Review

Does skeletal muscle oxidative stress initiate insulin resistance in genetically predisposed individuals?

Dorit Samocha-Bonet[1], Leonie K. Heilbronn[1], Dov Lichtenberg[2] and Lesley V. Campbell[1]

[1] Diabetes and Obesity Research Program, Garvan Institute of Medical Research, 384 Victoria Street, Darlinghurst, NSW 2010, Australia
[2] Department of Physiology and Pharmacology, Lady Davis Chair of Biochemistry, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv 69978, Israel

Reactive oxygen species (ROS) are postulated to be a common trigger of insulin resistance. For example, treatment of adipocytes with either tumor-necrosis factor-α or dexamethasone increases ROS before impairing glucose uptake. Similarly, treatment with mitochondria-specific antioxidants preserves insulin sensitivity in animal models of insulin resistance. However, it remains unclear whether ROS contribute to insulin resistance in humans. First-degree relatives (FDRs) of type 2 diabetes subjects are at increased risk of developing insulin resistance and type 2 diabetes. Here we review the documented metabolic impairments in FDRs that could contribute to insulin resistance via increased oxidative stress. We propose that lipotoxic intermediates and lipid peroxides in skeletal muscle interfere with insulin signaling and might cause insulin resistance in these 'at risk' individuals.

First-degree relatives of type 2 diabetes individuals – a model of prediabetes

Type 2 diabetes (T2D) is a disorder characterized by impaired insulin secretion from pancreatic β-cells and impaired insulin action on target tissues (termed insulin resistance). Both characteristics are influenced by genetic and environmental factors and predict the development of T2D [1,2]. T2D is associated with an imbalance between the normal (homeostatic) levels of pro- and antioxidative agents, commonly termed "oxidative stress" [3]. The prediabetic state is also associated with oxidative stress [4,5], and recently oxidative stress was suggested as the common cause of insulin resistance in certain human diseases [6], an issue addressed in recent reviews [3,7]. To study the role of oxidative stress in T2D, we can artificially separate the development of T2D into first, the processes responsible for the development of insulin resistance, and second the progression from insulin resistance to T2D. The contribution of oxidative stress in the second stage, i.e. the progression of insulin resistance into T2D, is currently better understood than the role it might play in the first stage, i.e. the development of insulin resistance.

The strong heritability of T2D is well established, and candidate gene variants have been identified that predominantly contribute to pancreatic β-cell dysfunction [8]. Studies in twins report high concordance [2], and increased prevalence of T2D occurs in certain ethnic populations [9,10]. Offspring of T2D subjects have a 1.5- to 6-fold higher risk of developing T2D, depending on parental transmission (paternal≤maternal<both parents) [11,12]. Thus, healthy first-degree relatives (FDRs) of T2D subjects are logical candidates for studies of factors involved in the pathogenesis of T2D. The earliest processes contributing to the genesis of insulin resistance can be evaluated in insulin-sensitive FDRs and compared with individuals of similar gender, age and overall adiposity but without a family history of T2D. In this review, we distinguish between studies of the "preinsulin resistant" stage where FDRs were insulin-sensitive, and the "prediabetic" stage where FDRs were already insulin-resistant (and/or glucose intolerant) but nondiabetic. Although the reviewed studies are not confounded by dyslipidemia, fasting hyperglycemia or excess adiposity, studies of insulin-resistant FDRs might be confounded by insulin resistance itself (and associated abnormalities).

Skeletal muscle plays a key role in determining systemic insulin sensitivity because in the postprandial state, a major proportion of glucose disposal occurs in skeletal muscle. Glucose uptake occurs in response to insulin binding to its receptor, stimulating the insulin signaling cascade, eventually allowing translocation of the glucose transporter GLUT4 to the plasma membrane. As with T2D subjects, prediabetic FDRs have reduced insulin-stimulated glucose uptake and nonoxidative glucose metabolism in skeletal muscle [13–17]. The reported molecular defect(s) mediating insulin resistance in skeletal muscle include impairments in insulin receptor substrate (IRS) phosphorylation, the phosphoinositol 3-kinase (PI3K)/Akt pathway and glycogen synthase activity (reviewed in detail elsewhere [18]). In Table 1, we highlight elements identified as defective to date in insulin-resistant FDRs.

In this review, we examine supporting evidence that oxidative stress induces insulin resistance in insulin-target cells and in animal models. We critically review the documented metabolic impairments that could contribute to insulin resistance via increased oxidative stress in FDRs

Corresponding author: Samocha-Bonet, D. (d.samochabonet@garvan.org.au).

