

TREADING THE TIGHTROPE OF
HEALTHY LIVING: PONDERING HEART
METABOLISM'S BALANCING ACT

Faadiel Essop
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Treading the tightrope of healthy living: pondering heart metabolism's balancing act

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ABOUT THE AUTHOR

Prof Faadiel Essop is the current chairperson of the Department of Physiological Sciences at Stellenbosch University. He hails from a strong rural background; he was born in Ceres and grew up in Paarl (Western Cape, South Africa). He completed his undergraduate studies (biochemistry and microbiology majors) and PhD degree (medical biosciences) at the University of Cape Town (UCT). He also completed (part-time basis) a BA Hons degree (Arabic) at the University of the Western Cape. After postdoctoral fellowship stints at UCT and the University of Leeds, he joined the Hatter Cardiovascular Research Institute at UCT's Faculty of Health Sciences (1998). During this time he

focused on mechanisms driving the onset of cardiac hypertrophy and the effects of hypoxia on the heart. He was also appointed to the board of the Medical Research Council of South Africa by the Minister of Health (1998–2000). Prof Essop was awarded the prestigious Fulbright fellowship (2005–2006) to spend time in Prof Heinrich Taegtmeyer's laboratory at the University of Texas-Houston Medical School. During February 2007, he joined the Department of Physiological Sciences at Stellenbosch University as an associate professor and was promoted to full professor in 2011. At Stellenbosch University he established the Cardio-Metabolic Research Group that focuses on altered fuel substrate metabolism and its contribution to the onset of type 2 diabetes and heart failure. Prof Essop and his students have received several awards over the last few years. He has published 42 peer-reviewed papers and supervised six PhD, five MSc and four postdoctoral students. He is married to Dr Rehana Essop and they have three children, Ziyaad, Aaliyah and Yasin.

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I wish to acknowledge – in keeping with the African spirit of Ubuntu – all those who played a significant role in my academic career thus far. Archbishop Desmond Tutu captured the concept of Ubuntu best with the following words: “Ubuntu speaks particularly about the fact that you can't exist as a human being in isolation. It speaks about our interconnectedness. You can't be human all by yourself, and when you have this quality – Ubuntu – you are known for your generosity.”

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TREADING THE TIGHTROPE OF HEALTHY LIVING: PONDERING HEART METABOLISM'S BALANCING ACT

THE VIRTUE OF MODERATION

The quest for balance and moderation is well entrenched in major belief systems, ancient traditions and philosophical musings. For example, Aristotle (384–322 BC) noted that “the virtue of justice consists in moderation” while Muslims are reminded in the Quran, “and thus We willed you to be a community of the middle way”. The Buddha (563–483 BC) also understood this when overhearing a lute player and realizing that the harmonious sounds produced depended on the lute strings’ not being tuned too tightly or too loosely. Likewise, the well-being of individual cells and organisms depends on a constant internal environment, referred to as ‘homeostasis’. The term ‘homeostasis’ derives from the Greek *homoios* (‘same’) and *stasis* (‘condition’) and was coined in 1929 by the American physiologist Walter Cannon.^{1,2} However, the concept was earlier recognized by others. The French scientist Claude Bernard (1813–1878) used the term *milieu interieur* and noted that “all the vital mechanisms, varied as they are, have only one object, that of preserving constant the conditions of life in the internal environment”.^{1,2} Moreover, some suggest that the celebrated physician Ibn Sina (Avicenna) (980–1037) was aware of intrinsic and extrinsic factors and their role in the development of disease, and also of the body’s interior milieu and homeostasis.^{3,4}

External environmental exposures depend largely on lifestyle choices (e.g. nutrition and physical activity) and the outside environment (e.g. urbanization and pollution). Organisms employ numerous mechanisms to ensure that their internal milieu is exquisitely balanced, for example through continuous monitoring of internal and external environments. If required, the body elicits the necessary adjustments to remain near its ‘set point’. Here the organism functions inside particular limits within specific environments/contexts. If these set limits are exceeded or not adequately met, it is sensed and organisms initiate the necessary adjustments to again operate within their set confines.² However, organisms are sometimes unable to maintain homeostasis, leading to cellular dysfunction and the onset of diseases. This may occur either due to deficiency (needs of cells not

met) or toxicity (cells poisoned by oversupply).² As a result of such disruption, organisms trigger various pathways within affected cells to limit damage caused. However, in the longer term, this disruption may also result in maladaptation and disease onset.

The human body consists of various systems (e.g. nervous, digestive, endocrine and cardiovascular) that function in concert to ensure overall well-being. Since no system operates as an independent unit, disruption of one or more systems may result in serious consequences for other systems of the body and threaten overall well-being.² For example, if homeostasis of the cardiovascular system is compromised due to internal and/or external exposures, it will result in serious consequences for the host organism.

IMPACT OF CONTEMPORARY EXTERNAL EXPOSURES

A World Health Organization report warns of the escalating global burden of cardiovascular diseases (CVD),⁵ and a recent study investigating the worldwide prevalence of CVD projects a marked increase for its incidence in developing nations (including South Africa) by 2020.⁶ Data suggest that the dramatic surge in CVD rates in developing countries is due to accelerated urbanization and associated lifestyle changes (e.g. poor nutritional choices, obesity and decreased physical activity).⁷ For example, urbanization is a growing problem in Africa, drastically altering lifestyle and thereby leading to changes in body composition and shape, which ultimately increases the risk for insulin resistance, type 2 diabetes and CVD.⁸ Demographic change in South Africa is also a major contributing factor; for example, the number of individuals aged 35–64 years old is projected to rise three-fold by 2025 despite the threat of HIV/AIDS.⁹ Moreover, antiretroviral treatment (protease inhibitors) is associated with increased onset of insulin resistance, dyslipidemia and lipodystrophy.⁹ Patients on chronic antiretroviral treatment will further swell the growing burden of cardio-metabolic diseases in developing countries such as South Africa. This sentiment is aptly echoed by Alafia Samuels

(University of the West Indies, Barbados) who state that “we have to be patient-focused. We cannot cure (a patient’s) HIV and then send them off to die with diabetes.”¹⁰ Projections show that the global incidence of type 2 diabetes will continue to rise, with the African continent facing ~50% increase in numbers.¹¹ Since cardiovascular complications are common in patients with type 2 diabetes, this will further increase the overall burden of disease.¹² The higher mortalities and morbidities associated with increased type 2 diabetes and CVD rates will have serious socio-economic implications, including disruption of family units, greater health care costs and diminished productivity. This will eventually threaten the sustainable development of South Africa and the rest of the African continent.

THE METABOLIC SYNDROME

An emerging paradigm suggests that a cluster of metabolic abnormalities, referred to as the metabolic syndrome, is associated with increased risk for the development of both type 2 diabetes and CVD.^{13,14} Here an underlying rationale is to provide a clinically accessible diagnostic tool that will allow for the early identification of individuals at risk for the development of type 2 diabetes and/or CVD. In broad terms, the metabolic syndrome is characterized by a ‘deadly quartet’, including impaired glucose regulation (hyperglycemia), poor lipid profile (dyslipidemia), hypertension and obesity.^{13,14} Since abdominal fat deposition is associated with more serious health implications than fat accumulating elsewhere,⁷ the International Diabetes Federation now includes increased waist circumference as a prerequisite for the diagnosis of the metabolic syndrome.^{13,14} Of note, central obesity is thought to be the single most important factor contributing to the development of the metabolic syndrome. Moreover, adipose tissue is now recognized to be an active metabolic organ, secreting hormones and cytokines that may have both paracrine and endocrine effects on different tissue types.

