# Work and Energy in Muscles

Why can't I sprint forever?

Silly question? Let us take a look at data from the 1964 summer Olympic Games. The participants were extremely motivated individuals. We can assume that they "gave all they had", running as fast as possible while still managing to come to the finish line. What lies behind the undisputable observation that those competing in short distance races ran faster than competitors in longer races?   Why must we reduce speed if we want to run long distances over extended time intervals? Even the most motivated athletes are bound by this simple rule. We can see this in the following graph.   Running speed is plotted against the duration of the race. Competitors running more than 30-40 seconds reduced their velocity markedly and a continual and gradual decrease occurred after about 2 minutes. Marathon runners ran a little more than half of the speed of sprinters.

Running Speed and Race Duration
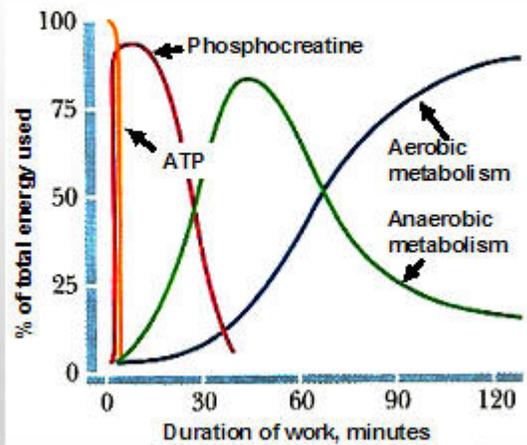Olympic Games, 1964
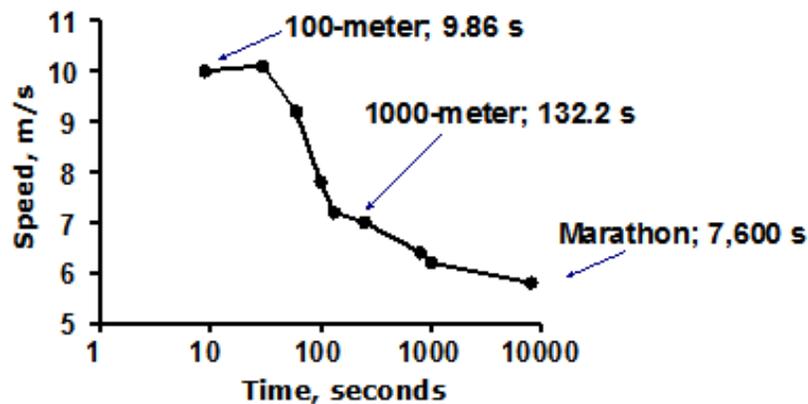
World Almanac, 1994

The explanation for this phenomena is that while the only direct fuel for muscles is ATP, we do not "use up" ATP while working. Even extremely hard work does not lower ATP concentrations by more than about 20 %.   Several differing energy sources are used by working muscles to maintain ATP levels. Phosphocreatine, muscle glycogen, blood glucose and fatty acids from adipose tissue are those possible energy sources.   Let us look at the striking differences between these.

 A 100-meter sprint takes less than 10 seconds to complete. During this very short period, the major driving forces are stored high-energy phosphates and anaerobic glycolysis. The runners can perform almost without breathing, using energy stored as ATP, creatine phosphate and glycogen (that is, anaerobic metabolism) in the active muscles. In contrast to long-distance runners, sprinters are often large, very muscular people. Sprinters have a dominance of so-called fast twitch or anaerobic muscle fibers. Those remarkably high speeds can only be

maintained while stored high-energy phosphate in the form of phosphocreatine is present. Almost all studies of phosphocreatine metabolism conclude that stores of phosphocreatine in skeletal muscle are emptied within the first 30 seconds of strenuous activity. After that, the very rapid rate of running must be reduced.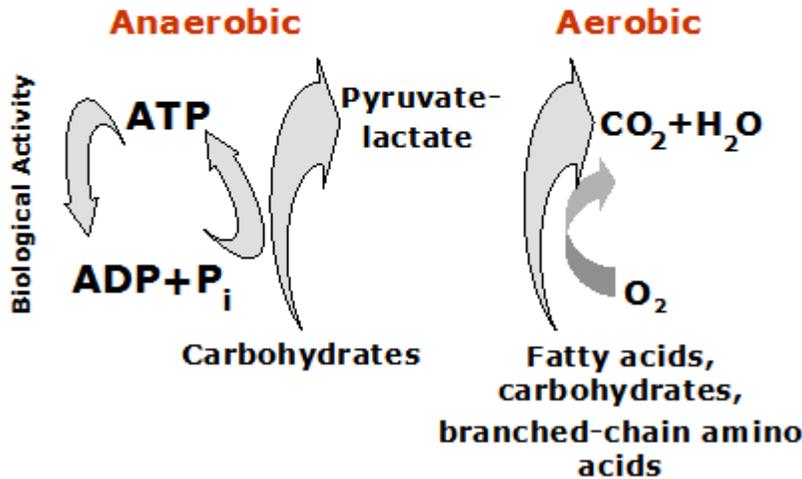 The energy supply for those who run from about 60 seconds to three minutes is primarily glycogen stored in muscles and blood glucose. These carbohydrates can be rapidly oxidized to pyruvate, lactate and CO_2 to provide the ATP required for muscle activity. However, the rate of ATP synthesis rate is far below that seen when using phosphocreatine as the phosphate donor. These differing sources of energy are summarized in the figure to the left. Please note that, while the source for

Energy Sources in Working Muscles

energy does swing extensively during work, the total amount of ATP in working muscles is surprisingly constant. While resting musculature has about 5 mmoles/liter of ATP, working muscles rarely experiences more than a 10-15 % fall in ATP concentration.

Metabolism of carbohydrates in muscle.

Energy production, that is ATP synthesis from ADP and P_i, can occur anaerobically (without use of oxygen) or aerobically (using mitochondrial reactions and oxygen). Use of ATP does not lead to major decreases in ATP levels, due to its rapid resynthesis. Extreme activity leads to no more than a 20% reduction in ATP concentration in muscles. Anaerobic glycolysis is far more rapid than aerobic metabolism. However, anaerobic processes can only use stored glycogen or blood sugar as substrates. Neither amino acids nor fatty acids can be processed without use of oxygen. Muscles differ in their ability to produce ATP through anaerobic and aerobic reactions. So-called fast-twitch white muscles with few mitochondria are the "anaerobic" fibers, producing ATP and yielding lactate acid as a temporary product. Aerobic fibers, those with many mitochondria, produce ATP using carbohydrates, fatty acids and branched-chain amino acids as substrates. Aerobic glycolysis is a very effective energy production, forming 36-38 ATP molecules for every glucose molecule that is oxidized to CO_2 and water. However, aerobic oxidation of all substrates and the linked ATP synthesis are relatively slow processes. Anaerobic glycolysis provides very rapid formation of ATP for short intervals while aerobic processes provide energy over longer time intervals. This explains the differing running rates seen in the example. Sprinters have a dominance of those rapid, lactate-producing white muscle fibers, while long-distance runners have an abundance of red oxidative muscle fibers.

Energy Sources in Working Muscle

Anaerobic ATP synthesis is coupled to formation of lactic acid from stored "glucose" (actually glycogen) or glucose from the blood (blood sugar). In the next figure you can see the progress of glucose metabolism following a meal and in a period with little work. Metabolism of glucose goes two differing ways in this situation. Some of it is oxidized, generating 2 ATP molecules for each glucose moiety that goes through anaerobic glycolysis. At the same time, some of the glucose can be stored as the polymeric anhydride, glycogen, using the energy in an ATP molecule (in the form of UTP) to split out water from the glucose molecules which form glycogen. We store part of

Glycogen Metabolism After a Meal
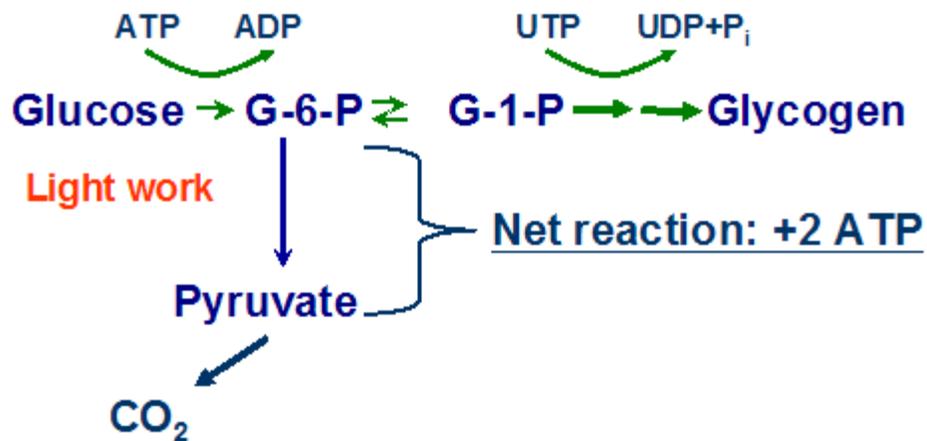
this energy in the anhydride bond that links glucosyl groups in the glycogen macromolecule. This is then used later to synthesize phosphorylated glucose (G-1-P) without utilization of ATP. Glycogen is made and stored at times of glucose excess and under rest or light work. We use that glycogen to cover future energy expenditures.

