energy balance in the muscle. The correlations suggest that the changes in PCr and muscle lactate mainly occur simultaneously during exercise, whereas IMP only accumulates when PCr levels are low. The decrease in ATP stores may contribute to fatigue.

Introduction

Regular resistance exercise is an essential component of effective intervention programs designed to improve strength in athletes and adults with chronic diseases and disabilities (Colberg *et al.*, 2010; Kraemer *et al.*, 2002). The response to resistance training program depends ultimately on pronounced metabolic and morphological adaptations of multiple cellular functions which depend to great extent on changes of a complex signaling network that is involved during each training session in response to contractile activity (Hancock *et al.*, 2006b; Winder & Hardie, 1999).

Whilst changes in muscle metabolites and power output during exhausting and non exhausting heavy intermittent cycling (McCartney *et al.*, 1986; Saltin & Essén, 1971), running (Cheetham *et al.*, 1986) or isometric knee extension (Edwards *et al.*, 1972) exercises are well characterized, little is known on substrate utilization and metabolic demand during consecutive sets of exhausting compared with non exhausting high-intensity dynamic resistance exercise. The adaptive response to strength training may be different when training leads to failure is compared to when it does not lead to failure, as different degrees of fatigue and muscle metabolite accumulation are elicited by the training (Spriet *et al.*, 1989). The purpose of this study was, therefore, to investigate the influence of the number of repetitions per set (leading to failure or not) on changes in muscle and blood metabolites and power output during high-intensity bilateral leg press exercise performed with the same initial load (~ 83% 1RM) in young trained men, while simultaneously examining the power output and fatigue developed throughout the exercise. These two exercise sessions are traditionally used for reaching specific training goals. Thus, the "leading to failure" exercise is characterized by a progressively decrease in load and power throughout the repeated sets and is primarily used for increasing muscular strength and hypertrophy (Burd *et al.*, 2012; Kraemer *et al.*, 1987). The "not leading to failure" exercise is characterized by the maintenance of load and average power throughout the sets and is used primarily for optimizing muscle power development (Izquierdo *et al.*, 2006b). To the author's knowledge, no study has analyzed the changes in muscle and blood metabolites during high-intensity dynamic exercise characterized by the maintenance of power output throughout the sets. Analyzing different conditions related to changes in power output may provide some clues to the understanding of the mechanisms by which the process of muscle contraction try to maintain an adequate function during dynamic resistance exercise (Sahlin & Broberg, 1990). A second purpose of the study was to examine the

relationship between the metabolic status of muscle and changes in power output. This kind of examination may enhance the understanding of factors that limit fatigue during leg press exercise, and thus give an indication of the regulation of the metabolic pathways and of how the anaerobic mechanisms are interrelated during dynamic resistance exercise in man.

Material and Methods

Subjects. Six healthy male volunteers participated in the study. Their mean (± SD) age, height, body mass, body mass index, estimated maximal oxygen uptake (VO_2max) in cycle ergometer and maximal strength (1RM) during bilateral leg press exercise were 34 ± 6 years, 179 ± 5 cm, 74.5 ± 7.2 kg, 23.3 ± 1.7 $Kg \cdot m^{-2}$, 57.1 ± 4.9 $ml \cdot kg^{-1} \cdot min^{-1}$ and 199 ± 43 kg, respectively. All were trained athletes, mainly in endurance events, but none trained for competition. The mean percentage of slow twitch (ST) fibers for these subjects was 65 ± 12%. The subjects were thoroughly informed of the purpose, nature, practical details and possible risks associated with the experiment, as well as the right to terminate participation at will, before they gave their voluntary written informed consent to participate. A medical examination was also completed by a physician. The present study is part of a project that has examined the metabolic, neural and training effects of leg press exercise and has been approved by the Institutional Review Committee of the Instituto Navarro del Deporte, according to the Declaration of Helsinki.

Experimental Protocol and Design. This study was designed to examine the influence of the number of repetitions per set (leading vs not leading to repetition failure) in changes in muscle metabolites and power output during high-intensity bilateral leg press exercise. To eliminate any possible effect of confounding factors, several variables such as initial load and total number of repetitions were controlled by equating their values between both exercise sessions. Each subject participated in two experiments on separate days, on which they performed 50 repetitions with the same initial load. This initial load (i.e., 154 ± 31 Kg or 83% 1RM, the heaviest load that could be correctly pressed only once using the correct technique) was the greatest weight which it was possible to complete 10 repetitions to failure (10RM) of leg press exercise. Failure was indicated by the inability to complete the next repetition. On one experimental day ("leading to failure protocol") the subjects performed 5 sets of 10 repetitions to failure (10REP), separated by 2 min of rest between each set. On this experimental day the subjects were able to finish all 10 repetitions at the initial load during the first set. Sometimes, however, the subjects could not lift the initial load during the following sets due to fatigue. Whenever subjects could not lift the load it was decreased by 15 Kg, thus allowing them to complete the experiment (50 repetitions). On the other experimental day ("not leading to failure protocol"), the subjects performed 10 sets of 5 repetitions

not to failure (5REP) with the same initial load as that of (10REP), separated by 2 min of rest between each set. Five repetitions per set were chosen during this non failure experimental day because this is the maximal number of repetitions at which maximal power production can be maintained or slightly (10-15%) decreased during a set of 10 repetitions to failure in leg extension exercise (Izquierdo *et al.*, 2006a). In 5REP all subjects were able to finish the entire protocol with the initial load and to maintain the average power production throughout the repeated sets. In 10 REP, however, all subjects decreased the average power production throughout the sets.

Intervention Period. All subjects participated in the two experiments in random order. Experiments were carried out at the same time of the day one to two months apart. No changes were observed in the subjects in maximal leg press strength (1RM) between the first (194 ± 25 Kg) and the second (185 ± 32 Kg) experimental day. To avoid disturbance of the subjects, they were instructed to record their normal diet for 48 hours prior the first experimental day and to repeat the same diet prior the second experimental day.