In our opinion, the alarming projections for the onset of type 2 diabetes and CVD necessitate a comprehensive strategy to deal with this problem and therefore our laboratory is coordinating a multi-pronged project to evaluate the metabolic syndrome within the southern African context. Here our focus is to 1) investigate the onset of metabolic risk factors in southern African communities, 2) gain insight into the basic mechanisms whereby metabolic risk factors actually trigger type 2 diabetes and CVD onset, 3) establish unique therapeutic

interventions to blunt the onset of insulin resistance/type 2 diabetes and CVD and 4) develop novel methods to detect the onset of diabetes and CVD.

The rest of this document will concentrate on selected research work conducted in our laboratory over the last years, emphasizing perturbed metabolism and its contribution to the onset of cardio-metabolic diseases. Our work focused particularly on unraveling the mechanisms underlying heart metabolism and function within the setting of type 2 diabetes and cardiac hypertrophy (thickened cardiac muscle). Here our rationale is that such an understanding will allow for the development of novel diagnostic tools and therapies to treat the growing burden of cardio-metabolic diseases in developed and developing nations.

ONSET OF METABOLIC SYNDROME IN SOUTH AFRICAN POPULATIONS

Of concern is that relatively limited data are available for the prevalence of the metabolic syndrome in South Africa and the African continent, thus limiting the development of effective strategies to deal with the increasing burden of disease for type 2 diabetes and CVD. In light of this, we began investigating the incidence of metabolic risk factors in a younger student population (< 30 years old) at Stellenbosch University. Here risk factors (4% of student population) presented at a much younger age than commonly expected.¹⁵ Our data showed some gender-based differences, with women displaying a greater prevalence of increased waist circumference while men exhibited higher blood pressures. Waist circumference in the female study population was positively associated with blood pressures and cholesterol levels. We propose that these differences are related to student behavioural patterns, in other words, male students displaying poor lifestyle choices (e.g. increased consumption of ‘junk food’) and female students exercising less than would normally be expected. We therefore recommend that it is imperative to screen young students in order to identify metabolic risk profiles relatively early on and to thereafter initiate appropriate lifestyle changes.

We are presently expanding our initial work in Stellenbosch to include a greater number of students and also the more senior campus population (> 30 years old). Our data show a marked incidence of metabolic risk factors in the older Stellenbosch campus population (38–58 years old) (see Table 1), with elderly men the most vulnerable group (unpublished data). Since obesity and poor lifestyle choices (older men) appear to be key

mediators of metabolic risk factor onset in this group, we advise improved body weight management (e.g. increased exercise) and sound lifestyle choices (e.g. lower alcohol intake). Together, these data show that metabolic risk factors present a) at a much earlier age than what would be commonly expected and b) at alarming rates in the older population. This therefore necessitates effective strategies to counter the predicted onset of type 2 diabetes and CVD. Moreover, it also requires further investigation and identification of mechanisms whereby risk factors eventually cause the development of cardio-metabolic diseases.

	Female students (< 30 years)	Female Staff (> 30 years)	P-values
Systolic BP (mmHg)	119	132.96	P < 0.001
Diastolic BP (mmHg)	77.65	88.61	
Glucose (mmol/L)	4.6	7.1	
Triglycerides (mmol/L)	1.47	2.78	
Total Cholesterol (mmol/L)	4.65	5.19	p < 0.05
BMI (kg/m ²)	20.98	27.99	P < 0.001

Table 1: Metabolic risk factors for female students and staff members at Stellenbosch University

Older Stellenbosch University female staff exhibits a marked increase in metabolic risk factors and anthropometric measures compared to the younger population. Here we found higher blood pressures and fasting levels of several metabolites, namely increased glucose, triglycerides and total cholesterol. Note: systolic blood pressures ≥ 130 and/or diastolic pressures ≥ 85 , fasting triglyceride ≥ 1.7 mmol/L and fasting glucose levels ≥ 5.6 mmol/L make up some of the criteria that constitute the metabolic syndrome, according to the International Diabetes Federation's definition. We are currently in the process of evaluating low-density lipoprotein and high-density lipoprotein levels in these populations. The body mass index (BMI) – marker of obesity – of older women was also higher than the cut-off value of 25 kg/m² and falls within the range that is regarded as pre-obese (n = 117).

THE REDUCTIONIST APPROACH AND BASIC SCIENCE

To better understand the underlying molecular mechanisms driving the onset of disease states, biomedical scientists currently adopt 'reductionism' as their operating paradigm. The basic rationale of reductionism is that a complex system, for example the onset of a particular disease state within a human being, can be understood by examining its basic elements. The approach is to reduce a specific disease condition to its basic cellular and molecular elements (e.g. genes, proteins and enzymes). Moreover, biomedical scientists employ various experimental systems (e.g. animal and cell-based models) in their laboratories to investigate and identify molecular events that may ultimately result in the onset of a particular disease.

Although this particular worldview has resulted in significant strides being made to develop novel therapies, its shortfalls are also increasingly being highlighted. For example, scientists are now facing 'information overload' with the overspecialization of disciplines often resulting in a plethora of molecular pathways with significant cross-talk a compounding factor. This may impair information flow and/or meaningful and coherent synthesis/understanding of the various molecular pathways and regulators continuously being identified. Oversimplification is another potential problem since therapeutic advances tested within the laboratory setting are not always effective when employed in the clinic. In agreement, Dr Claude Lenfant, former director of the National Heart, Lung, and Blood Institute (NHLBI), states, "Enormous amounts of new knowledge are barrelling down the information highway, but they are not arriving at the doorsteps of our patients."¹⁶ As a result, major institutions such as the National Institutes of Health in the United States of America have adjusted their strategic approach with a renewed interest in the more rapid 'translation' of laboratory-based science into the clinic and to also foster greater co-operation between basic scientists and clinicians. Furthermore, recent approaches such as 'systems biology' are also attempting to deal with the shortcomings of molecular reductionism. However, Joyner¹⁷ correctly points out that systems biology still employs a cell-centric focus with limited understanding and application beyond the cell.

Since some of these approaches are still rooted in the existing scientific worldview, it is likely that the current paradigm will need to be revised in future to reflect a more integrative approach. Physiology as a

discipline may indeed be able to help bridge the gap between molecular reductionism and complexity. Here the idea is put forward that physiology may fill the current intellectual void since its focus is to understand the integrated functions of organs/organisms within the larger context of homeostasis and regulation.^{17,18}

THE ABCS OF HEART METABOLISM

The main focus of our research work over the last years was on the role of altered metabolism of the heart and its impact on myocardial energy (adenosine triphosphate [ATP]) production and contractile function. The normal human heart requires between 3.5 and 5 kg of ATP per day to sustain its function.¹⁹ An adequate supply of oxygen and fuel substrates allows for their metabolism to generate enough ATP to keep the heart pumping. Fatty acids serve as the major fuel substrate for the normal, fasting adult mammalian heart, providing the majority of its energetic requirements.¹⁹ The rest of the heart's ATP is derived from glucose and lactate in nearly equal proportions, while ketone bodies are also utilized as a fuel substrate under certain conditions. After uptake by specific cardiac fatty acid transporters, long-chain fatty acids are esterified by fatty acyl-CoA synthetase and may either be stored as triglycerides or transported into the mitochondrion for fatty acid β -oxidation.