Breakdown of glycogen occurs when energy demands are increased. We now "get back" that UTP/ATP that we expended when energy demands were small. We split glycogen with an enzyme called glycogen phosphorylase. This uses inorganic phosphate to form G-1-P and G-6-P.   Remember, hexokinase required ATP to synthesize G-6-P from glucose. With glycogen and phosphorylase we can go directly to phosphorylated glucose (G-1-P) without the use of ATP! Therefore, we now get three ATPs for each glucose equivalent that goes through anaerobic glycolysis.   This is a 50 % increase in energy winning during times of need!
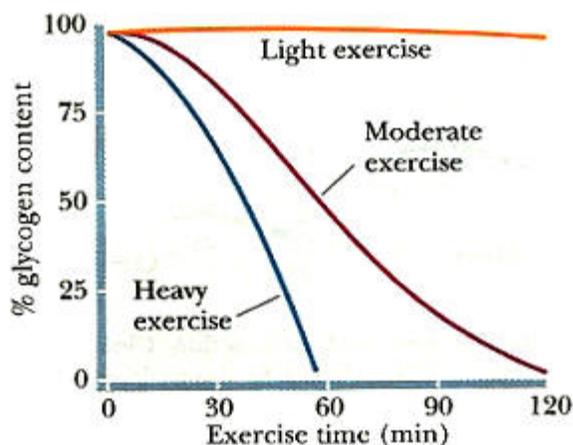
Glycogen Metabolism, Heavy Work

Glucose → G-6-P ⇌ G-1-P ← P_i Glycogen

Netto: +3 ATP

Pyruvate

CO_2 lactate
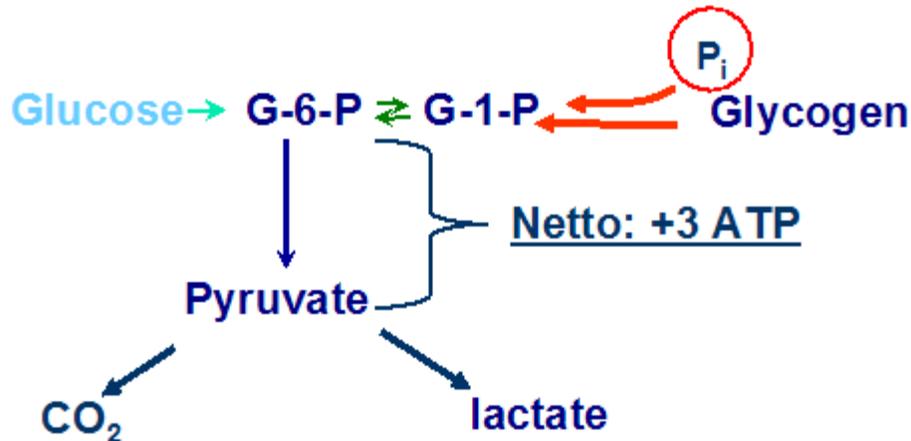
This is, of course, in marked contrast to aerobic oxidation where between 36 and 38 ATPs are formed for every glucose molecule that is oxidized to CO_2. So, which advantage do we gain by using anaerobic glycolysis with lactic acid as a temporary end-product? Why do people burning glycogen anaerobically run faster than those who use aerobic metabolism? The answer is simply that anaerobic oxidation is exceedingly rapid. Large quantities of ATP are synthesized during the three minutes or so that this process dominates muscle metabolism. However, glycogen reserves are rapidly exhausted and lactic acid accumulation quickly leads to muscle stiffness and pain. Continuing high-performance work after muscle glycogen is exhausted leads to massive uptake of glucose from the blood with a resulting fall in blood glucose levels. This leads to central effects, with a "black out" as the final consequence. Races between 100 and 2000 meters are a balance between too high and too little energy use; run slowly and lose, run too fast and pass out!

Another way to look at glycogen utilization is to see how this is coupled to work intensity. Small energy demands do not initiate glycogenolysis, as shown in the figure below. Lightly loaded muscles manage to cover their energy needs through oxidation of circulating glucose and fatty acids. However, increasing work loads demands more powerful contractions and ATP utilization. This increases the rate of glycogen breakdown to cover these needs. In other words, the harder we work, the sooner we become exhausted! Reducing the glycogen content of

Glycogen Utilization in Working Muscle

skeletal musculature does not decrease energy production. It merely shifts the substrate used from glycogen to blood glucose. If we press our bodies sufficiently, this will reduce blood sugar levels so much that we begin to lose vision and mental activity. More about this further on.

Lactate, energy metabolism's "blind path".

Why do anaerobically active muscles spew out lactate and pyruvate?   Part of the answer here is that the anaerobic part of glycolysis can proceed much faster than the aerobic reactions. Thus, pyruvate accumulates because the capacity of the mitochondrial oxidative reactions is greatly exceeded. But why is that pyruvate converted to lactate?
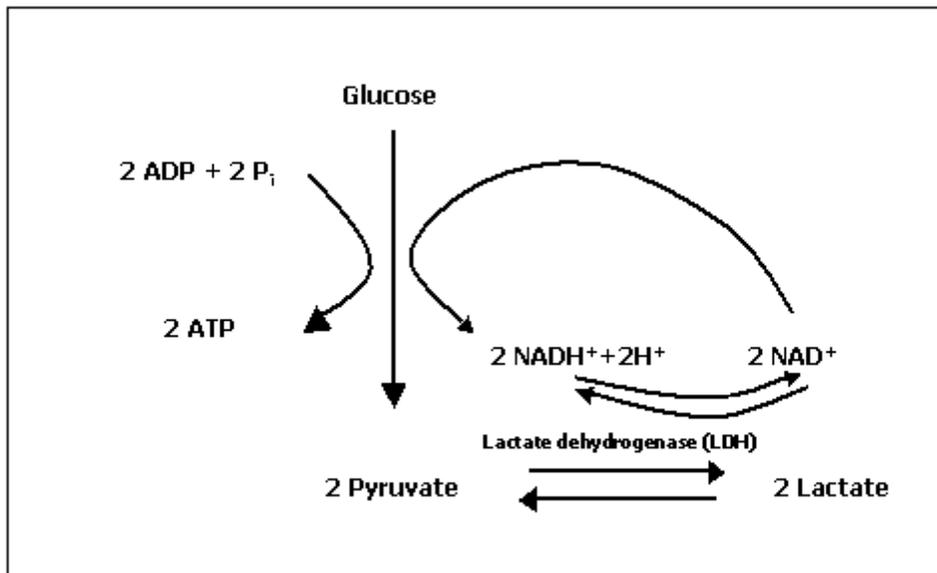
The key here is that glycolysis is completely dependent on a stable supply a metabolic oxidant to convert glucose and glycogen to pyruvate. Thus, two molecules of the nucleotide NAD^+ are converted to $NADH + H^+$ for each glucose or glycogen molecule that is oxidized. That is, conversion of 1 glucose to 2 pyruvate molecules requires 2 ADP and 2 NAD^+(check the figure at the left). So, in order to run anaerobic metabolism while the substrate is still there, muscles use lactic dehydrogenase to oxidize $NADH + H^+$ to NAD^+. There is no other rapid source for NAD^+ our organs! Production of lactate is not to form an acid byproduct. If it were not for the need for oxidation of NADH we could just pump out pyruvate (also an acid, by the way). We do, in fact excrete pyruvate from working muscles, but the ratio of pyruvate to lactate is determined of the ratio between NAD^+ and $NADH^+ +H^+$. There is much more NAD^+ than $NADH^+$ in cytosol, so lactate dominates the picture.

