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RESEARCH ARTICLE

UNCOUPLING OF CELL RESPIRATION AND OXIDATIVE PHOSPHORYLATION: IT'S POSSIBLE ROLE IN OBESITY

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ABSTRACT

The electron transport chain is the final phase of tissue respiration through which the energy from oxidation of nutrients is converted into an electrochemical gradient. This electrochemical gradient generates a proton motive force which is converted into high energy phosphoanhydride bonds of ATP within the mitochondria. Studies have shown that the transduction of electromotive force into phosphoanhydride bond is not perfect as there is some degree of uncoupling in which the electrochemical gradient is dissipated when protons are returned to the mitochondria not through ATP synthase. This can be in the form of low grade background uncoupling sometimes referred to as proton leakage or it can be due to naturally produced special proteins known as uncoupling proteins with in the inner mitochondrial membrane. Uncoupling tends to decrease ATP synthesis and the energy from oxidation that should have formed ATP is released as heat. Oxidation of nutrients is also increased due to a decrease in ATP synthesis. Here we review the possible role of this uncoupling process in the development of obesity.

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INTRODUCTION

Animals depend on flow of electrons directly or indirectly for generation of energy. The main source of these electrons are reduced nutrients such as carbohydrates, lipids and amino acids(Huttemann *et al*., 2007). When these nutrients are oxidized during catabolism, the high potential electrons are collected by special coenzyme electron carriers, NADH and $FADH_2$(Echtay, 2007). These electrons are then released in the electron transport chain where they flow along a path of enzyme complexes with increasing redox potential to the final electron acceptor oxygen. The electron transport chain basically consists of four enzyme complexes i.e. complex I, complex II, complex III, and complex IV; and in addition ubiquinone and cytochrome C, the two mobile electron carriers (Fillingame, 1997, Junge *et al*., 1997). The four enzyme complexes and the mobile electron carriers are all embedded in the inner mitochondrial membrane. Complex IV is the site where the final electron acceptor, oxygen is reduced to water. As electrons flow through the enzyme complexes from NADH and $FADH_2$ through complexes I and II respectively to complex IV they release energy. This energy translocates protons from the mitochondrial matrix through the inner mitochondrial membrane to the intermembrane space.

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Complexes I, III and IV are the proton pumps where translocation takes place. But because the inner mitochondrial membrane is impermeable to protons there is a build-up of proton concentration in the intermembrane space, which creates an electrochemical gradient. As a result of the increased electrochemical gradient the protons find their way into the mitochondrial matrix through special pores embedded in the inner mitochondrial membrane(Brand *et al*., 1994). These pores known as ATP synthase have enzymatic activity such that the proton motive force released as protons pass through the pores drives ATP synthesis in a process known as oxidative phosphorylation (Fig.1). Thus the electron transport chain and oxidative phosphorylation are coupled processes; the energy released from the flow of electrons is converted into high energy phosphoanhydride bond of ATP through the electrochemical gradient established by translocated protons. Oxidative phosphorylation is the source of most of the ATP formed in the body and it's an aerobic process.

What is the effect of uncouplers on tissue respiration and oxidative phosphorylation

Uncouplers are substances which dissipate the electrochemical gradient established by the flow of electrons in the electron transport chain. They can do this either by dissipating the chemical component of the electrochemical gradient by releasing protons in the mitochondrial matrix.

