PUBLICATIONS

Biomedicine & Pharmacotherapy 83 (2016) 785–791

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Swertiamarin ameliorates oleic acid induced lipid accumulation and oxidative stress by attenuating gluconeogenesis and lipogenesis in hepatic steatosis

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ARTICLE INFO

Article history:
Received 21 April 2016
Received in revised form 29 June 2016
Accepted 14 July 2016

Keywords:
Hepatic steatosis
Swertiamarin
Insulin signaling
AMPK
Oxidative stress

ABSTRACT

Swertiamarin, a bitter secoiridoid glycoside, is an antidiabetic drug with lipid lowering activity meliorates insulin resistance in Type 2 Diabetes condition. Therefore, the study was designed to explore the antioxidant and hypolipidemic activity of swertiamarin in ameliorating NAFLD caused due to hepatic lipid accumulation, inflammation and insulin resistance. Steatosis was induced in HepG2 cells by supplementing 1 mM oleic acid (OA) for 24 h which was marked by significant accumulation of lipid droplets. This was determined by Oil Red O (ORO) staining and triglyceride accumulation. Swertiamarin (25 μg/ml) decreased triglyceride content by 2 folds and effectively reduced LDH release (50%) activity by protecting membrane integrity thus, preventing apoptosis evidenced by reduced cleavage of Caspase 3 and PARP1. We observed that swertiamarin significantly increased the expressions of major insulin signaling proteins like Insulin receptor (IR), PI(3)K, pAkt with concomitant reduction in p307 IRS-1. AMPK was activated by swertiamarin action, thus restoring insulin sensitivity in hepatocytes. In addition, qPCR results confirmed OA up-regulated Sterol Regulatory Element Binding Protein (*SREBP*)-1c and fatty acid synthase (*FAS*), resulting in increased fatty acid synthesis. Swertiamarin effectively modulated PPAR-α, a major potential regulator of carbohydrate metabolism which, in turn, decreased the levels of the gluconeogenic enzyme *PEPCK*, further restricting hepatic glucose production and fatty acid synthesis. Cumulatively, swertiamarin targets potential metabolic regulators AMPK and PPAR-α, through which it regulates hepatic glycemic burden, fat accumulation, insulin resistance and ROS in hepatic steatosis which emphasizes clinical significance of swertiamarin in regulating metabolism and as a suitable candidate for treating NAFLD.

1. Introduction

Swertiamarin, a bitter secoiridoid glycoside, is a known potent compound for complementary and alternative medicine for many human diseases. Swertiamarin is the major compound found in *Enicostemma littorale* Blume. It's pharmacokinetic study suggested that it rapidly distributed in most of the tissues and was absorbed through the liver and eliminated by the kidney [1]. Evidences suggest that swertiamarin possesses multifactorial effects which are hypoglycemic, hypolipidemic, antioxidant, hepatoprotective and insulin sensitizing activity [2–5].

Central obesity, insulin resistance, and altered fat metabolism are causative factors associated with the development of NAFLD globally in 20–30% and 15% in western population and Asians respectively. Obesity leads to ectopic fat deposition in liver giving rise to NAFLD [6]. Ectopic fat deposition in hepatocytes enhance inflammatory responses as the activated macrophages of liver called Kupffer cells release pro-inflammatory cytokines like TNFα, IL6, *etc.* that provoke intrahepatic inflammatory response and oxidative stress [7]. Elevated PAT (perilipin, adipophilin and TIP47), a family of lipid droplet-associated proteins actively involved in droplet formation, and their turnover directs lipase to digest the neutral lipids and prevent lipolysis [8]. This leads to ectopic fat

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http://dx.doi.org/10.1016/j.biopha.2016.07.028
0753-3322/

deposition converting hepatocytes to bulky, signet shaped, leaky cells.

The disease progression, as observed from histopathological studies, categorizes it sequentially into macrovesicular steatohepatitis, microvesicular steatosis, steatohepatitis (NASH), NASH with fibrosis to cirrhosis and liver cancer [9,10]. To study the pathophysiology of the disease, various *in vivo* experiments have been carried out. Transcriptional factors regulating lipid metabolism and pro-inflammatory cytokines responsible for NAFLD in LIRKO mice elucidated the molecular mechanism of this disease. HepG2 cells (human hepatoblastoma cells) are considered as the best model for understanding the pathophysiology of this disease [11]. Oleic acid (OA) (monounsaturated), treated at pathophysiological dose for 24 h, has been used for induction of steatosis in hepatocytes by enhanced ROS, fatty acid synthesis, elevated triglyceride (TG) accumulation and hampered insulin sensitivity in the model cells which lead to hepatosteatosis [12].

Inflammation in hepatocytes activate PKCs and serine/threonine kinases that downregulate tyrosine kinase activity and insulin receptors of insulin signaling pathway, thus causing insulin resistance. Moreover, accumulated fatty acids activate PEPCK which produces hepatic glucose production thus, leading to hepatic glycemic burden [13,14]. SREBP-1c gets activated, further stimulating synthesis of lipids by activating the major transcriptional factor PPAR-α (predominant in hepatocytes) and lipogenic genes like FAS, ACC-1, fatty acid translocase (CD36) and fatty acid oxidizing Carnitoyl Palmitoyl Transferase I (CPT-1) enzyme [15,16]. Cumulatively the hepatocytes embark a sign of cell damage and injury that leads to loss in the cell membrane integrity, detected by percent release of Lactate dehydrogenase (LDH) and caspase activation [17,18]. 5′ AMP-activated protein kinase (AMPK), a metabolic sensor, controls (HGP) fatty acid synthesis and induces insulin signaling by regulating PEPCK, SREBP-1c translocation and AKT phosphorylation respectively [19].