Preliminary tests. Several pre-test sessions took place during the 3 weeks preceding the experiments. First, the subjects were familiarized with the experimental testing procedures about 2 weeks beforehand. Second, two weeks before the first experiment the subjects participated in a control testing day where resistance-load verifications for 1RM were determined in the leg press exercise machine. Then, after at least 10 min rest, the subjects performed a maximal repetitive set until failure with the load that theoretically should produce 10 repetitions to fatigue (~85% of 1RM). If the number of repetitions until failure was equal to 10, the load was defined as a 10RM and used during the experimental main tests. If the number of repetitions until failure was different from 10, several trials of a maximal repetitive set until failure were performed on different days with lower or higher loads during subsequent test sessions, in order to determine the load leading to failure in exactly 10 repetitions. Third, the maximum oxygen uptake (VO_2max) of each subject was estimated (Storer *et al.*, 1990) on a separate day by using a continuous incremental test until exhaustion on a friction-loaded cycle ergometer (Monark Ergomedic 818E, Varberg, Sweden). The first work load (60 W) was high enough to ensure that exhaustion would occur within 8–14 min, the load being increased by 30 W at the end of every min. Heart rate was continuously

monitored throughout the test (15 s) with a cardiotachometer (Sportester Polar, Kempele, Finland). Average power output at exhaustion was 347 ± 27 W.

Main tests. On the morning of the experiment, the subject arrived after a light breakfast and a 2 h fast period. On arrival at the laboratory subjects rested on a bed for 20 minutes so that small incisions could be made under local anesthesia (2 ml, 1% lidocaine) in the skin and fascia over the vastus lateralis muscle of one leg. The subjects then completed a warm-up period consisting of a set of 5 repetitions at 50%, three to four repetitions at 75% and 1 repetition at 90% of maximal bilateral leg press strength (1RM). Three to four subsequent attempts were made to determine the 1RM. The resting period between maximal attempts was always 2 min. After 10 min rest, a muscle biopsy (initial biopsy) was taken from the middle region of the muscle vastus lateralis (15 cm above the patella and approximately 3 cm below entry through the fascia) and after an arterialized blood sample was drawn from the earlobe, previously hyperemized with Finalgon® (Boehringer Ingelheim, Germany). Then they performed either 5 sets of 10 repetitions to failure (10REP) or 10 set of 5 repetitions not to failure (5REP) with the maximum load possible to achieve 10 repetitions during the first set (10RM). Subjects were instructed to always try to displace the weight as fast as possible. The duration of each repetition decomposed in its concentric and eccentric components was recorded. Repetitions were interspaced by ~1-s pauses to prevent stretch-shortening cycle enhancement of performance. The power output of each repetition was monitored continuously and measured during the concentric phase of leg press action. Immediately (within 5–10 s) after the last repetition of the first set and immediately after the last repetition of the last set muscle biopsies were taken in all subjects. Additional arterialized blood samples were drawn after 16 and 45 min of recovery to determine the post-exercise uric acid blood concentration. All participants were highly motivated and strong verbal encouragement was given to all subjects to motivate them to perform each repetition maximally and as rapidly as possible. Subjects remained fasted throughout the tests.

Equipment. The study was performed on a horizontal bilateral leg extension variable resistance machine (i.e. leg press action in a sitting position; Technogym, Gambettola, Italy). The sitting position was individually adjusted to minimize displacement between the lower back and backrest during muscular force exertion and, therefore, to avoid

posture changes. Subjects were instructed to put their feet in the same position on the force platform. The exercise machine incorporated four force transducers on a foot platform located below the subject's feet. The strain gauges recorded the applied force (N) to an accuracy of 1 Newton at 1000 Hz. The force platform and leg press plate all remained stationary throughout the lift, while the body moved away from the feet. In addition, a rotational encoder (Computer Optical Products Inc, California, USA) was attached to the weight plates to record the position and direction of the displacement to an accuracy of 0.2 mm at 1000 Hz. Customized software was used to calculate power (immediate product of displacement velocity and applied force) per repetition. After the end of the exercise, results were integrated over 1-ms intervals. The maximum 10-ms integral of applied force and displacement velocity during each repetition is referred to as ''peak power output''. The average 10-ms integral of applied force and displacement velocity over the total concentric contraction time of each repetition is referred to as ''mean power output''.

Muscle samples. Muscle biopsies were taken as described by Bergstrom (Bergstrom, 1975) from the right leg on each occasion. Muscle samples were immediately frozen (in 5–10 s) in liquid nitrogen and stored at -80ºC for subsequent metabolite assay and histochemical analysis, after being freed from visible fat and connective tissue.

Analysis. Muscle Phosphocreatine (PCr), creatine (Cr) and lactate, were analyzed by fluorometric analysis (Lowry & Passonneau, 1971). Skeletal muscle adenine nucleotides and inosine monophosphate (IMP) were analyzed by high-performance liquid chromatography (HPLC) (Norman *et al.*, 1991). All muscle metabolite concentrations are expressed as $mmol \cdot Kg^{-1}$ wet muscle. In addition, a piece of the biopsy taken before the first set was frozen separately, later used for serial cross-sectioning (10 µm) and stained for myofibrillar ATPase after alkaline and acid preincubation (Brooke & Kaiser, 1969) for fiber classification into slow-twitch (ST) and fast-twitch (FT) fibers.

Calculations. Cellular energy charge, a measure of the extent to which the total adenine nucleotide pool of the cell (ATP, ADP and AMP) is phosphorylated, was estimated using the following equation:

Energy charge = ([ATP] + 0.5 [ADP]) / ([ATP] + [ADP] + [AMP])

Blood samples. Capillary blood samples for determination of uric acid concentrations were obtained from a hyperemized earlobe at rest and 16 and 45 min after the end of the exercise protocol. According to the manufacturer's instructions, after cleaning and puncturing a single 28.5- 31.5-KL capillary sample was taken and placed over the strip (Reflotron uric acid) for an automatic reflectance photometry analysis (Reflotron; Boehringer Mannheim, Mannheim, Germany) within the first 2–3 min after obtaining the sample. The analyzer was calibrated (Reflotron Check) before every subject's capillary samples analysis.