1043-2760/$ – see front matter doi:10.1016/j.tem.2009.09.008 Available online 23 October 2009

Table 1. Insulin signaling and metabolic impairments in normoglycemic insulin-resistant and insulin-sensitive first-degree relatives (FDRs) of individuals with type 2 diabetes

		Insulin-resistant FDRs		Insulin-sensitive FDRs	
Insulin signaling[a]	**During fasting**	IRS-1-serine phosphorylation (inhibitory) [14] ↑ PKB/Akt Ser-phosphorylation (activating) [63] ↔ IRS-1 associated PI3K activation [63] ↑ pAS160 [13] ↔		–	
	During hyperinsulinemia (hyperinsulinemic clamp)	PKB/Akt Ser-phosphorylation (activating) [14,63] ↓ IRS-1-tyrosine phosphorylation (activating) [63] ↓ Glycogen synthase activity [16,64] ↓ Non-linear association between AS160 phosphorylation and glucose uptake in muscle strips [13]			
Skeletal muscle mitochondrial function		[17,41] ↓		[33,42] ↔	
Baseline skeletal muscle expression/activity of enzymes participating in substrate transport and oxidation		[14] ↓		[33,59] ↔	
Skeletal muscle expression of the PGC1 family		[65] ↓	[13,14] ↔	[33] ↔	
Skeletal muscle mitochondrial content		[14,17,41,65] ↓		[34] ↓	[42] ↔
Metabolic flexibility		[66,67] ↓		[33,34] ↓	[42] ↔
Tumor-necrosis factor-α		[68] ↑		[68] ↔	
Intramyocellular triglyceride content		[17,67,69,70] ↑	[71] ↔	[42] ↔	

Arrows indicate higher, lower or similar levels compared to insulin-sensitive control individuals with no relatives with T2D.
In cases where insulin sensitivity was not evaluated, normal glucose tolerant FDRs are compared with normal glucose tolerant control individuals.
[a]Only studied in insulin-resistant FDRs.

and highlight potential areas left to explore. This review is important because clear evidence that oxidative stress contributes to insulin resistance in humans is lacking.

Reactive oxygen species induce insulin resistance *in vitro* and in animal models

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated in all biological systems under aerobic conditions, either intentionally or as byproducts of physiological processes. An intentional generator of superoxide (O_2^-) is the membranous enzymatic complex NADPH-oxidase (NOX) that for many years was thought to be exclusive to phagocytes [3]. However, it is now known that multiple members of the NOX family are expressed in insulin-target cells, including adipocytes (NOX4 [19]) and muscle cells (NOX2 [20,21], NOX4 [21]). Transient bursts of low concentrations of ROS via NOX in various tissues occur in response to a variety of growth factors/hormones and enhance signaling pathways essential for normal cell function [22,23]. ROS generated by NOX in response to insulin binding to its receptor is such an example. Specifically, in insulin-sensitive cells, upon insulin binding, ROS released by NOX relieve negative inhibition of the insulin signaling cascade by oxidizing negative regulators of the pathway and facilitating the transduction of the insulin signal downstream [22]. However, NOX can also generate ROS upon activation by proinflammatory cytokines and saturated fatty acids that interact with the toll-like receptor, both implicated in insulin resistance-associated conditions in adipocytes [3]. When 3T3–L1 adipocytes are treated with glucose, fatty acids and proinflammatory cytokines, cellular glucose uptake is reduced through common pathways that are dependent on ROS generation. These include molecular targets of the insulin signaling cascade (Box 1) and stress pathways that act downstream of the insulin signaling pathway, including the nuclear factor-κB (NF-κB), NH_2-terminal Jun kinase (JNK), p38 mitogen-activated protein kinase (p38 MAPK) and protein kinase C (PKC) [3,6,7,24].

The majority of cellular ROS is produced by mitochondria during ATP synthesis. Specifically, resynthesis of ATP from ADP is coupled to the oxidation of the reducing equivalents NADH and $FADH_2$ generated by enzymatic pathways that metabolize fat, carbohydrates and proteins. An electrochemical gradient across the inner mitochondrial membrane is generated and the energy is used by ATPase to produce ATP from ADP [25]. Superoxide is generated by electrons leaking to oxygen from the electron transport chain (ETC), mainly through complexes I and III [26]. These anions are released into the mitochondrial matrix and/or into the intermembrane space, where they are dismutated rapidly into hydrogen peroxide (H_2O_2) by Mn–superoxide dismutase (SOD2) or Cu/Zn–SOD (SOD1), respectively. The antioxidative enzymes catalase and glutathione peroxidase (GPx) detoxify H_2O_2 by its conversion to water and molecular oxygen. Increased mitochondrial ROS generation in the vasculature, kidney, neurons, retina and pancreatic β-cells is implicated in T2D through hyperglycemia and increased glucose flux into the mitochondria [3].