Major objective of this study is to treat type 2 diabetes associated complications like NAFLD by potent insulin sentizer. However, chemically synthesized insulin senstizer drugs have side effects with high dosage. Therefore, herbal compounds are the best choice as alternative medicine. In our *in vivo* study, NA-STZ induced NIDDM Charles foster rats were treated with swertiamarin to understand it's potentials on insulin signalling proteins along with carbohydrate and lipid metabolism genes. The study depicted that swertiamarin, a bitter glycoside effectively reduced insulin resistance, restored carbohydrate and lipid metabolism in liver and adipose tissues of NIDDM rat. Henceforth, in the present study We used HepG2 cells in which hepatosteatosis was induced using OA, and explored the antioxidant and hypolipidemic activity of swertiamarin in ameliorating NAFLD in insulin resiatance condition.

2. Materials and methods

2.1. Chemicals and media

Dulbecco's modified eagle's medium (DMEM) low glucose, trypsin-EDTA, OA, fat free BSA and Oil Red O stain were purchased from Sigma Aldrich. Isolation and characterization of swertiamarin from *Enicostemma Littorale* Blume was carried out by recording melting point and UV spectrometry with a standard sample of swertiamarin. Purity of the sample was checked by HPTLC using ethyl Acetate:Methanol:Water (0.7:0.2:0.1) as solvent system [20]. Ultraviolet absorption spectrum showed λ_{max} in the range of 240–245 nm. Melting point of the compound was 190–192 °C. The mass fragmentation pattern of compound represented base peak *m*/*z* of 374 representing molecular weight and *m*/*z* of 212 (M-162), a characteristic peak after removal of sugar moiety from the compound. TLC profile observed under UV 254 nm; and Densitograms of tracks [4]. Fetal bovine serum (FBS) and Penicillin-Streptomycin were procured from Gibco, Life Technologies and cell culture experiments were carried out in tissue culture plastic wares (Nunclon). RNA extraction was performed using Trizol (Invitrogen), gene specific primers were designed from IDT and all the other reagents required for molecular biology experiments were procured from Life Technologies. TG and LDH kits were purchased from Reckon Diagnostics, Vadodara, India and all proteomics grade reagents were obtained from Bio-Rad and antibodies for protein expression were obtained from CST.

2.2. Cell culture

Human hepatocellular carcinoma cells (HepG2) obtained from National Centre for Cell Sciences (NCCS), Pune, India, were seeded (1×10^5cells/T25 Flask) and cultured in Dulbecco's Modified Eagle's Medium (DMEM) Low Glucose with 10% FBS and 1% antibiotic-antimycotic solution (10X) at 37 °C with 5% CO_2 (Thermo scientific, CO_2 incubator). Cells were subsequently passaged every four days by trypsinization with 0.25% trypsin-EDTA solution.

2.3. Induction of steatosis in hepatocytes

The experiment was carried out for 24 h, which was divided into three groups: (1) Control, (2) OA Treated; 1 mM OA was used for induction of steatosis in HepG2 cells [21] and (3) 25 ug/ml swertiamarin was added along with OA in HepG2 cells.

2.4. Qualitative and quantitative analysis of in vitro steatosis

At the end of the treatment period (24 h), cells were fixed in buffered 4% para-formaldehyde for 10 min at RT and were washed twice with PBS. 1.0 ml of Oil Red O solution (1% in isopropanol) was then added to each well and incubated at RT for 10 min. After removing the stain from each well, the cells were washed with PBS until the solution became clear. Wells were dried, mounted in glycerin and examined under phase contrast microscope, Nikon. After washing and drying completely, 1 ml of isopropanol (100%) was added to each well, incubated for 10 min and then transferred to 1.5 ml tube and the absorbance were read at 520 nm [21] using Microplate Reader (Multiskan, Thermo Co.).

2.5. TG accumulation assay

After 24 h of treatment, cells were washed thrice with PBS and lysed with 1% triton X-100 in PBS. The cell lysate were centrifuged at 10,000*g* for 2 min, the supernatant was collected, assayed for TG using a commercially available enzymatic kit (Reckon Diagnostics, Baroda, India). Results were expressed as percentage TG. These values were normalized to total protein in the extract, measured with the Bradford reagent method (Bio-Rad).

2.6. LDH release assay

Cytotoxicity was measured as the fraction of Lactate dehydrogenase (LDH) released into the medium. HepG2 cells were maintained in 6 well plates for 24 h as described above. After the collection of conditioning media, cells were washed with phosphate buffered saline (PBS) and lysed in 1% triton X-100 in PBS. Cell lysates were collected, vortexed for 15 s and centrifuged at 4000*g* for 5 min. LDH activity was measured in the supernatant and in the cell lysate by a commercially available kit (Reckon Diagnostics Ltd, Baroda, India). LDH activity was calculated for intracellular and supernatant separately from the

Table 1
List of primers sequences with its accession number and amplicon size.