Statistics. Standard statistical methods were used for the calculation of mean and standard deviation (SD). Student's paired t-test was used for comparisons of analytic values during the two different experimental conditions in this study, whereas one-way analysis of variance for repeated measures was used to examine the differences in performance indexes and metabolite concentrations over time. When a significant F-value was achieved ($P<0.05$), the means were compared using a LSD post-hoc test. For the purposes of comparison the power output for the second 5 repetitions of each set was compared with that of the first 5 repetitions. Coefficient of determination (R^2) was used to determine associations among variables taking both exercise conditions as a whole. Linear or non linear regressions were determined using either a linear or a second-degree polynomial form. A second-degree polynomial form was accepted if it resulted in a significant reduction in error variance as compared with the linear solution. Statistical significance was accepted at the $P<0.05$ level.

Results

Power and force production. During 5REP all the subjects were able to complete all the repetitions with the initially load assigned (154 ± 31 Kg; 83 ± 8% of 1RM). During 10REP, however, most of the participants were unable to complete all the repetitions with this starting load, due to failure. The load had to be reduced by 7.2 ± 3.8% after 27 ± 16 repetitions and was progressively reduced, reaching 85 ± 12% ($P<0.05$) of the initial load at the last repetition. Average load during the 50 repetitions of 10REP was 6.1 ± 6.3% lower ($P<0.05$) than during 5REP.

Average peak power during the first 5 repetitions of the first set was similar in both experimental conditions (Fig. 1). During each set of 5REP the highest value of average peak power was reached during the second or third repetition and thereafter average peak power decreased progressively (7-20%, $P<0.05$) between the second and the fifth repetition along each set. During each set of 10REP the highest value of average peak power was also reached during the second or third repetition and thereafter average peak power sharply decreased (35-45%, $P<0.01$) between the second and the tenth repetition along each set. During 10REP the magnitude of the decline in average peak power production between the first 5 repetitions of the first set and the last 5 repetitions of the last set was 33 ± 19% (from 821 ± 209 to 569 ± 159 W; $P<0.05$). In contrast, during 5REP average peak power output in groups of 5 repetitions was maintained between sets. When both types of exercise were compared in groups of 5 repetitions, the average peak power from repetitions 6th to 50th was 28 ± 12% lower ($P<0.01$) during 10REP than during 5REP. In both experimental conditions, average mean power output changes paralleled those of peak power output.

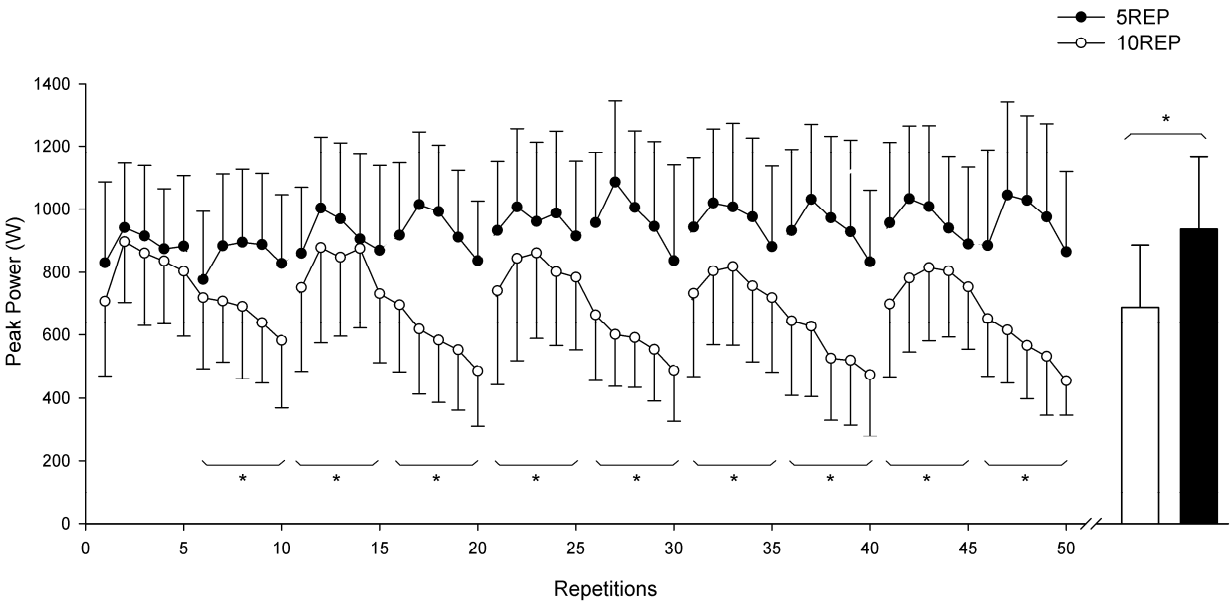


Figure 1. Peak power output profiles (average for n = 6 subjects) for each exercise during the two experimental conditions: when exercise was 5 bouts of 10 repetitions to failure (10REP; open circles), and when exercise was 10 bouts of 5 repetitions not to failure (5REP; filled circles). Boxes represent mean of the peak power output throughout 50 repetitions for 10REP and 5REP. *significant difference (P<0.05) between 10REP and 5REP (pooled from 5 to 5 repetitions). Values are means ± SD.

Muscle metabolites. Table 1 shows muscle metabolite concentrations before the first set (initial biopsy) and immediately after the first and last set during both experimental days. Initial metabolite concentrations were within the normal range for human skeletal muscle. At the end of the 10REP exercise PCr stores were almost depleted (85% fall, P<0.05), whilst ATP (21%), energy charge (4%), and the total amount of adenine nucleotides pool (ATP + ADP + AMP) (20%) where reduced (P<0.05). Concomitantly, IMP and lactate where increased. In contrast to 10REP, 5REP resulted in markedly lower decrease of the muscle PCr (~ 15% fall, P<0.05) and unchanged muscle ATP, IMP, energy charge and the total amount of adenine nucleotide pool concentrations, whereas muscle lactate was only slightly elevated above the initial levels. The changes observed in muscle ATP, total adenine nucleotide pool, IMP, PCr and lactate levels at the end of exercise were significantly higher (P<0.01-0.05) during 10REP compared with 5REP.