Now, there are two forms of lactate dehydrogenase, so-called isoenzymes. Skeletal muscle has the M_4 isoenzyme while heart muscle has the H_4 isoenzyme. Liver has a mixture of these. What is the functional difference between these? Well, both forms of LDH are inhibited by pyruvate, but the M_4 enzyme is less affected. This allows the M_4 enzyme to form lactate from pyruvate in anaerobically active muscles and keeps the NAD^+ production going. In the heart and liver, the H_4 and H_2M_2 forms may allow more rapid uptake of lactate which then can serve as a substrate for aerobic energy production.

Whole body lactate metabolism is more complicated than that which is described above. Click here for a more complete discussion of lactate metabolism.

Aerobic Energy Metabolism

Events that persist for more than roughly three minutes must be powered by aerobic metabolism. The glucose that goes through anaerobic glycolysis produces pyruvate. This is then taken up by mitochondria and completely oxidized to CO_2 and water. The total ATP produced per glucose molecule is over 10 times that produced in anaerobic metabolism. However, aerobic ATP production is a much slower process than direct phosphorylation of ADP by phosphocreatine or anaerobic glycolysis. The rate of muscle activity must be adjusted to the reduced tempo of high-energy phosphate synthesis. This is summarized in the following table. Here, the rate of synthesis of ATP by exchange with phosphate in phosphocreatine is set to 100. This is our most rapid ATP-synthesizing reaction. Anaerobic glycolysis, using glycogen as substrate, is about half as fast. Another 50% cut can be seen when we examine the rate of aerobic glycolysis starting with glucose from the circulation. And, once again, there is another 50 % reduction in the rate of

Rate of ATP Synthesis is Dependent upon Substrate

Substrate	Relative Rate	
Phosphocreatine	100	Direct ~P transfer, no substrate oxidation required.
Glycogen (anaerobic glycolysis)	55	Phosphorolysis, G-1-P formed without use of ATP yielding 3 ATP/glucosyl group, "tree-structure" gives many simultaneous reaction sites.
Glucose (aerobic glycolysis)	23	Limited by rates of membrane glucose transport, glycolysis and mitochondrial oxidation.
Fatty Acids	10	Lipolysis only at interface between oil droplet and cytosol, limited by transport and mitochondrial oxidation.

ATP synthesis if we begin with fatty acids from adipocytes.

Physical activity lasting over many minutes and hours cannot be supported by the limited bodily reserves of glucose and glycogen. Fatty acids from food and adipose tissue, therefore, supply most of the substrate used by muscle tissue working over time. Branched-chain amino acids can

also serve as substrates for mitochondrial ATP synthesis in muscle. It is striking that the substrates we have least of are metabolized swiftly, while those present in large amounts are slowly metabolized. These facts are directly related to the speed at which muscles can operate. Short intervals, high speed, long intervals, low speed!

Human Energy Stores.

Energy substrates in the human body are either carbohydrates, fat or proteins. Since energy production from carbohydrates is so much more rapid than that from fat, one might think that we

Energy Stores in Man

	kcal/g dry weight	Water g/g dry weight	kcal/g wet weight	Total energy stored, kcal
Carbohydrates	4	2-3	1-1,5	840
Triacylglycerol	9	0	9	135000
Protein	4	2-3	1-1,5	24000

should accumulate glycogen instead of fat. Nature has chosen fat as our main energy storage form for two reasons. Firstly, fatty acid are more reduced than carbohydrates. Therefore, the energy content of fat is over twice that of glycogen and proteins. Furthermore, both glycogen and proteins are hydrophilic, that is, they bind water. The result is that stored glycogen and protein have a much lower "energy density" than the hydrophobic lipids. While fat stores have 9 kcalories per gram wet weight, carbohydrates and proteins have only 1-1.5 calories per gram wet weight. Our bodies would have to be very much larger and heavier if we stored large amounts of energy as glycogen or proteins.

While proteins are not to any great extent burned as an energy-giving substrate in most of the bodies tissues, they are converted to glucose and can, therefore, support blood sugar levels and CNS metabolism over long periods. The "skin-and-bone" appearance of prisoners is clear evidence of the conversion of muscle protein to blood sugar that holds life in such unfortunates.

Consciousness and muscle activity

Muscle activity and running speed is closely coupled to the rate of synthesis of ATP in muscles. Fuels giving the most rapid rates of energy production are found in limited amounts. Long-term exercise must be supported through the use of fat as an energy source. Since lipid metabolism is relatively slow, long-term activities must progress at slower rate than high speed short-term activities.

The following figure shows the change in the choice of oxidative substrate during exercise continuing for several hours. We assume that the person described here starts the work period

Energy Sources in Working Muscle
Time Interval and Choice of Substrate
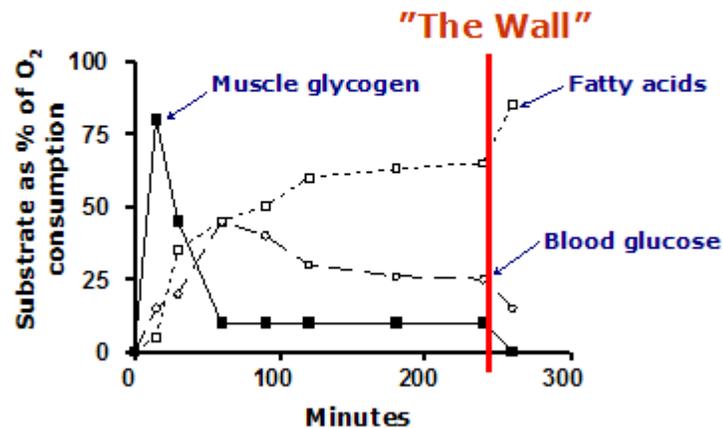
eagerly, using stored glycogen at the beginning of the work session. Muscle glycogen remains the major source of energy during the first half-hour. After this period we see that fatty acids and blood glucose take over as major energy sources since muscle glycogen stores have become depleted. Glucose continues as an important energy source throughout the experimental period. Remember that muscle must have some degree of anaerobic flux (are always dependent upon some degree of glucose utilization) and that blood cells and the brain are completely dependent upon glucose as their energy substrate. Muscle activity over longer periods can reduce blood glucose levels. There is not more than approximately 20 grams of glucose in blood and extracellular fluids.

Most of the circulating glucose comes from breakdown of liver glycogen and hepatic gluconeogenesis. Adrenalin, noradrenalin and glucagon activate liver phosphorylase and initiate glycogenolysis and gluconeogenesis. These same hormones activate hormone-sensitive lipase in adipose tissue, leading to a gradual increase in the fatty acid concentration in blood. In the model shown here we see that stabilizing of energy metabolism in time depends increasingly upon fatty acids as the source of fuel. Blood glucose utilization rises and falls thereafter to a stable level which is somewhat higher than that seen at rest. Blood sugar levels remain adequate for brain metabolism so long as the liver can produce enough glucose to balance that taken up and used in the working muscles. At some point we meet "the wall" (the red line), blood sugar

levels decline; we become dizzy, miss vision and, finally, collapse. Blood sugar levels have fallen below that needed to maintain CNS activity! This occurs because we are built such that muscle activity can exceed the rate of hepatic gluconeogenesis. The brain and retina, which are totally dependent on adequate levels of blood sugar for function, can no longer perform normally. When blood glucose levels fall below about 2-3mmol/l we lose consciousness (comatose before being eaten by a tiger?). Recovery does occur, but this takes time: lactate, amino acids and to some extent glycerol must be converted to glucose to normalize blood sugar levels.

Note that sudden increases in muscle activity such as spurting to the "finish line" in a race must be fuelled by anaerobic metabolism (that is, glycolysis from glucose or glycogen to lactate). This can result in a very rapid uptake of circulating glucose and quickly lead to a marked fall in the level of blood glucose. The trick in winning races is to balance carbohydrate "burning" with glucose production. That is, one must work at an intensity that gives maximal performance without a significant drop in blood sugar level before the finish. One problem with this is that our daily condition varies; knowing where today's "wall" is placed is something that we must "feel".

The Winter Olympics 2010; the 50 kilometer ski race.

A very striking example of carbohydrate use and physical exertion can be seen in the following pictures from this race. The main company of competitors managed to pace themselves very well. After 49.5 kilometers they still hang together in a tightly placed group. Then, the spurt to the finish line separated them. In the first picture we see the earlier leader had fallen. He then got up again but missed the lead. One can only guess that he had a reduced blood sugar level and was less observant than he should have been. He may just have "reached the wall".