Houstis *et al.* provided key evidence for a causal role of mitochondrial ROS in insulin resistance [6]. Specifically, H_2O_2 emission was detected before any decrease in insulin-stimulated glucose uptake in 3T3–L1 adipocytes treated with tumor necrosis factor-α (TNFα) or dexamethasone. Furthermore, transgenic overexpression of mitochondrial antioxidants in adipocytes partially prevented glucose uptake impairment [6]. The role of mitochondria in the induction of insulin resistance is also evidenced in animal models of insulin resistance when insulin sensitivity is

Box 1. The insulin signaling cascade, reactive species and insulin resistance

Insulin plays an essential role in metabolic homeostasis in mammals by mediating glucose disposal into muscle and adipose tissue in the postprandial state. The most downstream event in the cascade of events leading to glucose entry into the cell is the translocation of the glucose transporter GLUT4 from intracellular vesicles to the plasma membrane [60]. Defect(s) in any component of the pathway, from the insulin receptor to the translocation of GLUT4 to the plasma membrane, might contribute to insulin resistance [18,60]. Insulin binding to its receptor on the plasma membrane increases insulin receptor tyrosine kinase activity, resulting in the phosphorylation of insulin receptor substrates (IRSs) on tyrosine residues. Phosphorylated IRSs activate phosphoinositol 3-kinase (PI3K) that catalyzes the generation of phosphatidylinositol 3,4,5-triphosphate (PIP_3) that serves as docking sites for phosphoinositide-dependent protein kinase-1 (PDK1) and Akt. Akt is the kinase controlling most of the metabolic actions of insulin and is activated by phosphorylation at Thr308 and Ser473 by PDK1 and mammalian target of rapamycin (mTOR), respectively [60].

Putative molecular targets for modification by ROS or RNS in the insulin signaling pathway include the following possibilities: (i) oxidative modifications enhance IRS degradation [3,24,48] and increase IRS serine (inhibitory) phosphorylation [3]; (ii) oxidative modifications impair Akt activation and inhibit its downstream targets [3]; (iii) disruption of actin-facilitated spatial organization of components of the insulin signaling cascade [3]; (iv) *S*-nitrosylation of cysteine residues on IRSs and Akt to inhibit their activity [61,62]; and (v) unknown IRS-independent molecular targets downstream of Akt [60].

preserved by treatment with mitochondria-targeted antioxidants [6,27,28]. Specifically, *ob*/*ob* mice [6] or high-fat fed C57BL/6 mice [28] treated with the mitochondrial antioxidant Mn(III)–tetrakis (4-benzoic acid) porphyrin are more sensitive to insulin than untreated mice (by the insulin tolerance test, ITT). Treatment of high-fat fed rats with the mitochondrial-specific small peptide antioxidant SS31 also abolished the development of insulin resistance (measured by the homeostasis model assessment) [27]. Recently, the transgenic mice approach has linked muscle mitochondrial overproduction of ROS to insulin resistance when mice overexpressing mitochondrial antioxidants in muscle were partially protected from insulin resistance under conditions of high-fat feeding [27,28]. Specifically, overexpression of catalase in skeletal and cardiac muscle delayed the onset of insulin resistance during 6 weeks of high-fat feeding (measured by the hyperinsulinemic-euglycemic clamp) [27], and overexpression of Mn–SOD in muscle and adipose tissue partially preserved insulin sensitivity, measured by the ITT [28]. These data suggest a causal role for muscle mitochondrial ROS generation in insulin resistance and skeletal muscle mitochondria in particular.

Skeletal muscle metabolic inflexibility, reduced mitochondrial content and intramyocellular lipids in FDR

During fasting, skeletal muscle of healthy lean individuals utilizes fat as the main energy source and under insulin-stimulated conditions rapidly switches to carbohydrate oxidation. The ability of skeletal muscle to adapt rapidly to a change in fuel availability is termed "metabolic flexibility" [29]. Considerable variability in metabolic flexibility is found in healthy lean individuals and might stem from intrinsic properties of skeletal muscle [30]. In fact, myotubes separated from their endocrine milieu and cultured *ex vivo* preserve the metabolic characteristics of their donors, strengthening the hypothesis that metabolic flexibility is, at least in part, genetically (and/or epigenetically) determined [30]. Metabolic flexibility can be expressed in terms of the drop in the respiratory quotient in response to increasing fat intake or during an overnight fast and using this criterion, prediabetic FDRs are metabolically inflexible (Table 1), a trait also observed in obese [31] and T2D subjects [32]. Importantly, we and others reported that insulin-sensitive FDRs also already exhibit metabolic inflexibility in response to a single high-fat meal [33] or longer term (3 days) high-fat feeding [34], potentially predisposing these individuals to lipid accumulation in skeletal muscle and insulin resistance.

In response to high-fat feeding, generally proteins involved in fatty acid oxidation in skeletal muscle are upregulated in rodents [35–37]. In particular, increases are observed in the protein levels of the mitochondrial biogenesis master regulator, peroxisome proliferator-activated receptor coactivator-1α (PGC1α) as well as the complexes of mitochondrial ETC and proteins involved in fatty acid metabolism [35,37]. However, in humans, isocaloric high-fat feeding causes a coordinated downregulation of expression of *PGC1α*, *PGC1β* and ETC members [38]. Of note, these findings are based on mRNA expression and the translation to the active proteins might be different. In metabolically inflexible FDRs, we found an impaired ability to switch on fat oxidation in response to a single high-fat meal. Along with this, the muscle mRNA expression of *PGC1α* tends to increase in control subjects but not in FDRs; furthermore, other key genes involved in lipid metabolism such as acetyl CoA–carboxylase-2 (*ACC2*), which catalyzes the synthesis of malonyl–CoA, and fatty acid translocase (*FAT*/*CD36*), a membrane fatty acid transporter, were dysregulated in FDRs compared to controls [33]. A limitation of this study, however, was the low numbers of subjects examined, and owing to lack of sample protein levels were not evaluated.