Table 1. Effects of leg press exercise on muscle adenine nucleotides, IMP, PCr, Cr, lactate and energy charge at before the first set, after the last repetition of the first set and after the last repetition of the last set, during 10REP and 5 REP exercise.

	10REP			5REP		
	Pre	Post 1st set	Post last set	Pre	Post 1st set	Post last set
ATP	6.46 ± 0.56	6.42 ± 0.57	4.90 ± 0.39*†	6.58 ± 0.35	6.19 ± 0.59	6.09 ± 0.41#
ADP	0.83 ± 0.03	0.91 ± 0.10	0.92 ± 0.11	0.86 ± 0.04	0.89 ± 0.08	0.87 ± 0.08
AMP	0.07 ± 0.04	0.09 ± 0.03	0.09 ± 0.04	0.08 ± 0.04	0.08 ± 0.03#	0.08 ± 0.03
TAN	7.37 ± 0.59	7.42 ± 0.67	5.91 ± 0.44*†	7.52 ± 0.36	7.16 ± 0.66	7.04 ± 0.49#
IMP	0.01 ± 0.00	0.08 ± 0.11	0.87 ± 0.69*	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.02#
PCr	21.0 ± 8.86	7.75 ± 5.53	3.15 ± 2.88*	19.5 ± 4.06	11.68 ± 7.82*#	14.47 ± 7.24*#
Cr	8.93 ± 4.96	25.45 ± 3.80	22.90 ± 6.89*	8.40 ± 3.25*	16.97 ± 6.33*	15.57 ± 5.01*
PCr + Cr	29.91 ± 5.19	34.55 ± 6.23	26.06 ± 8.44	27.90 ± 3.65	30.56 ± 6.19	30.14 ± 8.46
La	1.70 ± 1.18	17.20 ± 3.50*	25.01 ± 8.09*	2.02 ± 1.05	7.10 ± 2.54*#	5.80 ± 4.62#
Energy Charge	0.933 ± 0.006	0.927 ± 0.004*	0.909 ± 0.014*†	0.932 ± 0.007	0.927 ± 0.006	0.928 ± 0.006

Values are expressed as mean ± SD in mmol•kg^{-1} wet wt muscle, except energy charge; n = 4-6. TAN, total adenine nucleotides (ATP + ADP + AMP); IMP, Inosine 5'- monophosphate; PCr, Phosphocreatine; Cr, Creatine; La, Lactate. For calculations of energy charge see METHODS. *significant difference (P<0.05) with pre exercise value. †significant difference (P<0.05) with post first set value. #significant difference (P<0.01-0.05) with 10REP exercise.

Compared with initial values, at the end of exercise the calculated ATP/ADP ratios decreased by 9% (P<0.05) in 5REP and by 30% (P<0.01) in 10REP (Table 2). The decrease of the ATP/ADP ratio in 10 REP was higher than the corresponding decrease observed in 5REP. The ATP/AMP ratio did not change at the end of 5REP but decreased (P<0.05) by 34% at the end of 10REP. Similarly, the ATP/IMP ratio did not change after 5REP but showed a pronounced decrease from 1049 to 13.4 (P<0.05) at the end of 10REP. No change occurred in the ADP/AMP ratio throughout exercises.

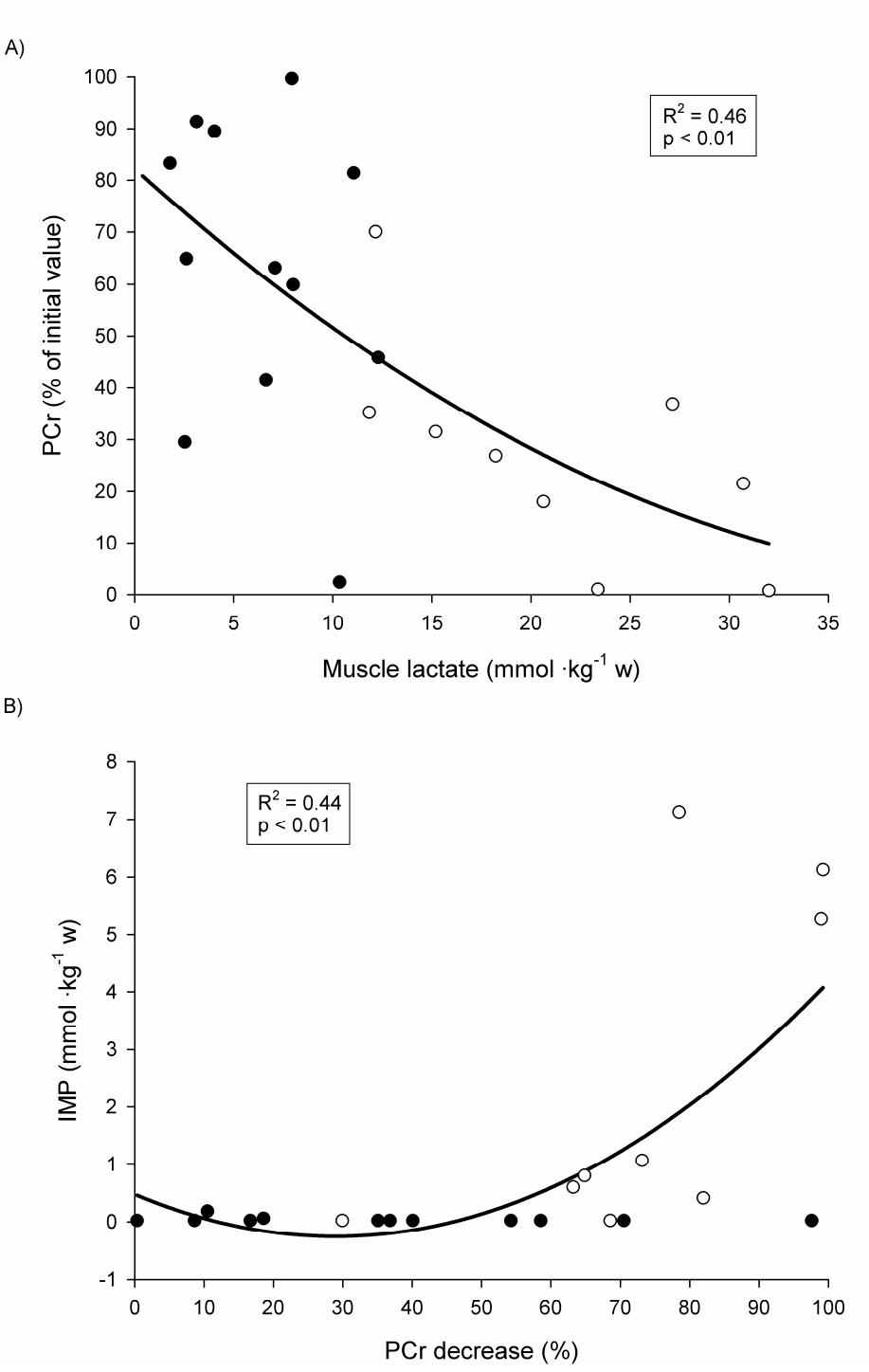
Table 2 Effects of leg press exercise on nucleotide metabolite ratios before the first set and during 10REP and 5REP exercise protocols.