Gene	Accession number	Sequence (5′→3′)	Product size (bp)
SREBP-1c	NM_001005291	**F:** TGCATTTTCTGACACGCTTC **R:** CCAAGCTGTACAGGCTCTCC	171
PPAR-γ	NM_138712	**F:** GTGGCCGCAGATTTGAAAGA **R:** TGATCCCAAAGTTGGTGGGC	138
ACC-1	NM_198834	**F:** TTTAAGGGGTGAAGAGGGTGC **R:** CCAGAAAGACCTAGCCCTCAAG	171
FAS	NM_004104	**F:** CACAGGGACAACCTGGAGTT **R:** ACTCCACAGGTGGGAACAAG	97
CPT-1	NM_001876	**F:** TCGTCACCTCTTCTGCCTTT **R:** ACACACCATAGCCGTCATCA	206
PCK	NM_002591	**F:** ATCCCCAAAACAGGCCTCAG **R:** GGTGAATCCGTCAGCTCGAT	179
β-Actin	NM_001101	**F:** ACTCTTCCAGCCTTCCTTCC **R:** CGTACAGGTCTTTGCGGATG	101

formula—ΔA/min X F (F = 3376) and % LDH release were calculated as per the formula—(Media/Total LDH activity) × 100.

2.7. RNA extraction and real time quantitative PCR

Total RNA was isolated from treatment groups using Trizol Reagent and 2 μg of total RNA was reverse transcribed into first strand cDNA, synthesized by using Reverse Transcription Kit (Fermentas INC, USA). Quantitative PCRs were performed in two independent experiments, in triplicates. 10 μl of total reaction volume containing 5 μl Power SYBR-Green master mix, 10pM of each forward and reverse primers (Table 1) using 100 ng cDNA was taken for Real Time PCR (ABI 7500 Real Time PCR). All qPCR results were normalized to the level of β-actin determined in parallel reaction mixtures to correct any differences in RNA input. Fold changes in qPCR gene expression was analyzed using 7500 Real time PCR software V.2.0.6 and Data assist software (Applied Biosystems Inc.) which led to a possible estimation of the actual fold change. The qPCR results are expressed as mean ± S.E.M of RQ values *versus* target gene.

2.8. Protein extraction and western blotting

Western blot was performed for expression of various proteins. Briefly, HepG2 cells were subjected to all respective treatments for 24 h, and then the cell pellets were lysed with 1 ml of the lysis

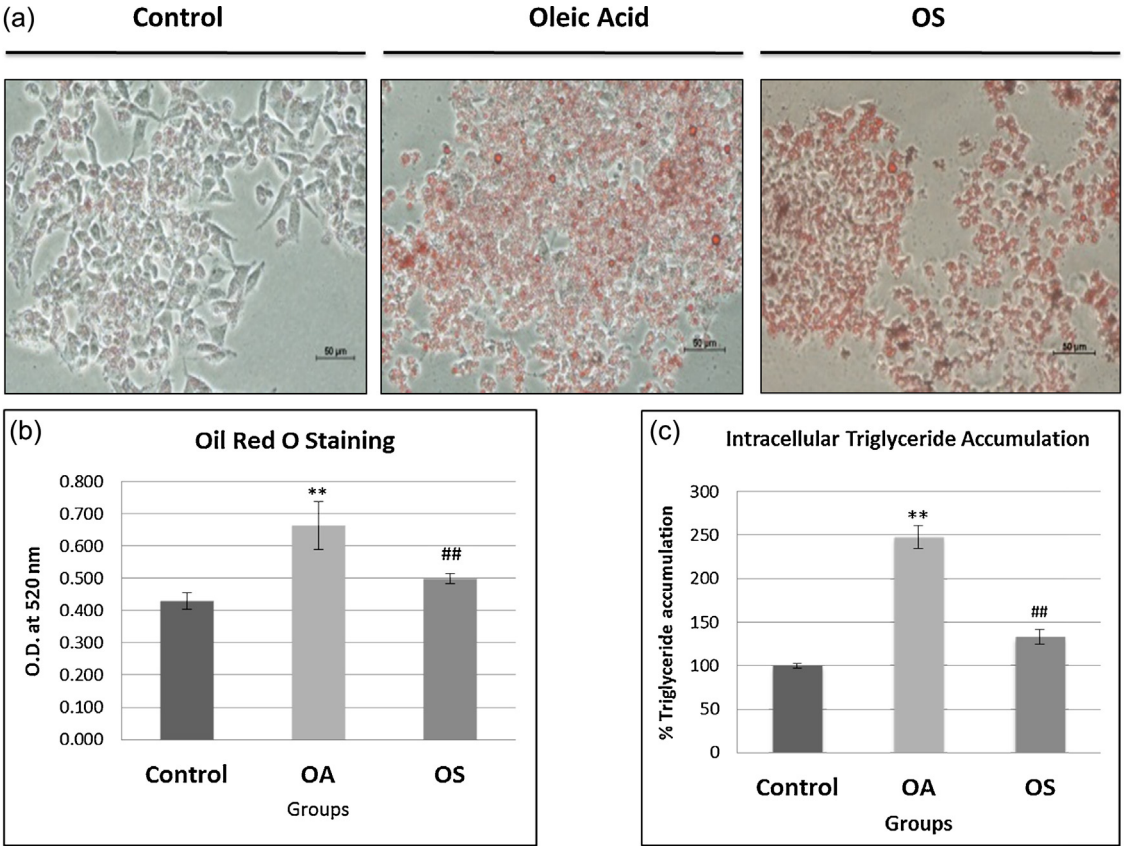

Fig. 1. OA-induced steatosis in HepG2 cells determined by Oil Red O staining and Triglyceride accumulation (a) Oil Red O staining observed at 20X magnification under Phase contrast microscope. (b) Quantification of Oil Red O stain (OD at 520 nm) after extraction procedure is represented in terms of % of Oil Red O stain compared to control; p-value $^{**} \leq 0.005$ as compared to control; p-value $^{\#\#} \leq 0.005$ as compared to OA, n = 3. (c) Intracellular Triglyceride accumulation values were normalized with the protein estimation and were represented as % Tg accumulation; p-value $^{**} \leq 0.005$ as compared to control; p-value $^{\#\#} \leq 0.005$ as compared to OA. n = 3.

