SP.235 / ESG.SP235 Chemistry of Sports

Spring 2009

SP.235 - Chemistry of Sports

Week 10: Energy Metabolism

Energy for your body

- You eat to provide your body energy
- How does your body metabolism the glucose?

Metabolism of Glucose

1 mole of glucose will be oxidized to make CO_2 and 30 ATP in the presence of O_2

Without O_2 the muscles will have incomplete oxidation to produce lactic acid and only 2 ATP, but the lactic acid can be further metabolized to produce more ATP (more on this later)

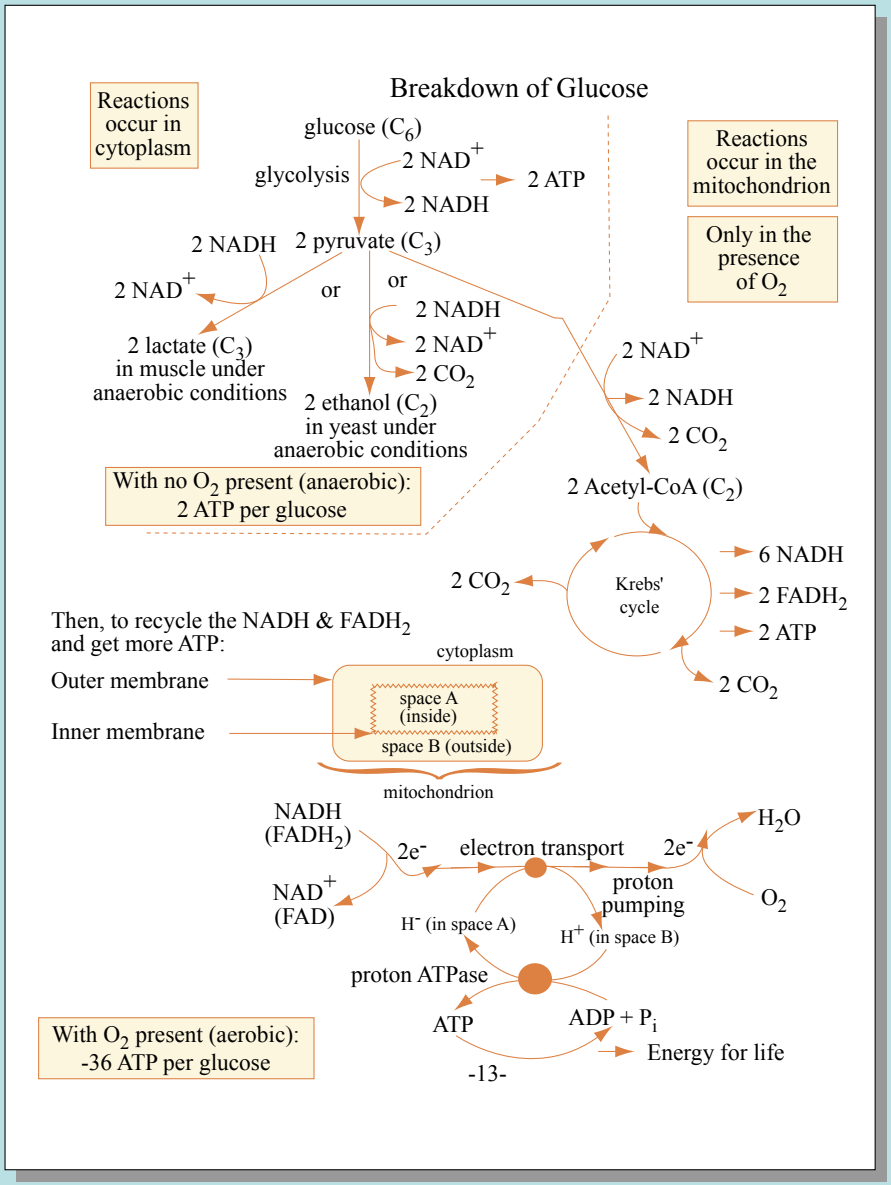
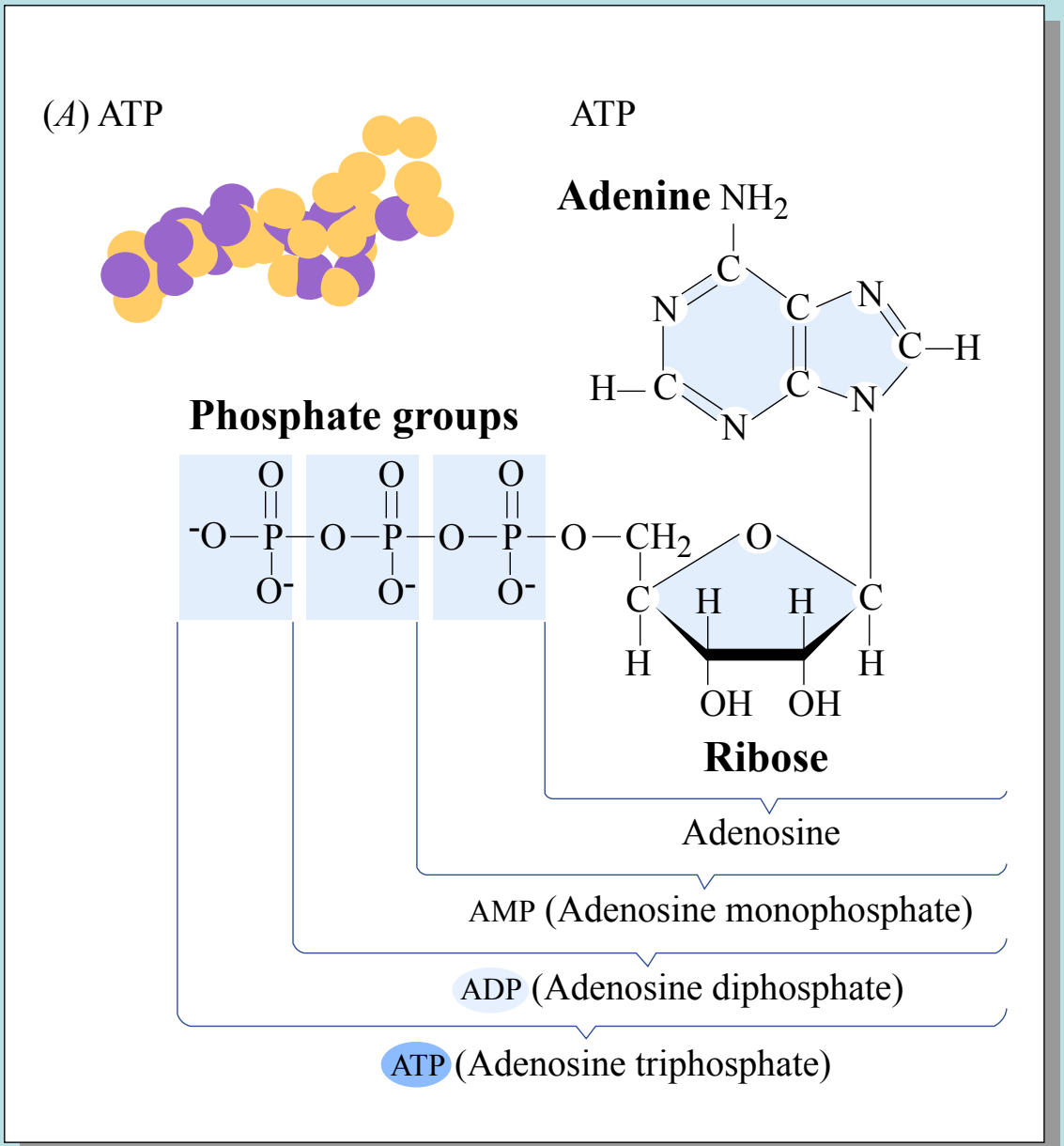