	10REP			5REP		
	Pre	Post 1st set	Post last set	Pre	Post 1st set	Post last set
ATP/ADP	7.7 ± 0.5	7.1 ± 0.2	5.4 ± 0.7 *†	7.7 ± 0.4	7.0 ± 0.3 *	7.0 ± 0.4 *#
ATP/AMP	100.5 ± 29.3	77.8 ± 19.7*	66.5 ± 27.2 *	91.2 ± 30.6	88.4 ± 24.8 #	89.1 ± 24.4 #
ADP/AMP	12.9 ± 3.6	10.9 ± 2.7	12.1 ± 3.9	11.8 ± 3.7	12.6 ± 3.3	12.7 ± 3.3
ATP/IMP	1049.3 ± 78.2	648.4 ± 553.8	13.4 ± 13.0 *	1053.3 ± 35.5	1055.3 ± 23.2	773.2 ± 406.2 #

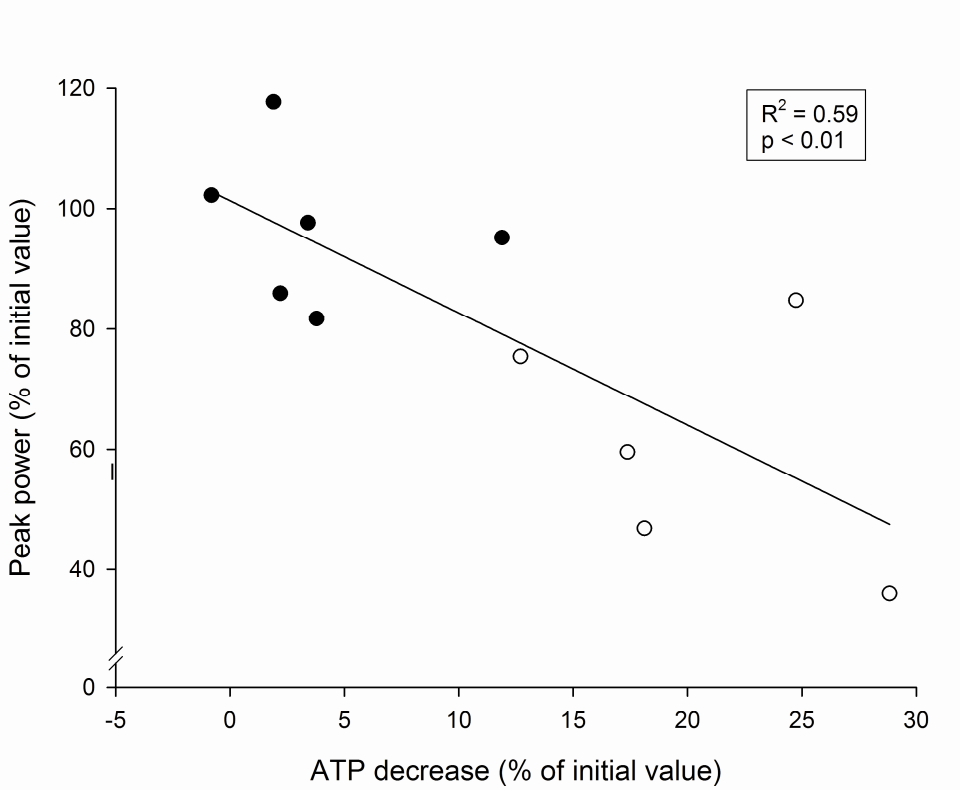
Values are expressed as mean ± SD. *significant difference (P<0.05) with pre exercise value. †significant difference (P<0.05) with post first set value. #significant difference (P<0.01-0.05) with 10REP exercise.

Blood uric acid. Peak blood uric acid concentration during recovery after the last set of 10REP (351 ± 96 µmol·l^{-1}) was 19% higher (P<0.05) than initial values (276 ± 52 µmol·l^{-1}). In 5REP the peak blood uric acid concentration after exercise (292 ± 74 µmol·l^{-1}) remained unchanged from initial values (286 ± 701 µmol·l^{-1}). The postexercise blood uric acid concentration value during 10REP was higher (P <0.05) than during 5REP.

