Wrestling with Weight Loss: The Dangers of a Weight-Loss Drug

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Part I – Too-Weighty-One

"I am tired of not making the wrestling team. I know I'm a good enough wrestler to do it," thought Connor as he walked to the gym one spring afternoon. "If I could just lose a few pounds, I would be able to make a lower weight class, and with my skills I would succeed!

"But how?" he wondered. Maybe his friends could help.

Over dinner in the dining hall, Connor asked his friends at the table if they had any suggestions for a quick way to lose weight.

"Well," said Becky, "I read about this stuff called 281 (pronounced "too-weighty-one") that they used to take in the 1930s before there were all these regulations on drugs. People lost weight quickly and easily, and the effects lasted as long as they took it."

"Is it still available?" asked Connor.

"Yeah, I saw an ad for it online. The website said it works great, but you have to be careful with dosage."

That night in his dorm Connor went online and found lots of websites offering to sell this diet pill, now called DNP (Harper, Dickinson, and Brand, 2001). According to the information, DNP had helped several people lose weight. Some of the side-effects included a fever, cataracts, and rashes. There were even some reports of deaths. DNP was said to be a "mitochondrial uncoupler."

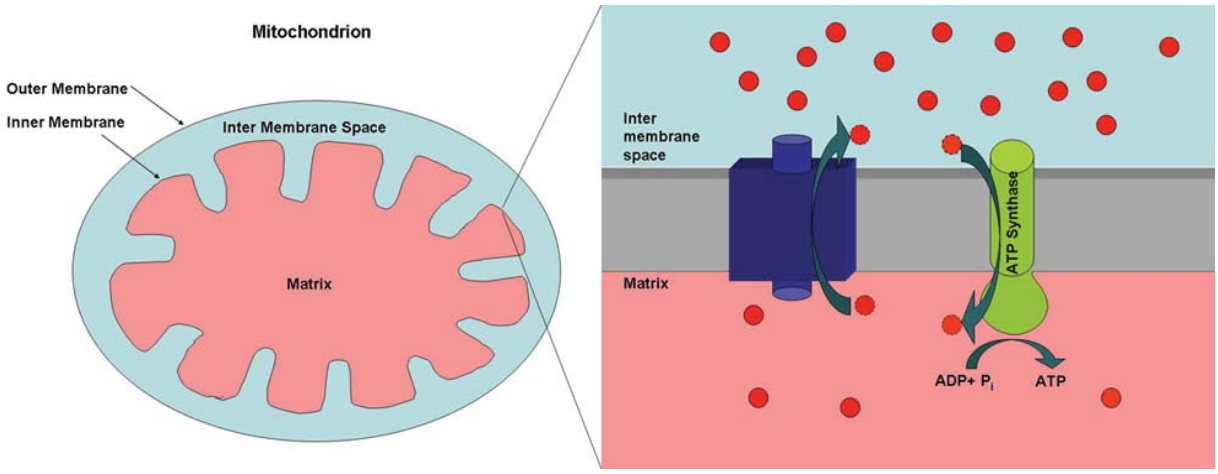
"What's that?" Connor wondered.

Questions

1. What do you know about mitochondria?

Part II – Mitochondrial Function

Here are some diagrams illustrating mitochondrial function.



The image on the left depicts the inside of a mitochondrion. Notice that there are two membranes. This creates two compartments within the mitochondrion. The inner-most space, called the matrix, is where ATP (the energy currency in the cell) is generated. The space between the inner and outer membranes is called the intermembrane space.

The image on the right is a magnification of the spaces on either side of the inner membrane. In the right-hand image, the red circles represent hydrogen ions (H^+, also called protons), the dark blue box shape represents the four complexes of the electron transport chain, and the green tube shape is ATP synthase.

Through the processes of glycolysis (in the cytosol) and the TCA cycle (in the mitochondrial matrix) energy captured in the breakdown of sugars and fats is used to generate a small amount of ATP. A much larger quantity of ATP can be produced by respiration. In this process, NADH and $FADH_2$ that were generated by glycolysis and the TCA cycle are used by the electron transport chain (ETC) to reduce oxygen to water and to generate a proton gradient across the inner mitochondrial membrane. In other words, sugars and fats provide the energy to pump protons to the intermembrane space. The ETC is the proton pumping mechanism.