buffer (1 mM EDTA, 50 mM Tris-HCl pH 7.5, 70 mM NaCl, 1% Triton, 50 mM NaF) supplemented with protease inhibitor cocktail (Fermentas). Protein estimation in all samples was carried out using Bradford reagent according to the manufacturer's suggestions (Bio-Rad). Cell lysate (20 μg) were separated on SDS-polyacrylamide gel using Mini-tetrapod electrophoresis system (Bio-Rad) and transferred onto nitrocellulose membrane (Thermo Inc). Blots were then incubated with blocking milk buffer (5% fat free skimmed milk with 0.1% Tween-20 in PBS). The blots were then probed for IR-β, pIRS-1, pAKT, PI(3)K, PPAR-α, pAMPK, AMPK, TNFα, Caspase 3 and PARP1 with primary antibodies (1:1000) and were incubated overnight at 4 °C. Anti-rabbit or anti-mouse IgG conjugated with HRP were used as secondary antibodies and the blots were developed using ultra-sensitive enhanced chemiluminiscence reagent (Millipore, USA) and image was captured by Alliance 4.7 UVI Tec Chemidoc.

2.9. Statistical analysis

The results were analyzed using one-way analysis of variance (ANOVA) and Student's T-test to determine the level of significance. $p < 0.05$ was considered to be significant. Results were expressed as mean ± SEM. The statistical analysis was carried out using the Graph Pad Prism 3.0 software.

3. Results

3.1. Confirmation of steatosis in OA induced HepG2 cells

After HepG2 cells were treated with 1 mM OA for 24 h, epithelial morphology of hepatocytes was found to be transformed into bulky lipid laden round cells, stained red due to Oil Red O stain. The stain was extracted with isopropanol and estimated spectrophotometrically at 520 nm, revealing that there was significant reduction in the lipid content in steatic hepatocytes treated with swertiamarin (25 μg/ml) when compared to OA induced steatic hepatocytes without SM treatment. This was the primary confirmatory step for hepatosteatosis (Fig. 1a and b).

3.2. Swertiamarin lowers the triglyceride (TG) content

Spectrophotometric analysis of triglyceride content in steatotic hepatocytes treated with swertiamarin showed significant reduction in the accumulated (50%) triglyceride content (Fig. 1c).

3.3. Swertiamarin rescues membrane integrity, apoptotic cleavage of PARP and caspase 3 activation of inflammatory mediated apoptotic pathway

Swertiamarin treatment significantly reduced (50%) LDH activity when compared to HepG2 cells treated with OA (Fig. 2a). Swertiamarin treatment significantly reduced the pro-inflammatory cytokine levels and thus, proved its anti-inflammatory activity. Swertiamarin significantly reduced the cleavage of Caspase 3 and PARP1 (Fig. 2b).

3.4. Effect of swertiamarin on insulin signaling proteins in insulin resistant HepG2 cells

Immunoblotting of various insulin signaling proteins from hepatosteatosis cells revealed that swertiamarin reduced insulin resistance by decreasing the expression levels of phosphorylated ser-307 IRS1 with concomitant increase in the levels of IR-β, PI(3)K and pAKT, proteins of insulin signaling. Swertiamarin reduced the expression of PPAR-α, the master regulator which was found to be

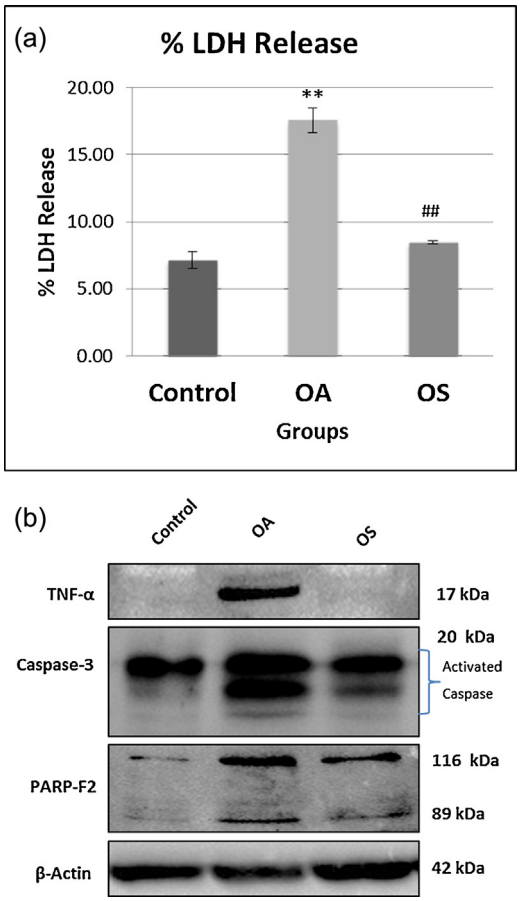

Fig. 2. Protective effect of swertiamarin on membrane integrity and cellular activation of apoptosis. (a) Effect of swertiamarin on % LDH release. p-value $^{**} \leq 0.005$ as compared to control; p-value $^{\#\#} \leq 0.005$ as compared to OA. n = 3 (b) Western blotting for checking protective effect of swertiamarin by analyzing TNFα, activated Caspase 3 and its most well-known death substrate PARP1 that is cleaved to an apoptotic signature 89 kDa fragment when compared to OA treated group.

elevated in OA induced insulin resistant hepatocytes (Fig. 3a and b).