Reduced oxidative capacity in insulin-sensitive FDRs might also stem from depletion of fatty acid oxidation machinery, namely mitochondrial content of skeletal muscle. Mitochondrial size and content are reduced in skeletal muscle of T2D, obese [39,40] and prediabetic FDR patients (Table 1). In the latter, depleted mitochondrial content was associated with reduced substrate oxidation [41] and ATP production [17], as evaluated by magnetic resonance spectroscopy (MRS) (Table 1). Ukropcova *et al.* reported reduced muscle mitochondrial content by mitochondrial DNA (mtDNA) copy number in insulin-sensitive FDRs, which correlated with metabolic flexibility and insulin sensitivity [34]. However, the results regarding mitochondrial content in insulin-sensitive FDRs are inconsistent, and even in diabetic subjects mitochondrial content of skeletal muscle was similar to that of controls [42]. These inconsistencies might stem from differences in physical activity between cohorts, which is a major driver of mitochondrial biogenesis [42], and from the large variation in results obtained from the mtDNA copy number

and citrate synthase activity assays used to quantify mitochondria in muscle. Ultimately, even if not coupled with reduced mitochondrial content, the observed metabolic inflexibility might contribute to accumulation of intramyocellular triglycerides (IMTGs) and lipotoxic intermediates in sedentary individuals with a genetic predisposition to T2D (Figure 1).

In healthy individuals, IMTGs are a readily available reservoir of substrates to be oxidized by active skeletal muscle. Accordingly, exercise training increases IMTG content and is associated with increased insulin sensitivity [43,44]. By contrast, in obese T2D patients [45] and in lean insulin-resistant elderly individuals [46], higher IMTG is associated with lower insulin sensitivity. It is now accepted that muscle triglycerides are metabolically harmless but correlate with, and reflect the accumulation of, other lipid intermediates such as diacylglycerol (DAG), ceramide and long-chain fatty acyl–CoA (LCFA–CoA) [47]. Each of these lipid species impairs insulin signaling and causes insulin resistance by activating stress-signaling pathways including NF-κB, JNK, p38 MAPK and PKC [44,48,49] and/or through ROS production [6]. An efficient coupling of lipolysis of intramyofibrillar lipid droplets and β-oxidation in adjacent mitochondria prevents the accumulation of lipotoxic intermediates in skeletal muscle and is the focus of recent research into IMTG accumulation in health and disease [44,47,50].

Lipid peroxides in skeletal muscle impair insulin sensitivity in FDRs

In sedentary individuals, the IMTG pool is more prone to peroxidation owing to its decreased depletion/repletion rate and its colocalization with mitochondria generated ROS [44,51]. Russell *et al.* found that endurance-trained and obese individuals had similar IMTG content, but lipid peroxide content was 4-fold higher in obese individuals [52]. Lipid peroxidation products were suggested to induce insulin resistance through NF-κB activation and increased TNFα [53]. This might be the case in severely obese individuals [52]; however, circulating TNFα is not increased in insulin-sensitive FDRs (Table 1). In any case, oxidative enzymes of the mitochondrial matrix are sensitive to peroxidation; thus, mitochondrial oxidative capacity is further impaired by ROS in a vicious circle [51]. Mild uncoupling of mitochondrial substrate oxidation from ATP production lowers the mitochondrial proton gradient and decreases mitochondrial ROS production [51]. The mitochondrial uncoupling protein-3 (UCP3) exports fatty acid anions from the mitochondrial matrix and is induced by the lipid peroxide product 4-hydroxy nonenal [51]. Decreased UCP3 levels in glucose intolerant and T2D subjects might contribute to increased lipid peroxides in skeletal muscle in these subjects [54]. However, UCP3 levels in skeletal muscle of FDRs have not yet been examined. Skeletal muscle lipid content is evaluated in most studies by MRS, histology and lipid extraction [55] and the level of the lipotoxic intermediates DAG and ceramide have only recently been measured in humans [47,56,57]. In fact, skeletal muscle ceramide [47,56] and DAG [47] content were elevated in obese insulin-resistant subjects and correlated with insulin resistance evaluated by the hyperinsulinemic-euglycemic clamp. In FDRs, increased IMTG was found in some, but not all, insulin-resistant cohorts (Table 1). Further studies assessing DAG, ceramide, LCFA and lipid peroxides in skeletal muscle of insulin-sensitive FDRs are required.