Mitochondrial fatty acid uptake is controlled by malonyl-CoA, a potent allosteric inhibitor of carnitine palmitoyltransferase-1 (CPT-1), the rate-limiting enzyme regulating this process.²⁰ The rates of synthesis and degradation of malonyl-CoA in the heart are stringently controlled by acetyl-CoA carboxylase (ACC) and malonyl-CoA decarboxylase (MCD), respectively. Two ACC isoforms with distinct functional roles have been identified in mammals, namely ACC α and ACC β .^{21,22} ACC α is enriched in lipogenic tissues where it plays a key role in fatty acid biosynthesis. Conversely, ACC β is abundantly expressed in oxidative tissues (e.g. heart and skeletal muscle) and is physically associated with mitochondria.²³ ACC β is therefore strongly implicated in the control of mitochondrial fatty acid β -oxidation. In support, we found decreased myocardial malonyl-CoA levels and increased cardiac mitochondrial fatty acid β -oxidation in ACC β mutant mice (displaying reduced ACC β activity).²⁴

We also performed studies on transcriptional mechanisms that regulate ACC β gene expression to identify novel 'on' and 'off' switches that regulate this process. This approach was adopted since both decreased and increased fatty acid β -oxidation rates are implicated in the onset of insulin resistance and diabetes-related CVD. We hypothesized that this apparent contradiction may be clarified when viewing such findings within particular contexts, for example experimental models employed and also the stage of disease progression. Thus the identification of both activators and inhibitors of ACC β gene expression offers therapeutic promise depending on the particular context. Here we identified a unique transcriptional activator, upstream stimulatory factor 1 (USF1), that increases cardiac ACC β gene expression.²⁵ Furthermore, we also identified nuclear respiratory factor-1 (NRF-1) as a novel inhibitor of ACC β gene expression.²⁶ We therefore propose that both USF1 and NRF-1 are useful targets to control malonyl-CoA levels and thereby up- or downregulate cardiac mitochondrial fatty acid β -oxidation, depending on the particular intracellular milieu (see Figure 1).

Upstream, 5'-AMP-activated protein kinase (AMPK) is a pivotal fuel sensor that is activated in response to environmental stress (e.g. oxygen lack and nutrient deprivation) with the primary aim to restore both cellular and whole body energy balance.²⁷ AMPK activates both myocardial glucose and fatty acid metabolic pathways to ultimately ensure increased production of myocardial ATP when required. It is well established that AMPK can phosphorylate and inhibit ACC β activity, thereby lowering malonyl-CoA levels and stimulating mitochondrial fatty acid β -oxidation. We also found, for the first time as far as we are aware, that AMPK dose-dependently reduces ACC β gene promoter activity²⁶ (see Figure 1). AMPK is therefore able to inhibit ACC β at both transcriptional and enzyme activity levels. After its mitochondrial uptake, the fatty acyl-CoA enters the β -oxidation spiral that successively shortens fatty acids by two carbons (per cycle) and generates NADH and FADH₂. The reducing equivalents generated subsequently donate electrons to the mitochondrial electron transport chain for ATP production. Mitochondrial fatty acid β -oxidation provides ~60–90% of the total energy requirements of the heart.¹⁹

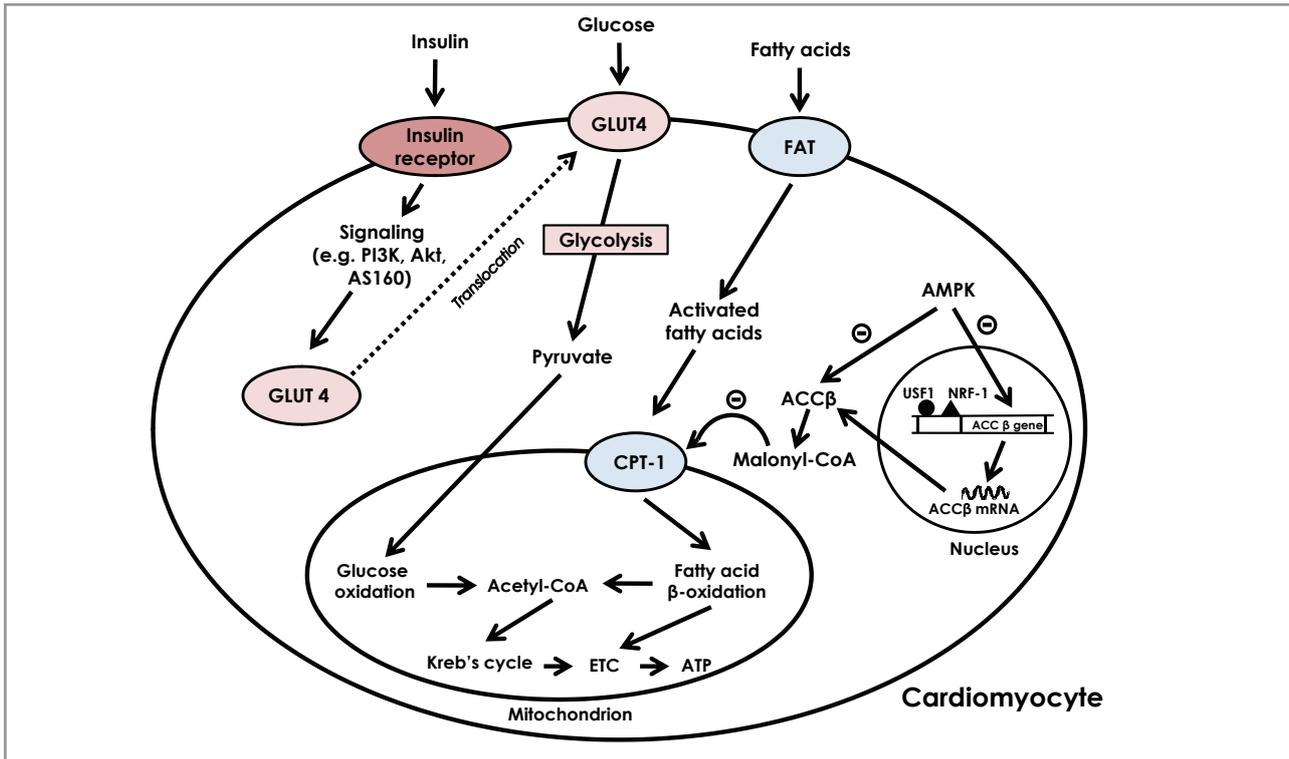


Figure 1: Basic scheme of cardiac fatty acid and glucose metabolism

Fatty acids are taken up by specific transporters (e.g. FAT – fatty acid transporter) and activated by the addition of coenzyme A. Mitochondrial uptake is controlled by carnitine palmitoyltransferase-I (CPT-I). Fatty acid β -oxidation subsequently occurs within the mitochondrial matrix to produce reducing equivalents that can enter the electron transport chain (ETC) to generate ATP for work. Mitochondrial fatty acid uptake is regulated by malonyl-CoA, a potent inhibitor that is synthesized by acetyl-CoA carboxylase- β (ACC β). We found that upstream stimulatory factor I (USF1) and nuclear respiratory factor-1 (NRF-1) can increase or decrease ACC β gene expression, respectively, and thus control cardiac malonyl-CoA levels. This, in turn, will regulate CPT-I activity and cardiac mitochondrial fatty acid uptake. We also found that AMPK activation can decrease ACC β gene expression. Circulating insulin regulates glucose uptake by promoting GLUT4 storage vesicles to migrate to the cell membrane where increased GLUT4 availability can now mediate glucose uptake. After its uptake, glucose is catabolized (in several steps) into pyruvate that can be taken up by mitochondria to be further metabolized. Reducing equivalents produced by the Krebs cycle can generate ATP via the ETC.

