NAD+ the disregarded molecule in cardiac metabolism

mechanisms underlying acute coronary syndromes. In line with the research tradition of the Center for Molecular Cardiology, the lecture spanned from the latest molecular insights to the most interesting clinical evidence emerging from large clinical trials. At the end of the lecture and after a most exciting discussion, Professor Crea was awarded with an engraved plate.

The '*Paul Vanhoutte Lecture*' series will carry on being organized in the future so as to maintain the memory of Prof. Vanhoutte as vivid as ever. The plan is to hold it once per year in Zurich and future invitees will always be first-class scientists who devoted their career to studying cardiovascular disease from a translational perspective. In this regard, the choice of Professor Crea for the inaugural lecture was perfectly fitting for the scope and the organizers are convinced that Prof. Vanhoutte would have felt the same.

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References

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Introduction

Advanced age and cardiovascular diseases are related to alterations in cellular metabolism.[1,2] The accumulation of fat, a phenomenon observed in ageing, causes a disturbance in glucose and lipid metabolism altering cardiovascular health.[3,4]

Current therapeutic approaches focus on the treatment of 'classic' risk factors such as arterial hypertension, diabetes mellitus, and hyperlipidaemia. It is believed that at least 90% of cardiovascular diseases could be preventable, if the right targets for prevention can be identified.[5] Numerous studies are suggesting that it is possible to delay the ageing process by manipulating cellular metabolism and thus reverse or delay age-associated diseases including cardiovascular diseases.[2,6,7] Experimental drugs that can interfere with myocardial metabolism have been tested experimentally and in clinical trials.[8–10] Most of them aim at switching the energy substrate of the myocardium in oxygen (O_2) restricted diseases to optimize O_2 consumption at the expense of optimized energy production. Although some of these therapies showed promising results, the side effects of switching energy substrates can be detrimental to the myocardium.[9,11,12] Clearly, a better understanding of the metabolic processes and their role and interactions with the development of cardiovascular diseases is needed.

In this regard, nicotinamide adenine dinucleotide (NAD^+) has gained renewed interest among researchers based on newly discovered functions[13–17] that make it one of the most critical and exciting molecules in the body. NAD^+ is required in over 500 enzymatic reactions that play a critical role in the regulation of almost all major biological processes.[7,18]

Here, we provide an overview of the cellular metabolism, the alterations to cardiac metabolism in the context of cardiovascular diseases, and the role of NAD^+ as a potential therapeutic goal in several inflammatory conditions, in particular with respect to cardiovascular diseases.

Understanding cellular metabolism

Cellular metabolism/respiration in the eukaryotic cells comprises a series of catabolic reactions to generate chemical energy in the form of adenosine triphosphate (ATP). Energy substrates commonly used during this process include glucose, proteins (amino acids), and triglycerides (fatty acids). For this review, we will focus on glucose and triglycerides due to their relevance in diabetes and obesity, the primary metabolic disorders in cardiovascular diseases.

Cellular respiration will start with the cytosolic *synthesis of pyruvate*, followed by the synthesis of acetyl CoA (coenzyme A) through *pyruvate oxidation*. Acetyl CoA will then enter the *citric acid cycle* in the mitochondrial matrix, where ATP and, more importantly, the nicotinamide adenine dinucleotide + one hydrogen atom (NADH) and flavin adenine dinucleotide + two hydrogen atoms ($FADH_2$)—the reduced forms of NAD^+ and FAD—will be produced. The NADH and $FADH_2$ molecules will then enter *oxidative phosphorylation*, where they will be stripped away of their electrons (oxidation) in *the electron transport chain* (ETC) located at the mitochondrial membrane. During this process, energy is released and used to pump protons (H^+) out of the mitochondrial matrix; protons will then flow back into the mitochondrial matrix through the enzyme ATP-synthase, producing ATP. Of note, during oxidative phosphorylation, oxygen (O_2) is required. Lack of O_2 during this step of cellular respiration will indeed produce energy; however, at the expense of increased lactic acid synthesis.[14,18–20] Furthermore, the majority of ATP will be produced by the oxidation of NADH and $FADH_2$ during the *oxidative phosphorylation* phase.

