Endocr (2009) 36:524–529
DOI 10.1007/s12020-009-9270-3

ORIGINAL ARTICLE

Variation in gene expression of presenilins-associated rhomboid-like protein and mitochondrial function in skeletal muscle of insulin-resistant rats

Huiqin Tang · Jie Liu · Li Niu · Wenbo He · Yancheng Xu

Received: 18 June 2009 / Accepted: 21 September 2009 / Published online: 27 October 2009

Abstract Multiple factors promote insulin resistance. In this study, we evaluated the mRNA levels of presenilins-associated rhomboid-like protein (PARL) and mitochondrial content and enzyme activity from skeletal muscle isolated from insulin-resistant rats. Rats fed a high-fat diet for 35 days developed moderate insulin resistance, which was determined by an increase in plasma glucose and insulin concentrations following an oral glucose tolerance test. The PARL mRNA level was lower in the insulin-resistant rats than in control animals, and is associated with low mitochondrial content and reduced mitochondrial enzyme activity in the skeletal muscle from the insulin-resistant rats. The results suggest that high-fat-diet-induced insulin resistance is associated with mitochondrial dysfunction in skeletal muscle, and may be the result of the decreased expression of the *PARL* gene, which encodes the protein with functional significance in mitochondria.

Keywords Gastrocnemius muscle · High-fat diet · Insulin resistance · Mitochondrial function · Presenilins-associated rhomboid-like protein

Introduction

Insulin resistance (IR), which is related to obesity, decreases the insulin response and glucose metabolism in a variety of tissues. This can lead to diabetes, abnormal lipid metabolism, and related complications such as cardiovascular disease, hypertension, and inflammation of peripheral tissues. Therefore, preventing insulin resistance is important for reducing the risk of developing these diseases. The role of skeletal muscle mitochondrial dysfunction in the development of insulin resistance has been a topic of intense interest recently. There are a number of studies that support the hypothesis that an overall reduction in the capacity of mitochondria to mediate oxidation phosphorylation occurs in the elderly, and this may also mediate insulin resistance [1].

The presenilins-associated rhomboid-like protein (PARL) gene is a newly discovered gene, which belongs to the Rhomboid superfamily of membrane proteins. This protein superfamily is highly conserved among eukaryotes, archaea, and bacteria. Recent studies have demonstrated that, in addition to regulating mitochondrial dynamics, PARL mediates mammalian-specific, developmentally regulated mitochondria-to-nuclei signaling via proteolysis of its N-terminus and the release of the Pβ peptide [2]. Due to the previous finding that PARL is associated with mitochondrial function, the aim of this study is to investigate the relationship between the expression of PARL and mitochondrial function in rats with high-fat-diet-induced insulin resistance when compared to normal rats.

Huiqin Tang and Jie Liu contributed equally to this work.

H. Tang · J. Liu · L. Niu · Y. Xu (✉)
Department of Endocrinology, Zhongnan Hospital of Wuhan University, 430071 Wuhan, Hubei, People's Republic China
e-mail: othq@hotmail.com

H. Tang
e-mail: thuiqintop@yahoo.com.cn

J. Liu
e-mail: olj1@yahoo.cn

L. Niu
e-mail: type1dm@126.com

W. He
Medical School of Wuhan University, 430071 Wuhan, Hubei, People's Republic China
e-mail: 416997363@qq.com

Results

High-fat-diet-induced insulin resistance results in statistically significant changes in blood glucose and insulin levels

Prior to introducing the experimental diets, all animals were subjected to an oral glucose tolerance test (OGTT) to assess their glucose metabolism. Blood glucose concentrations during OGTT did not differ significantly between the rats (data not shown). A second OGTT was performed 35 days after the high-fat diet was introduced to determine if the high-fat diet promoted insulin resistance (Fig. 1). After 35 days, the rats fed with the high-fat diet, group IR, had significantly higher glucose concentrations than control rats, group N, at 30 and 120 min after glucose administration (185.3 ± 6.2 vs. 210.3 ± 8.3 mg/dl and 136.1 ± 9.3 vs. 165.1 ± 9.4 mg/dl, respectively, $P < 0.05$) (Fig. 1a). IR rats also had significantly greater blood insulin concentrations than the control animals at 0, 60, 120, and 180 min after glucose administration (2.0 ± 0.3 vs. 4.1 ± 0.6 ng/ml, 2.3 ± 0.2 vs. 5.0 ± 1.2 ng/ml, 2.1 ± 0.3 vs. 5.1 ± 1.0 ng/ml, 2.0 ± 0.3 vs. 4.2 ± 0.8 ng/mL, respectively, $P < 0.01$) (Fig. 1b).