Myocardial glucose uptake is mediated by glucose transporter (GLUT) isoforms, namely GLUT1 and GLUT4. GLUT1 is enriched during the fetal stages of development, while GLUT4 is postnatally induced, insulin-independent and the major transporter in the adult heart.¹⁹ After insulin binds to the membrane-bound insulin receptor, it triggers signaling cascades (e.g. PI3 kinase, Akt and AS160) that allow GLUT4 to migrate from intracellular vesicular stores to the sarcolemma, thereby increasing myocardial glucose uptake.^{1,19} (see Figure 1). Glucose subsequently enters the glycolytic pathway and is ultimately converted to pyruvate with the production of two ATP molecules (per glucose molecule) without any oxygen requirements (anaerobic glycolysis). Additional metabolic fates of glucose include

its conversion to glycogen (for rapid utilization at a later stage) and flux through the polyol, hexosamine biosynthetic and pentose phosphate pathways. Pyruvate subsequently enters the mitochondrion where it is further catabolized via the Krebs cycle to generate reducing equivalents (NADH and FADH₂) that can eventually enter the electron transport chain to produce mitochondrial ATP. The complete breakdown of glucose (glycolysis and glucose oxidation) accounts for ~10–40% of the heart's energy requirements.¹⁹

NADH and FADH₂ generated by glucose and fatty acid metabolism can initiate electron transfer through the mitochondrial electron transport chain. Electrons are passed through complex I (NADH dehydrogenase) and complex II (succinate dehydrogenase) to coenzyme Q.

Reduced CoQ then transfers electrons to complex III (ubiquinol cytochrome c reductase) that donates it to oxidized cytochrome c. Thereafter reduced cytochrome c passes electrons to complex IV (cytochrome c oxidase) that reduces molecular oxygen to water in the final step. During such electron transfer (between complex I and complex IV), a proton gradient is established across the inner mitochondrial membrane and exploited for ATP production. As protons move back into the mitochondrial matrix via complex V (ATP synthase) mitochondrial ATP is produced.

Cardiac fuel substrate utilization is dynamic and may be altered according to the prevailing physiologic or pathophysiologic milieu. For example, after meal intake (fed state), circulating insulin levels rise to promote myocardial glucose uptake. During the fasted state (several hours after a meal), fatty acid utilization predominates with a concomitant decrease in carbohydrate utilization and circulating insulin levels. Furthermore, high-altitude natives, such as the Himalayan Sherpas and the Andean Quechuas, display enhanced myocardial glucose uptake.²⁸ Here increased glucose utilization at high altitude (relatively hypoxic environment) may occur since it is a more oxygen-efficient fuel substrate to generate ATP compared to fatty acids. In certain instances, myocardial fuel substrate switches may also result in maladaptive effects, thereby leading to impaired cardiac contractility. The latter has been the focus of our laboratory during the last years, namely to delineate the effects of altered metabolism in hypertrophied and diabetic hearts. We employed several animal and cell-based experimental systems to investigate our hypothesis that chronically high fuel substrate (fatty acids and glucose) and lower oxygen levels trigger maladaptive pathways, leading to decreased mitochondrial ATP generation and increased cell death. This, in turn, would be expected to lead to impaired cardiac contractile function.

THE PHYSIOLOGIC HYPERTROPHIED HEART

To gain insight into the link between physiologic and pathophysiologic cardiac hypertrophy, we established and characterized a rat model of hypobaric hypoxia-induced right ventricular hypertrophy (physiologic).^{29–32} Relatively few studies have separately examined the metabolism and function of the right and left ventricles in response to hypertrophy. This model also allowed us to separately examine the effects of hypoxia *per se* on the heart, namely by focusing on the non hypertrophied

left ventricle.^{33–40} However, these findings will not be discussed in this particular document. Rats were typically exposed to hypobaric hypoxia (11% oxygen) for various lengths of time (1–12 weeks), and we found a robust hypertrophic response (increased right ventricular weight and myocyte size) as early as one week. No fibrosis was detected in the hypertrophied right ventricle (see Figure 2).

We found coordinate induction of several genes regulating mitochondrial function, and increased citrate synthase activity and mitochondrial DNA levels in the hypertrophied right ventricle (at the two-week and four-week time points).^{31,32} In parallel, mitochondrial respiratory capacity and right ventricular contractile function were both sustained (see Figure 3). Thus the physiologic hypertrophied right ventricle maintains its output by increasing mitochondrial respiratory capacity and ATP production. We propose that pathophysiologic hypertrophy may occur when mitochondrial respiratory capacity diminishes, thereby leading to decreased cardiac output and eventually heart failure. In agreement, Neubauer⁴¹ proposed that during end-stage heart failure, the heart runs out of fuel since both glucose and fatty acid oxidation are downregulated. These data therefore indicate that the restoration of mitochondrial respiratory capacity in failing hearts may be a useful therapeutic strategy to pursue. However, the pitfalls of reductionism should be borne in mind and such treatments be tailored to be employed within specific contexts, for example end-stage heart failure in this instance.

THE DIABETIC HEART

We investigated cardiac contractile function and mitochondrial respiratory capacity in a rat model of diet-induced prediabetes, in other words, “metabolic syndrome-like”.⁴² Here intake of a sub-optimal diet induced obesity together with insulin resistance, increased visceral fat, dyslipidemia and higher plasma insulin levels. Moreover, prediabetic rats exhibited reduced cardiac contractile function at baseline together with decreased mitochondrial bioenergetic capacity. We also found increased myocardial damage when isolated rat hearts were challenged with an ischemic insult (representing a simulated heart attack). In agreement, isolated heart mitochondria from prediabetic rats displayed decreased mitochondrial respiration in response to acute oxygen deprivation. In a separate study, we also found attenuated mitochondrial respiratory capacity in a mouse model of type I diabetes.⁴³