Glucose and triglycerides as energy substrates differ in the amount of ATP production and O_2 consumption. While a molecule of glucose is capable of producing between 32 and 38 molecules of ATP,[19,20] palmitic acid, a triglyceride synthesized in the liver, produced up to 107 molecules of ATP; this, however, at the expense of increased O_2 consumption.[3,21] Notably, triglycerides required up to 10–15% more O_2

to generate a similar amount of ATP when compared with glucose.[22] Due to the high energy demand of the heart, cardiac metabolism prefers triglycerides as a primary source of energy substrate, obtaining 60–90% of its energy through fats.[23,24]

Altered metabolism in cardiovascular diseases

Metabolic disorders have always been of interest in cardiovascular diseases. Recent discoveries have underscored the importance of targeting cardiac metabolism in the context of cardiac hypertrophy, heart failure, and ischaemia, including cardiac reperfusion injury.[24–26]

Fatty acids ensure a better cardiac energy efficiency; this, however, is at the expense of increased O_2 demand that is detrimental in the context of cardiac hypertrophy, cardiovascular ischaemia, and heart failure. Substrate selection in cardiac metabolism is flexible and can choose different energy substrates according to its needs.[9,24,27] Current research in heart failure has shown an adaptive change of energy substrate in the adult heart.[23,28] When the healthy heart suffers from mechanical overload—i.e. in conditions such as hypertension, valvular disease, or post-myocardial infarction—it will lead to depletion of the fatty acid reserves forcing cardiac metabolism to switch the energy substrate towards glucose optimizing O_2 consumption at the expense of energy efficiency.

An indirect correlation was found between fatty acid translocase (FAT/CD36) and GLUT4 two essential transmembrane proteins responsible for the uptake of fatty acids and glucose, respectively. The failing heart will present with down-regulation of FAT/CD36 and up-regulation of GLUT4, which correlates with the energy substrate change observed in mechanical overload and hypertrophy. The correlation between FAT/CD36 and GLUT4 has also been observed in patients presenting with aortic valve stenosis.[29]

Cardiomyocytes cultured in high glucose developed glucotoxicity, structural, and functional damage to beta cells and target tissues of insulin, resulting in insulin resistance, a characteristic of heart failure.[14,15,38] In contrast, transgenic mice with cardiac-specific overexpression of glucose transporters showed a substantial increase in glucose uptake and glycolysis, maintaining normal cardiac function and lifespan, suggesting no significant damage through glucotoxicity.[23,24]

The diabetic and obese population exhibit increased supply of substrates in the heart leading to an increase in fatty acid oxidation with a subsequent decrease in glucose oxidation resulting in an impaired cardiac efficiency. These groups of patients end in a lipotoxic cardiomyopathy, characterized by cellular dysfunction and cell-death mediated organ dysfunction.[30,31]

The reciprocal inter regulatory relationship between these two substrates becomes apparent in ischaemia,[25,29,32] where pyruvate will be converted to lactate by the enzyme lactate dehydrogenase, to generate the NAD^+ required to maintain anaerobic *glycolysis*.[14,25,33] As a consequence, there is an increase in H^+ and (free) fatty acids that have been lost from the triglycerides or break away from oil molecules. Once restoration of circulation and O_2 supply occur, fatty acids are dramatically oxidized, inhibiting glucose oxidation and leading to a further dramatic H^+ production resulting in an impaired recovery of cardiac function.[21,23] This process represents ischaemia–reperfusion injury and is one perfect example of the Randle—glucose-fatty acid—cycle initially described by Phillip Randle in 1963.[34,35]

The role of NAD^+ in cardiovascular metabolism

ATP-synthesis is the most important mechanism to restore cardiac function regardless of substrate selection.[23,32] NAD^+/NADH and FAD/$FADH_2$ redox couples synthesize most of ATP; thus, these redox couples have come into focus in current research. In particular, the role of NAD^+ is now seen beyond its role in metabolic regulation.[13,14,17,36–38]

The discovery of NAD^+ happened in the early 1900s; however, its role in redox reactions remained unknown until the mid-1930s.[13] The importance of NAD^+ in the homeostasis of the immune system has been underscored recently,[14,15,36,37,39] but the role of NAD^+ in the balance of metabolism in cardiovascular health has been scarcely investigated. Interestingly, the amount of NAD^+ present in the heart is the second-highest concentration, only preceded by the liver.[14,33] NAD^+/NADH pools are highly compartmentalized due to the availability of precursors and biosynthetic enzymes and show different sensitivity in response to different stimuli.[14,28]