Relationships between muscle metabolites. To examine the relationships between variables, both exercise conditions were taken as a whole. A significant curvilinear negative relationship was observed between muscle lactate content after the first and the last set and the corresponding levels of muscle PCr (in % of the initial levels) (R^2 = 0.46, P< 0.05) (Figure 2A). Similarly, a significant curvilinear relationship was observed between the relative decrease in muscle PCr levels (in % of the initial levels) and the levels of muscle IMP (R^2 = 0.44, P< 0.01) (Figure 2B). From these curvilinear relationships it can be estimated that a 60% decrease in PCr below rest values is required to elicit muscle IMP accumulation.



Relationships between muscle metabolites and changes in power output. A significant linear negative correlation was observed between the average changes in peak power output observed during the last two repetitions (expressed in percent of the initial two repetition values) and the decreases in ATP levels (expressed in percent of initial value) (R^2 = 0.59, P< 0.01) (Figure 3). Furthermore, a significant curvilinear negative correlation was observed between the average peak power output changes observed during the last two repetitions of the first and last sets (expressed in percent of the initial two repetition values) and the corresponding levels of muscle lactate (R^2 = 0.64, P< 0.01) (Figure 4). The curvilinear nature of the curve seems to indicate that when muscle lactate levels do not exceed the upper limit of 10-15 mmol·Kg^{-1} wet muscle, power output changes little from maximum values. However, when muscle lactate values exceed this upper level, the power output decreases sharply.



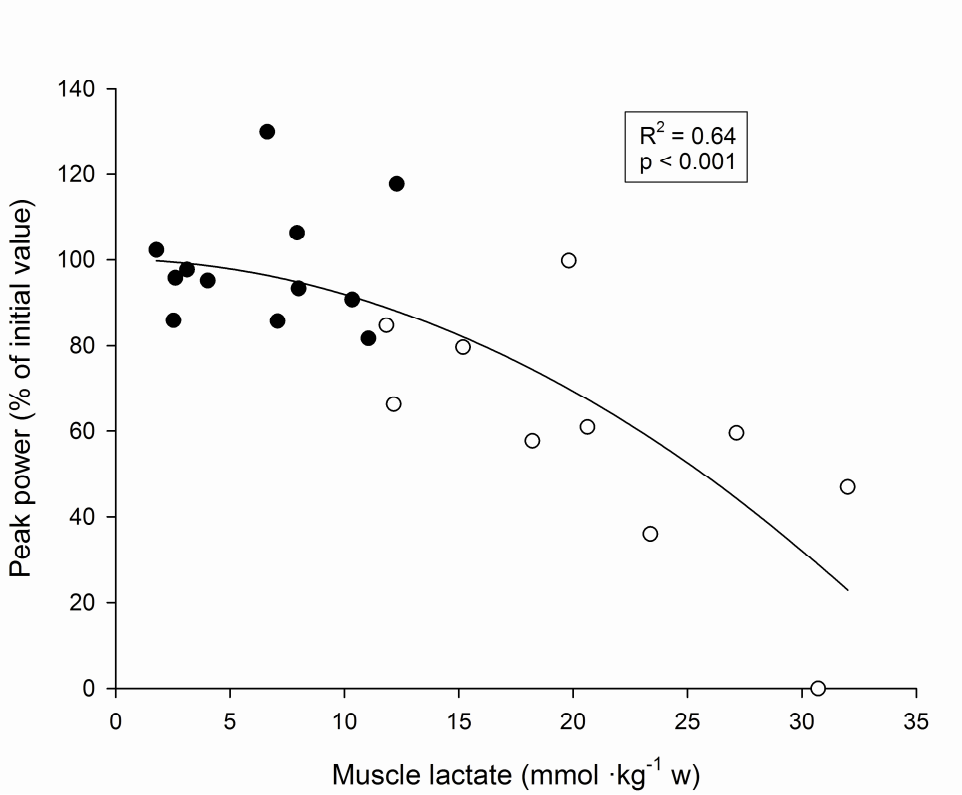


Figure 4. Individual relationships between the relative average peak power output changes (expressed in percent of initial value) between the first and the last two repetitions of the first bout and between the first and last two repetitions of the exercise, and muscle lactate concentrations, during 10REP (open circles), and 5REP (filled circles).

Discussion

Muscle metabolism. The 10REP leg press exercise demanded a maximal effort from the subjects, as reflected by the marked decrease in power output that took place during the last 5 repetitions of each set. The use of this model of 10REP caused a state of energy deficiency and a decline in the phosphate potential resulting in a near-complete depletion of PCr stores, a significant reduction in ATP (21%) and the size of muscle total adenine nucleotide pool (TAN), and in marked increases in lactate and IMP accumulation in the muscle as well as high levels of uric acid in the blood. The elevated levels of muscle lactate indicate that anaerobic glycolysis is extensively activated during this type of exercise. The increased muscle IMP originates from degradation of ATP and appears to reflect the failure of ATP resynthesis to match ATP hydrolysis rates (Sahlin & Broberg, 1990). The elevated plasma accumulation of uric acid after 10RP suggests that IMP was not reaminated back to AMP after exercise, and that there instead was a dephosphorylation of IMP to inosine, and consequently hypoxanthine and uric acid (Hellsten *et al.*, 1998). The net result would have been a wasteful loss of purines from muscle (Hellsten *et al.*, 1998) that would require replacement by either the purine nucleotide cycle or the novo synthesis (Winder & Hardie, 1999). The extent of anaerobic energy production and the fall in peak power output with 10REP is higher than the changes found previously in anabolic steroids user body–builders following an exercise regimen comprising 5 sets of 10RM each of front squats, back squats, leg presses and knee extensions (Tesch *et al.*, 1986). However, it is qualitatively and quantitatively similar to what has been reported previously by others during and after high intensity intermittent cycling (McCartney *et al.*, 1986; Saltin & Essén, 1971), knee isometric (Edwards *et al.*, 1972), or isokinetic (Jansson *et al.*, 1987) exercises leading to exhaustion between 6 and 60s. These changes have been also associated with large reductions in muscle glycogen (Saltin & Essén, 1971) and ATP (Jansson *et al.*, 1987; Saltin & Essén, 1971), particularly in Type II fibers (Casey *et al.*, 1996), with the most pronounced changes being located in type IIx fibers (Koopman *et al.*, 2006), whereas blood and muscle pH can reach values as low as 7.1 (McCartney *et al.*, 1986) and 6.6 (Bogdanis *et al.*, 1996) respectively. However, the experimental protocol in previous studies was not the same as in the present study in which repetitive isoinertial contractions using leg press actions were used. Thus, it appears that 10REP causes a marked disruption to the energy balance in the muscle.