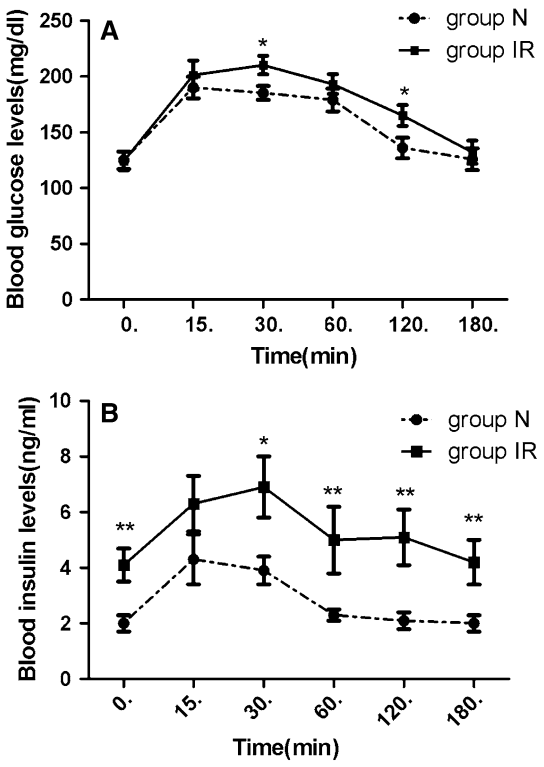

Fig. 1 Variation in the plasma glucose (**a**) and insulin (**b**) concentrations after feeding high-fat diet for 35 days. Values are expressed as means ± SD ($n = 15$). * $P < 0.05$, ** $P < 0.01$ vs. group N

Rats with high-fat diet-induced insulin resistance express lower levels of PARL mRNA in the gastrocnemius muscles

To determine if insulin resistance altered PARL mRNA expression in the gastrocnemius muscle, real-time RT-PCR was performed using total RNA extracted from the gastrocnemius muscle. PARL mRNA expression was significantly lower in the gastrocnemius muscles from the IR rats when compared to normal muscle (0.81 ± 0.18 vs. group N, $P < 0.01$) (Fig. 2).

Effects on mitochondrial DNA and protein in gastrocnemius muscle

For both the heavy and the light strands of mitochondrial DNA (mtDNA), the D-loop is a major site of transcription initiation. The ratio of mtDNA D-loop to nuclear DNA 18S rRNA in IR rats was significantly lower than in the normal rats (0.83 ± 0.19 vs. group N, $P < 0.01$) (Fig. 3a). If mitochondrial DNA content is decreased, there should be a concomitant decrease in viable mitochondria and mitochondrial components, including electron chain transport complex enzymes. Therefore, we evaluated the impact of insulin resistance on mitochondrial complexes I and II. The expression of both the complexes (Fig. 3b) was significantly lower in IR rats than in normal rats (0.91 ± 0.15 vs. normal group, $P < 0.05$; 0.91 ± 0.12 vs. normal group, $P < 0.05$, respectively).

Insulin resistance induces reduced mitochondrial complex activity in the gastrocnemius muscle

A reduction in mitochondrial complex enzymes would lead to changes in the overall activity of these enzymes. Therefore, we evaluated complex enzyme activity in

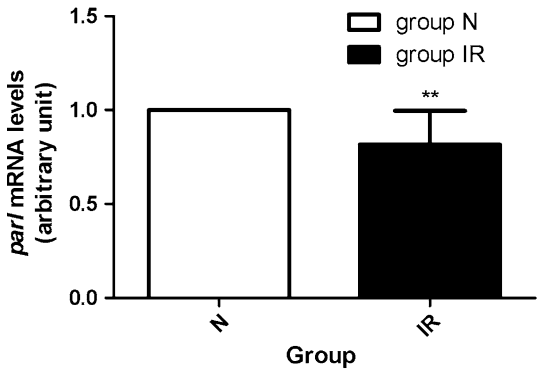

Fig. 2 PARL mRNA in the gastrocnemius muscle from the group IR rats is reduced when compared to the mRNA level of normal muscle. Values are expressed as means ± SD ($n = 15$). ** $P < 0.01$ vs. group N