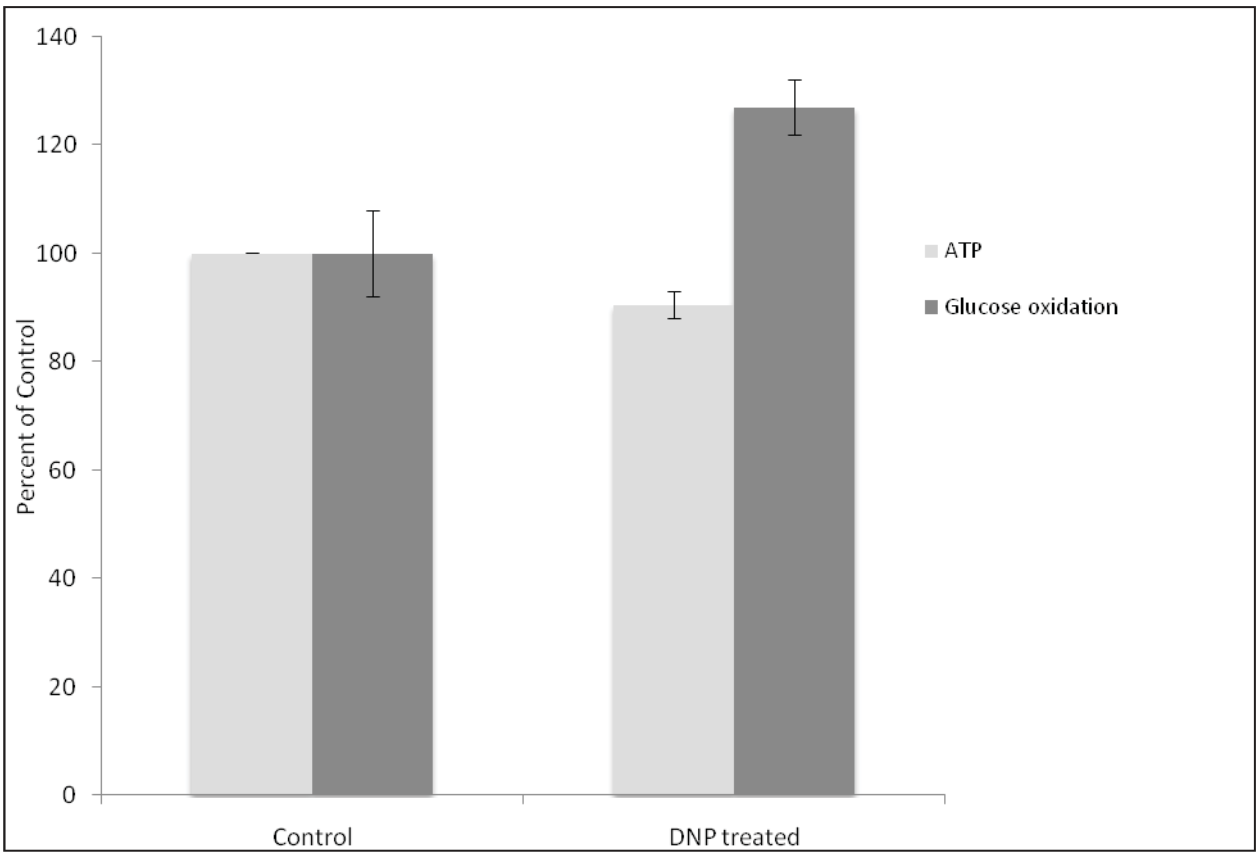
A protein within the inner membrane called ATP synthase acts as a turbine through which protons can move down their concentration gradient back to the matrix. The dissipation of the proton gradient (traveling from the intermembrane space to the matrix) releases energy. ATP synthase captures the energy released by the protons and uses it to create ATP. Note that the synthesis of ATP requires energy, which is provided by the movement of protons, so this is an energy transfer reaction. The movement of protons back and forth across the inner membrane is one way that the energy released from the breakdown of fats and sugars is *coupled with* the production of ATP.

Questions

2. What are the consequences of a proton gradient and how could a gradient be used in the mitochondrion? List all the possibilities that come to mind.
3. What must be an important characteristic of the inner membrane in order for this gradient to be established and maintained?
4. If you "poke a hole" in the inner membrane such that protons can freely move across it, what would happen:
   a. To the proton distribution across the inner membrane?
   b. To the amount of ATP produced by the mitochondria?
   c. To the energy released in the movement of the protons?
5. Most ATP is consumed soon after its production. The cell has ways of detecting how much ATP is produced and needs to keep its supply constant. If you poke a hole through the inner membrane, what might the cell do to try to adapt to the change and reestablish previous levels of ATP? List all the possibilities.

Part III – What Does This Have to Do with DNP?