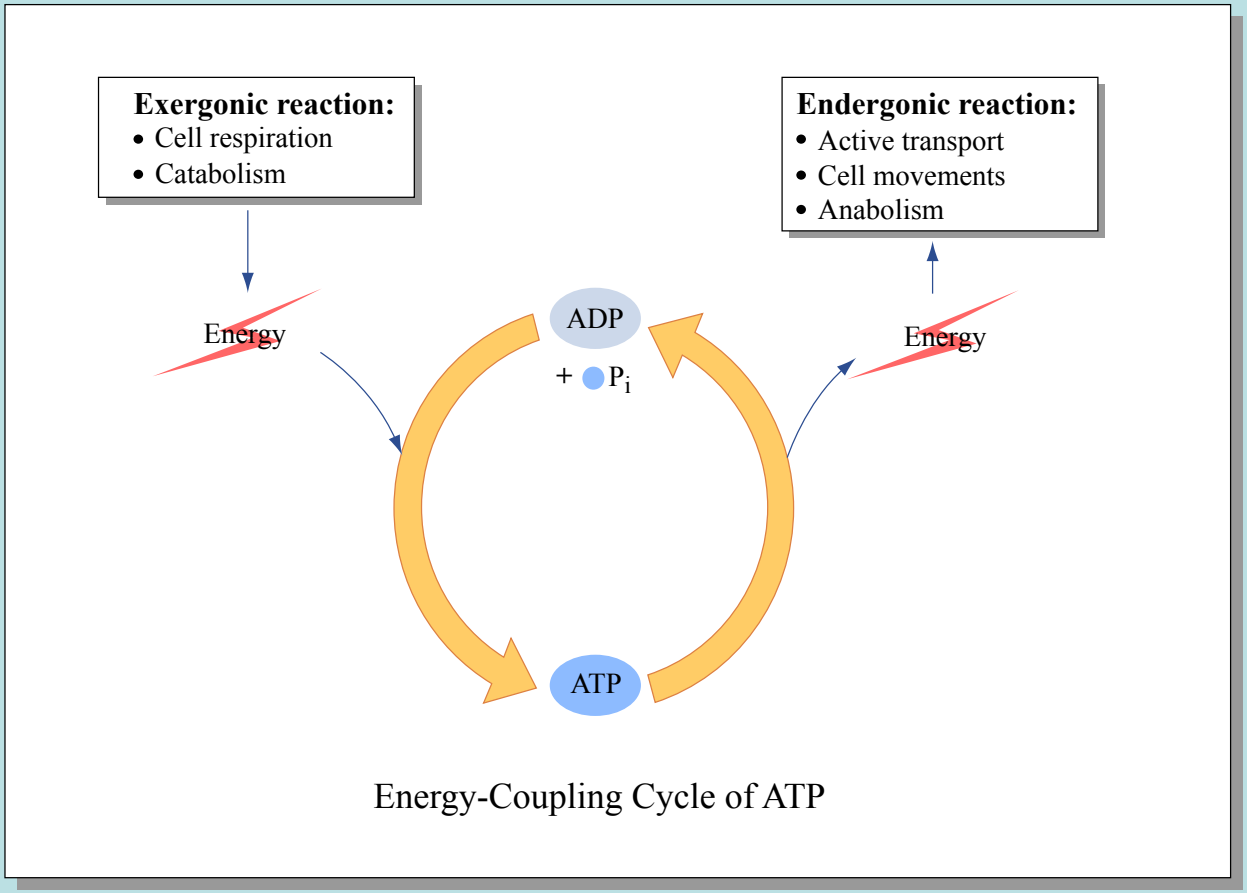
Figure by MIT OpenCourseWare. Adapted from Purves, William K., David Sadava, Gordon H. Orians, and Craig Heller. *Life: The Science of Biology*. 7th ed. New York, NY: Sinauer Associates and W.H. Freeman, 2003. ISBN: 9780716798569.

Structure of ATP

- Why is ATP used as energy currency?