Concluding remarks and future directions

Reactive species have a Janus-like action in modulating insulin action, with both a facilitating role in insulin signal transduction as well as the capability to trigger insulin resistance. On the one hand, a global reduction of hydrogen

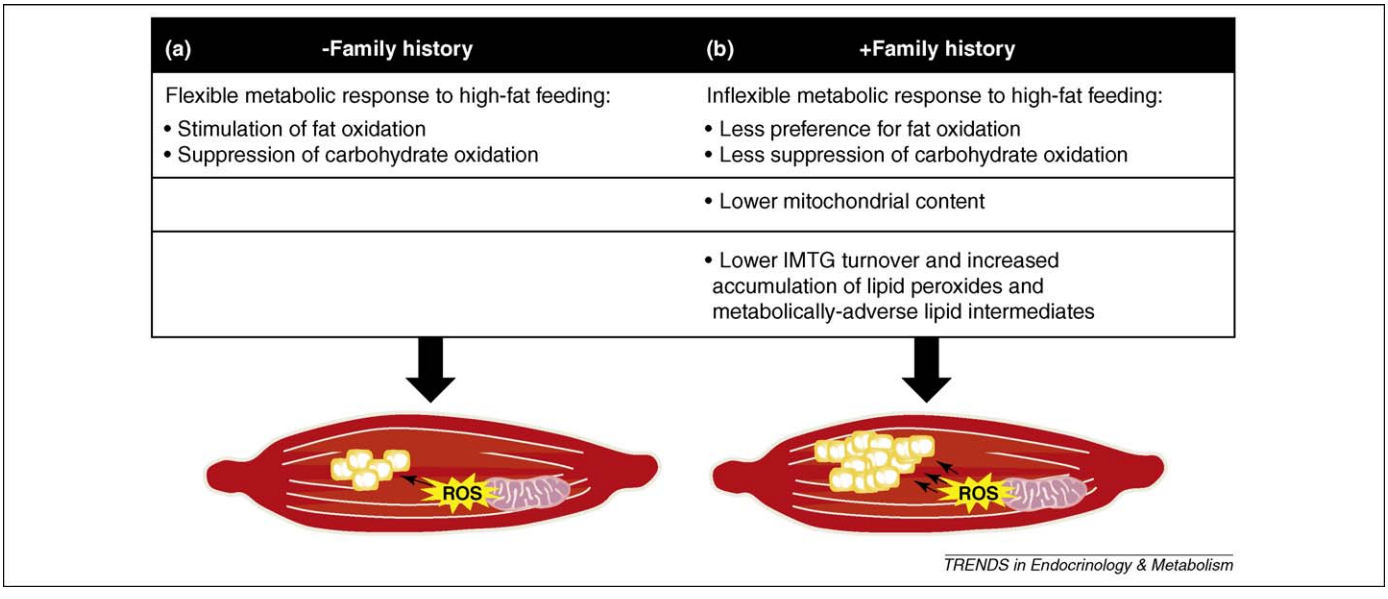

Figure 1. Generation of reactive oxygen species (ROS) in skeletal muscle of subjects with a genetic predisposition to type 2 diabetes. **(a)** Flexible metabolic response to high-fat feeding in a nonobese sedentary individual without a family history (−Family history) of type 2 diabetes. A fine coupling between lipid availability and lipid oxidation in mitochondria results in increased turnover of IMTGs in skeletal muscle and low levels of lipid peroxidation. **(b)** Inflexible metabolic response to high-fat feeding in a nonobese individual with a genetic predisposition to type 2 diabetes (+Family history) results in a mismatch between lipid availability and lipid oxidation in mitochondria, accumulation of IMTGs and lipid intermediates and an increased oxidative stress in skeletal muscle.

peroxide in animals induces insulin resistance, as shown in mice overexpressing the intracellular enzyme GPX1 [58], strengthening the pivotal role of ROS in insulin signaling. On the other hand, recent evidence suggests ROS as a common trigger of insulin resistance in cells and animal models [6,7,27,28]. However, oxidative stress results from multiple underlying mechanisms and currently there is no unifying measure, and therefore evidence of oxidative stress in key target tissues in insulin resistance-associated disorders is not consistent [3]. Currently, research efforts are dedicated to understanding the underlying mechanisms responsible for IMTG and lipid accumulation in skeletal muscle [47,50], and gold standard methods are applied to measure the lipotoxic species in humans [47]. To this panel of measures, studies in the field would benefit from adding a measure of lipid peroxidation products.

We propose that a mismatch between lipid supply and mitochondrial oxidation capacity in skeletal muscle in insulin-sensitive nonobese sedentary FDRs in the high-fat fed state favors the accumulation of lipotoxic lipid species in skeletal muscle. This lipid pool is enriched by reduced turnover and is susceptible to peroxidation by ROS emerging from the colocalized mitochondria (Figure 1). Thus, insulin resistance develops because lipotoxic intermediates and lipid peroxides interfere with insulin signaling. The beneficial effect of exercise training on insulin resistance in these subjects is in accordance with this proposal [59]. Future studies should explore the underlying mechanisms by which lipid peroxides in skeletal muscle induce insulin resistance. Further studies in insulin-sensitive FDRs are necessary to evaluate lipid species and peroxidation products in skeletal muscle. Similarly, it will be important to challenge these 'at risk' individuals with prolonged high-fat feeding to test the long-term effects of metabolic inflexibility on insulin sensitivity, mitochondrial function and skeletal muscle lipid intermediates and peroxidation products. Such prolonged challenges to skeletal muscle might magnify potential defects and mechanisms that predispose these individuals to insulin resistance.