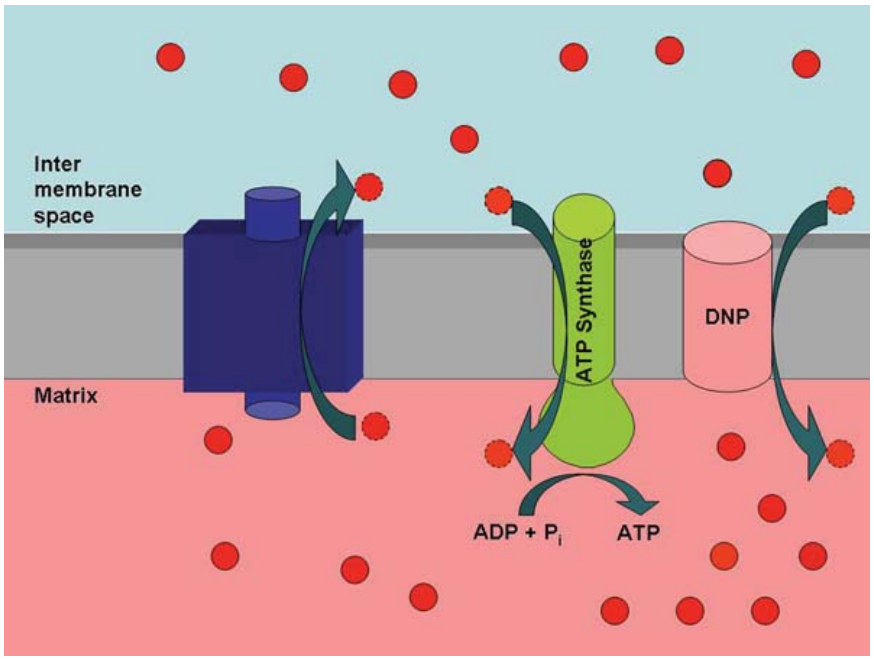
The graph below shows data on the effect of DNP on mitochondria function in human myotubule cells (the data are extracted from Figures 1 and 3 in Gaster, 2007). Myotubules are the progenitors of muscle cells. Muscle cells are heavy users of ATP. For this experiment, human myotubules were grown in the presence ("DNP treated") or absence ("Control") of DNP. The levels of ATP and glucose oxidation were measured. Glucose oxidation is a measure of the rate of glycolysis (initial breakdown of sugars). The data are expressed as a percent of the control level. The error bars show the standard error (n= 3 for ATP analysis, n=8 for glucose oxidation).



Questions

6. Summarize what happens to ATP and glucose oxidation levels in response to treatment with DNP.
7. What could cause the effects shown in the graph? Propose as many mechanisms as possible.
8. Remember that DNP is a "mitochondrial uncoupler." An uncoupler is a chemical that disconnects two linked biological processes. Draw a diagram showing the linked processes that lead to mitochondrial ATP synthesis. Mark your diagram to show where DNP could uncouple.
9. Review the list of physiological effects that DNP has on the body (list as many as possible). Which of these effects are energy-related? Using your proposed mechanism of action of DNP, can you explain them?
10. Which linked processes do you think DNP is uncoupling taking into account the physiological effects you listed in Question 9?

Part IV – What Should Connor Do?



DNP (2,4-dinitrophenol) inserts into the inner mitochondrial membrane and shuttles protons between the intermembrane space and the matrix. The energy released as the protons move down their concentration gradient is dissipated as heat. This is manifested physiologically by a rise in body temperature (a fever). Without the protein gradient, ATP synthase is not able to maintain ATP production.

The mitochondrion tries to adjust its ATP production by increasing the rate of glycolysis and burning more energy reserves (fats). The energy from the fat burning is used to pump protons to the inter membrane space. However, as long as DNP is in the membrane, this is a futile effort as many of the protons flow back into the matrix. This is how DNP works as a weight-loss drug. It uncouples the energy derived from the breakdown of fats and sugars (which pumps protons to the matrix) from the production of ATP.

DNP is but one known mitochondrial uncoupler. Acetylsalicylic acid (Aspirin™) and the drug 3,4-methylenedioxy-N-methamphetamine (MDMA, or ecstasy) are also mitochondrial uncouplers (Mingatto et al., 1996; Rusyniak et al., 2005). In human cells, there are also proteins (called UCPs—uncoupling proteins) that can uncouple the breakdown of sugars and fats from the synthesis of ATP. Variant forms of these proteins have been linked with obesity (Crowley & Vidal-Puig, 2001).

In the course of his web research, Connor encounters a 2006 report of two people dying as a consequence of DNP use.

"Surely, they wouldn't be able to sell DNP online if it were dangerous. I'll bet they had another condition."

But the report in the *Journal of Analytical Toxicology* states that "Death in both cases was attributed to 2,4-DNP toxicity" (Miranda et al., 2006).

Questions

11. Based on your understanding of the mechanism of action of DNP, how dangerous do you think DNP really is?
12. For an athlete, what are the consequences of ATP depletion?
13. Should Connor take DNP to lose weight?

References

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