Energy Coupling cycle of ATP



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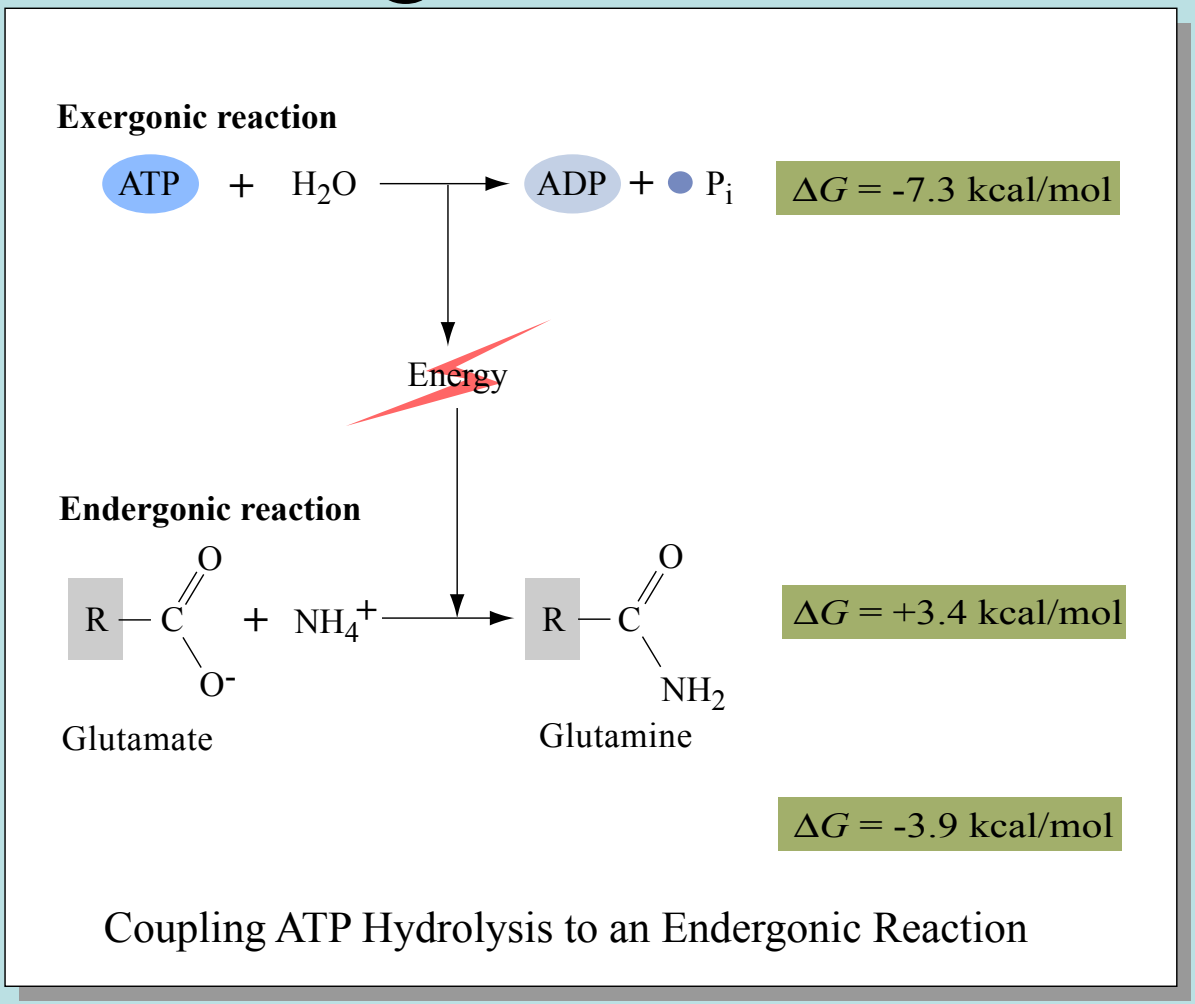
Coupling ATP hydrolysis to an endergonic reaction



Coupling ATP Hydrolysis to an Endergonic Reaction

Control and Regulation of Fuel Metabolism

- Absorptive process-food is present in the gut and nutrients are being absorbed. This period lasts until the stomach and small intestine are empty
- Postabsorptive period - the processes of energy metabolism and biosynthesis must run on internal reserves.
- Nutrient traffic must b e controlled so that reserves accumulate during the absorptive period and are used appropriately during the postabsorptive period

Liver

- The liver directs the traffic of the nutrients that fuel metabolism
- When fuel molecules are abundant in the blood, the liver stores them as glycogen and fats. (liver also synthesizes blood plasma proteins from circulating amino acids)
- When the availability of fuel molecules in the blood declines, the liver delivers them back to the blood

Liver

- Has the ability to interconvert fuel molecules
- Can convert monosaccharides into either glycogen or fats and vice versa
- Can convert certain amino acids can synthesize glucose (from some amino acids, pyruvate and lactate) through a process called gluconeogenesis (this is the science behind the Low carb diets)

Liver and Lipoproteins

- Lipoproteins are the way that the body gets the hydrophobic fats into the aqueous blood.
- Lipoprotein - a particle made up of a core of fat and cholesterol and a covering of protein that makes it water-soluble
- Largest lipoprotein is the chylomicrons (we talked about these last week - they are formed in the intestine and are how fats are absorbed from the food into the lymphatic system

Lipoproteins are synthesized in liver

- Fat has a low density, so the more fat a lipoprotein has, the lower its density
- HDL -high density lipoproteins
- LDL - low density lipoproteins
- VLDL - very low density lipoproteins

Lipoproteins are synthesized in liver

- HDL -high density lipoproteins
  - Acceptors of cholesterol (25 % composition)
  - Believed to remove cholesterol from tissues and carry it to the liver, where it can be used to synthesize bile
- LDL - low density lipoproteins
- VLDL - very low density lipoproteins

Lipoproteins are synthesized in liver

- HDL -high density lipoproteins
- LDL - low density lipoproteins
  - 50 to 60 % cholesterol, which they transport to tissues around the body for use in biosynthesis and for storage
- VLDL - very low density lipoproteins
  - Contain mostly triglyceride fats which they transport to fat cells in tissues around the body

LDL vs HDL

- LDL - bad cholesterol
- HDL - good cholesterol
- High ratio of LDL to HDL in a person's blood is a risk factor for atherosclerotic heart disease
- Regular exercise increases HDL
- Should have your LDL/HDL checked during your annual physical

Insulin and Glucagon

- Insulin is secreted by the β cells of the pancreas and it plays a major role in directing glucose to where it will be used or stored
- Insulin enhances the uptake of glucose from the blood and its conversion to glycogen by liver and muscle cells
- Insulin stimulates fats cells to take up glucose form the blood and convert it to stored fat and it stimulates cells in most tissues of the body to preferentially use glucose as their metabolic fuel