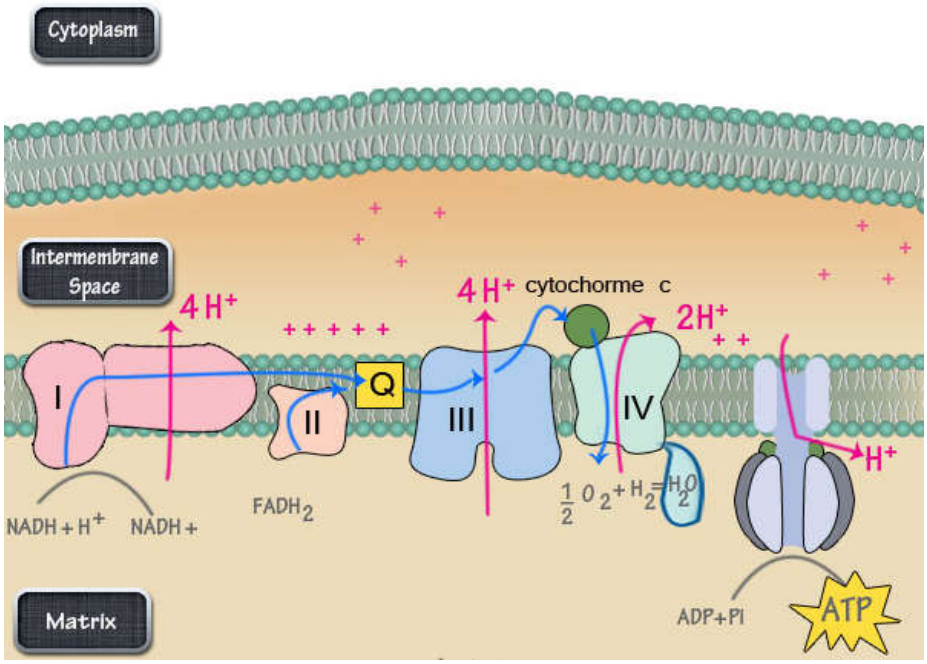

Fig. 1. Outline of the electron transport chain showing flow of electrons from reduced coenzymes NADH + H^+ and $FADH_2$ along enzyme complexes to the final electron acceptor O_2. As electrons flow the energy they release translocate protons through enzyme complexex I, III, IV to the intermembrane space forming an electrochemical gradient between the matrix and the intermembrane space. As protons flow back into the matrix through ATP synthase their proton-motive force drives synthesis of ATP. Figure from http://www.dbriers.com/tutorials/about/

They can also dissipate the electrical component of the electrochemical gradient by creating pores in the inner membrane through which charged ions can cross. By dissipating the electrochemical gradient protons returning into the matrix through ATP synthase do not generate enough proton motive force to drive ATP synthesis. In an effort to sustain the electrochemical gradient so that there can be enough proton motive force for ATP synthesis, the electron transport chain speeds up the rate of NADH and $FADH_2$ oxidation. In order to replenish NADH and $FADH_2$ which are being oxidized at a fast rate by the electron transport chain, there is increased oxidation of nutrients such as carbohydrates, lipids and amino acids. As a result of reduced ATP synthesis in the presence of increased oxidation, the redundant energy of electron flow in the electron transport chain tends to be released as heat. It has also been observed that even though the inner mitochondrial membrane is impermeable to protons there is still residual leakage of protons into the mitochondrial matrix depending upon the fatty acid composition of the inner mitochondrial membrane lipid bilayer. Studies have shown that there isacorrelation between mitochondrial proton conductance and composition of fatty acids in the inner mitochondrial membrane (Porter *et al*., 1996, Brand *et al*., 2003). In addition it has also been shown that mitochondrial proton conductance may be in part due to abundance and not activity of adenine nucleotide translocase, independent of its role in facilitating exchange of ATP and ADP nucleotides (Brand *et al*., 2005). As a result of mitochondrial proton conductance in the inner mitochondrial membrane, some protons may bypass the ATP synthase pores (Brand, 1990, Brown and Brand, 1991). This non-productive leakage of protons is significant as it accounts for about a quarter of the basal metabolic rate (Rolfe and Brand, 1996, Rolfe and Brand, 1997). The physiological role of proton-leak reactions is not well known. Studies previously done have shown existence of proton-leaks in both ectothermic and endothermic animals (Brand *et al*., 1991) and energy consumption of proton-leaks and other oxygen consuming processes from the same organs were comparable (Brookes *et al*., 1998). The above observations suggest that the main function of proton-leaks is not thermogenesis. The proton leaks may still have a significant role to play in regulation of metabolism, body mass and reactive oxygen species (ROS) (Mailloux and Harper, 2011, Cannon *et al*., 2006). Drastic changes in these protein leaks may predispose to pathological conditions due to disruption of metabolism. For instance the background uncoupling due to protein leaks may actually be a natural safeguard against development of obesity since the net effect is be increased catabolism and conversely absence of the background uncoupling could be a predisposing factor for obesity as background catabolism gets diminished.

The inducible uncoupling is mainly carried out by special resident proteins within the inner mitochondrial membrane known as uncoupling proteins (UCPs)(Divakaruni and Brand, 2011). These are anion-carrier proteins that carry protons across the inner mitochondrial membrane into the matrix dissipating the electrochemical gradient in the process(Liu *et al*., 2013). There has been a lot of interest in UCPs because of their potential as players in the etiology of conditions like diabetes mellitus, obesity, malignancy and cardiovascular diseases. So far 5 UCPs have been described and these are UCP1, UCP2, UCP3, UCP4, and UCP5(Krauss *et al*., 2005). This review will focus on UCP1, UCP2 and UCP3 because these are the ones that have been well studied by different research teams. UCP1 is found in brown adipose tissue mitochondria and has a very important role in non-shivering

