



Identification of defective mechanisms in specific forms of type 2 diabetes

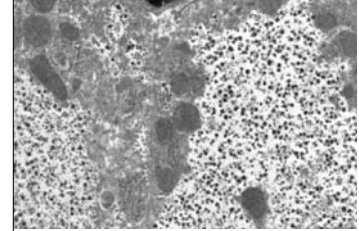
It has been estimated that between 200 million and 300 million people worldwide currently meet World Health Organization diagnostic criteria for diabetes mellitus. This epidemic of predominantly type 2 diabetes is largely mediated by our shift toward a more sedentary lifestyle, which predisposes us to obesity and insulin resistance. Individuals affected by type 2 diabetes may also exhibit an array of associated undesirable effects, such as hypertension, dyslipidemia, and hypercoagulability, which lead to morbidity and mortality from atherosclerotic vascular disease. The co-existence of several of these disorders with insulin resistance constitutes the metabolic syndrome. The major factors proposed to participate in the development of insulin resistance are inflammation, excessive lipid availability, oxidative stress, endoplasmic reticulum stress and mitochondrial dysfunction. A key step towards a complete understanding of type 2 diabetes is the identification of insulin resistance susceptibility genes, which will lead to the acquisition of therapeutic targets for future drug design. Our global aim is to determine the molecular mechanisms involved in the development of insulin resistance, and to identify novel susceptibility genes for insulin resistance and type 2 diabetes. The specific research projects are as follows: i) Analysis of the relationship between mitochondrial activity and insulin signalling. Role of mitochondrial dynamics proteins in metabolic homeostasis and in the control of insulin resistance; ii) Autophagic machinery, and metabolism; iii) Role of regulators of nuclear gene expression in adiposity and in insulin resistance; iv) Identification of novel targets and development of new compounds for the treatment of diabetes.

Muscle mitochondrial metabolism is reduced in type 2 diabetes. This type of diabetes is characterised by insulin resistance, which affects skeletal muscle and other insulin-sensitive tissues, and by defective insulin secretion. Muscle insulin resistance occurs as a result of alterations in intracellular signalling and is manifested by a reduced capacity of insulin to stimulate glucose uptake. In addition to these alterations, insulin-resistant subjects show a reduced muscle capacity to properly oxidise substrates -glucose and lipids- during fasting conditions and after a meal. The switch between glucose and lipid oxidation, depending on energy requirements, is referred to as 'metabolic flexibility'. In this regard, type 2 diabetic subjects show metabolic inflexibility since they present a higher capacity to oxidise lipids in insulin-stimulated conditions, instead of switching to glucose oxidation.

Insulin-resistant conditions are characterised by alterations in mitochondrial activity in skeletal muscle. Elderly insulin-resistant subjects show a reduction in mitochondrial oxidative and phosphorylation activity, as assessed by *in vivo* by $^{13}\text{C}/^{31}\text{P}$ NMR spectroscopy, and also increased fat accumulation in muscle and

liver. The skeletal muscle of type 2 diabetic patients shows a decrease in the activity of the Krebs cycle and of the respiratory chain. In keeping with these observations, plasma levels of lactate are enhanced and the rate of whole-body lactate production is also increased in these patients. In addition, it has been reported that oral administration of dichloroacetate to diabetic patients reduces fasting hyperglycemia, and plasma lactate, cholesterol and triglycerides without affecting circulating insulin. Several lines of evidence suggest that the alterations in mitochondrial metabolism in skeletal muscle occur before the development of type 2 diabetes. Thus, offspring of type 2 diabetic parents show reduced ATP synthesis, which was the first indication that this may be an inherited defect.