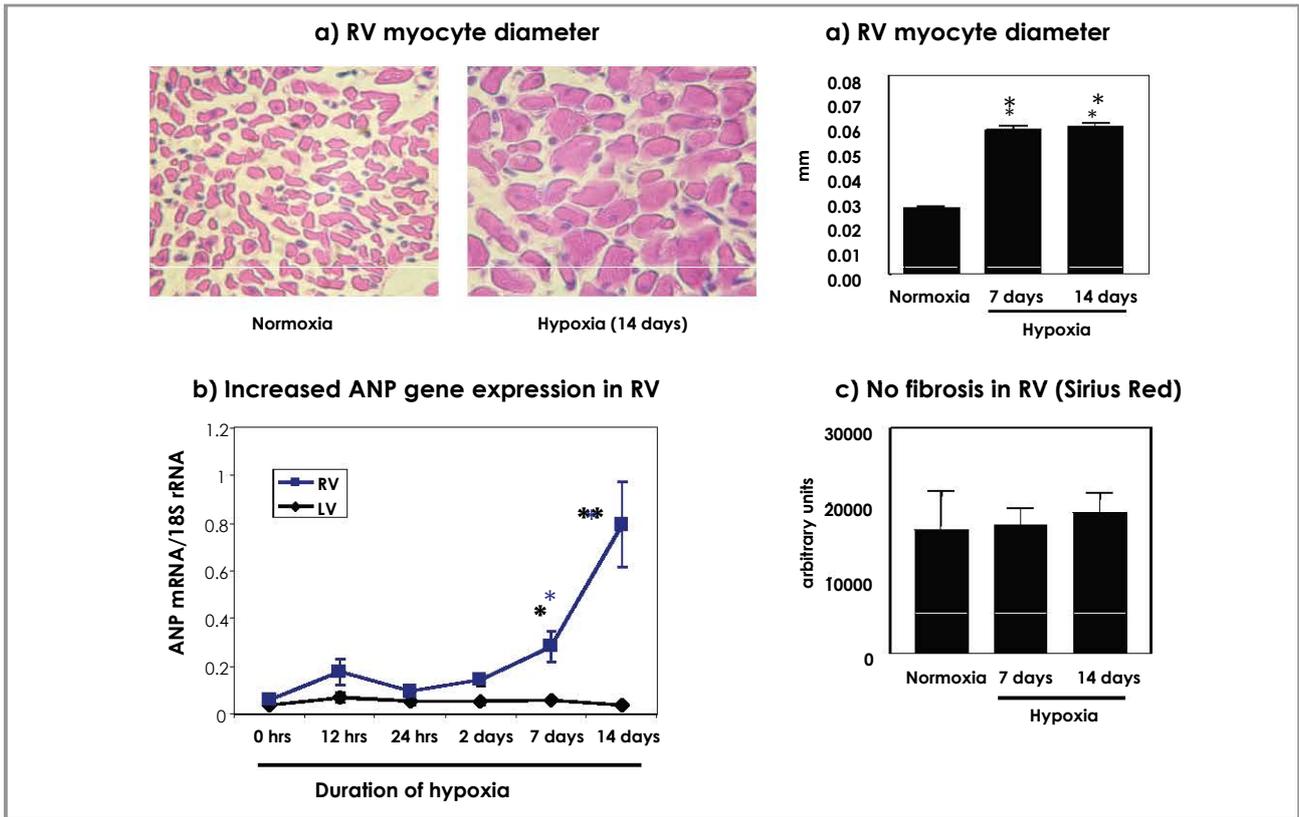


Figure 2: Robust hypertrophic response in right ventricles exposed to hypobaric hypoxia

Male Wistar rats were exposed to 14 days of hypobaric hypoxia (45 kPa, 11% O₂), and the degree of right ventricular (RV) cardiac hypertrophy was assessed versus normoxic controls. Histologic analysis of RV heart cells revealed markedly increased cell size – refer a) (magnification 40 x). Moreover, gene expression levels of atrial natriuretic peptide (ANP), a well-known marker for cardiac hypertrophy, increased significantly after 14 days – refer b). Sirius Red staining of heart tissues detected no fibrosis in the hypertrophied RV. *p < 0.05 vs. matched controls.

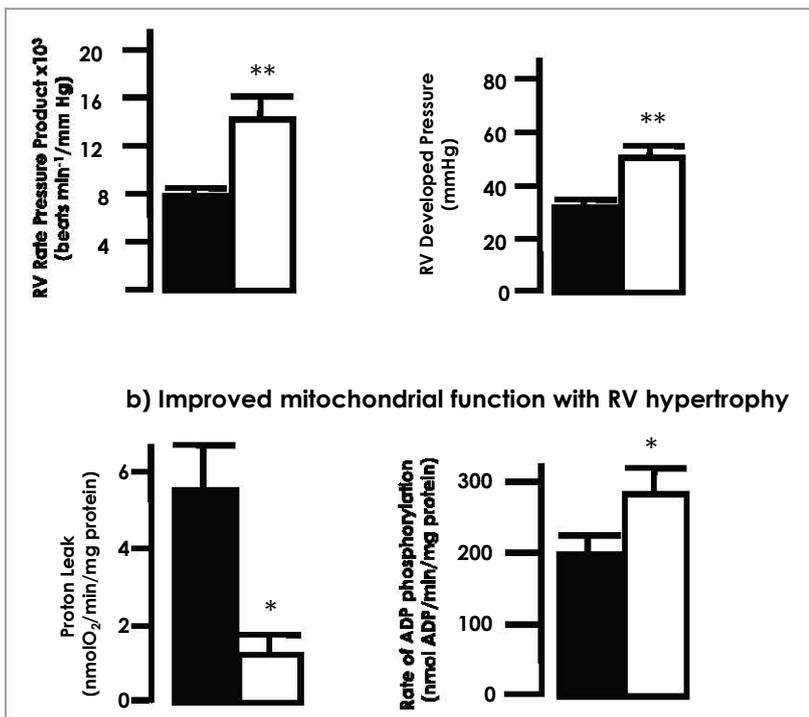


Figure 3: Improved heart function and mitochondrial respiratory capacity in the hypertrophied right ventricle

After 14 days, hearts were isolated and *ex vivo* Langendorff perfusions performed to assess contractile function. Here the hypertrophied RV displayed increased function – refer a). In separate experiments, mitochondria were isolated and purified to evaluate respiratory capacity. These data show decreased mitochondrial proton leak and increased ADP phosphorylation – refer b). Thus these mitochondria become more efficient in terms of mitochondrial ATP production. *p < 0.05 and **p < 0.01 vs. matched controls, respectively.

These data therefore demonstrate that attenuated mitochondrial respiratory function and bioenergetic capacity contribute to the onset of decreased cardiac contractile function at baseline and in response to an ischemic insult in the prediabetic state.

Proteomic studies

To gain further insight into the mechanisms responsible for impaired mitochondrial function and contractile dysfunction, we examined alterations in the mitochondrial proteome with the onset of type 2 diabetes. We employed the db/db transgenic mouse model that is characterized by deficient leptin receptor activity, which subsequently leads to obesity and the onset of type 2 diabetes. The db/db mice display hyperphagia (overeating) since the fat-specific hormone leptin is now unable to perform its usual function, in other words, acting on leptin receptors in the hypothalamus to suppress appetite.

We employed 2D-polyacrylamide gel electrophoresis (PAGE) studies and found several differences between control and obese hearts that broadly fall into two categories, namely related to energy metabolism and related to contraction/cytoskeleton, respectively.⁴⁴ Here we found a significant decrease in peptide levels of ubiquinol cytochrome-c reductase core protein I, a subunit of complex III of the electron transfer chain that catalyzes transfer of electrons from coenzyme Q to cytochrome c. Despite attempts by the obese heart to augment mitochondrial ATP production, attenuated ubiquinol cytochrome-c reductase core protein I peptide levels likely contribute to impaired mitochondrial ATP production. We also found co-ordinated down-regulation of key contractile/cytoskeletal proteins in the obese heart, namely α -smooth muscle actin, α -cardiac actin, myosin heavy chain (MHC)- α and MyBP-C. These peptides play a crucial role to ensure sustained myocardial contractile function and cytoskeletal support. These data are consistent with previous work that found an MHC isoform switch during the onset of diabetes, namely decreased MHC α and increased MHC β expression.⁴⁵ MyBP-C is a thick filament-associated protein and provides an additional regulatory step to myocardial contraction. MyBP-C gene mutations can cause hypertrophic cardiomyopathy⁴⁶ while its absence (cMyBP-C null mice) significantly attenuates *in vivo* left ventricular function.⁴⁷ We therefore propose that the prediabetic milieu elicits transcriptional changes (decreased ex-

pression of respiratory chain complex components and contractile proteins) that contribute to impaired respiratory capacity and contractile function observed in the diabetic heart.