The ratio between NAD^+/NADH has been shown to be the most crucial factor in different disease models.[12,14,17] An estimated ratio of 500:1 is considered normal, and any alteration of this relationship can result in cellular damage during hypoxic stress.[28,40] An accumulation of mitochondrial NADH and $FADH_2$ blocking oxidative phosphorylation via the ETC causing reductive stress and accumulation of reactive oxygen species (ROS) are observed. Reactive oxygen species are known to play an important role in a variety of inflammatory responses including those observed and leading to heart failure.[28] Reactive oxygen species accumulation is strongly associated with an increase in the NADH/NAD^+ ratio and accumulation of L-2-hydroxyglutarate, a reductive metabolite that buffers reductive stress through inhibition of glycolysis and the citric acid cycle.[14,41] The intracellular increase in NADH through hypoxic stress will create a reverse electron transfer, producing higher levels of succinate—the only enzyme that participates in both the citric acid cycle and the ETC—and therefore increasing oxidation and subsequently ROS levels.[14,29] A similar process is observed during mechanical overload in the heart.[32,42] In diabetic, obese, and elderly subjects, a decrease in NAD^+ which alters the NAD^+/NADH ratio can cause insulin resistance, decreased glucose metabolism and an increase of fatty acid metabolism.[43]

The previous observations suggest that the addition of NAD^+ may re-establish the NAD^+/NADH ratio and thus reduce cardiovascular damage. However, NAD^+ and NADH have been thought to be incapable of crossing the cellular membranes until very recently.[14,44] Investigators have shown an increase in intracellular and mitochondrial-specific NAD^+ and NADH in several experimental systems after the addition of exogenous NAD^+ and NADH.[14,28,37,45] Also, nanomolar concentrations of NAD^+ and its precursors have been detected in different body fluids and plasma.[46] Our group has shown the influence of exogenous NAD^+ in the regulation and homeostasis of the immune system in different disease models.[13,15,37,39] The exact mechanisms by which exogenous NAD^+ and NADH work remain elusive. The possibility of specific extracellular receptors exists[26,37]; however, alternative mechanisms might come into play. An interesting hypothesis for myocardial cells, is based on the concept that NAD^+ is metabolized into its cell-permeable precursors, which could then enter the cell through specific carrier proteins.[14,47]

Intrinsic NAD^+ production is governed by at least four known complex pathways capable of regulating NAD^+ synthesis.[48,49] In short, these pathways are known as the Preiss-Handler pathway, *de novo* synthesis