Several mechanisms contribute to the reduction of mitochondrial activity in insulin-resistant conditions, namely changes in mitochondrial density or intrinsic alterations in mitochondrial metabolism. In this regard, there is evidence that insulin-resistant obese individuals with type 2 diabetes have approximately 30% fewer mitochondria in their skeletal muscles than age-matched healthy controls. Thus, the skeletal muscle of type 2 diabetic



patients shows a lower mitochondrial DNA content, and these patients also present reduced citrate synthase activity. It is likely that the decreased mitochondrial mass is a defect in both obesity and type 2 diabetes. Consequently, no differences in muscle mitochondrial DNA copy number or in citrate synthase are found in muscle of obese type 2 diabetic patients compared to non-diabetic obese subjects. A reduced mitochondrial density has been demonstrated in muscle of insulin-resistant offspring of type 2 diabetic parents.

Several studies have reported that the mitochondrial alterations found in muscle of type 2 diabetic patients reflect a functional impairment of mitochondria since these alterations are present even after correction by mitochondrial mass. However, other studies have not detected differences in electron transport chain or in oxygen consumption after correction by surrogates of mitochondrial mass. In all, current data support the view that mitochondrial mass is decreased in skeletal muscle in obesity and in type 2 diabetes, and some evidence supports the notion of a functional impairment of mitochondria in these conditions.

Alterations in the density and function of mitochondria have been demonstrated in muscle of insulin resistant-offspring of type 2 diabetic subjects. This observation points to the presence of inherited defects that lead to mitochondrial dysfunction; however, solid demonstration of this hypothesis is still pending.

A reduction in the expression of genes encoding for oxidative phosphorylation has been proposed to explain the alterations in mitochondrial metabolism in type 2 diabetes. In addition, the expression of the nuclear co-activators PGC-1 α and PGC-1 β is reduced in muscle of type 2 diabetics and in offspring of insulin-resistant subjects with this disease. In this regard, hypermethylation of PGC-1 α within non-CpG nucleotides has been detected in skeletal muscle of type 2 diabetic patients. The reduced activity of PGC-1 α and PGC-1 β may explain, at least in part, the defective expression of genes encoding respiratory chain subunits and the lower mitochondrial biogenesis that occurs in muscle in type 2 diabetes. In contrast to these findings, no changes in PGC-1 α or PGC-1 β expression have been reported in diabetic Asian Indians.

Type 2 diabetes is also associated with reduced expression of genes involved in oxidative metabolism as well as with the repression of Mfn2. Mfn2 loss-of-function decreases glucose oxidation and mitochondrial membrane potential in muscle and non-muscle

Research Group Members

Group Leader:
Antonio Zorzano

Postdoctoral Fellows:

M^a Àngels Díaz, Saska Ivanova, Iliana López, Pablo Muñoz, Deborah Naon, Montserrat Romero, Jana Sánchez, Manuela Sánchez, David Sebastián, Eleonora Sorianello

PhD Students:

Victor Francis, Maria Isabel Hernández, Caroline Mauvezin, Eduard Noguera, David Sala, Ana Sancho, Jessica Segalés, Sonia Veiga

Project Manager:

Olga Bausà

Lab Technician:

Ignacio Castrillón, Juan Carlos Monasterio

Visiting Student:

Guilherme Alves (Brazil)



cells. This observation suggests that Mfn2 dysregulation plays a relevant role in the pathophysiology of type 2 diabetes. It is unlikely that the dysregulation of Mfn2 expression is a consequence of reduced insulin action. Thus, Mfn2 expression in healthy, obese or type 2 diabetic subjects is not altered in response to 3 hours of hyperinsulinemia during euglycemic-hyperinsulinemic clamps nor is the expression of this protein affected when cultured muscle cells are incubated for up to 48 hours with supramaximal insulin concentrations.

Furthermore, it has been shown that Mfn2 is induced by PGC-1 α or by PGC-1 β through interaction with the transcription factor ERR α . This may be particularly relevant since it has been reported that the nuclear co-regulators PGC-1 α and PGC-1 β are repressed in type 2 diabetes.