The effects of increased free fatty acids

To further understand how altered circulating fuels may alter cardiac metabolism and heart function, we also focused on the effects of elevated free fatty acids usually found during the prediabetic and the diabetic state. We determined whether cardiac efficiency, that is the ratio of cardiac work to myocardial oxygen consumption (MVO₂), is diminished in diabetic hearts. Our data showed decreased cardiac efficiency in db/db hearts, namely an 86% increase in myocardial oxygen consumption (increased mitochondrial respiration and cardiac fatty acid β -oxidation).⁴³ The extra oxygen cost of increased fatty acid β -oxidation relative to glucose oxidation will, however, make a minor contribution since there is only a theoretical 11% decrease in the efficiency of hearts shifting from complete glucose oxidation to complete fatty acid β -oxidation. We therefore propose that intracellular futile metabolic cycles and upregulation of mitochondrial uncoupling proteins (induced by elevated plasma fatty acids) could dissipate the proton gradient across the inner mitochondrial membrane.⁴⁸ Since an early increase in fatty acid β -oxidation precedes the onset of contractile dysfunction such pronounced 'oxygen wastage' may compromise heart function when oxygen demand is high (e.g. elevated workloads) or when oxygen delivery is limited (e.g. an ischemic insult).

The effects of elevated glucose levels

We are also focusing on the damaging effects of elevated glucose levels (hyperglycemia), a risk factor that forms part of the metabolic syndrome, on the heart. There is a growing shift in the paradigm for the role of dietary macronutrient composition in the incidences of CVD, as studies show that there is no reduction in CVD with a low-fat/high-carbohydrate diet but there is rather reduced risk with a diet low in sugar and rapidly absorbed starches and high in polyunsaturated fatty acids.^{49,50} Moreover, there is increasing evidence from animal studies showing that a diet with a high glycemic load, typical of highly processed foods, accelerates the development and progression of CVD⁵⁰ (see Figure 4).

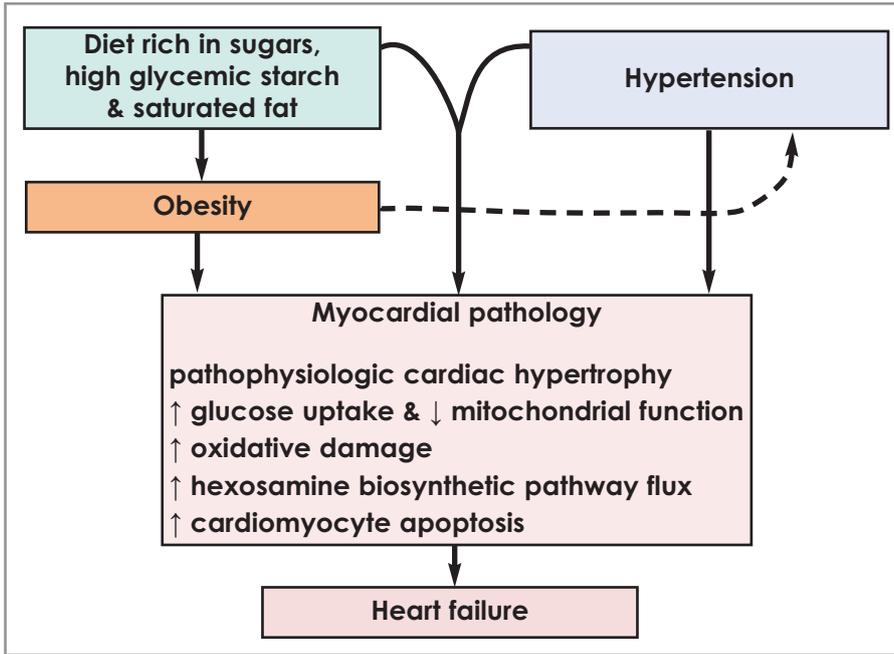


Figure 4: Flow diagram depicting the interaction between a 'junk food' diet, obesity and hypertension

The diagram depicts an evolving paradigm characterizing the effects of diet on left ventricular remodelling. A diet high in sugar and saturated fats promotes myocardial dysfunction through hypertension-dependent and independent pathways (adapted from 50).

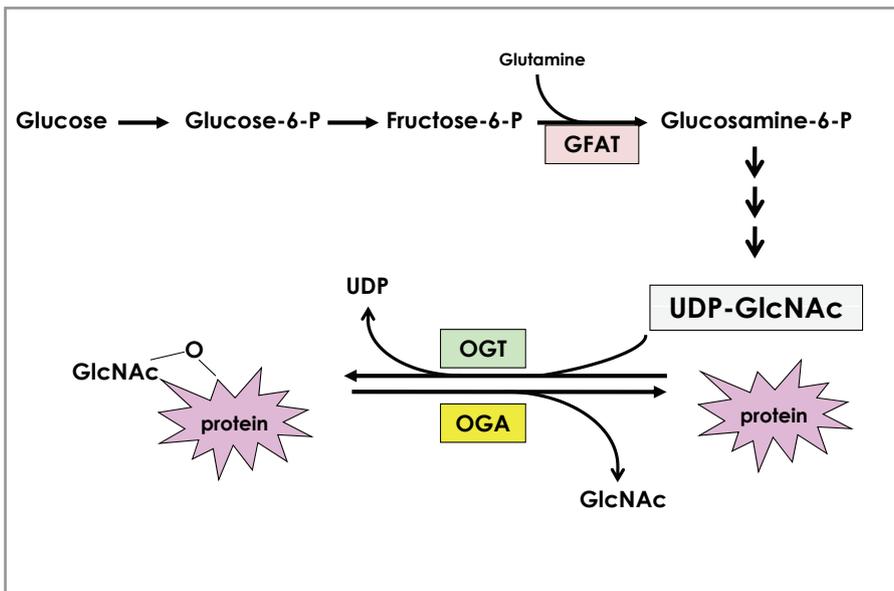


Figure 5: The hexosamine biosynthetic pathway (HBP)

Production of glucosamine-6-phosphate is catalyzed by glutamine: fructose-6-phosphate amidotransferase (GFAT). Glucosamine 6-phosphate is ultimately converted to UDP-N-acetylglucosamine (UDP-GlcNAc), the HBP's end product. O-GlcNAc transferase (OGT) catalyzes the O-linked transfer of GlcNAc to target proteins while O-GlcNAcase (OGA) catalyzes the reverse reaction.