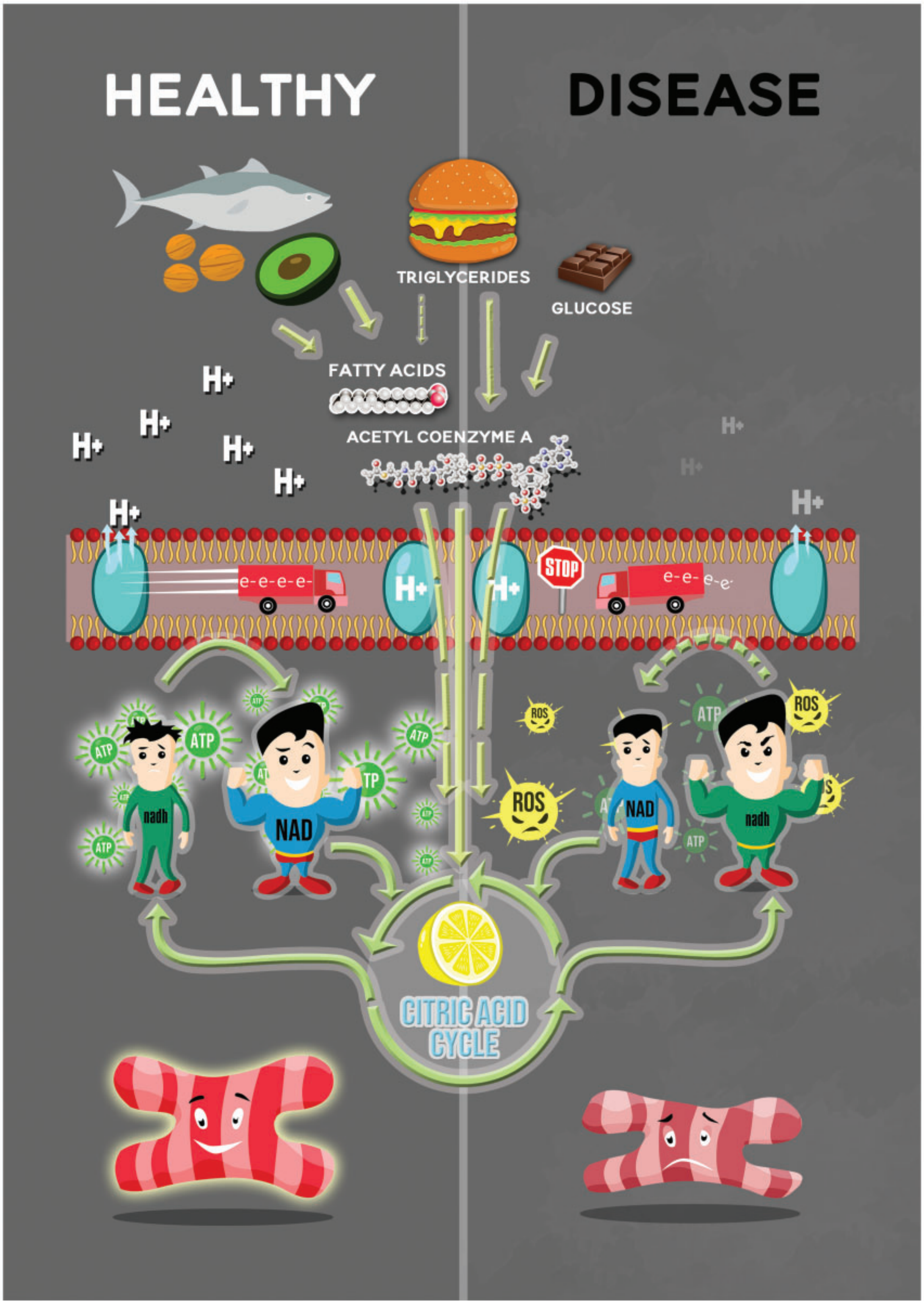

Figure 1 Cardiac metabolism and changes in cardiovascular diseases. Cardiac cellular respiration uses fat and sugars as energy substrates. Due to the high cardiac energy demand, cardiac metabolism prefers fats over sugars at the expense of higher O_2 demand; however, this preference is flexible and adjusts to metabolic needs. The balance of the NAD^+/NADH ratio is paramount to keep metabolic homeostasis. In cardiovascular diseases an imbalance in NAD^+/NADH ratio will block oxidative phosphorylation at the electron transport chain causing reductive stress and accumulation of reactive oxygen species, known to cause a variety of inflammatory responses including a myriad of cardiovascular diseases.

(through tryptophan, an essential amino acid), glutarate/citric acid pathway, and finally, the NAD^+-salvage pathway. Each of these pathways takes advantage of these precursors to produce NAD^+. These precursors have been a crucial dormant player in metabolic regulation and are gaining momentum in current research. There is a variety of precursors for NAD^+; The NAD^+-salvage pathway and its precursors NAM (nicotinamide), NMN (NAM mononucleotide), and NR (NAM riboside) have shown promising experimental results in cardiovascular diseases and metabolic syndrome models by re-establishing the NAD^+/NADH redox state improving glucose metabolism, reducing insulin resistance, and improving muscle motor performance.[14,16,33,50]

Conclusions and outlook

Several efforts are made to improve cardiac health by regulating metabolism. Current experimental treatments have shown promising results yet at the expense of detrimental side effects. NAD^+ is a critical player in cellular metabolism; and deserves attention for its multiple properties including enzyme synthesis, cell signalling, extracellular function, and anti-inflammatory properties during ischaemia–reperfusion injury.

A stable ratio of NAD^+/NADH is essential to maintain cellular homeostasis. Any alteration in the ratio of NAD^+/NADH will cause oxidative or reductive stress which may have detrimental effects on a cellular level. Bioactive NAD precursors hold a promise as potential therapeutic goals for the treatment of cardiovascular disease and heart failure. The duality and the dynamics of the process that keeps the adequate ratio of NAD^+/NADH pools, make any therapeutic approach challenging.

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The Russian National Congress of Cardiology 2019

The Russian National Congress of Cardiology is held annually in different cities throughout Russia. In 2019, the meeting returned to Ekaterinburg—the fourth-largest city of the country named after the Russian Empress Catherine I. The Congress held on 24–26 September and gathered 4617 delegates from 28 countries. Speakers and participants from Bulgaria, Finland, Germany, Greece, Italy, Kazakhstan, Portugal, Serbia, Spain, Turkey, UK, and other countries joined the event. Nearly 2500 people joined the online broadcasting and 1500 of them applied for Russian CME credits.

The scientific programme of the Congress was developed according to the needs of the diverse medical audience: plenary sessions, satellite symposia, round tables and special discussions for cardiologists, physicians, cardiovascular surgeons, basic scientists and researchers, healthcare organizers, and others. Simultaneous translation into English was organized for foreign participants.

The European Society of Cardiology (ESC) was represented by the President Prof. Barbara Casadei, President-Elect Prof. Stephan Achenbach, Vice-President Prof. Jose-Luis Zamorano, and Secretary/