3.5. Swertiamarin activates AMPK in OA induced insulin resistance in HepG2 cells

Swertiamarin was co-treated with OA for 24 h in HepG2 cells, phosphorylation of AMPK at Thr-172 verses AMPK protein was noted by immunoblotting with total AMPKα antibody. AMPK phosphorylation at threonine 172 is currently accepted as a marker of AMPK activity. Exposure of HepG2 cells to OA (1 mM) decreased phosphorylated AMPK (Thr 172) by 39% without a change in total AMPKα protein. Swertiamarin conserved phosphorylation of AMPKα (Thr 172) to the normal levels in insulin-resistant hepatocytes (Fig. 3c and d).

3.6. Swertiamarin reduces fat accumulation by regulating fat metabolic enzymes and HGP in steatotic hepatocytes

Swertiamarin treatment significantly reduced the expressions of major lipogenic genes like FAS, ACC-1. Also the levels of transcriptional factors like SREBP-1c and PPAR-γ were significantly down regulated in swertiamarin treated NAFLD cells.

Swertiamarin treatment markedly decreased expressions of this gene, with reduction in *CPT-1* levels, thus altering fatty acid oxidation. There was also a significant decrease in the expression

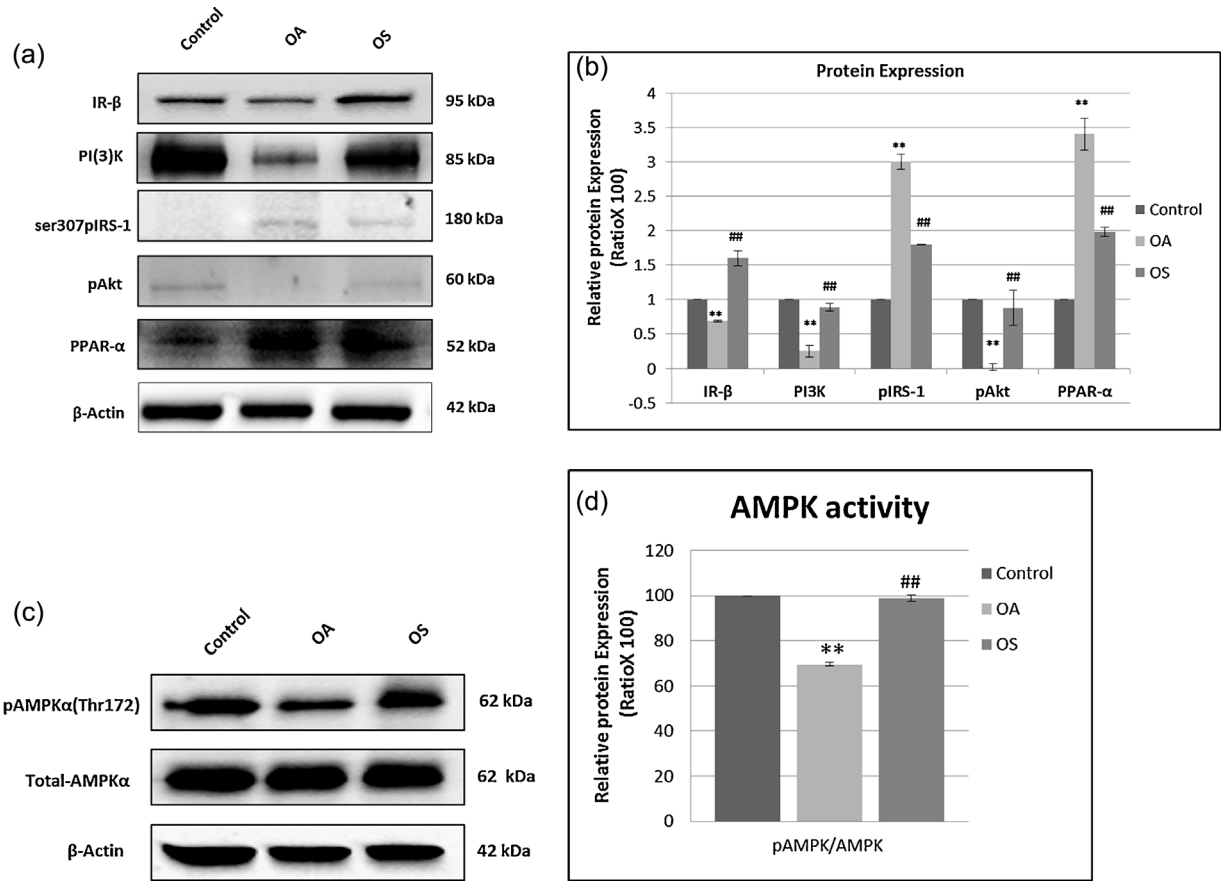

Fig. 3. Effect of swertiamarin on signaling pathways using insulin resistant HepG2. (a) Western blot study showing the effect of SM treatments on the expression of PPAR-α and Insulin signaling proteins: IR-β, $_{ser307p}$IRS-1, pAKT and PI(3)K in the HepG2 as compared to OA treated group. β-actin was taken as an internal control. (b) Densitometric analysis for the Insulin signaling proteins done using Image J software. (c) Swertiamarin on AMP-Kinase activation using insulin resistant HepG2. Western blot study showing the effect of SM treatments on the expression of phopho-AMPKα(Thr172) and total AMPKα in the HepG2 as compared to OA treated group. (d) Densitometric analysis for AMPKα activity done by Image J software, Data presented as Mean ± SEM of n = 3. p-value $^{**} \leq 0.005$ as compared to control; p-value $^{\#\#} \leq 0.005$ as compared to OA. p-value; ns > 0.05 as compared to control & OA. (20 μg protein).