Acknowledgements

Research in the laboratories of D.S.B., L.K.H. and L.V.C. is funded by the National Health and Medical Council of Australia (NHMRC) and the Diabetes Australia Research Trust (DART). Research in the laboratory of D.L. is supported by the Lady Davis Chair of Biochemistry. We would like to thank Nigel Turner for his critical comments on this manuscript.

References

1 Jenkins, A.B. and Campbell, L.V. (2004) The genetics and pathophysiology of diabetes mellitus type II. *J. Inherit. Metab. Dis.* 27, 331–347
2 Beck-Nielsen, H. *et al.* (2003) Metabolic and genetic influence on glucose metabolism in type 2 diabetic subjects – experiences from relatives and twin studies. *Best Pract. Res. Clin. Endocrinol. Metab.* 17, 445–467
3 Bashan, N. *et al.* (2009) Positive and negative regulation of insulin signaling by reactive oxygen and nitrogen species. *Physiol. Rev.* 89, 27–71
4 Meigs, J.B. *et al.* (2007) Association of oxidative stress, insulin resistance, and diabetes risk phenotypes: the Framingham Offspring Study. *Diabetes Care* 30, 2529–2535
5 Menon, V. *et al.* (2004) Oxidative stress and glucose levels in a population-based sample. *Diabet. Med.* 21, 1346–1352
6 Houstis, N. *et al.* (2006) Reactive oxygen species have a causal role in multiple forms of insulin resistance. *Nature* 440, 944–948
7 Eriksson, J.W. (2007) Metabolic stress in insulin's target cells leads to ROS accumulation – a hypothetical common pathway causing insulin resistance. *FEBS Lett.* 581, 3734–3742
8 Prokopenko, I. *et al.* (2008) Type 2 diabetes: new genes, new understanding. *Trends Genet.* 24, 613–621
9 Lillioja, S. *et al.* (1993) Insulin resistance and insulin secretory dysfunction as precursors of non-insulin-dependent diabetes mellitus. Prospective studies of Pima Indians. *N. Engl. J. Med.* 329, 1988–1992
10 Zimmet, P. *et al.* (1981) The prevalence of diabetes in the rural and urban Polynesian population of Western Samoa. *Diabetes* 30, 45–51
11 Bjornholt, J.V. *et al.* (2000) Type 2 diabetes and maternal family history: an impact beyond slow glucose removal rate and fasting hyperglycemia in low-risk individuals? Results from 22.5 years of follow-up of healthy nondiabetic men. *Diabetes Care* 23, 1255–1259
12 Meigs, J.B. *et al.* (2000) Parental transmission of type 2 diabetes: the Framingham Offspring Study. *Diabetes* 49, 2201–2207
13 Karlsson, H.K. *et al.* (2006) Insulin signaling and glucose transport in skeletal muscle from first-degree relatives of type 2 diabetic patients. *Diabetes* 55, 1283–1288
14 Morino, K. *et al.* (2005) Reduced mitochondrial density and increased IRS-1 serine phosphorylation in muscle of insulin-resistant offspring of type 2 diabetic parents. *J. Clin. Invest.* 115, 3587–3593
15 Rothman, D.L. *et al.* (1995) Decreased muscle glucose transport/phosphorylation is an early defect in the pathogenesis of non-insulin-dependent diabetes mellitus. *Proc. Natl. Acad. Sci. U. S. A.* 92, 983–987
16 Schalin-Jantti, C. *et al.* (1992) Impaired activation of glycogen synthase in people at increased risk for developing NIDDM. *Diabetes* 41, 598–604
17 Petersen, K.F. *et al.* (2004) Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. *N. Engl. J. Med.* 350, 664–671
18 Frojdo, S. *et al.* (2009) Alterations of insulin signaling in type 2 diabetes: a review of the current evidence from humans. *Biochim. Biophys. Acta* 1792, 83–92
19 Mahadev, K. *et al.* (2004) The NAD(P)H oxidase homolog Nox4 modulates insulin-stimulated generation of H2O2 and plays an integral role in insulin signal transduction. *Mol. Cell. Biol.* 24, 1844–1854
20 Javesghani, D. *et al.* (2002) Molecular characterization of a superoxide-generating NAD(P)H oxidase in the ventilatory muscles. *Am. J. Respir. Crit. Care Med.* 165, 412–418
21 Bedard, K. and Krause, K.H. (2007) The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. *Physiol. Rev.* 87, 245–313
22 Goldstein, B.J. *et al.* (2005) Redox paradox: insulin action is facilitated by insulin-stimulated reactive oxygen species with multiple potential signaling targets. *Diabetes* 54, 311–321
23 Rhee, S.G. (2006) Cell signaling. H2O2, a necessary evil for cell signaling. *Science* 312, 1882–1883
24 Evans, J.L. *et al.* (2002) Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. *Endocr. Rev.* 23, 599–622
25 Turner, N. and Heilbronn, L.K. (2008) Is mitochondrial dysfunction a cause of insulin resistance? *Trends Endocrinol. Metab.* 19, 324–330
26 Andreyev, A.Y. *et al.* (2005) Mitochondrial metabolism of reactive oxygen species. *Biochemistry* 70, 200–214
27 Anderson, E.J. *et al.* (2009) Mitochondrial H2O2 emission and cellular redox state link excess fat intake to insulin resistance in both rodents and humans. *J. Clin. Invest.* doi:10.1172/JCI37048
28 Hoehn, K.L. *et al.* (2009) Insulin resistance is a cellular antioxidant defense mechanism. *Proc. Natl. Acad. Sci. U. S. A.*, doi:10.1073/pnas.0902380106
29 Galgani, J.E. *et al.* (2008) Metabolic flexibility and insulin resistance. *Am. J. Physiol. Endocrinol. Metab.* 295, E1009–E1017
30 Ukropcova, B. *et al.* (2005) Dynamic changes in fat oxidation in human primary myocytes mirror metabolic characteristics of the donor. *J. Clin. Invest.* 115, 1934–1941
31 Kelley, D.E. *et al.* (1999) Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. *Am. J. Physiol.* 277, E1130–E1141