In the next picture we see the very happy winner just at the finish line. While he was extremely happy you can be sure that he was "painfully" aware of his enormous anaerobic exertion. Both muscle glycogen and blood glucose had been converted to lactic acid.

Two or three seconds after finishing he collapsed and breathed extremely rapidly. And, as we can see from the third picture, his eyes rolled upward and he was near to losing consciousness.   After a few minutes he was on his feet and "back in business" though dead beat!

So, what do we see here? The secret of winning this kind of long-term race is to go a rapidly as possible without exhausting muscle glycogen and blood sugar. Oxidative glycolysis and fat metabolism must cover energy requirements during the race's sustained long distance period. Long training is really essential here! The final spurt requires maximal speed and endurance. Anaerobic metabolism covers the extreme energy requirements during that short period but with the danger of exhausting muscle carbohydrate stores (glycogen) with ensuing hypoglycemia. Anaerobic glycolysis yields large amounts of lactic acid. This must be converted to CO_2 and "blown off" during those final and painful minutes after the race.

NB: All three photos; Dag W. Grundseth, Aftenposten 06.03.2010

Muscle Fiber Types; Genetics and Performance.

Our skeletal muscles account for 40-50 % of normal body weight. These are comprised of three main fiber types:
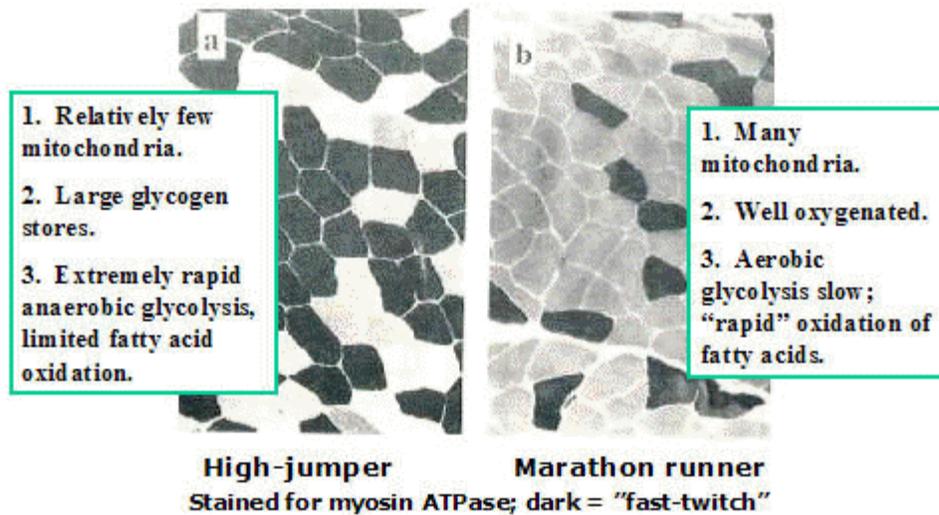
Type I   Slowly contracting red fibers in which aerobic metabolism dominates.

Type IIA   Fibers of intermediate contractility where both anaerobic and aerobic processes are active.

Type IIB   Rapidly contracting white fibers where anaerobic metabolism is the major energy supply.

The organization of fibers in each of us is determined genetically. A physiologist I knew used to say "give me a leg muscle biopsy and I'll tell you whether you should be a sprinter or a marathon runner"". In other words, the composition of fibers in our muscles determines how fast and how long we can run. People with a large proportion of type I fibers are not especially fast runners, but can continue activity

Quadriceps Fiber Composition in Athletes

1. Relatively few mitochondria.

2. Large glycogen stores.

3. Extremely rapid anaerobic glycolysis, limited fatty acid oxidation.

1. Many mitochondria.

2. Well oxygenated.

3. Aerobic glycolysis slow; "rapid" oxidation of fatty acids.

High-jumper   **Marathon runner**

Stained for myosin ATPase; dark = "fast-twitch"

Modified from: K. N. Frayn, Metabolic Regulation, a Human Perspective, 2 ed. page 106

far longer than those with a dominance of white fibers. The latter are quicker, but they drain their carbohydrate stores much earlier. Careful choice of parents is mandatory!

The figure above, taken by permission from Professor Keith Frayn's "Metabolic Regulation, a Human Perspective" illustrates this. Fast twitch fibers are darkly stained in these sections. The marked difference between muscle types in two athletes can be clearly seen:

1. High jumpers, weight lifters and sprinters perform almost explosively. Their competitive presentations are over in a minute or less. For top performance, they must have a dominance of type IIB "fast twitch" fibers in the muscles involved. They utilize phosphocreatine and anaerobic glycolysis from muscle glycogen and blood glucose to supply energy for their efforts.

2. Marathon runners use mitochondrial ATP production as their major source of energy in a competitive event. They are dependent upon a good portion of "slow twitch" type I fibers. That is, they use oxygen-supported lipid and carbohydrate oxidation to support their activity. They can run for hours, but at much lower rates than sprinters.

Key Skeletal Muscle Enzymes

Fiber Type	Hexokinase	Phosphofructo-kinase	Citrate Synthase	Carnitine palmitoyl-Transferase
	Enzyme Activity, µmol/min/g			
I (slow)	1.6	20	23	0.6
IIB (fast)	0.6	96	10	0.1
IIA (intermediate)	1.5	72	35	0.7

If we look at key enzymes in these muscle types we can easily understand the basis for the differing metabolic function. In the first table we see enzyme values in "normal" muscles. Two key enzymes from anaerobic metabolism, hexokinase and phosphofructokinase and two from aerobic mitochondrial metabolism are shown here (data from Newsholme and Leech, 1992). The rate-limiting step in glycolysis starting from glycogen is that catalyzed by phosphofructokinase (PFK). We can clearly see that type IIA and IIB fibers have a far greater PFK activity than type I fibers. This permits a rapid glycolysis with formation of ATP and lactate in these fibers until the substrate is used up. In contrast to this, type I fibers have higher levels of citrate synthase (the beginning of aerobic handling of pyruvate) and of carnitine-palmitoyl transferase, the enzyme which is the starting point for aerobic metabolism of fatty acids. Note that all of these fiber types have hexokinase activity and that this is highest in the slow and intermediate fibers. All types of human skeletal muscle can utilize blood glucose under stress.

Now, perhaps the most interesting part of this story is the effect of training on the levels of these enzymes. Newsholme led subjects through a vigorous conditioning period and then took muscle biopsies. The most striking finding here was that one

Key Skeletal Muscle Enzymes
After Training

Fiber Type	Hexokinase	Phosphofructo-kinase	Citrate Synthase	Carnitine palmitoyl-Transferase
	Enzyme Activity, μmol/min/g			
I (slow)	2.4	24	41	1.2
IIB (fast)	0.7	88	18	0.2
IIA (intermediate)	4.1	58	70	1.2

trained up the aerobic system; phosphofructokinase activity was relatively unaffected following the training period. Citrate synthase and carnitine-palmitoyl transferase activities were approximately doubled in all fiber types. Hexokinase, which is essential for use of blood sugar, was also markedly increased in both type I and type IIA fibers.

This coordinates well with previous work showing that training boosts muscle mass and the capillary bed surrounding the conditioned muscles. This increases gas exchange and supports the rise in mitochondrial and oxidative capacity.

"On the move for the sake of science"

The most striking picture of the effects of training on muscle metabolism that I am aware of can be found in National Geographic, September 2000 in an article entitled "The Unbeatable Body: Pushing the Limit". A simplified picture and explanation follow.

"On the move for the sake of science, subjects in a Yale University fitness study are measured against each other. Thirty-seven-year-old Rich (left) leads in VO2, that is, maximal oxygen uptake. He uses less anaerobic metabolism while running. 60-year-old Larry (center), has a reduced VO2 and narrower arteries than Rich, in spite of regular training. His diminished condition is related to the aging process. 35-year-old Salvatore (right) leads a sedentary life that negatively affects his cardiovascular and respiratory fitness. His VO2 is the lowest of these three. He could not run on the treadmill but had to "walk" the "experimental hour". His arteries are visibly smaller. The yellow colors in his leg show that his muscles had to work anaerobically to complete an hour on the treadmill *. Click on the thumbprint below for more graphic details.

The point? Our bodies are programmed to

exercise.