Mitochondrial dysfunction and insulin resistance

The association found between insulin-resistant states and mitochondrial dysfunction has led to the proposal that a reduced mitochondrial metabolism causes insulin resistance. Several intervention studies have tested this hypothesis and have generated discordant findings. Obese/overweight subjects underwent a 4-month intervention in which they performed physical exercise and had further weight loss induced by dietary restriction. This intervention led to increased mitochondrial size and stimulated ETC, citrate synthase and succinate dehydrogenase in muscle, in parallel to ameliorated insulin sensitivity. Similar findings were reported in type 2 diabetic subjects in response to weight loss/physical activity intervention. These studies illustrate that the amelioration of mitochondrial metabolism and improved insulin sensitivity run in parallel (Zorzano *et al*, 2009a).

In contrast, several other studies indicate that the amelioration of insulin resistance can occur in the absence of changes in mitochondrial metabolism. Thus, dietary restriction for 16 weeks caused improved insulin sensitivity in obese/overweight subjects in the absence of changes in mitochondrial metabolism or cardiolipin content. In addition, treatment for 8 weeks with rosiglitazone decreased insulin resistance in type 2 diabetic patients, without any improvement in mitochondrial function. Comparison of mitochondrial content and insulin sensitivity in a range of ethnic groups also casts doubts on a strict relationship between mitochondrial dysfunction and insulin resistance. Thus, Asian Indians displaying higher mtDNA content and increased oxidative enzyme activity are more insulin-resistant than age-, sex- and BMI-matched North American counterparts.

In all, the data available on humans indicate that not all interventions that improve insulin sensitivity are a consequence of parallel changes in muscle mitochondrial activity.

Specific alterations in morbidly obese type 2 diabetic subjects

Weight reduction and physical exercise are the best approaches to ameliorate insulin sensitivity; however, compliance with lifestyle changes has proven to have little beneficial effect. In recent decades, bariatric surgery has emerged as a potential

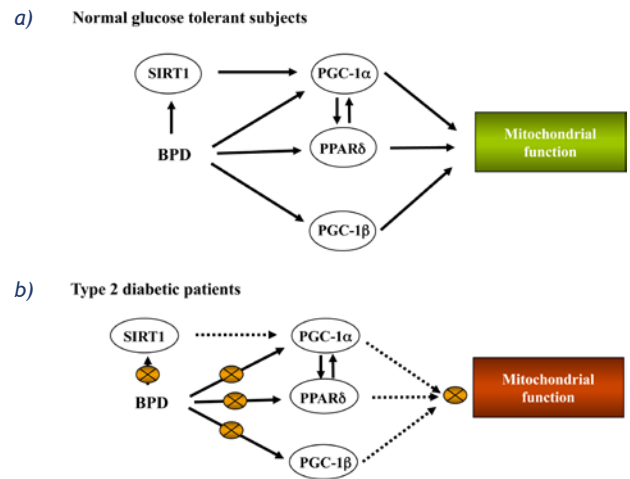


Figure 1. Scheme of the changes in gene expression that occur in non-diabetic (panel a) and type 2 diabetic subjects (panel b) in response to bilio-pancreatic diversion (BPD).

therapy for diabetes. Bilio-pancreatic diversion (BPD) is a bariatric surgical technique characterised by a massive weight loss mainly as a result of lipid malabsorption. BPD causes a net improvement in insulin sensitivity, long before the normalisation of body weight. In addition, BPD regulates substrate oxidation and modulates the expression of genes involved in lipid synthesis and oxidation in both muscle and adipose tissue.

BPD surgery improves insulin sensitivity both in type 2 diabetic and in nondiabetic subjects. We had previously described increased Mfn2 mRNA expression in skeletal muscle of morbidly obese subjects with normal glucose tolerance (NGT) after BPD. In the studies reviewed here, we aimed to determine whether the expression of genes involved in mitochondrial biogenesis/function was induced in response to BPD. To this end, we selected nuclear genes that regulate mitochondrial biogenesis such as PGC-1 α , PGC-1 β , and PPAR δ ; genes that regulate mitochondrial metabolism and fusion such as Mfn2; genes that regulate PGC-1 α such as SIRT1; and genes that encode for constitutive proteins such as Porin or Citrate synthase. In addition, to focus on the mechanisms leading to reversal of diabetes after BPD, we analysed the effect of comparable weight loss caused by BPD on the expression of the aforementioned genes and whether there were differences in the response between morbidly obese NGT subjects and morbidly obese type 2 diabetic subjects. All patients were characterised before and after weight loss with respect to insulin sensitivity by the euglycemic-hyperinsulinemic clamp, and glucose and lipid oxidation, as assessed by the respiratory chamber.