32 Kelley, D.E. and Simoneau, J.A. (1994) Impaired free fatty acid utilization by skeletal muscle in non-insulin-dependent diabetes mellitus. *J. Clin. Invest.* 94, 2349–2356
33 Heilbronn, L.K. *et al.* (2007) Impaired fat oxidation after a single high-fat meal in insulin-sensitive nondiabetic individuals with a family history of type 2 diabetes. *Diabetes* 56, 2046–2053
34 Ukropcova, B. *et al.* (2007) Family history of diabetes links impaired substrate switching and reduced mitochondrial content in skeletal muscle. *Diabetes* 56, 720–727
35 Hancock, C.R. *et al.* (2008) High-fat diets cause insulin resistance despite an increase in muscle mitochondria. *Proc. Natl. Acad. Sci. U. S. A.* 105, 7815–7820
36 Hoeks, J. *et al.* (2008) Mitochondrial function, content and ROS production in rat skeletal muscle: effect of high-fat feeding. *FEBS Lett.* 582, 510–516
37 Turner, N. *et al.* (2007) Excess lipid availability increases mitochondrial fatty acid oxidative capacity in muscle: evidence against a role for reduced fatty acid oxidation in lipid-induced insulin resistance in rodents. *Diabetes* 56, 2085–2092
38 Sparks, L.M. *et al.* (2005) A high-fat diet coordinately downregulates genes required for mitochondrial oxidative phosphorylation in skeletal muscle. *Diabetes* 54, 1926–1933
39 Toledo, F.G. *et al.* (2008) Mitochondrial capacity in skeletal muscle is not stimulated by weight loss despite increases in insulin action and decreases in intramyocellular lipid content. *Diabetes* 57, 987–994
40 Ritov, V.B. *et al.* (2005) Deficiency of subsarcolemmal mitochondria in obesity and type 2 diabetes. *Diabetes* 54, 8–14
41 Befroy, D.E. *et al.* (2007) Impaired mitochondrial substrate oxidation in muscle of insulin-resistant offspring of type 2 diabetic patients. *Diabetes* 56, 1376–1381
42 Phielix, E. *et al.* (2008) Lower intrinsic ADP-stimulated mitochondrial respiration underlies in vivo mitochondrial dysfunction in muscle of male type 2 diabetic patients. *Diabetes* 57, 2943–2949
43 Kelley, D.E. and Goodpaster, B.H. (2001) Skeletal muscle triglyceride. An aspect of regional adiposity and insulin resistance. *Diabetes Care* 24, 933–941
44 Moro, C. *et al.* (2008) Determinants of intramyocellular triglyceride turnover: implications for insulin sensitivity. *Am. J. Physiol.* 294, E203–E213
45 Kelley, D.E. *et al.* (1991) Skeletal muscle density: effects of obesity and non-insulin-dependent diabetes mellitus. *Am. J. Clin. Nutr.* 54, 509–515
46 Petersen, K.F. *et al.* (2003) Mitochondrial dysfunction in the elderly: possible role in insulin resistance. *Science* 300, 1140–1142
47 Moro, C. *et al.* (2009) Influence of gender, obesity, and muscle lipase activity on intramyocellular lipids in sedentary individuals. *J. Clin. Endocrinol. Metab.* 94, 3440–3447
48 Evans, J.L. *et al.* (2003) Are oxidative stress-activated signaling pathways mediators of insulin resistance and beta-cell dysfunction? *Diabetes* 52, 1–8
49 Savage, D.B. *et al.* (2007) Disordered lipid metabolism and the pathogenesis of insulin resistance. *Physiol. Rev.* 87, 507–520
50 Liu, L. *et al.* (2009) Paradoxical coupling of triglyceride synthesis and fatty acid oxidation in skeletal muscle overexpressing DGAT1. *Diabetes*. (in press)
51 Schrauwen, P. and Hesselink, M.K. (2004) Oxidative capacity, lipotoxicity, and mitochondrial damage in type 2 diabetes. *Diabetes* 53, 1412–1417
52 Russell, A.P. *et al.* (2003) Lipid peroxidation in skeletal muscle of obese as compared to endurance-trained humans: a case of good vs. bad lipids? *FEBS Lett.* 551, 104–106