* The color scale also shows the relative use of aerobic (blue) and anaerobic metabolism (yellow). Interested readers are urged to go to the original article for insight in muscle metabolism, blood flow and condition. You can download a more informative version of this

figure from National Geographic by clicking on the thumbnail. Be patient, the file is large and downloading takes time.   Important physiological data are included in this figure. The men to the left are 37 and 60 years old respectively. Both train daily and completed the one hour running period on a treadmill. The younger man ran faster and longer. If you examine the date you will see that the older man had a reduced vital capacity and iliac artery diameter. These are natural effects of the aging process. Salvatore, at the right, was only 35 years old. He was a sedentary worker and followed no training program. His vital capacity and iliac artery diameter (and presumably blood flow to his legs) were the lowest of these three men. He walked through the exercise period. We need to be physically active to maintain good health, strength and the ability to work!

"Our bodies are programmed to exercise"

This quote from the article above underlines a major point that has been all to often forgotten in modern times. Physical condition is dependent upon daily exercise.

Figures from two studies that have emphasized this follow. In the first of these we can see

# Effect of Training and Inactivity on mitocondrial Enzyme Activity
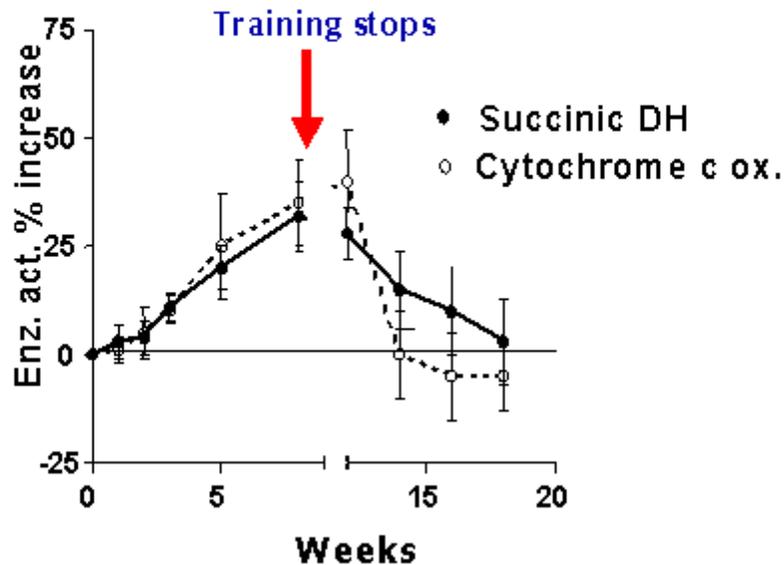
increasing succinic dehydrogenase and cytochrome c oxidase activity found in biopsies taken during a 5-week training period. These are key enzymes in the mitochondrial production of ATP from carbohydrates and lipids. The volunteer's conditioned limbs were then held more or less inactive during the following 5-week period. The trained muscles soon lost the conditioning effect of training. Cytochrome c oxidase levels fell below normal! This emphasizes the fact that daily activity is the best way to keep fit.

Most experts suggest that walking between 30 minutes to a hour daily is necessary for good fitness and health, Gym visits are fine, but daily activity seems to be even more important.

Another study showing the effects of training on citrate synthase is shown in the next figure. This

## Training and mitochondrial capacity
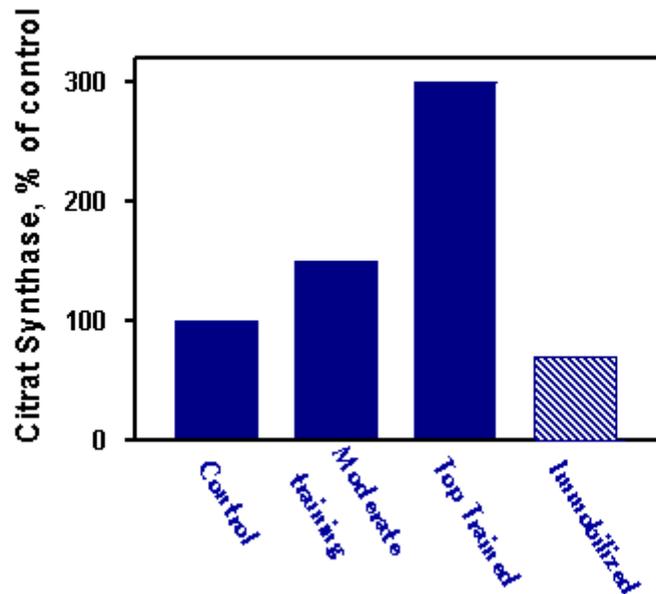
is the "entrance" enzyme to aerobic metabolism for both lipids and carbohydrates. Once again, we can see that training increases enzyme activity and mitochondrial oxidative capacity. The striking point here is that immobilization reduced enzyme activity markedly. All of us that have gone with a cast for weeks have experienced loss of muscle mass and the pronounced weakness that follows and that this figure indicates.

I may perhaps be accused of overdriving, but once more will I emphasize that the global surge of overweight with the illnesses that this brings with it is the result of reduced exercise and training. The urban life style that is being adopted by ever-increasing numbers is cause of world-wide poor health. Lack of motion leads to muscle weakness which leads to even less motion...

Canadian Indians exercise less too.

We can see another example of the effects of modern life on Canadian Indians. These people

Maximal Oxygen Consumption
Canadian Inuits 1970-1990
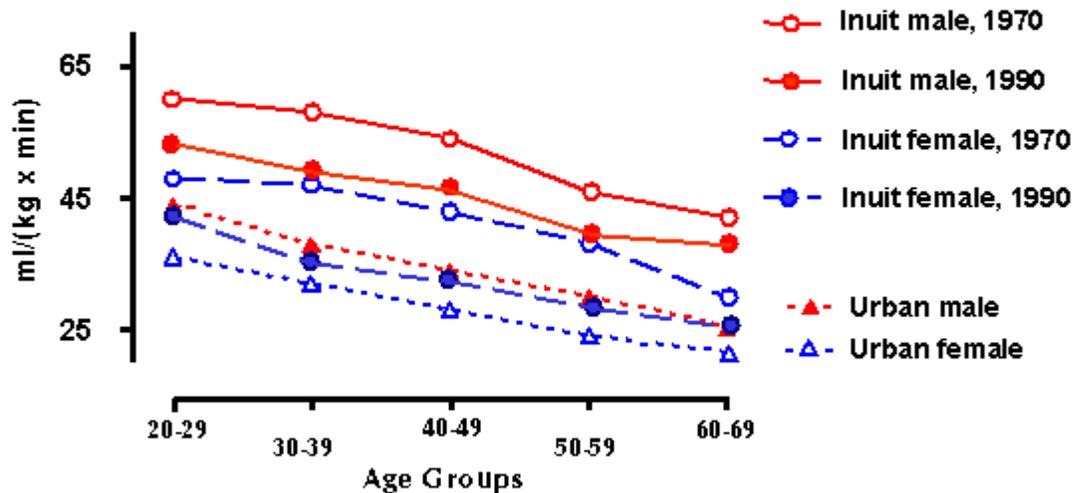
L. Cordain et al, Int. J. Sports Med 19 (1998)

live in the Hudson Bay area. There is a film about their modern life made in the late 1920s entitled Nanuk of the North. Even at that time, they were much less active than previously. The data I have is from 1970-1990. One measured maximal oxygen uptake in women and men of differing age-groups. As would be expected, capacity decreased with age, and was larger in men than women. The striking here is that even among these people who live in a simple but demanding milieu, we find a decrease in physical condition.

The triangles give values for urban men and women. The men have a vital capacity approximately identical to that of the 1990 Indian women while the vital capacities of urban females were significantly lower.

Fitness in native and industrialized societies

mI/(kg × min)

70
60 — Excellent fitness
50 — Average fitness
40
30 — Poor fitness
20
10
0

Inuit
Bushman
Venezuelian Indians
New Guinea Lumi
Mexican Indians
Finnish Sami
Tanzanian Masai
Modern 20-29 years
Modern 30-39 years
Modern 40-49 years
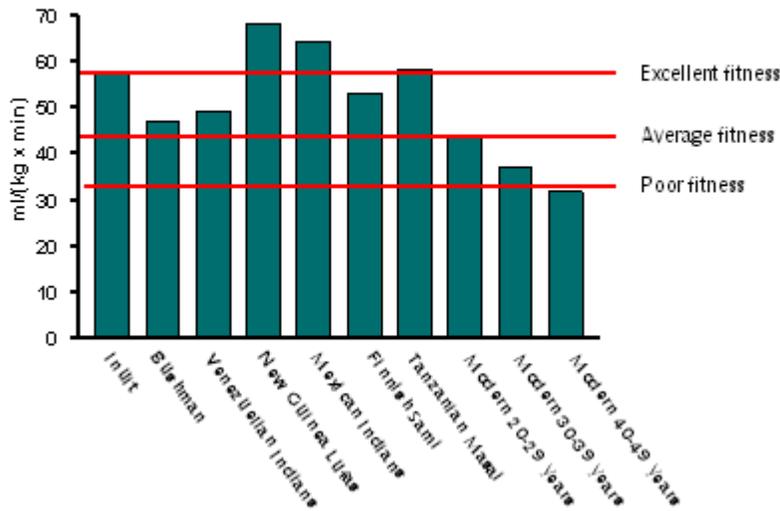
L.Cordain et al. Int. J. Sports Med 19 (1998)

There are, in fact, many studies that take up the differing fitness of native and urban people. Another that I value is shown in the next figure. Here, the authors have looked at oxygen uptake in native people around the world and compared these values with city people in differing age groups. The vital capacity of modern urban men was defined as "average fitness". Clearly, vital capacity declined among urban people with increasing age, with "poor fitness "being characteristic for older city people. None of the native groups fell under "average fitness". Go to the original article for details.