NGT and type 2 diabetic morbidly obese subjects responded to BPD by losing weight to a similar extent, improving insulin sensitivity, and lowering glycemia, insulinemia, and the plasma concentration of cholesterol and triglycerides. Under these conditions, in which both groups showed a similar clinical biochemistry, NGT subjects showed a higher rate of glucose oxidation

and lower lipid oxidation than diabetic patients. Furthermore, in response to BPD, non-diabetic subjects showed an induced expression of genes encoding for mitochondrial proteins (Mfn2, citrate synthase or porin), and regulatory proteins (PGC-1 α , PGC-1 β , PPAR δ , or SIRT1). However, under similar conditions, diabetic patients did not show any increase in the expression of these genes (Figure 1).

We propose that the pattern of changes in gene expression detected in skeletal muscle of non-diabetic subjects in response to BPD serves to trigger mitochondrial biogenesis in skeletal muscle, thereby favouring increased glucose oxidation under conditions of reduced lipid availability. This notion requires confirmation by direct analysis in muscle but is consistent with

observations that whole body glucose oxidation is increased in subjects after BPD. Subjects undergoing BPD show reduced lipid availability provided that BPD causes lipid malabsorption. This observation may account for the detection of lower whole-body lipid oxidation after BPD in non-diabetic subjects.

In addition, our findings suggest that weight loss induced by BPD exerts a beneficial effect on insulin sensitivity via mechanisms that are independent of the skeletal muscle expression of genes involved in mitochondrial biogenesis/function. Furthermore, the observation that gene expression is not altered with weight loss in type 2 diabetic patients while it is induced in subjects with NGT points to the contribution of a heritable component.

Scientific output

Publications

Chavey C, Lazennec G, Lagarrigue S, Clapé C, Iankova I, Teyssier J, Annicotte JS, Schmidt J, Mataka C, Yamamoto H, Sanches R, Guma A, Stich V, Vitkova M, Jardin-Watelet B, Renard E, Strieter R, Tuthill A, Hotamisligil GS, Vidal-Puig A, Zorzano A, Langin D and Fajas L. CXC ligand 5 is an adipose-tissue derived factor that links obesity to insulin resistance. *Cell Metab*, 9(4), 339-49 (2009)

González-Muñoz E, López-Iglesias C, Calvo M, Palacín M, Zorzano A and Camps M. Caveolin-1 loss of function accelerates glucose transporter 4 and insulin receptor degradation in 3T3-L1 adipocytes. *Endocrinology*, 150(8), 3493-502 (2009)

Hernández-Alvarez MI, Chiellini C, Manco M, Naon D, Liesa M, Palacín M, Mingrone G and Zorzano A. Genes involved in mitochondrial biogenesis/function are induced in response to bilio-pancreatic diversion in morbidly obese individuals with normal glucose tolerance but not in type 2 diabetic patients. *Diabetologia*, 52(8), 1618-27 (2009)

Liesa M, Palacín M and Zorzano A. Mitochondrial dynamics in mammalian health and disease. *Physiol Rev*, 89(3), 799-845 (2009)

Ortega FJ, Moreno-Navarrete JM, Ribas V, Esteve E, Rodriguez-Hermosa JI, Ruiz B, Peral B, Ricart W, Zorzano A and Fernández-Real JM. Subcutaneous fat shows higher thyroid hormone receptor-alpha1 gene expression than omental fat. *Obesity*, 17(12), 2134-41 (2009)

Yraola F, Zorzano A, Albericio F and Royo M. Structure-activity relationships of SSAO/VAP-1 arylalkylamine-based substrates. *ChemMedChem*, 4(4), 495-503 (2009)