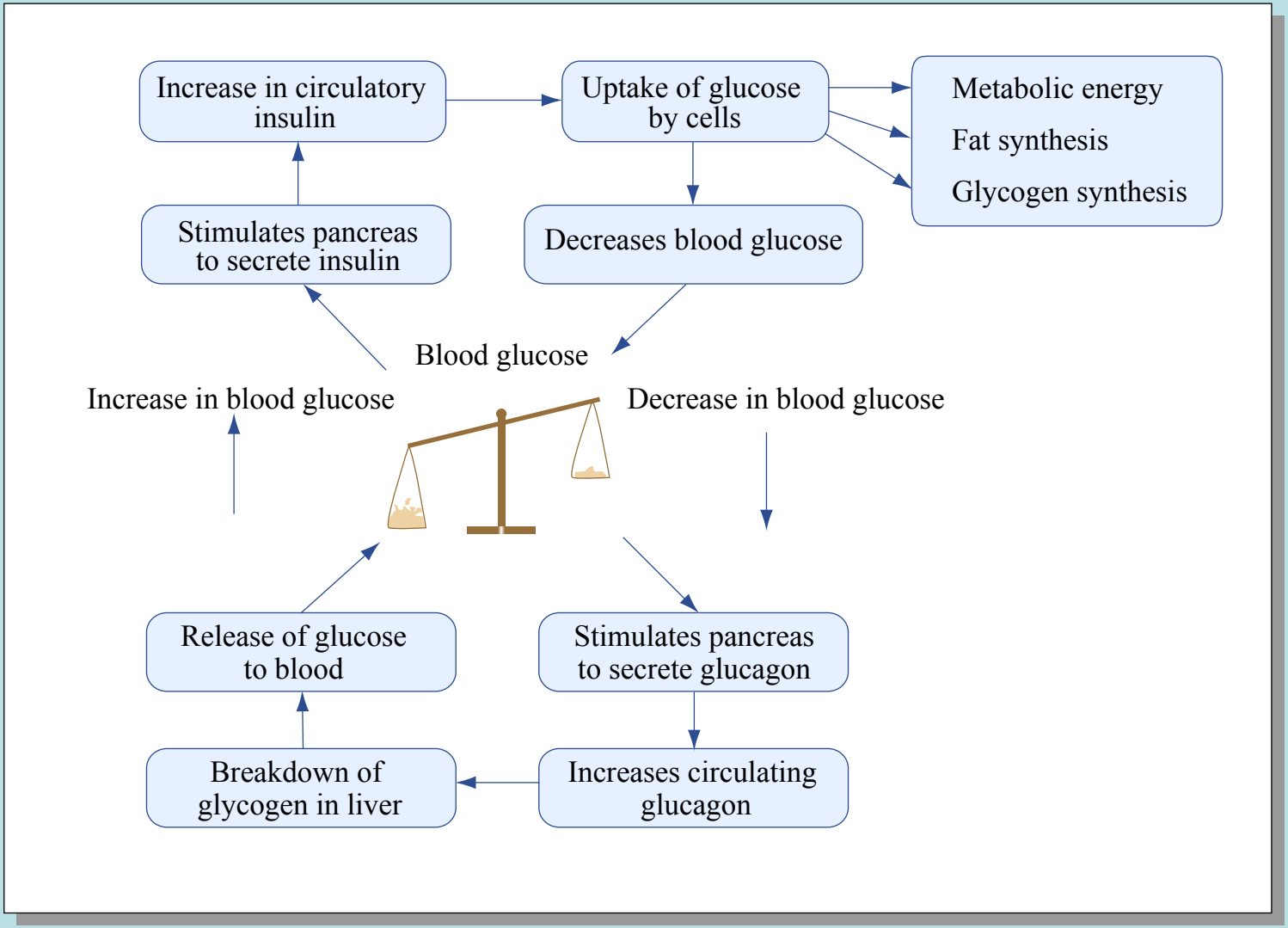
Glucagon

- Opposite affect than Insulin
- It stimulates liver cells to break down glycogen and carry out gluconeogenesis
- The liver then produces glucose and releases it into the blood

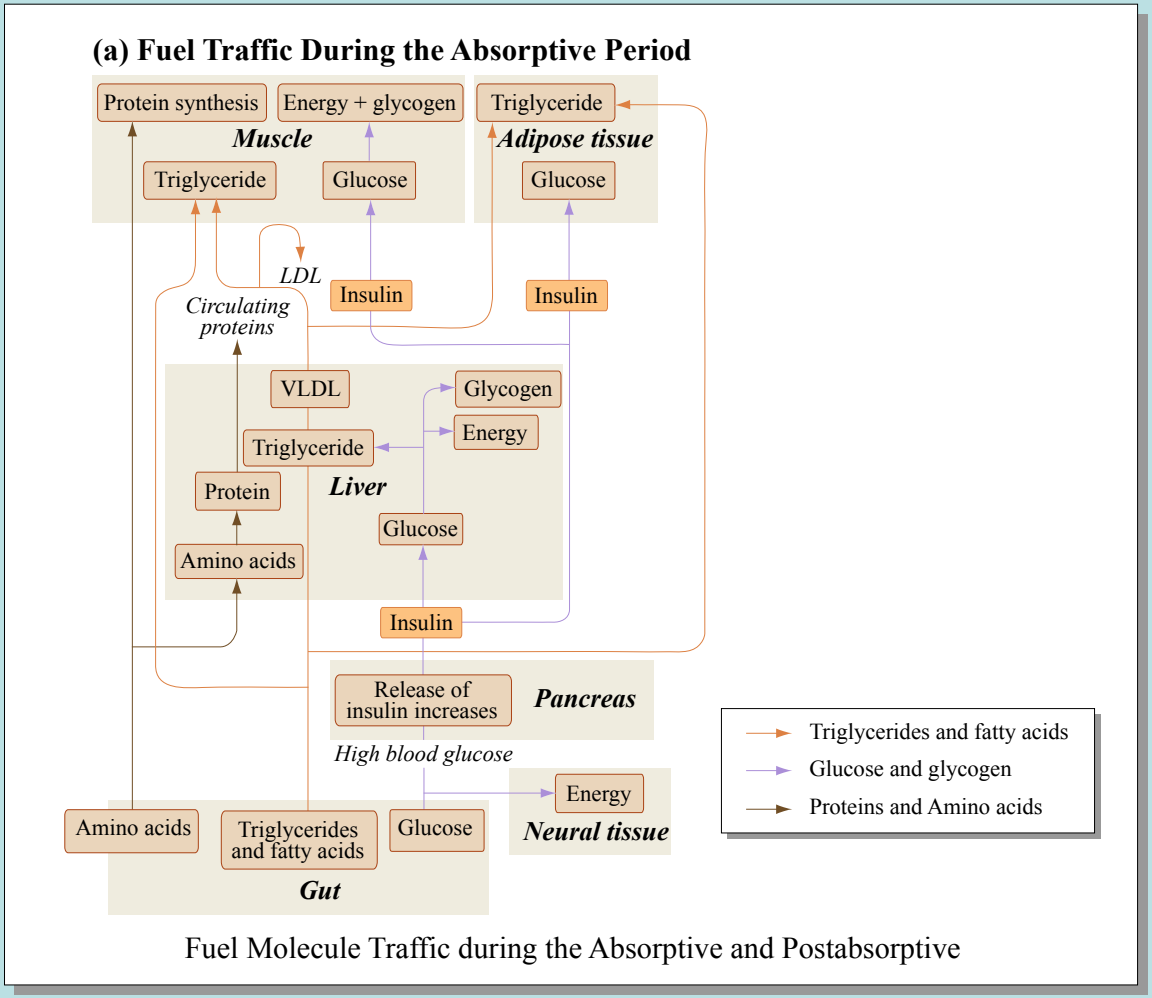
Postabsorptive period

- Blood glucose is falling, release of insulin is decreasing, uptake of glucose by most cells is curtailed
- The liver is breaking down glycogen to supply glucose to the blood, the liver and the adipose tissues supply fatty acids to the blood and most of the cell so the body preferentially use fatty acids as their metabolic fuel (except the nervous system which can only use glucose)

Regulating glucose levels in the blood



Fuel Traffic curing the absorptive period



Fuel Molecule Traffic during the Absorptive and Postabsorptive

Figure by MIT OpenCourseWare. Adapted from Purves, William K., David Sadava, Gordon H. Orians, and Craig Heller. *Life: The Science of Biology.* 7th ed. New York, NY: Sinauer Associates and W.H. Freeman, 2003. ISBN: 9780716798569.