What are the sources of energy in working muscles?

Muscle has several possible energy substrates and these are listed in the next table. (The data in the following three tables are from R.W. McGilvery, <u>Biochemistry, a Functional Approach</u>, W. B. Saunders, Philadelphia 1970).

ATP <u>IS</u> the "currency" of energy metabolism. Muscle contraction, that is coupling between actin

Energy Sources in Working Muscle

	ATP	Creatine Phosphate	Anaerobic Glycolysis	Aerobic Glycolysis	Fatty acid Oxidation
Amount	5 mmoles/kg	17 mmoles/kg	350 g Muscle Glycogen	440 g Glycogen Muscle/Liver	9000-15000 g
Total ~P available (mmoles)	220	660	6700	84000/19000	>4,000,000
Maximum Rate of ATP synthesis	-	73 mmoles/sec	40 mmoles/sec	17/6 mmoles/sec	7 mmoles/sec
Duration		4 seconds	2-3 minutes	1-2 hours	Many hours
Comments		Most rapid ATP source	Rapid, limited by H^+ formation and substrate amount	Rate limited by O_2 and pyruvate transport to mitochondria	Slow, ATP/O_2 ratio lower than carbohydrates. Slow FFA transport

and myosin is powered by ATP (and ONLY ATP). There is only a small lager of this material in muscle cells but this is backed up by several buffer systems. The most rapid of these is the creatine phosphate/creatine phosphokinase system. This is also the smallest reserve and at maximum utilization it is exhausted in about 4 seconds (newer data suggests 30 seconds). This is a major source of high-energy phosphate for sprinters. The next largest energy source is anaerobic glycolysis. Only glycogen stored in muscles and blood glucose can serve as substrates for anaerobic glycolysis. In quantity, aerobic glycolysis follows, being able to supply enough energy for muscle activity over several hours (dependent upon intensity). Fatty acid oxidation has the largest ATP-producing capacity. This is relatively slow but can produce energy over many hours if work intensity corresponds to the rate of ATP production. It is fascinating to note that the most rapid sources of energy are also the most limited. This simple fact underlies the common observation that running speed falls off with the duration of a race. We can step up the running rate, exceeding the ATP delivery rate from aerobic metabolism even when glycogen reserves are used up. Muscle then takes glucose from the blood. The problem with this is that blood sugar levels then fall and we lose consciousness. To work at a maximum exertion over time, the load must be in step with aerobic energy production.

Energy Source, 100 Meter Sprint

ATP utilization ca. 60 mmol/kg			
	At start	After race	Net ATP used
	mmoles/kg		
ATP	5	4	1
Creatine Phosphate	25	7	18
Glycogen*	56	42	42

* As glucose

We can look at two examples of this, where the work intensity has been adjusted according to the estimated duration of a race.

A 100 meter sprint is the first case. Here, speed is maximal and the runner has drawn on his ATP pool, creatine phosphate and glycogen to replenish the high-energy phosphate used in muscle contraction. This is an extreme example and a decrease in ATP is seen. A prominent observation is that ATP decreased only 20% in spite of the physical effort. Most of the energy used came from the ATP-buffer systems creatine phosphate/creatine kinase and anaerobic glycolysis. Around 14 mmoles of glycogen (as glucose) times 3 (3 ATPs from each 6-carbon fragment from glycogen) gave 42 ATP from glycolysis. Eighteen ATPs came from creatine phosphate.

The next example is from an experimental situation which resembles a marathon. Here, the runner had to keep going for about three hours. The speed in this kind of a race is quite a bit lower than in a sprint. It is based on aerobic metabolism which gives us a "slow" but constant flow of ATP production coupled to $O_2$ reduction. During the first two hours carbohydrates (blood glucose and glycogen).   With time there was a switch in the substrate utilized. A gradual stimulation of lipolysis led to increases in circulating fatty acid levels and a concurrent increased use of fatty acids as the substrate for aerobic metabolism.

Energy Sources in Working Muscle
"Maraton"

Running time, minutes	% Contribution to O_2 Uptake		
	Glucose	Fatty acids	Muscle glycogen
40	27	37	36
90	41	37	22
180	36	50	14
240	30	62	8

From Felig and Wahren, 1975

To summarize, creatine phosphate and anaerobic glycolysis supply energy for intense, short work sessions while aerobic metabolism of both carbohydrates and fat supply energy for longer work sessions. So, if we could just build up creatine phosphate reserves we really could run fast and forever? No, in spite of many many advertisements this does not work. Click here if you want to know more about creatine supplements.

The teenage weakling; glycogen storage disease

Clearly, the oxidation of sugar and glycogen in anaerobic metabolism is a very important contributor to the energy supply of skeletal muscle. Mutations of the enzymes in glycolysis can inhibit this system. In the following case we will see that reduction in the level of phosphofructokinase, the pace-setting enzyme in glycolysis, leads to muscle weakness.

"The Teenage Weakling"
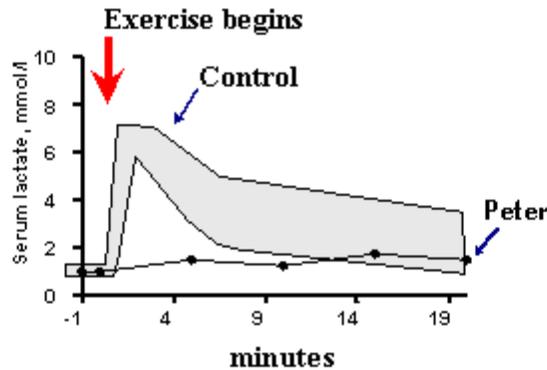
	Glycogen mg/g	G-6-P µmol/g	F-6-P µmol/g	F-1,6 Bis fosfat, µmol/g
Peter	43.8	9.2	1.6	0.02
Control	10	0.5	0.1	0.6

· Biochemistry for the Medical Sciences, Higgins, Turner and Wood, 1994

"Peter" had a long history of muscle weakness. He was more or less normal while resting, but experienced severe muscle pain under hard work. The figure shows serum lactate accumulation during exercise in a control group and in Peter's case. While there was an abrupt production of lactate in the control group, this was absent in Peter's instance.