Zorzano A. Regulation of mitofusin-2 expression in skeletal muscle. *Appl Physiol Nutr Metab*, 34(3), 433-39 (2009)

Zorzano A, Liesa M and Palacín M. Mitochondrial dynamics as a bridge between mitochondrial dysfunction and insulin resistance. *Arch Physiol Biochem*, 115(1), 1-12 (2009)

Zorzano A, Liesa M and Palacín M. Role of mitochondrial dynamics proteins in the pathophysiology of obesity and type 2 diabetes. *Int J Biochem Cell Biol*, 41(10), 1846-54 (2009a)

Zorzano A, Palacín M, Martí L and García-Vicente S. Arylalkylamine vanadium salts as new anti-diabetic compounds. *J Inorg Biochem*, 103(4), 559-66 (2009)

Zorzano A, Sebastián D, Segalés J and Palacín M. The molecular machinery of mitochondrial fusion and fission: An opportunity

for drug discovery? *Curr Opin Drug Discov Devel*, 12(5), 597-606 (2009)

Research networks and grants

Adipose tissue: a key target for prevention of the metabolic syndrome

European Science Foundation, BM0602 (2007-2011)

Principal investigator: Antonio Zorzano

Ajuts a grups de recerca reconeguts

Agency for Administration of University and Research Grants (AGAUR), 2009-SGR215 (2009-2013)

Principal investigator: Antonio Zorzano

CIBERDEM (Diabetes y Enfermedades Metabólicas Asociadas)

Carlos III Health Institute (2007-2011)

Principal investigator: Antonio Zorzano

Determinantes genéticos de las alteraciones metabólicas de la obesidad y diabetes de tipo 2

Spanish Ministry of Science and Innovation, SAF2008-03803 (2008-2013)

Principal investigator: Antonio Zorzano

Integration of the system models of mitochondrial function and insulin signalling and its application in the study of complex diseases (MITIN)

European Commission, HEALTH-F4-2008-223450 (2008-2011)

Principal investigator and coordinator: Antonio Zorzano

Transnational cooperation for technological innovation in the development of molecules for the treatment of diabetes and obesity

Interreg-IVB, DIOMED, SOE1/P1/E178 (2009-2011)

Principal investigator and coordinator: Antonio Zorzano

Collaborations

Aquaporins in adipose tissue

Graça Soveral, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa (Lisbon, Portugal)

Early-onset type 2 diabetes, exercise and mitochondrial function

John Nolan, St James' Hospital, Trinity College Dublin (Dublin, Ireland)

Effect of salicylate conjugate compounds in obese and diabetic mice

Alec Mian and Luc Martí, Genmedica Therapeutics (Barcelona, Spain)

Expression of genes in human adipose tissue

Joan Vendrell, Hospital Joan XXIII (Tarragona, Spain)

Functional analysis of adipose cell proteins

José Manuel Fernández-Real, Trueta Hospital (Girona, Spain)

Functional role of DOR homologue genes

Aurelio Teleman, German Cancer Research Center—DFKZ (Heidelberg, Germany)

Generation of a screening platform

Fernando Albericio, IRB Barcelona (Barcelona, Spain)

Generation of a screening platform

Mabel Loza, Universidad de Santiago de Compostela (Santiago de Compostela, Spain)

In vivo role of neuregulins

Anna Gumà, University of Barcelona (Barcelona, Spain)

Mitochondrial dynamics in cardiac cells

Sergio Lavandero, Universidad de Chile (Santiago, Chile)

Regulation of gene transcription in skeletal muscle

Ubiratan F Machado, Institute of Biomedical Sciences, University of São Paulo (São Paulo, Brazil)

Role of mitofusin 2 in endoplasmic reticulum

Luca Scorrano, Venetian Institute of Molecular Medicine (Padova, Italy)

Structural analysis of DOR protein

Sandra Macedo Ribeiro, Institute for Molecular and Cell Biology (Porto, Portugal)

Type 2 diabetes in morbid obesity and mitochondrial function

Geltrude Mingrone, Catholic University, School of Medicine (Rome, Italy)