In contrast to 10 repetitions with a 10RM load (10REP), the energy balance was maintained during 5 repetitions with a 10RM load (5REP), despite the accumulation of sets. Thus, compared with 10REP, 5REP resulted in a markedly lower decrease in muscle PCr content (~ 15% vs 80% fall) with only modest increases in muscle lactate and no measurable changes in muscle levels of ATP and IMP and in blood levels of uric acid. These minor metabolite changes observed in 5REP, despite a high ATP turnover rate, suggest that ATP synthesis matched ATP utilization and that cellular homeostasis was maintained, thereby demonstrating that the rate of AMP deamination was low (Sahlin & Broberg, 1990). Consequently, peak power output was maintained only during the 5REP exercise. The absence of blood uric acid increase during 5REP is in line with the finding of unchanged ATP levels. These results indicate that reducing the number of repetitions per set by 50% maintains power output and energy balance in the muscle throughout sets.

The changes in the ATP/ADP and ATP/AMP ratios during the contraction process have been widely studied because the relative levels of the adenine nucleotides are more important metabolic regulators for maintenance of adequate cellular functions than the absolute concentration of ATP (Sahlin & Broberg, 1990). At the end of 5REP the calculated ATP/ADP ratio was decreased by 9% compared with initial values, and was coincidental with no changes in the calculated energy charge, AMP, ATP/AMP and ATP/IMP ratios, with a 7-20% decrease in the average peak power between the second and the fifth repetition of each set, and with moderate decreases (30%) in PCr levels. The dichotomy between changes in the ATP/ADP ratio and changes in the ATP/AMP ratio during exercise is not without precedent. Thus, increases in the estimated muscle-free ADP content, without any changes in estimated free AMP, have been observed during 15 s sprint isokinetic cycling exercise (Parolin *et al.*, 1999) or after 10 minutes of cycling at 65% of maximal oxygen uptake (Howlett *et al.*, 1998), indicating that an slight initial increase in ADP availability does not displace the adenylate kinase favoring the formation of AMP (ADP + ADP ↔ AMP + ATP), when the decrease in power production and the changes in muscle PCr or P_i levels are moderate. The activity of adenylate kinase was still low after 5REP, probably because the dephosphorylation of ADP to AMP is buffered by PCr (Casey *et al.*, 1996). In this situation the AMP-activated protein kinase (AMPK) may be only slightly activated by the increased ADP availability (Xiao *et al.*, 2011). At the end of 10REP, however, a further decrease (30%)

in the ATP/ADP ratio, higher to that seen after 5 REP, was accompanied by decreases in the ATP/AMP and ATP/IMP ratios, in parallel with further decreases (33%) in power production and with almost depleted PCr levels. The decreased ATP/AMP and ATP/IMP ratios indicate that, as opposed to 5REP, during 10REP the adenylate kinase and the AMP deaminase were significantly activated. The very low levels of PCr and the activation of adenylate kinase could amplify the activation of AMPK, that acts as a fuel-sensing enzyme monitoring cellular energy levels to prevent the catastrophic consequences of larger decreases in energy state (Hancock *et al.*, 2006; Hardie & Carling, 1997; Tullson & Terjung, 1991). This dual mechanism (initial decreases in ATP/ADP ratio followed by later decreases in ATP/AMP and ATP/IMP ratio) would allow AMPK to sense energy deficit progressively over a wide range of energy availability (Koopman et al., 2006).

The described repetition-related differences in acute metabolic response to repeated sets of leg press exercise should reflect two different stimuli for training-induced adaptations occurring after heavy-resistance training. Thus, some studies have shown that high-intensity resistance training not to failure of the knee extensor muscles enables a favorable environment for achieving greater enhancements in maximal strength and power output compared with training to failure (Izquierdo *et al.*, 2006b; Kraemer *et al.*, 1997; Sanborn *et al.*, 2000). Taken together, the results of these studies and the present one suggest that a program of dynamic knee extension resistance exercise characterized by low metabolite accumulation and maintenance of cellular homeostasis and energy balance may be a more effective, efficient and safe option compared with training to failure designed to maximize fatigue/metabolite accumulation. Under this assumption, it would be time to replace the classical “no pain, no gain” training philosophy to a more rational and ecologically based “no pain, more gain” one.

Relationships between muscle metabolites. Previous studies have shown that during fatiguing and not fatiguing isometric knee extension and cycling exercise average absolute PCr content changed curvilinearly and negatively with respect to the absolute muscle lactate content (Harris *et al.*, 1977). In the present study a significant curvilinear negative relationship was observed throughout the exercises between the percent decrease of muscle PCr and the corresponding muscle lactate content. This agrees with the findings of Karlsson and co-workers (Karlsson *et al.*, 1970) during maximal and

submaximal cycling exercise and with the close relationship between the logarithm of mass-action ratio of the creatine kinase reaction and muscle pH reported in men after isometric knee extension exercise (Sahlin *et al.*, 1975). Furthermore, it indicates that the changes between the PCr and lactate mainly occur simultaneously during exercise, supporting the observations of others that the anaerobic glycolysis is initiated in the muscle at the onset of heavy exercise (Gollnick & Hermansen, 1973). In agreement with our results, accumulation of IMP matched quantitatively by a decline in intramuscular ATP has been reported during submaximal and maximal cycling exercise when PCr levels drop below 40% of the resting levels (Hultman, 1967; Karlsson *et al.*, 1970; Sahlin & Broberg, 1990; Spriet *et al.*, 1987).