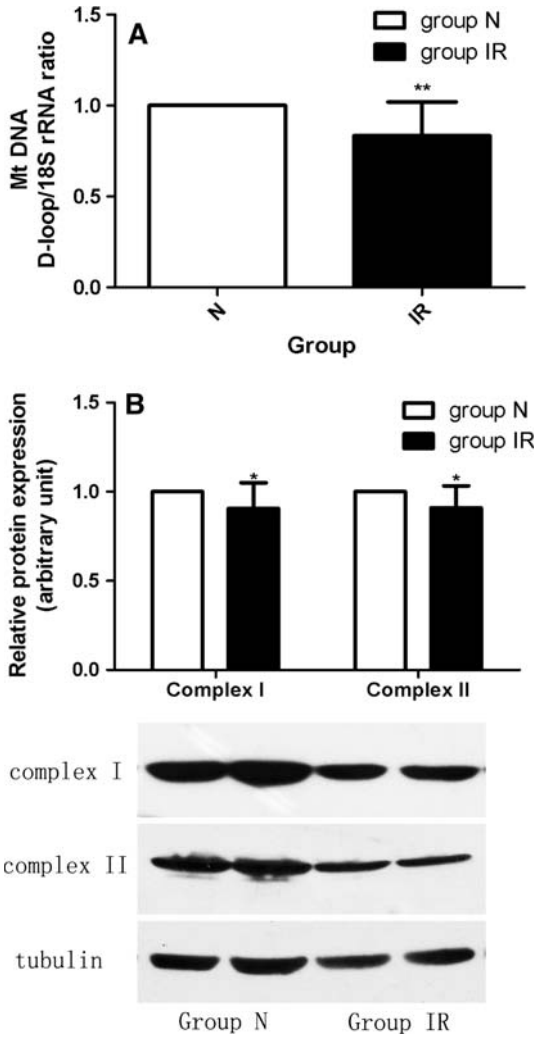

Fig. 3 High-fat-diet-induced insulin resistance decreases mitochondrial DNA content and protein level in the gastrocnemius muscle. **a** The content of mtDNA and nuclear 18Sr RNA gene (18Sr DNA) in each group. **b** Protein levels of tubulin, mitochondrial electron transport complexes I and II in each group. Values are expressed as mean ± SD ($n = 15$).* $P < 0.05$ vs. group N, ** $P < 0.01$ vs. group N

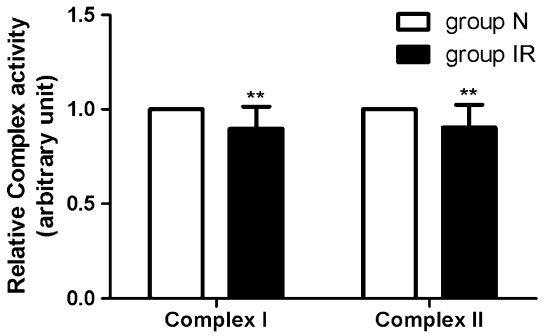

Fig. 4 High-fat-diet-induced insulin resistance reduces the activities of the complexes I and II enzymes in the gastrocnemius muscle. Values are expressed as means ± SD ($n = 15$). ** $P < 0.01$ vs. group N

mitochondria isolated from the gastrocnemius muscle of IR rats when compared to normal rats. Similar to complex enzyme expression, the activity of both mitochondrial complexes I and II (Fig. 4) was significantly lower in the IR rats when compared to control animals (0.89 ± 0.12 vs. normal group, $P < 0.01$; 0.90 ± 0.12 vs. normal group, $p < 0.01$, respectively) (Fig. 4).

Discussion

Insulin resistance is associated with obesity and Type-2 Diabetes (T2DM). The precise factors that lead to insulin resistance have not been fully elucidated. Recently, some reports have demonstrated that mitochondrial dysfunction occurs during insulin resistance [3–6], suggesting that mitochondrial dysfunction may play a role in the development to insulin resistance. Skeletal muscle is the major site of peripheral IR and defects in insulin signaling in the skeletal muscle can contribute to increased blood glucose levels [7]. However, the mechanisms leading to mitochondrial damage during insulin resistance are currently unknown.