level of PPAR-α, the master transcriptional regulator responsible for glucose oxidation. OA treated hepatocytes showed increased expressions of *PEPCK*, an enzyme responsible for production of glucose that caused hepatic glucose production, which was found to be reduced in swertiamarin treated group (Fig. 4).

4. Discussion

Central obesity with dysregulated adipocyte metabolism is causative factor for NAFLD, worldwide. Excess of fat accumulation in hepatocytes activates cytotoxic macrophages that cause inflammation in the hepatocytes which worsens the pathophysiology by enhanced fibrosis and liver damage [22]. Insulin sensitizers are better choice of treatment. However, to overcome their side effects, swertiamarin, a potent anti-diabetic drug as proven from our *in vivo* study, was further studied to understand its molecular mechanism in ameliorating NAFLD in OA induced steatosis in HepG2 cells. Current study states that swertiamarin inhibits translocation and accumulation of free fatty acids into OA treated hepatocytes, which prevent insulin resistance [6,23]. Hepatocytes were prevented from damaging their cytoskeletal organization, thus preventing their conversion to signet bulky and leaky cells resembling the myofibroblastic phenotype [11,22]. Significant reduction in LDH release provides evidence that swertiamarin prevents oxidative stress and disruption of cell membrane, hence, reducing cytotoxicity in hepatocytes. All the alterations evidenced cumulatively in the OA induced steatosis in hepatocytes activated cleavage of caspase and PARP1 [24]. Swertiamarin treatment significantly reduced caspase and PARP-1 cleavage.

AMPK acts as a nutrient sensor and plays a central role in promoting catabolism by regulating PPARs, the transcriptional

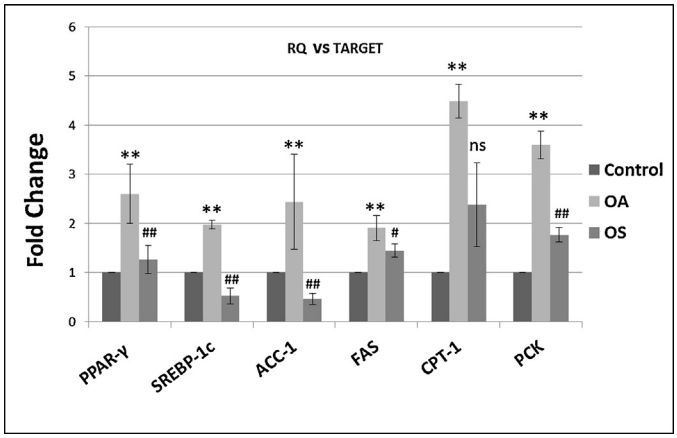

Fig. 4. Effect of swertiamarin on the expression of fat metabolism genes and HGP in liver steatosis. The expression levels of fat metabolic genes *SREBP-1c, PPAR-γ, ACC-1, FAS, CPT-1* and *PEPCK(PCK)* were checked using quantitative PCR. Data presented as Mean ± SEM of n = 3. p-value $^{**} \leq 0.005$ as compared to control; p-value $^{\#\#} \leq 0.005$ as compared to OA.

factors responsible for lipogenesis and fatty acid oxidation. It regulates SREBP-1c nuclear translocation, thus inhibiting the PPAR-γ mediated activation of lipogenic genes like *ACC-1, FAS* and *CD36*, hence decreasing fat accumulation and mobilization [19,25,26].

Swertiamarin treatment potentially increased the levels of AMPK with concomitant reductions in lipogenic enzymes. Also, with increased fat production, fatty acid oxidation is elevated in NAFLD by increased *CPT-1* expressions, which were found down-regulated upon swertiamarin treatment by regulating PPAR-α transcription, thus providing a clear line of evidence that swertiamarin would be a potential drug used for treating hepatic steatosis. PPAR-α is predominantly expressed in liver and senses fatty acid. The normal function of PPAR-α/PGC-1 pathway is to maintain fasting to feeding state by maintaining balance between fatty acid oxidation to hepatic glucose production, which is also controlled by AMPK. Carbohydrate metabolism in liver is tightly regulated by many key enzymes among which *PEPCK* is the key regulatory enzyme of gluconeogenesis and increased ketosis [15]. Swertiamarin treatment significantly reduced the expressions of *PEPCK*, thus preventing hyperglycemic burden and toxicity in hepatocytes. Effects of swertiamarin with respect to fat and carbohydrate metabolism, delineates it as a potent regulator of fat and carbohydrate metabolism in hepatocyte in steatosis. The present study supports that swertiamarin exhibits hypolipidemic and cholesterol reducing activity at systemic level *in vivo* [4,27].