Our major focus is on the detrimental effects of increased activation of a glucose-based metabolic pathway, the hexosamine biosynthetic pathway (HBP). The HBP usually acts as a 'fuel sensor' under normal conditions, in other words, sensing glucose and free fatty acid availability and repartitioning fuel substrates into suitable storage depots within the body (reviewed in 51 and 52). After uptake, glucose is rapidly converted to glucose-6-phosphate and thereafter to fructose-6-phosphate. Fructose-6-phosphate is the entry point for the HBP, forming glucosamine-6-phosphate. Production of glucosamine-6-phosphate is catalyzed by glutamine: fructose-6-phosphate amidotransferase (GFAT), the rate-limiting enzyme of the HBP (see Figure 5). Through

a series of reactions, glucosamine 6-phosphate is ultimately converted to UDP-N-acetylglucosamine (UDP-GlcNAc), the end product of the HBP. UDP-GlcNAc is a substrate for O-GlcNAc transferase (OGT) that catalyzes the O-linked transfer of GlcNAc to specific serine/threonine residues of target proteins. Conversely, O-GlcNAcase (OGA) catalyzes the reverse reaction, namely the removal of O-GlcNAc residues from target proteins. O-GlcNAc modification is implicated in a diverse range of intracellular reactions and pathways, including altering protein degradation and intracellular localization, modulating protein-protein interactions and regulating transcriptional mechanisms.^{51,52}

and CVD. In light of this, our work during the last few years has focused on developing novel therapies to blunt the effects of chronic HBP activation and its downstream effects, namely onset of insulin resistance and cardiac dysfunction.

THE DEVELOPMENT OF METABOLIC THERAPIES

The development of novel therapies to treat cardio-metabolic diseases usually takes a considerable length of time, sometimes decades. This route usually originates with conception of the original idea, basic experimentation and further evaluation in smaller and larger clinical settings. When criteria of safety are satisfactorily met, drugs are finally approved for treatment of various illnesses. However, unexpected side-effects can sometimes occur after chronic treatment with specific drugs. For example, although antiretroviral treatment dramatically decreases HIV/AIDS morbidity and mortality, the long-term effects may include metabolic derangements and the onset of CVD. This example demonstrates the limitation of the reductionist approach to biomedical science. As a result, additional studies are required to effectively treat associated cardio-metabolic diseases associated with chronic antiretroviral treatment. Our laboratory hypothesized that protease inhibitor treatment elevates oxidative stress that attenuates contractile function at baseline and in response to myocardial ischemia. We are currently investigating this postulate by establishing a unique rat model of chronic protease inhibitor utilization (lopinavir/ritonavir treatment for eight weeks). Here our data show that protease inhibitor-treated rats exhibited increased body weights and low-density lipoprotein cholesterol levels. We also found attenuated contractile function at baseline following eight weeks of protease inhibitor treatment and increased myocardial cell death (unpublished data). Decreased contractile function persisted during the reperfusion period (after an experimentally induced ischemic insult). We are currently testing inhibitors of maladaptive pathways that can eventually be coadministered with antiretroviral drugs.

In light of these difficulties, we have focused on natural agents as a potential therapeutic strategy since this may result in a more rapid translation into the clinic and also prove to be more cost-effective, especially within the developing world context. Previous work demonstrated that diabetes results in decreased levels of thiamine (Vitamin B₁), which is essential for normal

functioning of the heart, muscles and nerves.⁵⁶ Deficiencies in the B-series vitamins are among the key causative factors leading to diabetic organ damage.⁵⁷ Hence, thiamine and its lipophilic analogue benfotiamine can reduce some of the complications associated with diabetes, for instance cardiomyopathy, retinopathy and neuropathy.⁵⁸ Since benfotiamine can decrease HBP flux, we hypothesized that it is a cardioprotective agent. Our preliminary data show that benfotiamine treatment blunts the damaging effects of hyperglycemia and significantly improves the heart's functional recovery in response to ischemia-reperfusion (unpublished data). Thus these data demonstrate that benfotiamine is a promising cardioprotective agent that may ultimately benefit pre- and full-blown diabetic patients suffering from cardiovascular disease complications.

We also tested whether oleanolic acid (a clove extract) possesses antioxidant properties. Our *in vitro* data demonstrated that oleanolic acid blunted hyperglycemia-induced oxidative stress and apoptosis in heart cells (unpublished data). It also markedly improved functional recovery of hearts perfused in response to ischemia-reperfusion under hyperglycemic conditions.

We also performed studies on oxygen lack (hypoxia) as a cardioprotective strategy. We propose that a moderate lack of oxygen (physiologic hypoxia) triggers protective signaling pathways, unlike a severe impairment of oxygen supply (pathophysiologic hypoxia) that may exceed the host organism's defense apparatus, resulting in a maladaptive cardiac phenotype (reviewed in 38). Physiologic hypoxia triggers various defense mechanisms, for example erythropoiesis and angiogenesis, to increase red blood cell mass and oxygen delivery to the heart. Here we propose that physiologic hypoxia activates regulatory pathways that mediate long-term cardiac metabolic remodeling, particularly at the transcriptional level. The proposal is that physiologic reactive oxygen species (ROS) play a central role by modulating the activity of redox-sensitive transcription factors to induce a fetal gene program (increased carbohydrate and decreased fatty acid metabolism) and increased mitochondrial biogenesis in the heart.³⁸ These programs will thus increase the efficiency of energy production and augment mitochondrial bioenergetic capacity to sustain cardiac contractile function. Collectively these studies show promise, and the next step would be to translate some of our findings into the clinic, in other words, small pilot studies.

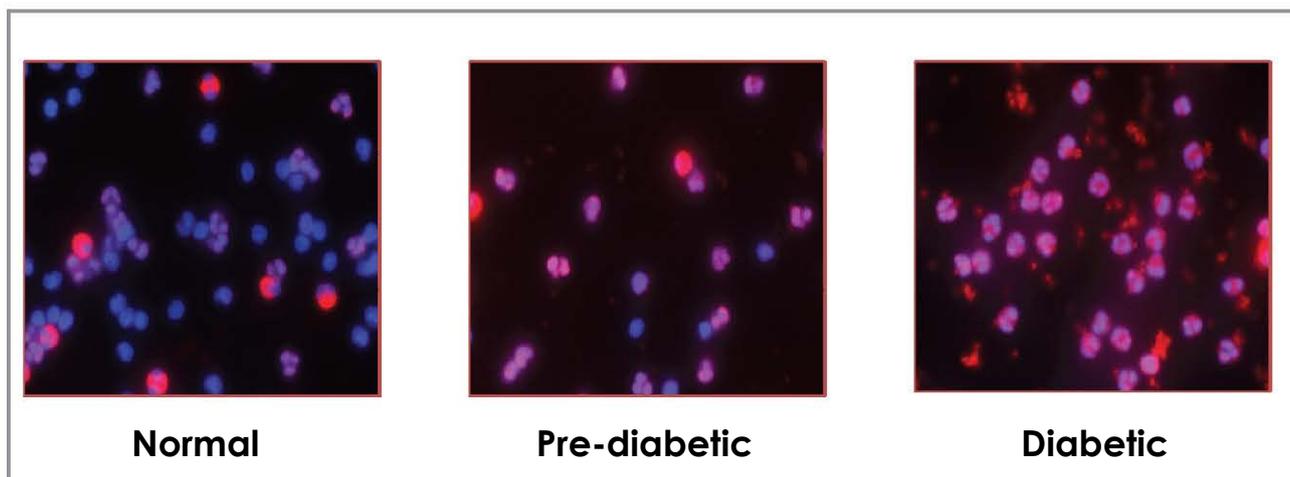


Figure 7: Increased HBP activation parallels fasting glucose levels
 Leukocytes stained for *O*-GlcNAc (Texas red) and cell nuclei (Hoechst blue dye) demonstrate increased red staining (*O*-GlcNAc) for prediabetic and diabetic individuals.