To determine if insulin resistance alters the expression PARL mRNA and mitochondrial function in the gastrocnemius muscle, rats were fed a diet rich in saturated fatty acids to induce insulin resistance [8, 9]. In this study, rats fed a high-fat diet for 35 days developed insulin resistance as demonstrated by significant elevations in blood glucose and insulin concentrations in response to orally administered glucose (Fig. 1).

In this study, we observed that insulin resistance is associated with low mitochondrial DNA content and reduced mitochondrial enzyme activity in skeletal muscle. Defective mitochondria may be important factors for the development or maintenance of IR. The internal structure and cytosolic organization of mitochondria are controlled by many proteins, such as the large dynamin-related GTPases, Mitofusin (Mfn) 1 and 2, and the cytosolic dynamin-related protein 1 (Drp1) [10].

In this study, PARL mRNA expression was reduced in the gastrocnemius muscle from insulin-resistant rats when compared to normal muscle. PARL is a mitochondrial protein involved in mitochondrial membrane remodeling and belongs to the highly conserved rhomboid-like protein family. The *PARL* gene maps to a quantitative trait locus (3q27) that is associated with metabolic traits [11]. A number of rhomboid proteases have been implicated in the regulation of mitochondrial membrane remodeling. In fact, studies in *Saccharomyces cerevisiae* have demonstrated that PCP1P is required to cleave the dynamin-related

GTPase, Mgm1p, which is an inter-membrane protein that participates in mitochondrial fusion events. The yeast PCP1P protein belongs to a subfamily of mitochondrial rhomboid proteases typified by the PARL protein, which is the human ortholog of PCP1P [12–15]. Proteolysis of PARL generates a cleaved form that modulates the shape of the mitochondrial reticulum. This cleavage depends on the phosphorylation state of the cleavage site, implicating mitochondrial kinases and phosphatases in the regulation of mitochondrial shape [16].

Insulin resistance may partially be the result of an imbalance between fatty acid beta-oxidation and glucose decomposition and oxidation in impaired mitochondria. This would lead to further damage to the oxidative potential of muscle, which would increase the severity of IR and, eventually, results in T2DM. It is likely that the insulin-resistance model used in this study is an example for moderate insulin resistance. Future studies should be performed in which PARL mRNA and protein levels are evaluated in animal models of severe insulin resistance.

The data examining the relationship between PARL gene polymorphisms and metabolic disease are complicated. Powell et al. [17] reported that no significant associations were observed between the PARL rs3732581 genetic variant and plasma insulin or glucose levels, BMI or metabolic syndrome. However, another recent study demonstrated that the Leu262Val polymorphism of PARL was associated with early onset Type-2 Diabetes and an increase in the urinary microalbumin to creatinine ratio in a case–control study of an Irish population [18]. Therefore, a role for PARL in metabolic conditions cannot be excluded and more comprehensive genetic studies are warranted.

In conclusion, the results of this study suggest that high-fat-diet-induced insulin resistance may impact mitochondrial function by decreasing the expression of PARL, which is important for maintaining normal mitochondrial structure and function. These results merit further investigation of the possible connection of *PARL* gene expression in skeletal muscle and insulin resistance in human populations.

Materials and methods

Animals

Thirty male Sprague–Dawley rats (6 weeks old) were obtained from the Laboratory Animal Center of Wuhan University (Wuhan, China). The rats were maintained at a stable temperature (22 ± 2°C) and humidity level (55 ± 5%), with a 12-h light/dark cycle (light 07:00–19:00 h). Rats were fed a diet of standard laboratory chow and water for 6 days prior to administration of an OGTT. According to the results of the first OGTT, fasting plasma glucose levels and levels at 2 h post-glucose administration, as well as body weight, were similar among all animals. The animals were divided into two groups of 15. The first group of rats (group N) was maintained on a standard rat diet (gross energy content, 13.35 kJ/g, including 19% fat) for 35 days. The second group (group IR) was fed a high-fat diet (gross energy content, 18.71 kJ/g, including 57% fat) for 35 days to induce IR. A second OGTT was performed 5 weeks after the introduction of the experimental diets. All the rats were killed by an anesthetic overdose (pentobarbitone, 120 mg/kg; Sigma–Aldrich, St. Louis, MO, USA), and the gastrocnemius muscle was excised and snap frozen in liquid nitrogen. Samples were stored at −80°C. The Wuhan University Animal Welfare Committee approved the use of SD rats in these studies. All experiments were conducted according to strict guidelines established by the National Health and Medical Research Council and Wuhan University Animal Welfare Committee.