What causes fatigue during consecutive sets of leg press exercise? Several authors have suggested that the capacity to regenerate ATP at the required rates and thus decrease force and power production during short duration maximal exercise may by related to an inability to maintain the required rate of anaerobic ATP production from PCr and glycogen degradation, mainly in type II fibers (Hultman & Greenhaff, 1991;Karatzaferi *et al.*, 2001), a corresponding increase in inorganic phosphate (Pi) and its diprotonated form, H_2PO^{4-}, increases in $[H^+]$, alterations in Ca^{2+} transport (Nakamaru & Schwartz, 1972), K^+ efflux from the muscle (Juel, 1988) or impaired neuromuscular transmission or failure of membrane excitation (Edwards, 1981). In the present study the fall in power production during both exercises as a whole was strongly correlated to the fall in ATP stores and to the lactate levels in mixed muscle homogenates. The association between changes in power output and changes in muscle lactate accumulation are in agreement with the above mentioned studies. The association observed between the loss of ATP stores and the relative decline in power output supports the idea that ATP depletion in a small percentage of fibers may lead to their failure to power production (Karatzaferi *et al.*, 2001). This idea is in agreement with some studies reporting post-exercise ATP levels in individual fibers as low as 1 to 2.4 $mmol \cdot Kg^{-1}$ wet muscle following maximal knee extension (Jansson *et al.*, 1987), or isokinetic cycling (Karatzaferi *et al.*, 2001) exercise. Hence, it is not unreasonable to suggest that some biochemical changes, such as a decrease in ATP stores and increases in lactate and by-products of ATP (H^+, P_i, ADP) of individual muscle fibers may contribute to fatigue during successive sets of leg press exercise.

Study limitations. This study had some limitations. First, it was characterized by a low number of experimental subjects. However, the strong statistically significant differences observed in muscle metabolites and power between the two exercises indicates that it is unlikely these differences occurred by chance. Future longitudinal biopsy studies should seek to recruit larger numbers of experimental subjects in order to reduce the potential risk of type II errors. Second, it may also be argued that the experimental setup could have been strengthened by matching the same load throughout sets and the total rest period between protocols (e.g. 1-min rest between sets in 5REP and 2-min rest between sets in 10REP). This would enable comparisons between two types of equivolumic exercise. It is obvious that, in this case, the magnitude of the differences between exercises would be lower than that observed in the present study. However, it has to be emphasized that the main goal was to compare metabolic and power changes during two types of resistance exercises traditionally used for reaching specific training outcomes ("hypertrophy" versus "optimal strength improvement"), but not to compare two equivolumic exercises. Finally, this study was performed in endurance trained men with a high proportion (65%) of type I fibers. Care should be taken when generalizing the results of this study to other populations (e.g. power athletes with high proportion of type II fibers). Despite these limitations, the findings do provide important and new information about the metabolic characteristics of two of the most popular high-intensity resistance exercises designed to increase muscular strength, power and hypertrophy in athletes and adults with chronic diseases and disabilities.

In summary, the main results of this study were, first, that reducing the number of repetitions during sets from ten to five while maintaining the same initial load and recovery periods in between induced markedly smaller demands on the high-energy phosphates system and on glycolytic energy supply, allowed ATP synthesis to match ATP utilization, to maintain power output and energy balance along the sets and to experience much less fatigue and discomfort during bilateral leg press exercise. Second, in mixed muscle homogenates the changes in muscle PCr during both exercises were correlated with muscle lactate and IMP content. These correlations suggest that the changes between the PCr and muscle lactate mainly occur simultaneously during exercise whereas IMP only accumulates when PCr levels are low. Finally, the fall in power production was strongly correlated to the fall in ATP stores and to the muscle

lactate levels. This suggest that a decrease in ATP stores and increases in lactate and by-products of ATP (H^+, P_i, ADP) of individual muscle fibers may contribute to fatigue during successive sets of leg press exercise.

Author Contributions

Conceived and designed the experiments: EG YH JALC MGI JI MI. Performed the experiments: EG RC JALC CG JI MI. Analyzed the data: EG INA RC YH JALC MG CG MGI JI MI. Contributed reagents/ materials/analysis tools: EG INA RC YH JALC MG CG MGI JI MI.

Wrote the paper: EG INA RC YH JALC MG CG MGI JI MI.

Disclosures

No conflicts of interest are declared by the authors.

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CHAPTER 4

Blood ammonia and lactate as markers of muscle metabolites during leg press exercise

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Abstract

To examine whether blood lactate and ammonia concentrations can be used to estimate the functional state of the muscle contractile machinery with regard to muscle lactate and ATP levels during leg press exercise. Thirteen men (age 34 ± 5 yr, 1RM leg press strength 199 ± 33 kg) performed either 5 sets of 10 repetitions to failure (5x10RF), or 10 sets of 5 repetitions not to failure (10x5RNF) with the same initial load (10 RM) and inter-set rests (2 min) on two separate sessions in random order. Capillary blood samples were obtained before, and during exercise and recovery. Six subjects underwent vastus lateralis muscle biopsies at rest, before the first set and after the final exercise set. 5x10RF resulted in a significant and marked decrease in power output (37%), muscle ATP content (24%) and high levels of muscle (25.0 ± 8.1 $mmol \cdot kg^{-1}$ wet wt) and blood lactate (10.3 ± 2.6 $mmol \cdot L^{-1}$) and blood ammonia (91.6 ± 40.5 $\mu mol \cdot L^{-1}$). During 10x5RNF no or minimal changes were observed. Significant correlations were found between: 1) blood ammonia and muscle ATP (r=-0.75), 2) changes in peak power output and blood ammonia (r=-0.87) and blood lactate (r=-0.84), and 3) blood and muscle lactate (r=0.90). Blood lactate and ammonia concentrations can be used as extracellular markers for muscle lactate and ATP contents, respectively. The decline in mechanical power output can be used to indirectly estimate blood ammonia and lactate during leg press exercise.

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