Fuel Traffic
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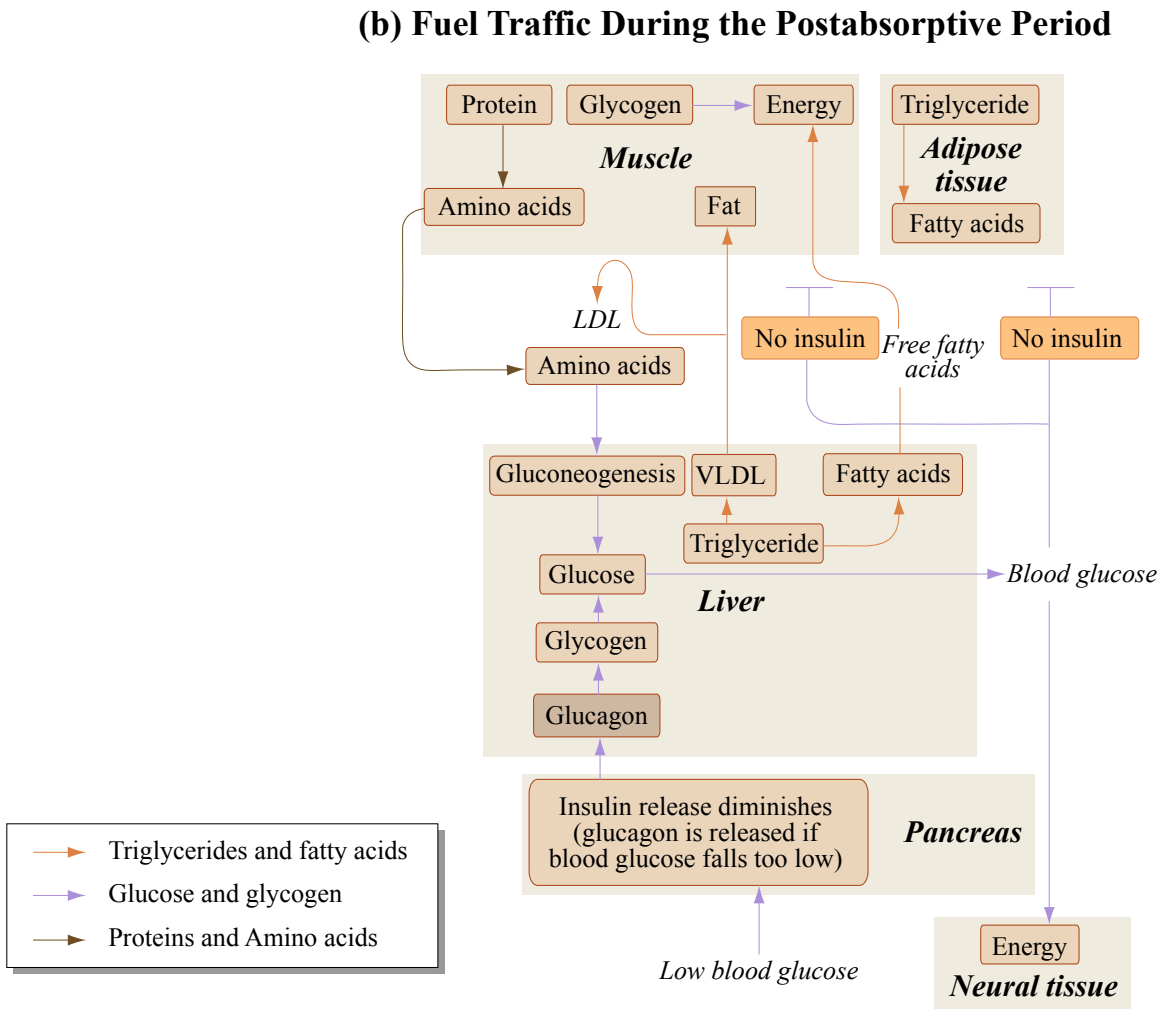

Fuel Molecule Traffic during the Absorptive and Postabsorptive

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What is needed to get muscles to move?

- Remember from week 2:
- Myosin heads have sites that can bind to actin and thereby form cross-bridges between the myosin and the actin filaments
- Myosin also bind ATP and hydrolyzes ATP.
- Hydrolysis of ATP provides the energy to change the conformation and therefore the orientation of the myosin head.

What is needed to get muscles to move?

- Remember from week 2:
- Muscles require ATP to stop contracting. As long as there is a constant supply of ATP, the muscle will have the ability to stop contracting (when you die there is no ATP, so you are in a state on constant contraction, rigor mortis)

chemical reaction for muscle utilization of ATP

$$\text{ATP} + \text{Actin} + \text{Myosin} \xrightarrow{Ca^{+2}} \text{Actomyosin} + \text{Pi} + \text{ADP} + \text{Energy (heat + work)}$$

Energy sources of muscular work for different types of work

	Power	Speed	Endurance
Duration	0 to 3 sec	4 to 50 sec	> 2 min
Enzyme system	Single enzyme	Glycolysis	Glycolysis, +cellular respiration
Enzyme location	Cytosol	Cytosol	Cytosol + mitochondria
Fuel storage site	Cytosol	Cytosol	Cytosol, blood, liver, adipose tissue
Rate of process	Immediate, very rapid	rapid	Slower but prolonged
Storage form	ATP, creatine phosphate	Muscle glycogen and glucose	Muscle and liver glycogen, glucose, muscle, blood and adipose tissue lipids, muscle, blood and liver amino acids
Oxygen involved	no	no	yes

Immediate source of energy

- Hydrolysis of ATP $ATP + H_2O \xrightarrow{ATPase} ADP + Pi$
- Creatine Phosphate (CP)also called phosphocreatine
  - exists in five to six times greater concentration in resting muscle than does ATP
  - Serves as a reserve of phosphate energy to regenerate ATP and creatine (C)

$$CP + ADP \xrightarrow[kinase]{Creatin} ATP + C$$

- Adenylate kinase (myokinase)
  - Has the ability to generate one ATP from 2 ADP

$$ADP + ADP \xrightarrow[kinase]{Adenylate} ATP + AMP$$

Effect of Creatine Supplementation on Exercise

- Creatine Supplementation does increase phosphocreatine
- Creatine supplementation appears to be most effective in short-term, high intensity exercise lasting up to 3 minutes in duration.
- Creatine appears to be especially helpful if the high-intensity activity is repeated with only a brief recovery period. This translates into athletes reporting that they can train harder in the gym or track because they recover faster after each set or repeat.