OGTT and insulin assay

OGTTs were performed on the rats before the experimental diets were introduced and 35 days after. Rats were fasted overnight prior to receiving, orally, a glucose solution (2 g/kg of body weight, 10 ml/kg of body weight). Blood samples were collected via the tail vein at 0 (fasting), 15, 30, 60, 120, and 180 min after glucose administration. Samples were centrifuged at 1,500×*g* for 15 min and the plasma was collected for use in determining specified parameters. Plasma insulin levels were measured using a Rat Insulin-specific RIA kit.

RNA extraction and quantification reverse transcription PCR

Total RNA was extracted from the rat skeletal muscle using the TRIzol reagent (Invitrogen Corp., Carlsbad, CA, USA) and purified using RNeasy columns per the manufacturer's instruction (QIAGEN, Cologne, Germany). For the cDNA, 1 μg of RNA was reverse transcribed into cDNA prepared by RT-PCR using the Superscript III Reverse Transcription for RT-PCR kit (Invitrogen). cDNA was diluted in DNase-free water (1:25) before quantification by real-time quantitative PCR. The primers used for PARL and GAPDH RT-PCR: *PARL* forward 5′-CCAATGCTGCTTTCCACG-3′ and reverse 5′-GACCCAGAATGTTCACGATG-3′; *GAPDH* forward 5′-GATGGGTGTGAACCACGAGAAA-3′ and reverse 5′-ACGGATACATTGGGGGTAGGA-3′.

Gene expression levels were quantified using the SYBR Green real-time PCR reagent and the ABI Prism 7700 Sequence Detection System (Applied Biosystems). Real-time RT-PCR was performed using a SYBR Green master

mix kit (Applied Biosystems). Each PCR reaction was performed in triplicate. The rat GAPDH gene served as the endogenous reference gene. The PCR conditions were: 50°C for 2 min, 95°C for 10 min, and 40 cycles of 95°C for 15 s and 60°C for 1 min. Relative differences in the PCR products obtained from the group IR samples and the products from the group N samples were evaluated using the ΔΔCt method. The reciprocal of $2^{-\Delta\Delta Ct}$ for each target gene was normalized to GAPDH and compared to the relative value of control cells. Final results are presented as a percent of the control.

Isolation of skeletal muscle mitochondria

The gastrocnemius muscles were removed from each leg. One portion was frozen in liquid N_2 and used for total RNA and protein extractions. Another portion was used immediately for mitochondrial isolation. All operations were carried out at 4°C or in ice. The gastrocnemius muscles were quickly excised, cleaned, rinsed, chopped with a pair of scissors and placed into ice-cold buffer (solution 1) consisting of (in mM) 120 NaCl, 20 HEPES, 2 $MgCl_2$, 1 EGTA, and 5 g/l bovine serum albumin, pH 7.4. Mitochondrial suspensions were prepared according to the methods of Birch-Machin et al. [19]. The disrupted muscle was resuspended 20-fold (wt/vol) in solution 1, and homogenized at 40% power (9,500 rpm) in an Ultra Turrax homogenizer (model T-25; IKA, Wilmington, NC, USA) for 10 s. The homogenate was centrifuged at 700×*g* for 10 min at 4°C. The supernatant was filtered through double-layered gauze and centrifuged at 12,000×*g* for 10 min at 4°C to pellet the mitochondria. The pellet was resuspended in the initial volume of solution 1 and again submitted to centrifugation at 12,000×*g* for 10 min at 4°C. The pellet was gently resuspended in 10 volumes of a buffer (solution 2) consisting of (in mM) 300 sucrose, 2 HEPES, 0.1 EGTA, pH 7.4, and centrifuged (3,500×*g*, 10 min, 4°C). The resulting pellet, which contained the mitochondria from the gastrocnemius muscle, was suspended in 0.5 ml of solution 2 and stored at −70°C until analyzed.