THE DEVELOPMENT OF DIAGNOSTIC TOOLS FOR CLINICAL APPLICATION

We are also pursuing the development of novel diagnostic tools to allow for more sensitive and earlier detection of cardio-metabolic diseases. Although there are currently tests available to screen for the onset of diabetes, for example glycosylated hemoglobin (HbA1c) levels, oral glucose tolerance and fasting blood glucose levels tests, these methods have limitations. For example, Saudek et al⁵⁹ propose that HbA1c is not always an effective diagnostic tool to identify pre-diabetes. At the end of their article the authors state, “There are serious deficiencies in the current criteria for diagnosing diabetes, including the requirement that the patient be fasting, and the lack of agreed-on screening criteria. These deficiencies make it unnecessarily inconvenient for clinicians to diagnose diabetes, thereby delaying the diagnosis and contributing to avoid-

able morbidity and mortality.” Thus there is a need for the development of novel diagnostic tools to detect prediabetes and diabetes in a timely, sensitive and cost-effective manner.

Although rapid protein *O*-GlcNAcylation occurs within white blood cells,⁶⁰ there is no literature regarding *O*-GlcNAcylated leukocyte proteins within the pre-diabetic setting. In light of this, we hypothesized that there is increased oxidative stress and *O*-GlcNAcylation in white blood cells in the prediabetic milieu and that this may offer a novel diagnostic tool to predict the onset of insulin resistance. Here our data (flow cytometry and fluorescence microscopy generated) show that *O*-GlcNAcylation of leukocyte proteins changes in parallel with increasing fasting glucose levels (see Figure 7) (unpublished data). The data show early promise for the establishment of a sensitive diagnostic test that may help improve the detection and management of type 2 diabetes.

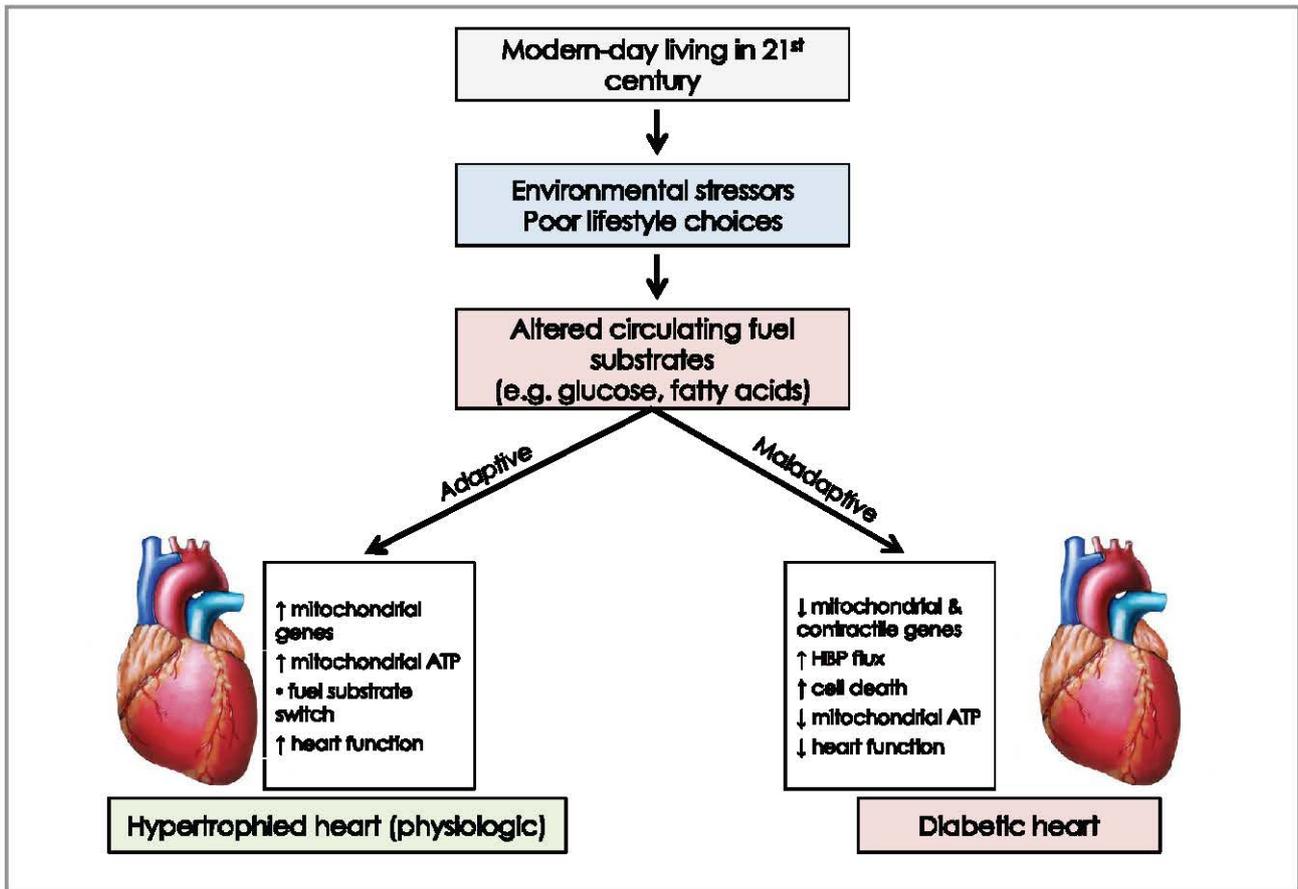


Figure 8: The impact of external and internal challenges on the heart's metabolism

We are of the opinion that markers of the HBP may result in novel diagnostic tools to assess prediabetes, while HBP inhibition within the prediabetic context may also provide cardioprotection.

HEART METABOLISM'S BALANCING ACT: *QUO VADIS?*

Treading the tightrope of healthy living in the 21st century is an arduous task. The global economy is characterized by great wealth disparities with serious health implications. Moreover, the prevailing economic model struggles to promote equity, efficiency and environmental sustainability. Also, production for profit and related consumerism translate into an environmental imbalance in terms of healthy living, for example pollution, stress, nutritional excess and sedentary lifestyles. Communities are therefore exposed to an increasing number of external stressors that can trigger internal changes, for example altered myocardial fuel substrate metabolism.

It therefore becomes a daunting balancing act for the heart's metabolism to sustain its energetic production and output in the midst of prolonged external and internal challenges. In some instances the heart is able

to successfully adapt, for example increased mitochondrial respiratory capacity that sustains the physiologic hypertrophied heart. However, it is hard to continue its balancing act(s) in the long-term. Chronically high circulating fuel substrates (e.g. glucose and fatty acids) trigger maladaptive pathways that result in cell death, decreased mitochondrial ATP production and the onset of type 2 diabetes and CVD. Our research work has identified several mechanisms whereby high circulating fatty acid and glucose levels lead to impaired cardiac contractility (see Figure 8). Moreover, we have discovered several molecular targets that can be exploited for diagnostic and therapeutic purposes. If future translational studies are successful, this should ultimately improve overall quality of life and well-being and help diminish the growing global burden of type 2 diabetes and CVD. However, it is crucial that such efforts go together with the development of a more integrative scientific research approach and a better balanced economic framework.

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