53 Russell, A.P. (2004) Lipotoxicity: the obese and endurance-trained paradox. *Int. J. Obes. Relat. Metab. Disord.* 28 (Suppl. 4), S66–S71
54 Schrauwen, P. *et al.* (2006) Reduced skeletal muscle uncoupling protein-3 content in prediabetic subjects and type 2 diabetic patients: restoration by rosiglitazone treatment. *J. Clin. Endocrinol. Metab.* 91, 1520–1525
55 Kelley, D.E. (2005) Skeletal muscle fat oxidation: timing and flexibility are everything. *J. Clin. Invest.* 115, 1699–1702
56 Adams, J.M., II *et al.* (2004) Ceramide content is increased in skeletal muscle from obese insulin-resistant humans. *Diabetes* 53, 25–31
57 Haus, J.M. *et al.* (2009) Plasma ceramides are elevated in obese subjects with type 2 diabetes and correlate with the severity of insulin resistance. *Diabetes* 58, 337–343
58 McClung, J.P. *et al.* (2004) Development of insulin resistance and obesity in mice overexpressing cellular glutathione peroxidase. *Proc. Natl. Acad. Sci. U. S. A.* 101, 8852–8857
59 Ostergard, T. *et al.* (2006) Impact of exercise training on insulin sensitivity, physical fitness, and muscle oxidative capacity in first-degree relatives of type 2 diabetic patients. *Am. J. Physiol. Endocrinol. Metab.* 290, E998–E1005
60 Hoehn, K.L. *et al.* (2008) IRS1-independent defects define major nodes of insulin resistance. *Cell Metab.* 7, 421–433
61 Nomiyama, T. *et al.* (2004) Reduction of insulin-stimulated glucose uptake by peroxynitrite is concurrent with tyrosine nitration of insulin receptor substrate-1. *Biochem. Biophys. Res. Commun.* 320, 639–647
62 Yasukawa, T. *et al.* (2005) S-nitrosylation-dependent inactivation of Akt/protein kinase B in insulin resistance. *J. Biol. Chem.* 280, 7511–7518
63 Storgaard, H. *et al.* (2001) Insulin signal transduction in skeletal muscle from glucose-intolerant relatives of type 2 diabetic patients [corrected]. *Diabetes* 50, 2770–2778
64 Vaag, A. *et al.* (1992) Decreased insulin activation of glycogen synthase in skeletal muscles in young nonobese Caucasian first-degree relatives of patients with non-insulin-dependent diabetes mellitus. *J. Clin. Invest.* 89, 782–788
65 Patti, M.E. *et al.* (2003) Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: potential role of PGC1 and NRF1. *Proc. Natl. Acad. Sci. U. S. A.* 100, 8466–8471
66 De Pergola, G. *et al.* (2003) Fuel metabolism in adult individuals with a wide range of body mass index: effect of a family history of type 2 diabetes. *Diabetes Nutr. Metab.* 16, 41–47
67 Lattuada, G. *et al.* (2005) Reduced whole-body lipid oxidation is associated with insulin resistance, but not with intramyocellular lipid content in offspring of type 2 diabetic patients. *Diabetologia* 48, 741–747
68 Ruotsalainen, E. *et al.* (2006) Changes in inflammatory cytokines are related to impaired glucose tolerance in offspring of type 2 diabetic subjects. *Diabetes Care* 29, 2714–2720
69 Jacob, S. *et al.* (1999) Association of increased intramyocellular lipid content with insulin resistance in lean nondiabetic offspring of type 2 diabetic subjects. *Diabetes* 48, 1113–1119
70 Perseghin, G. *et al.* (1999) Intramyocellular triglyceride content is a determinant of in vivo insulin resistance in humans: a 1H-13C nuclear magnetic resonance spectroscopy assessment in offspring of type 2 diabetic parents. *Diabetes* 48, 1600–1606
71 Kriketos, A.D. *et al.* (2005) Intramyocellular lipid is not significantly increased in healthy young insulin resistant first-degree relatives of diabetic subjects. *Diabetes Care* 28, 2332–2333