Western blot assay of complexes I and II

Muscles were homogenized in ice-cold buffer containing the following: 65-mmol/l Tris (pH 7.4), 150-mmol/l NaCl, 5-mmol/l EDTA, 1% (v/v) NP-40, 1 mg/ml each of aprotinin, leupeptin, and pepstatin, 10-mmol/l sodium fluoride, and 1-mmol/l phenylmethylsulfonylfluoride. Protein concentration was determined using Lowry Method. 10.0 μg/lane of protein was applied to a series of two 10% sodium dodecyl sulfate–polyacrylamide gels (SDS–PAGE) for electrophoresis. One gel was stained with Coomassie blue to visualize the protein distribution. Protein present on the second gel was electroblotted onto a nitrocellulose membrane (0.45 μm). The nitrocellulose membranes were blocked with 5% non-fat milk/TBST for 1 h at room temperature. Membranes were incubated overnight with primary antibodies directed against tubulin (Santa Cruz, 1:4000), complex I (Santa Cruz, 1:1500), and complex II (Santa Cruz, 1:1500), in 2.5% milk/TBST at 4°C. After incubation with the appropriate secondary antibody, bands were visualized by ECL and quantified by densitometry (GE).

Assays for mitochondrial complexes I and II activities

The mitochondrial fraction obtained from gastrocnemius muscles was used to measure the activity of complexes I and II. Assays of complex I (NADH-CoQ oxidoreductase) and complex II (succinate-CoQ oxidoreductase) were performed as described [20, 21]. Complex I activity was tested by monitoring the reduction of 2,6-dichlorophenol indophenol at 600 nm upon addition of assay buffer (0.05-M Tris–HCl, pH 8.1, 0.1% BSA (w/v), 1-mM antimycin A, 0.2-mM NaN_3 and 0.05-mM coenzyme Q1), Complex II activity was tested spectrophotometrically and the reaction was scanned at 600 nm for 2 min at 30°C using succinate as the substrate, the assay buffer contain 0.05-M phosphate buffer, pH 7.8, 0.1% BSA, 1-mM antimycin A, 0.2-mM NaN_3, and 0.05-mmol/l coenzyme Q1.

Total DNA isolation and real-time PCR

Total DNA was extracted using QIAamp DNA Mini Kit (Qiagen). Extraction proceeded as per manufacturer's instructions. The final DNA pellet was suspended in 20-μl water (ddH_2O) and was diluted 1:20 to make a working solution. Real-time PCR was performed using mitochondrial DNA and genomic NA-specific primers on a 72-well Rotor-Gene RG-3000 QPCR machine (Corbett Life Science, Sydney, Australia). The rat 18S rRNA gene served as the endogenous reference gene. Water blank was included as a negative control in each experiment. The standard curve method was used for relative quantification. The melt curve was assessed to confirm primer specificity and that no unspecific products were produced during the reaction. Results are expressed as the mitochondrial D-loop/18S rRNA ratio [22].

Statistical analysis

Data are presented as the mean ± SD. Statistical differences were determined using Student's paired *t* test and $P < 0.05$ was considered statistically significant.

Acknowledgment This study was supported by Zhongnan Hospital of Wuhan University.

Disclaimer statement There is no inaccurate or misleading data, opinion or statement appears in this article, the data and opinions appearing in the articles are the sole responsibility of the contributor concerned. Accordingly, the publishers and editors and their respective employees, officers and agents accept no responsibility or liability whatsoever for the consequences of any such inaccurate or misleading data, opinion or statement.