Effect of Creatine Supplementation on Exercise

- Some believe that creatine increases body weight and strength gains, but this is not supported by the literature (cited in Houston, Biochemistry primer for Exercise science, p51)
- Houston also points out:
  - Many creatine supplementation enthusiasts may be unaware that only a fraction of the large supplemental doses are actually retained by the body. Out kidneys can filter excess levels of creatine from the blood, with the excess excreted in the urine.

Immediate source of energy

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- **Adenylate kinase (myokinase)**
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Adenylate kinase (myokinase)

- During exercise, the rate of ATP hydrolysis is high, so the ADP concentration will increase.
- Keeps the ADP concentration from building up to high
- The increase in [AMP] also stimulates glucose metabolism (upregulates phosphofructokinase, one of the key regulating enzymes in glycolysis)

Nonoxidative (Glycolytic) Energy Sources

- Since glycolysis does not use oxygen, it is referred to as nonoxidative
- Glycolysis - breakdown of glucose
- Glycogenolysis - breakdown of glycogen
- This includes both glycolysis and fermentation
- Glycolysis produces pyruvate which is then fed into the fermentation process

$$\text{Glucose} \xrightarrow[\text{Fermentation}]{\text{Glycolysis}} 2\ \text{ATP} + 2\ \text{Lactate}^- + 2\ \text{H}^+$$

Production of Lactate

- Lactate dehydrogenase takes the pyruvate and produces lactate (and in the process reoxidizes NADH which is fed back into glycolysis)

Nonoxidative metabolism of glucose

- There are 10 times as much lactate in resting muscles than pyruvate
- The muscle uses the lactate to produce pyruvate, which then enters the oxidative metabolism of glucose
- This is called the lactate shuttle

Oxidative Energy sources

- This involves both glycolysis and cellular respiration
- The starting materials can be
  - glucose (from the blood),
  - glycogen (from the liver),
  - fats (from the muscle itself and from around the body)
  - amino acids (from the muscle itself and from around the body)

Oxidative Energy systems

$$\text{Glucose} + O_2 \xrightarrow[\text{cellular respiration}]{\text{glycolysis}} 36\ \text{ATP} + 6\ CO_2 + 6\ H_2O$$

$$\text{Palmitate} + O_2 \xrightarrow[\text{metabolism}]{\text{Oxidative}} 129\ \text{ATP} + CO_2 + H_2O$$

Palmitate ($C_{16}H_{32}O_2$) is the first fatty acid produced during lipogenesis (synthesis of fatty acids) and is used to synthesize larger fatty acids

$$\text{alanine} + \alpha\text{-ketoglutarate} \xrightarrow[\text{pyruvate transaminase}]{\text{Glutamate}} \text{Pyruvate} + \text{Glutamate}$$

Blood Glucose concentration during rest

- Normal blood glucose is postabsorptive humans is approximately 100 mg per dL (5.5 mM) - this level is required for normal cell functioning
- RDA for dietary carbohydrates is 130 g/day (or 520 kcal/day)

Blood Glucose during exercise

- Maintenance of this minimum blood [glucose] is now difficult when you have muscles requiring more glucose
- The glucose gets released from the liver (from its glycogen stores).
- Normally, the liver releases 1.8 mg (kg body weight)$^{-1}$ min^{-1}
- At 50 % VO_2 the rate is now about 3.5 mg. kg^{-1} min^{-1}

Glycolysis in Muscle

- Pale or white skeletal muscle contain large quantities of glycolytic enzymes
- Red muscle fibers are capable of rapid glycolysis

Confusion in terminology

- For physiologists - the formation of lactic acid was historically thought to be produced because there was no oxygen present in the cells (and since the formation of lactic acid does not require oxygen) it was called anaerobic
- This is not true.
- Lactate is the preferred fuel during exercise

Brooks et al in Exercise physiology

- They prefer to use the terms to describe energy metabolism as either:
  - "rapid" for anaerobic or
  - "slow" for aerobic

Back to Lactate

- The lactate produced in the cytosol during rapid glycolysis is then shuttled into the mitochondria and is oxidized into pyruvate by mitochondrial lactate dehydrogenase (this pyruvate is then fed into the cellular respiratory chain)
- Now the working muscle can use oxygen to generate much more ATP

Back to Lactate

- Lactate is polar so transport across membranes happens via a family of monocarboxylate transport (MCT) proteins.
- There are a number of different isoforms of the MCT proteins (labelled MCT1, MCT2, MCT3 and MCT4)

Dubouchaud, Hervé, Gail E. Butterfield, Eugene E. Wolfel, Bryan C. Bergman, and George A. Brooks. "Endurance training, expression, and physiology of LDH, MCT1, and MCT4 in human skeletal muscle." *Am J Physiol Endocrinol Metab* 278, no. 4 (April 2000): E571-E579

Abstract:

"To evaluate the effects of endurance training on the expression of monocarboxylate transporters (MCT) in human vastus lateralis muscle, we compared the amounts of MCT1 and MCT4 in total muscle preparations (MU) and sarcolemma-enriched (SL) and mitochondria-enriched (MI) fractions before and after training. To determine if changes in muscle lactate release and oxidation were associated with training-induced changes in MCT expression, we correlated band densities in Western blots to lactate kinetics determined in vivo…."

Key words: Vastus lateralis muscle - outer part of quad

sarcolemma is the cell membrane of a muscle cell

Dubouchaud, Hervé, Gail E. Butterfield, Eugene E. Wolfel, Bryan C. Bergman, and George A. Brooks. "Endurance training, expression, and physiology of LDH, MCT1, and MCT4 in human skeletal muscle." *Am J Physiol Endocrinol Metab* 278, no. 4 (April 2000): E571-E579

Abstract (continued):

"…Nine weeks of leg cycle endurance training [75% peak oxygen consumption (VO2 peak)] increased muscle citrate synthase activity (+75%, $P < 0.05$) and percentage of type I myosin heavy chain (+50%, $P < 0.05$); percentage of MU lactate dehydrogenase-5 (M4) isozyme decreased (-12%, $P < 0.05$). MCT1 was detected in SL and MI fractions, and MCT4 was localized to the SL. Muscle MCT1 contents were consistent among subjects both before and after training; in contrast, MCT4 contents showed large interindividual variations. MCT1 amounts significantly increased in MU, SL, and MI after training (+90%, +60%, and +78%, respectively), whereas SL but not MU MCT4 content increased after training (+47%, $P < 0.05$). Mitochondrial MCT1 content was negatively correlated to net leg lactate release at rest ($r = -0.85$, $P < 0.02$). Sarcolemmal MCT1 and MCT4 contents correlated positively to net leg lactate release at 5 min of exercise at 65% VO2 peak ($r = 0.76$, $P < 0.03$ and $r = 0.86$, $P < 0.01$, respectively)."