thermo genesis by dissipating the mitochondrial electrochemical proton gradient (Heaton *et al*., 1978, Rafael and Heldt, 1976) which is a very important mechanism for keeping new-born mammals warm. UCPI gene expression is increased by thyroid hormones, epinephrine and norepinephrine, cold and retinoids (Silva and Rabelo, 1997) and UCPI is activated by non-esterified fatty acids and inhibited by purine nucleotides (Brand *et al*., 1999). Even though brown adipose tissue tends to decrease in quantity and activity after birth, some recent studies have shown that there is still metabolically significant amounts of brown fat in adult human beings(Saito *et al*., 2009, Cypess *et al*., 2009, van Marken Lichtenbelt *et al*., 2009, Virtanen *et al*., 2009). This suggests that UCP1 which is found in brown adipose tissue may still be a major player in metabolic processes in adults. UCP2 is found in many body organs like the spleen, pancreas, liver, and UCP3 is found mostly in skeletal muscles.UCP2 and UCP3 may not have a significant thermogenic role despite the fact that like UCP1 they also dissipate the electrochemical gradient in the inner mitochondrial membrane. This may be due to the fact that they are present in very low concentrations in the inner mitochondrial membrane and transport protons only when they are activated by specific molecules such as products of membrane phospholipid peroxidation (Echtay *et al*., 2003). Some studies have revealed evidence of thermogenesis by UCP2 and UCP3; UCP3 overexpression in mouse models caused same level of uncoupling which suggests that under certain conditions UCP3 is significantly thermogenic (Echtay *et al*., 2002). Other documented functions of UCP2 and UCP3 include reduction of mitochondrial ROS production(Rolfe and Brand, 1997), mediation of ROS signaling and insulin secretion(Rutter, 2001, Chan *et al*., 1999, Chan *et al*., 2001), and export of fat acid and fatty acid peroxides(Himms-Hagen and Harper, 2001). In this review the focus is on the role of uncoupling proteins in the etiology of obesity and other important functions will merely be mentioned and not discussed in detail.

Obesity and uncoupling proteins

Chemical uncouplers like 2,4-dinitrophenol can accelerate energy expenditure resulting in weight loss and that is the main reason why they are sometimes abused by people to lose weight despite the high rate of side effects(Colman, 2007). It is fairly logical to assume that UCPs can have the same effect as they accelerate metabolism and release excess energy as heat. Being proteins UCPs are prone to polymorphism as a result of genetic variations in the coding regions. The polymorphisms can potentially give rise to variations in the performance of theses UCPs such that some would have defective uncoupling capabilities and others would have enhanced uncoupling capacity. Perhaps it is these genetic polymorphisms that can explain in part why some individuals may be obese and others lean even when both groups have comparable levels of physical activity. Since fatty acids composition is also important in proton conductance it could also be that the variations in the profile of mitochondrial inner membrane lipids may in part be a factor that contributes to the effectiveness of uncoupling by the proteins. Various studies have been carried out in animal models to investigate the role of UCP1, UCP2 and UCP3 in obesity and how this is affected by genetic polymorphism. The role of UCP1 in metabolism is well established primarily as an uncoupler for non-shivering thermogenesis but the roles of UCP2 and UCP3 in metabolism have not been clearly established because to some extent research findings have been largely inconclusive(Ricquier and Bouillaud, 2000, Boss *et al*., 2000). Part of the problem is that UCPs genes are under polygenic control and the phenotypes are subject to controls from many genes which may be under control of other genes (Dalgaard and Pedersen, 2001). Transgenic and knock-out mouse models were used in many studies to assess the effect of genetic variations. UCP1 knockout mice showed increased sensitivity to cold and there was no uncoupling in brown adipose tissue of mice with deleted UCP1 gene compared to the wild type(Enerback *et al*., 1997).When over- expression of UCP1was induced in white adipose tissue the mice didn't become lean but developed resistance against diet induced obesity (Baumruk *et al*., 1999). UCP2 knockout mice were found to be not obese or resistant to diet induced obesity with normal temperature(Arsenijevic *et al*., 2000) and similarly UCP3 knockout mice didn't express the expected diet induced obesity(Vidal-Puig *et al*., 2000) suggesting that thermo genesis may not be the primary function of UCP2 and UCP3. Some studies have shown an association between UCP2 polymorphism and energy metabolism but this association does not translate into association of UCP2 with obesity (Esterbauer *et al*., 2001, Evans *et al*., 2001).