Analysis of a muscle biopsy clearly demonstrated that Peter had much higher glycogen, G-6-P and F-6-P levels than control persons. Furthermore, he had very low levels of fructose 1,6 bis phosphate. This metabolic "crossover" is indicative of a lack of phosphofructokinase, the enzyme which catalyses conversion of fructose-6-phosphate to fructose 1.6 bis phosphate. This enzyme is essential and rate-limiting for ATP production in anaerobic glycolysis. Peter's case demonstrates the important role of anaerobic glycolysis. He was unable to utilize muscle glycogen or blood glucose as an energy source. Here, aerobic oxidation of fatty acids (an almost unlimited but slow process) had to drive production of skeletal muscle ATP.
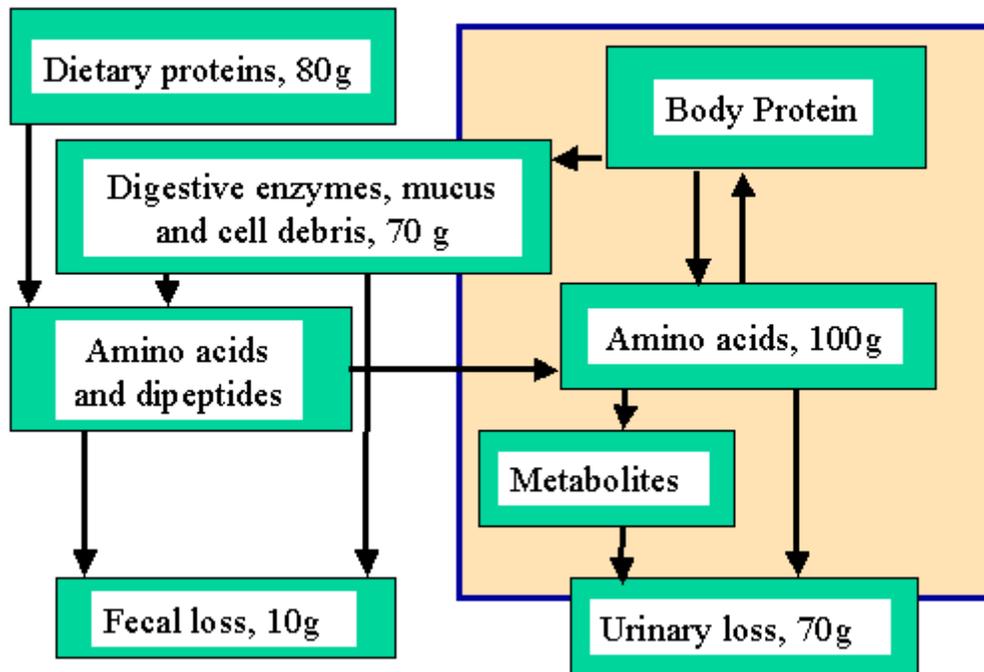
The present case presents one of several forms of glycogen storage disease, mainly affecting skeletal muscle. Other forms affect both liver and/or muscle and can be fatal.

Should I Eat More Protein?

The undeniable fact that muscles are very rich in protein has led to a huge dietary protein supplement industry. A quick internet search gave 74,023 pages with stuff about this. Body-builders and others who desire impressive bodies eat massive amounts of protein-rich products and amino acid supplements to support their muscle-building activities. This seems very logical, but has no basis in well-known facts about protein nutrition.

Arguments about the need for proteins in the diet begin with what we call nitrogen balance. This is the difference between protein intake (our daily source of nitrogen-containing compounds) and loss of nitrogen in feces and urine. Also involved is the

Overview of Protein Metabolism

Dietary proteins, 80g

Digestive enzymes, mucus and cell debris, 70 g

Amino acids and dipeptides

Body Protein

Amino acids, 100g

Metabolites

Fecal loss, 10g

Urinary loss, 70g

David A. Bender, Introduction to Nutrition and Metabolism, 2.ed., 1997

large amount of cellular debris and digestive enzymes which we process in our intestines. As you can see from the figure, the normal situation is that intake and loss are more or less equal; nitrogen balance lies at "0". We have no storage form of amino acids. Synthesis of new protein is supported by the amino acid "pool" in blood and extracellular fluid. This amounts to around 100g. There are many studies and reports that have calculated the "dose" of protein needed to support maintenance of the body under a variety of conditions. One of the most extensive is from the National Academy Press, Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients) (2002). Chapter 10 of this report takes up protein and amino acid metabolism for almost all ages and life conditions. It can be read gratis online but is very long and technical. The main conclusion is that normal adults require about 0.8-1.0 grams of dietary protein per kilogram of body weight per day. Adolescent athletes require most protein and several studies suggest between 1.5 and 2 g/kg/day for this group. Body-builders and some athletes take up to 4g/kg of protein daily. The use of such high protein intake levels is not medically recommended nor supported. Excessive protein (more than 2g/kg/day) is often associated with dehydration, urinary calcium loss and inadequate carbohydrate intake. This can lead to weight loss. A summary of current knowledge can be found in a Medscape article; click here. Amino acids (and therefore proteins) are not good energy suppliers either. Muscles can utilize branched-chain amino acids as an energy source, but they must be transaminated and the amino groups converted to urea in the liver. This is a slow energy-

demanding process. Conversion of amino acids to glucose has the same disadvantage; formation of urea brakes gluconeogenesis.

Once again, there is no basis for the enormous protein supplementation market that has evolved during the past decade! A good, normal diet with a variable composition gives all the protein that is required for a physically active life.

How do I choose that diet?

Origin of nonessential amino acids

Alanine	from pyruvate
Aspartic acid	from citric acid cycle intermediates
Asparagine	
Arginine	
Glutamic acid	
Glutamine	
Proline	
Serine	from 3-phosphoglycerate
Glycine	from serine
Cysteine	from serine (Sulfur from methionine)
Tyrosine	from phenylalanine

Baynes and Dominiczak, Medical Biochemistry, Mosby 1999

Proteins are built up of chains of amino acids that are freed and adsorbed in the intestine. These are divided into groups; nonessential amino acids that we can make ourselves and essential amino acids that we cannot synthesize and must eat. These essential amino acids are present in varying amounts in body proteins. Therefore, the physiological state of the organism in question is an important factor in establishing just how "essential" these different amino acids are. If you look at the following tables you will see that cysteine is among the non-essential amino acids. We can make it from serine, also a non-essential amino acid. BUT, we must have methionine, en essential amino acid, for synthesis of cysteine. Things are not so easy!

The essential amino acids are "essential" according to the situation the body experiences. Starved rats must have nine different essential amino acids to gain

weight. They do not have to eat the "essential" amino acid arginine. They have enough arginine in their own "amino acid pool" to build up their bodies without arginine in their diet in short periods.

What is "essential" on a daily basis does not necessarily include all of the amino

Essential amino acids

"Standard" list	For weight gain in protein-starved adult rats	For positive nitrogen balance in adult humans
Phenylalanine	Phenylalanine	Phenylalanine
Valine	Valine	Valine
Threonine	Threonine	Threonine
Tryptophan	Tryptophan	Tryptophan
Isoleucine	Isoleucine	Isoleucine
Methionine	Methionine	Methionine
Histidine	Histidine	
Arginine		
Leucine	Leucine	Leucine
Lysine	Lysine	Lysine

G. I. Zubay, Biochemistry, 1998, Wm. C. Brown, N.Y.

acids that we cannot produce ourselves. Normal human adults do not need arginine or histidine in their short-term diet to hold a positive nitrogen balance.

So, how do you choose a protein source? The problem can be expressed as "how do I cover my need for essential amino acids? The reason for this is that every protein we make has a fixed composition. If we have all the amino acids (building blocks) we need but one, the protein will not be made! All the pieces have to be available at the right place and the right time.

Proteins in food differ in their amino acid makeup.   This variation results in differing

Biological Value of Some Protein-Rich Foods

	Ile	Leu	Val	Thr	Met + Cys	Trp	Lys	Phe + Tyr	His	Biological Value
Egg, chicken	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0	0,94
Milk, human	1,1	1,4	1,0	1,0	1,1	1,6	1,0	1,0	0,9	0,95
Milk, Cow	1,1	1,3	1,0	0,9	0,7	1,3	1,3	0,9	1,1	0,90
Muscle, beef	0,8	0,9	0,7	0,9	0,9	0,9	1,4	0,7	1,6	0,76
Soybeans	1,0	0,9	0,8	0,8	0,6	1,3	1,1	1,0	1,4	0,75
Rice	0,8	0,9	0,9	0,8	0,9	1,2	0,5	1,2	0,8	0,75
Wheat	0,6	0,8	0,6	0,7	0,8	1,1	0,4	0,8	1,0	0,67
Potatoes	0,6	1,1	0,8	1,3	0,6	1,9	1,4	0,8	1,1	0,67
Oats	0,8	0,8	0,8	0,7	0,6	1,2	0,6	1,0	1,1	0,66
Corn	1,0	1,7	0,8	0,7	1,1	0,5	0,4	1,0	1,0	0,60

McGilvery, Biochemistry, 1970

"biological" valves as amino acid sources. Food types that have proteins most like our own are most "valuable". Once again, it is the essential amino acid content that determines a food's biological value.   Chicken egg protein has a high biological value for humans. In the following table chicken egg essential amino acid content is set at 1.0. The biological value of egg protein is 0.94. That is, this protein source gives an almost perfect mixture of amino acids as a substrate for production of human proteins. Human milk has just about the same rating. Cow milk has a somewhat lower methionine and cysteine content; its biological value is, therefore, a bit lower than chicken egg and human milk. Similarly, beef has a relative lack of phenylalanine and tyrosine, with a corresponding lower biological value. The result of this is that one must eat more beef muscle than chicken egg to obtain the same amount of essential amino acids.