Key words: SL - cell membrane of muscle, MI-mitochondria, MU - total muscle

Dubouchaud, Hervé, Gail E. Butterfield, Eugene E. Wolfel, Bryan C. Bergman, and George A. Brooks. “Endurance training, expression, and physiology of LDH, MCT1, and MCT4 in human skeletal muscle.” *Am J Physiol Endocrinol Metab* 278, no. 4 (April 2000): E571-E579

- Results support the conclusions that
- 1) endurance training increases expression of MCT1 in muscle because of insertion of MCT1 into both sarcolemmal and mitochondrial membranes,
- 2) training has variable effects on sarcolemmal MCT4, and
- 3) both MCT1 and MCT4 participate in the cell-cell lactate shuttle, whereas MCT1 facilitates operation of the intracellular lactate shuttle.

Bergman, Bryan C., Eugene E. Wolfel, Gail E. Butterfield, Gary D. Lopaschuk, Gretchen A. Casazza, Michael A. Horning, and George A. Brooks. "Active muscle and whole body lactate kinetics after endurance training in men." *J Appl Physiol* 87, no. 5 (November 1999): 1684-1696.

Abstract:

"We evaluated the hypotheses that endurance training decreases arterial lactate concentration ([lactate]a) during continuous exercise by decreasing net lactate release (L) and appearance rates (Ra) and increasing metabolic clearance rate (MCR). Measurements were made at two intensities before [45 and 65% peak VO2 consumption (VO2 peak)] and after training [65% pretraining VO2 peak, same absolute workload (ABT), and 65% posttraining VO2 peak, same relative intensity (RLT)]. Nine men (27.4 ± 2.0 yr) trained for 9 wk on a cycle ergometer, 5 times/wk at 75% VO2 peak. Compared with the 65% VO2 peak pretraining condition (4.75 ± 0.4 mM), [lactate]a decreased at ABT (41%) and RLT (21%) ($P < 0.05$). decreased at ABT but not at RLT. Leg lactate uptake and oxidation were unchanged at ABT but increased at RLT. MCR was unchanged at ABT but increased at RLT. We conclude that *1*) active skeletal muscle is not solely responsible for elevated [lactate]a; and *2*) training increases leg lactate clearance, decreases whole body and leg lactate production at a given moderate-intensity power output, and increases both whole body and leg lactate clearance at a high relative power output."

Key words: lactate shuttle; exertion; glycogen; glucose; stable isotopes

Lactate dehydrogenase expression on mitochondria: Dubouchaud et al data

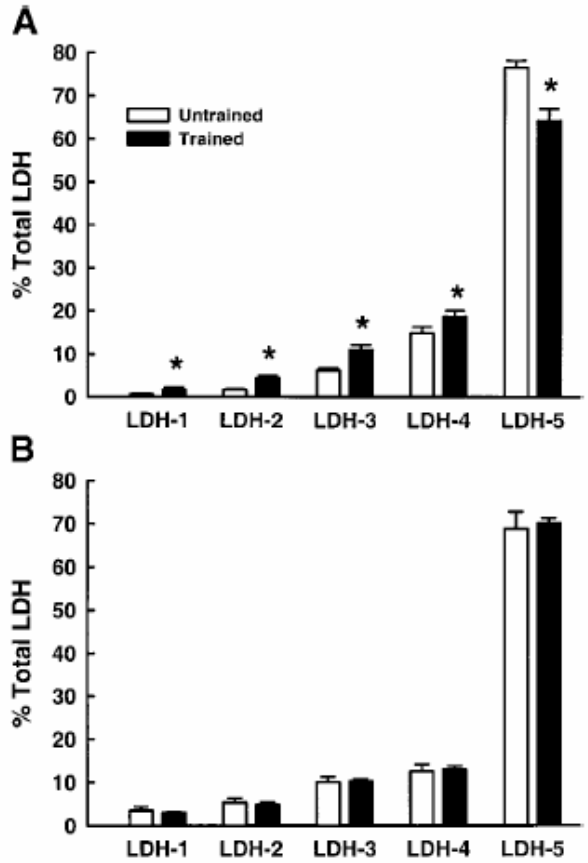

Fig. 2. Effect of leg cycle endurance training on the relative distribution of the lactate dehydrogenase (LDH) isozymes in human vastus lateralis. *A*: LDH distribution in total muscle homogenate expressed as percentage of the total LDH content. *B*: LDH distribution in mitochondria-enriched fraction expressed as percentage of the total LDH content. LDH isozymes are identified as LDH-1, LDH-2, LDH-3, LDH-4, and LDH-5. * Significantly different after training, $P < 0.05$. Values are means ± SE of 7 subjects.

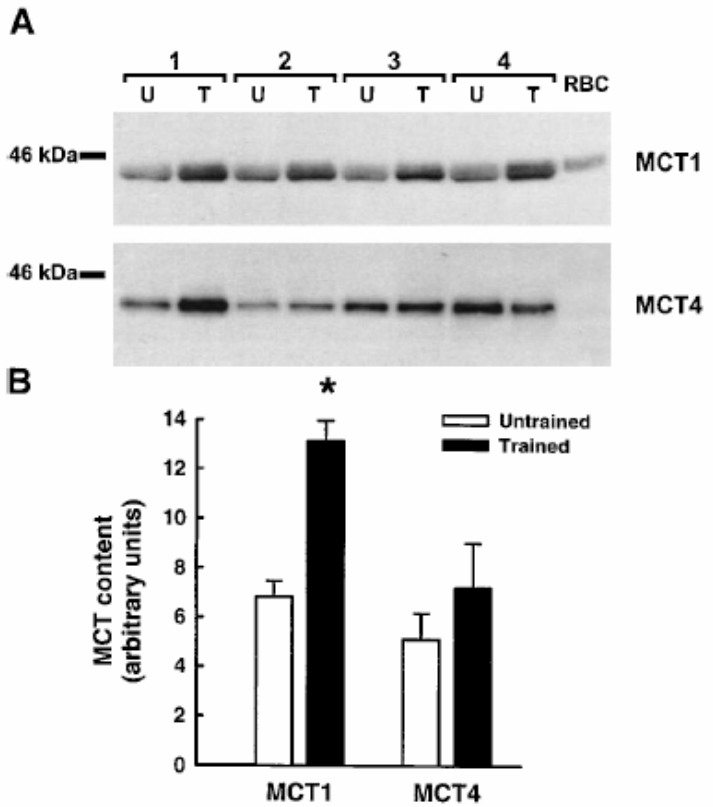

Fig. 3. Effect of leg cycle endurance training on the monocarboxylate transporter (MCT) 1 and MCT4 expression. *A*: representative Western blots showing the expression of MCT1 and MCT4 in total muscle homogenates from 4 subjects before and after training. RBC, human red blood cells. *B*: comparison of the MCT1 and MCT4 contents in total muscle homogenates before and after training. * Significantly different after training, $P < 0.05$. Values are means ± SE of 7 subjects. Results are expressed in arbitrary units (1 = MCT1 signal measured on 5 μg of human RBCs in the same conditions).