In Type 2 DM, gluconeogenesis and fatty acid oxidation are elevated through CREB-dependent induction of PPAR-α/PGC-1 pathway stimulating the mammalian tribbles homolog TRB-3 which interferes with serine-threonine kinase AKT/PKB of insulin signaling cascade [28]. Excess of free fatty acids are activators of various protein kinases (PKCs) that leads to Ser 307 phosphorylation of IRS-1 and downregulation of tyrosine phosphorylation [29–34], which is also found in OA induced hepatosteatosis in hepatocytes. Western blot analysis in the current study depicted that swertiamarin significantly reduced the levels of IRS-1 (ser 307) phosphorylation, a hallmark of insulin resistance with increase in IR-β, PI(3)K and pAKT which elevates insulin sensitivity and could probably restore glycogen storage [7]. The increased expression of the insulin signaling proteins in the swertiamarin treated steatotic hepatocytes strengthens the evidence that shows swertiamarin as a potential insulin sensitizer [4].

The present study explores that swertiamarin activates AMPK which govern the transcriptional regulators SREBP-1c/PPAR-γ and hence, controls lipogenic genes, ACC-1 and FAS that reduces Tg accumulation, hence, preventing LDH release and ROS generation. Transcriptional regulator PPAR-α and insulin signaling proteins are restored back to their normal levels which delineates swertiamarin among the group of recent drugs implicated for amelioration of Diabetes [35]. AMPK activation by swertiamarin reduced hepatic glucose production, lipogenesis and insulin resistance by controlling the major transcriptional factors PPARα, PPARγ along with insulin signaling proteins respectively. Thus, the study provides a strong evidence that swertiamarin potentially targets all the master regulators like AMPK, PPAR (α and γ) and oxidative stress. Thus, swertiamarin proves to be a potent AMPK activator and Insulin sensitizer signifying a powerful drug for the treatment of hepatosteatosis and insulin resistance in obesity (Graphical abstract).

Declaration of interest

The authors declare that there is no duality of interest associated with this manuscript.

Author contributions

SG, TP—Conceived and designed the experiments; SS,TP, SG—Isolation and characterization of compound; TP—Performed all experiments; TP, SG—Analyzed the data; TP, KR, SG.—Wrote the paper.

Acknowledgements

We thank DBT-MSUB-ILSPARE program for Central Instrumentation Facility, The M.S. University of Baroda. We thank DBT for providing fellowship as Research Associate in DBT-Basic Project.

References

[1] H.L. Li, X.J. Peng, C.J. He, E.F. Feng, G.L. Xu, G.X. Rao, Development and validation of a LC-ESI–MS/MS method for the determination of swertiamarin in rat plasma and its application in pharmacokinetics, J. Chromatogr. B 879 (2011) 1653–1658.
[2] R.D. Sonawane, S.L. Vishwakarma, S. Lakshmi, M. Rajani, H. Padh, R.K. Goyal, Amelioration of STZ-induced type 1 diabetic nephropathy by aqueous extract of Enicostemma littorale Blume and swertiamarin in rats, Mol. Cell. Biochem. 340 (1–2) (2010) 1–6.
[3] S. Saravanan, V. Hairul Islam, N. Prakash Babu, P. Pandikumar, K. Thirugnanasambantham, M. Chellappandian, C. Simon Durai Raj, M. Gabriel Paulraj, S. Ignacimuthu, Swertiamarin attenuates inflammation mediators via modulating NF-κβ/I κB and JAK2/STAT3 transcription factors in adjuvant induced arthritis, Eur. J. Pharm. Sci. 56 (2014) 70–86.
[4] T.P. Patel, S. Soni, P. Parikh, J. Gosai, R. Chruvattil, S. Gupta, Swertiamarin an active lead from enicostemma littorale regulates hepatic and adipose tissue gene expression by targeting PPAR-gamma and improves insulin sensitivity in experimental NIDDM rat model, Evid. Based Complement. Altern. Med. 2013 (2013) 358673.
[5] S.B. Joseph, B.A. Laffitte, P.H. Patel, M.A. Watson, K.E. Matsukuma, R. Walczak, J. L. Collins, T.F. Osborne, P. Tontonoz, Direct and indirect mechanisms for regulation of fatty acid synthase gene expression by liver X receptors, J. Biol. Chem. 277 (13) (2002) 11019–11025.
[6] D.G. Tiniakos, M.B. Vos, E.M. Brunt, Nonalcoholic fatty liver disease: pathology and pathogenesis, Annu. Rev. Pathol. 5 (2010) 145–171.
[7] M. Asrih, F.R. Jornayvaz, Inflammation as a potential link between nonalcoholic fatty liver disease and insulin resistance, J. Endocrinol. 218 (3) (2013) R25–R36.
[8] N. Anderson, J. Borlak, Molecular mechanisms and therapeutic targets in steatosis and steatohepatitis, Pharmacol. Rev. 60 (3) (2008) 311–357.
[9] J.K. Reddy, M.S. Rao, Lipid metabolism and liver inflammation. II. Fatty liver disease and fatty acid oxidation, Am. J. Physiol. Gastrointest. Liver Physiol. 290 (5) (2006) G852–8.
[10] S. Bellentani, F. Scaglioni, M. Marino, G. Bedogni, Epidemiology of non-alcoholic fatty liver disease, Dig. Dis. 28 (1) (2010) 155–161.
[11] S. Srivastava, Z. Li, X. Yang, M. Yedwabnick, S. Shaw, C. Chan, Identification of genes that regulate multiple cellular processes/responses in the context of lipotoxicity to hepatoma cells, BMC Genomics 8 (2007) 364.
[12] M. Kohjima, N. Higuchi, M. Kato, K. Kotoh, T. Yoshimoto, T. Fujino, M. Yada, R. Yada, N. Harada, M. Enjoji, SREBP-1c, regulated by the insulin and AMPK signaling pathways, plays a role in nonalcoholic fatty liver disease, Int. J. Mol. Med. 21 (4) (2008) 507–511.
[13] K. Copps, M. White, Regulation of insulin sensitivity by serine/threonine phosphorylation of insulin receptor substrate proteins IRS1 and IRS2, Diabetologia 55 (10) (2012) 2565–2582.
[14] V.T. Samuel, Z.-X. Liu, X. Qu, B.D. Elder, S. Bilz, D. Befroy, A.J. Romanelli, G.I. Shulman, Mechanism of hepatic insulin resistance in non-alcoholic fatty liver disease, J. Biol. Chem. 279 (31) (2004) 32345–32353.
[15] C. Postic, J. Girard, Contribution of de novo fatty acid synthesis to hepatic steatosis and insulin resistance: lessons from genetically engineered mice, J. Clin. Invest. 118 (3) (2008) 829–838.
[16] P. Degrace, L. Demizieux, J. Gresti, J.-M. Chardigny, J.-L. Sébédio, P. Clouet, Hepatic steatosis is not due to impaired fatty acid oxidation capacities in C57BL/6J mice fed the conjugated trans-10, *cis*-12-isomer of linoleic acid, J. Nutr. 134 (4) (2004) 861–867.
[17] R.N. Jadeja, M.C. Thounaojam, R.V. Devkar, A.V. Ramachandran, Clerodendron glandulosum. Coleb extract prevents in vitro human LDL oxidation and oxidized LDL induced apoptosis in human monocyte derived macrophages, Food Chem. Toxicol. 49 (6) (2010) 1195–1202.
[18] J.R. Lewis, S.R. Mohanty, Nonalcoholic fatty liver disease: a review and update, Dig. Dis. Sci. 55 (3) (2010) 560–578.
[19] M.M. Mihaylova, R.J. Shaw, The AMPK signalling pathway coordinates cell growth, autophagy and metabolism, Nat. Cell Biol. 13 (9) (2011) 1016–1023.
[20] S. Vishwakarma, M. Rajani, M. Bagul, R. Goyal, A rapid method for the isolation of swertiamarin from Enicostemma littorale, Pharm. Biol. 42 (6) (2004) 400–403.
[21] W. Cui, S.L. Chen, K.-Q. Hu, Quantification and mechanisms of oleic acid-induced steatosis in HepG2 cells, Am. J. Transl. Res. 2 (1) (2010) 95.