Vegetables and cereals as a group have lower biological values than animal meat, milk and eggs. We can see that several essential amino acids are less available here. Vegetarians must eat more protein than meat-eaters to obtain the amino acids they need for body maintenance.

It is because of this difference in the amino acid composition in food that we should strive after variation in the daily food source. The recommendation of around 0.8 gram protein/kg/day takes this into account. Growing and very active adolescents may have a requirement for double as much protein in periods. However, adults do not need more protein than they get in a normal diet. Increasing physical activity increases appetite and protein intake is therefore enhanced accordingly. Supplement

with large amounts of protein mixtures and amino acid supplements is unnecessary and can lead to illness (kidney damage)! There is no medically sound basis for the enormous "protein supplement" market!
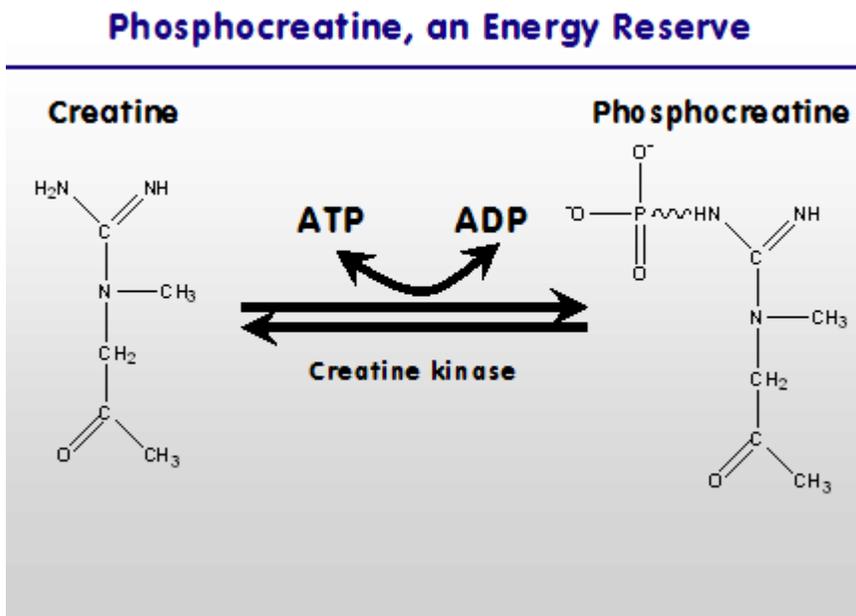
Bigger and stronger with creatine?

 A recent Internet search for creatine supplements yielded over 1,200,000 "hits". Most of these are announcements about creatine dietary supplementation aimed to build bigger and better bodies. What is this all about?

## Creatine and creatine phosphate's role in muscle metabolism.

As I have discussed earlier, all muscle work is powered by ATP. It is the power in the high-energy gamma bond that drives actin-myosin coupling and muscle contraction. In spite of this, ATP levels are unusually stable even in working muscles due to several ATP-buffering systems. Click here to review this.

The creatine phosphate-creatine phosphokinase system is the most rapid of these ATP-buffering systems. The equilibrium between creatine, creatine phosphate, ADP and ATP is summarized in the figure to the left.   ATP concentration in skeletal muscle lies around 5 mmol/kg while one finds approximately 15-20 mmol/kg of creatine phosphate. Thus small changes in the ATP/ADP ratio are quickly evened out by use of creatine phosphate.

Phosphocreatine, an Energy Reserve

Creatine — ATP ⇌ ADP — Phosphocreatine

Creatine kinase

This has been decisively shown in the human forearm through the use of magnetic resonance as shown in the next figure. Here we can see the concentrations of inorganic phosphate, creatine phosphate and the three phosphate groups in ATP. Measurements were taken at rest and during
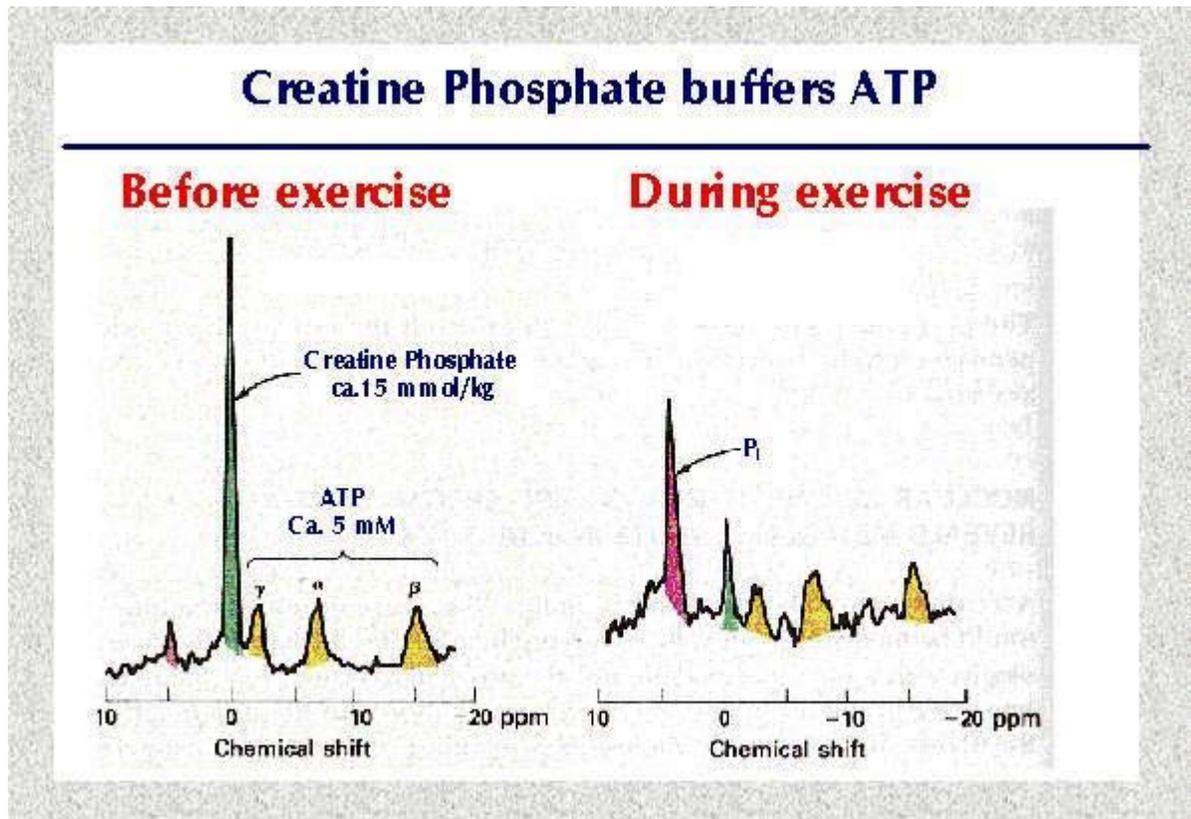
a vigorous exercise period. The yellow peaks represent ATP's phosphate groups. These remain unchanged while exercising while creatine phosphate levels were markedly reduced (green peak) and inorganic phosphate (red peak) was markedly increased. (Figure from G. K. Radda, Science 233, 641, 1986 as presented in Lubert Stryer's Biochemistry)

A logical conclusion might be that, if one could just increase creatine phosphate levels in muscles, one would be able to carry out more muscle work. The problem with this is that most studies of the effects of creatine supplementation have not shown enhanced strength. This is not surprising of several grounds. First, creatine and creatine phosphate are ionized compounds at physiological pH levels. Accumulation of large amounts of these in muscle would increase osmolarity and lead to muscle water uptake and swelling.   Secondly, creatine phosphate has an extremely rapid turnover and an eventual increase in creatine phosphate would be quickly exhausted. Current evidence suggests that creatine can possibly increase performance lasting for no more than 30 seconds. Creatine supplementation does not appear to increase muscle mass. Please go to a good review article by Paddon-Jones et al, "Potential Ergogenic Effects of Arginine and Creatine Supplementation" Journal of Nutrition 134:2888S-2894S, October 2004 for more information.

A recent (2007) internet search for "creatine" yielded more than 7,000,000 hits, most of which concern creatine as a dietary supplement. This in spite of the fact that most studies have not shown significant effects of creatine on muscle strength. The market for creatine products is very large. According to Paddon-Jones, creatine sales in the USA alone exceeded $100 million in 1998. It is tempting to use an earlier comment concerning protein dietary supplements: the use of creatine supplements represents "<u>very expensive urine</u>"!

The bottom line: there is little or no basis for dietary supplementation with creatine!