Source: Dubouchaud, Hervé, Gail E. Butterfield, Eugene E. Wolfel, Bryan C. Bergman, and George A. Brooks. "Endurance Training, Expression, and Physiology of LDH, MCT1, and MCT4 in Human Skeletal Muscle." *Am J Physiol Endocrinol Metab* 278, no. 4 (April 2000): E571-E579. [E:4/2000p571]

Lactate dehydrogenase expression on mitochondria: Dubouchaud et al data

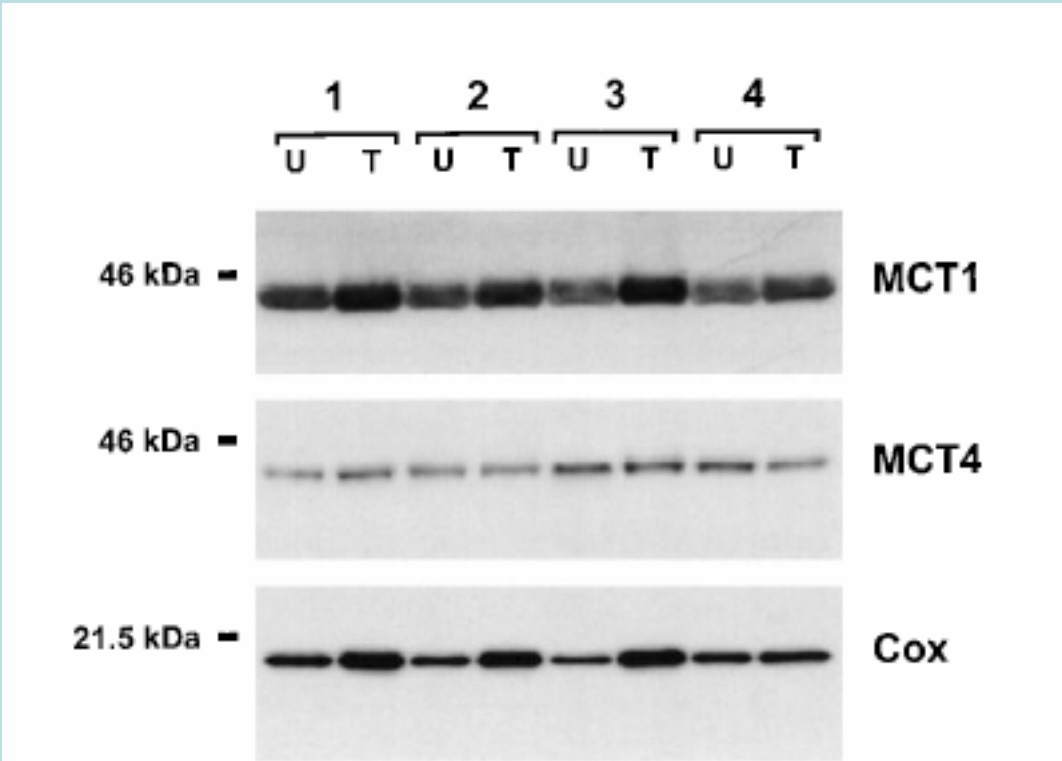

Fig. 7. Representative Western blots showing the MCT1 and MCT4 contents in the mitochondria-enriched fractions before and after leg cycle endurance training in 4 subjects.

Source: Dubouchaud, Hervé, Gail E. Butterfield, Eugene E. Wolfel, Bryan C. Bergman, and George A. Brooks. "Endurance Training, Expression, and Physiologyof LDH, MCT1, and MCT4 in Human Skeletal Muscle." *Am J Physiol Endocrinol Metab* 278, no. 4 (April 2000): E571-E579. [E:4/2000p571]

Lactate dehydrogenase expression on mitochondria: Dubouchaud et al data

In summary, our results suggest that endurance training increases expression of MCT1 in muscle because of insertion of MCT1 into both SL and MI membranes. In contrast, training of the type that shifts the profile of contractile and metabolic proteins toward slow-twitch, high-oxidative fibers has variable effects on expression of the sarcolemmal lactate transporter MCT4. Both MCT1 and MCT4 appear to participate in the lactate exchange among cells, tissues, and organs, whereas MCT1 facilitates lactate uptake and oxidation in cells with high mitochondrial densities.

Source: Dubouchaud, Hervé, Gail E. Butterfield, Eugene E. Wolfel, Bryan C. Bergman, and George A. Brooks. "Endurance Training, Expression, and Physiology of LDH, MCT1, and MCT4 in Human Skeletal Muscle." *Am J Physiol Endocrinol Metab* 278, no. 4 (April 2000): E571-E579. [E:4/2000p571]

Now to Patti's confusion

- Go to the human genome site:

MCT search

Lactate dehydrogenase

Mitochondrial expression?

Patti's enlightenment

These proteins are synthesized off genes in the nucleus and then transported into the mitochondria

Which makes sense - evolutionary these prokaryotic cells (mitochondria precursors) did not have internal organelles, so they were not capable of the lactate shuttle.

Effect of training on Glycolysis

- Endurance training has little effect on glycolysis enzymes but increases the total number of mitochondria present, so the rate of lactate accumulation decreases (because any lactate that is produced is shuttled into the numerous mitochondria for further processing)

Now to the papers:

See:

Bacon, L., and M. Kern. "Evaluating a Test Protocol for Predicting Maximum Lactate Steady State." *The Journal of Sports Medicine and Physical Fitness* 39 (1999): 300-308.

Vobejda, C., K. Fromme, W. Samson, and E. Zimmermann. "Maximal Constant Heart Rate - A Heart Rate Based Method to Estimate Maximal Lactate Steady State in Running." *International Journal of Sports Medicine* 27 (2006): 368-372.

Next week's fun

- Swimming