In one study using UCP2 mRNA expression it was demonstrated that there was a direct relationship between UCP2 and metabolism and the UCP2 expression was enhanced by thyroid hormones. In addition some studies in human beings have shown that UCP2 expression is reduced in obesity (Oberkofler *et al*., 1998, Nordfors *et al*., 1998). So even though UCP2 may not show any direct association with obesity it may still play a significant role in metabolism. Thyroid hormone administration, a situation that increases metabolic rate and thermogenesis has been associated with increased UCP3 gene expression(Schrauwen and Hesselink, 2002). In addition UCP3 gene expression has been found to be reduced after endurance training (Boss *et al*., 1998) or weight loss (Vidal-Puig *et al*., 1999). Under normal circumstances endurance training would be expected to reduce uncoupling in muscles so that energy is not wasted in thermogenesis but used for physical activity. This would explain reduced expression of UCP3 in muscles subjected to endurance training. The loss of UCP3 gene expression after endurance training and weight loss is an indirect evidence to support the hypothesis that UCP3 has an important role to play in metabolism. The above findings suggest that both UCP2 and UC3 through their uncoupling capacity have a role in thermogenesis and metabolism even though this may be secondary to other functions. Therefore UCP2 and UCP3 may be good targets for pharmacological and gene manipulation in the treatment of obesity and other associated conditions like type 2 diabetes mellitus.

The possible link between obesity and oxidative stress

The fact that UCP2 and UCP3 have a role in thermogenesis and metabolism, though secondary, may explain in part why conditions like obesity and diabetes mellitus type 2have

increased levels of oxidative stress(Giacco and Brownlee, 2010, Ramadan *et al*., 2014). Oxidative stress is mainly due to the imbalance between the production of ROS and removal of ROS by antioxidants like vitamin C, vitamin E and antioxidant enzymes like glutathione peroxidase.ROS are molecules or ions formed by the incomplete one-electron reduction of *oxygen and mainly originate from the mitochondria.* Other minor sources of ROS include reactions catalyzed by enzymes like NAD(P)H oxidase and xanthine oxidase(Babior, 1999, Freeman and Crapo, 1982). In the electron transport chain ROS are formed mainly in complex I and complex III especially when there is interruption in the flow of electrons(Turrens and Boveris, 1980, Sugioka *et al*., 1988). Formation of ROS in mitochondria starts when there is partial reduction of molecular oxygen to form a superoxide anion which is converted by the superoxide dismutase into hydrogen peroxide (H_2O_2). In the presence of ferrous ions the Fenton reaction converts H_2O_2 into highly toxic hydroxyl radicals (Mates, 2000). ROS through their reactivity can cause damage to important macromolecules like lipids, proteins and nucleic acids(Reaume *et al*., 1996, Levine *et al*., 1994). Even though these ROS are well known for the oxidative stress they cause and are implicated in etiology of non-communicable diseases like hypertension, type 2 diabetes mellitus and morbid obesity, they have very important functions in the body such as cell signaling, bactericidal effects and modulation of many other physiological processes(Saitoh *et al*., 1998, Adachi *et al*., 2004). Mitochondrial ROS formation is increased when there is high proton motive force and vice versa(Brand *et al*., 2002). Hence UCP2 and UCP3 by lowering the proton motive force decrease ROS formation(Nicholls and Budd, 2000).The magnitude of the electrochemical gradient determines the rate at which ROS are formed in the mitochondria. High electrochemical gradient tends to have a suppressant effect on flow of electrons in the electron transport chain.

When the flow of electrons slows down it gives chance for electron carriers like semi Quinone to donate single electrons to molecular oxygen. Therefore speeding up electron flow doesn't give time for electron carriers to donate electrons to molecular oxygen, and as a result ROS production can be slowed(Dalgaard and Pedersen, 2001). By reducing the electrochemical gradient, uncouplers reduce the rate of ATP synthesis by cells. Therefore in order to maintain the electrochemical gradient in an effort to sustain ATP synthesis there is increased oxidation of nutrients which results in increased generation of NADH and FADH2. These reduced coenzymes are oxidized in the mitochondria via the electron transport chain and the flow of electrons toward oxygen is increased giving electron carriers little time to donate single electrons to oxygen. What this means is that in the presence of normal activity of uncouplers, obesity would be avoided as energy gets wasted in thermogenesis and at the same time production of ROS can be reduced. In absence or presence of defective uncouplers, catabolism would be slow resulting in obesity and production of ROS would be increased. This may in part explain why in other conditions closely linked to obesity such as hypertension, and type 2 diabetes mellitus there is increased oxidative stress. In conclusion there is likelihood proton leaks may play a role in etiology of obesity as individuals with high proton leaks are likely to be lean where as those with no proton leaks are likely to be obese. There is a universal agreement that the main function of UCP1 is non-shivering thermogenesis whereas the role of UCP2 and UCP3 in non-shivering thermogenesis is equivocal, though current literature seems to suggest that there is a metabolic role which doesn't conclusively translate into obesity. The reduction of ROS by UCP2 and UCP3 may explain why obese individuals tend to have increased oxidative stress. So, UCPs are potential targets for pharmacological and genetic manipulation in the treatment of obesity.

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