References

1. K.F. Petersen, D. Befroy, S. Dufour et al., Mitochondrial dysfunction in the elderly: possible role in insulin resistance. Science **300**(5622), 1140–1142 (2003)
2. A. Sik, B.J. Passer, E.V. Koonin, L. Pellegrini, Self-regulated cleavage of the mitochondrial intramembrane-cleaving protease PARL yields Pbeta, a nuclear-targeted peptide. J. Biol. Chem. **279**(15), 15323–15329 (2004)
3. C. Bonnard, A. Durand, S. Peyrol et al., Mitochondrial dysfunction results from oxidative stress in the skeletal muscle of diet-induced insulin-resistant mice. J. Clin. Invest. **118**(2), 789–800 (2008)
4. M. Jove, J. Salla, A. Planavila et al., Impaired expression of NADH dehydrogenase subunit 1 and PPAR gamma coactivator-1 in skeletal muscle of ZDF rats: restoration by troglitazone. J. Lipid Res. **45**(1), 113–123 (2004)
5. L. Lionetti, M.P. Mollica, R. Crescenzo et al., Skeletal muscle subsarcolemmal mitochondrial dysfunction in high-fat fed rats exhibiting impaired glucose homeostasis. Int. J. Obes. (Lond) **31**(10), 1596–1604 (2007)
6. L.M. Sparks, H. Xie, R.A. Koza et al., A high-fat diet coordinately downregulates genes required for mitochondrial oxidative phosphorylation in skeletal muscle. Diabetes **54**(7), 1926–1933 (2005)
7. G.M. Reaven, Pathophysiology of insulin resistance in human disease. Physiol. Rev. **75**(3), 473–486 (1995)
8. Y. Kirino, T. Kamimoto, Y. Sato, K. Kawazoe, K. Minakuchi, Y. Nakahori, Increased plasma dipeptidyl peptidase IV (DPP IV) activity and decreased DPP IV activity of visceral but not subcutaneous adipose tissue in impaired glucose tolerance rats induced by high-fat or high-sucrose diet. Biol. Pharm. Bull. **32**(3), 463–467 (2009)
9. H. Chang-Quan, D. Bi-Rong, H. Ping, L. Zhen-Chan, Insufficient renal 1-alpha hydroxylase and bone homeostasis in aged rats with insulin resistance or type 2 diabetes mellitus. J. Bone Miner. Metab. **26**(6), 561–568 (2008)
10. L. Scorrano, Multiple functions of mitochondria-shaping proteins. Novartis Found. Symp. **287**, 47–55 (2007). (discussion 55–59)
11. A.H. Kissebah, G.E. Sonnenberg, J. Myklebust et al., Quantitative trait loci on chromosomes 3 and 17 influence phenotypes of the metabolic syndrome. Proc. Natl Acad. Sci. USA **97**(26), 14478–14483 (2000)
12. M. Herlan, F. Vogel, C. Bornhovd, W. Neupert, A.S. Reichert, Processing of Mgm1 by the rhomboid-type protease Pcp1 is required for maintenance of mitochondrial morphology and of mitochondrial DNA. J. Biol. Chem. **278**(30), 27781–27788 (2003)
13. G.A. McQuibban, S. Saurya, Freeman M, Mitochondrial membrane remodelling regulated by a conserved rhomboid protease. Nature **423**(6939), 537–541 (2003)
14. E.V. Koonin, K.S. Makarova, I.B. Rogozin, L. Davidovic, M.C. Letellier, L. Pellegrini, The rhomboids: a nearly ubiquitous family of intramembrane serine proteases that probably evolved by multiple ancient horizontal gene transfers. Genome Biol. **4**(3), R19 (2003)
15. L. Pellegrini, B.J. Passer, M. Canelles et al., PAMP and PARL, two novel putative metalloproteases interacting with the COOH-terminus of Presenilin-1 and -2. J. Alzheimers Dis. **3**(2), 181–190 (2001)
16. L. Pellegrini, L. Scorrano, A cut short to death: Parl and Opa1 in the regulation of mitochondrial morphology and apoptosis. Cell Death Differ. **14**(7), 1275–1284 (2007)
17. B.L. Powell, S. Wiltshire, G. Arscott et al., Association of PARL rs3732581 genetic variant with insulin levels, metabolic syndrome and coronary artery disease. Hum. Genet. **124**(3), 263–270 (2008)
18. M. Hatunic, M. Stapleton, E. Hand, C. DeLong, V.E. Crowley, J.J. Nolan, The Leu262Val polymorphism of presenilin associated rhomboid like protein (PARL) is associated with earlier onset of type 2 diabetes and increased urinary microalbumin creatinine ratio in an Irish case-control population. Diabetes Res. Clin. Pract. **83**(3), 316–319 (2009)
19. M.A. Birch-Machin, H.L. Briggs, A.A. Saborido, L.A. Bindoff, D.M. Turnbull, An evaluation of the measurement of the activities of complexes I-IV in the respiratory chain of human skeletal muscle mitochondria. Biochem. Med. Metab. Biol. **51**(1), 35–42 (1994)
20. K.M. Humphries, Y. Yoo, L.I. Szweda, Inhibition of NADH-linked mitochondrial respiration by 4-hydroxy-2-nonenal. Biochemistry **37**(2), 552–557 (1998)
21. L. Sun, C. Luo, J. Long, D. Wei, J. Liu, Acrolein is a mitochondrial toxin: effects on respiratory function and enzyme activities in isolated rat liver mitochondria. Mitochondrion **6**(3), 136–142 (2006)
22. W. Shen, K. Liu, C. Tian et al., R-alpha-lipoic acid and acetyl-L-carnitine complementarily promote mitochondrial biogenesis in murine 3T3-L1 adipocytes. Diabetologia **51**(1), 165–174 (2008)