[22] S.L. Friedman, Mechanisms of hepatic fibrogenesis, Gastroenterology 134 (6) (2008) 1655–1669.
[23] S. de Ferranti, D. Mozaffarian, The perfect storm: obesity, adipocyte dysfunction, and metabolic consequences, Clin. Chem. 54 (6) (2008) 945–955.
[24] W. Cui, S.L. Chen, K.Q. Hu, Quantification and mechanisms of oleic acid-induced steatosis in HepG2 cells, Am. J. Transl. Res. 2 (1) (2010) 95–104.
[25] R.A.K. Srivastava, S.L. Pinkosky, S. Filippov, J.C. Hanselman, C.T. Cramer, R.S. Newton, AMP-activated protein kinase: an emerging drug target to regulate imbalances in lipid and carbohydrate metabolism to treat cardio-metabolic diseases. Thematic Review Series: New Lipid and Lipoprotein Targets for the Treatment of Cardiometabolic Diseases, J. Lipid Res. 53 (12) (2012) 2490–2514.
[26] M.S. Sozio, C. Lu, Y. Zeng, S. Liangpunsakul, D.W. Crabb, Activated AMPK inhibits PPAR-α and PPAR-γ transcriptional activity in hepatoma cells, Am. J. Physiol. Gastrointest. Liver Physiol. 301 (4) (2011) G739–G747.
[27] H. Vaidya, M. Rajani, V. Sudarsanam, H. Padh, R. Goyal, Swertiamarin: a lead from *Enicostemma littorale* Blume. for anti-hyperlipidaemic effect, Eur. J. Pharmacol. 617 (1) (2009) 108–112.
[28] S.-H. Koo, H. Satoh, S. Herzig, C.-H. Lee, S. Hedrick, R. Kulkarni, R.M. Evans, J. Olefsky, M. Montminy, PGC-1 promotes insulin resistance in liver through PPAR-α-dependent induction of TRB-3, Nat. Med. 10 (5) (2004) 530–534.
[29] L. Rui, V. Aguirre, J.K. Kim, G.I. Shulman, A. Lee, A. Corbould, A. Dunaif, M.F. White, Insulin/IGF-1 and TNF-α stimulate phosphorylation of IRS-1 at inhibitory Ser 307 via distinct pathways, J. Clin. Invest. 107 (2) (2001) 181–189.
[30] R.H. Unger, Lipid overload and overflow: metabolic trauma and the metabolic syndrome, Trends Endocrinol. Metab. 14 (9) (2003) 398–403.
[31] G. Boden, G. Shulman, Free fatty acids in obesity and type 2 diabetes: defining their role in the development of insulin resistance and β-cell dysfunction, Eur. J. Clin. Invest. 32 (s3) (2002) 14–23.
[32] J. Tamburini, N. Chapuis, V. Bardet, S. Park, P. Sujobert, L. Willems, N. Ifrah, F. Dreyfus, P. Mayeux, C. Lacombe, Mammalian target of rapamycin (mTOR) inhibition activates phosphatidylinositol 3-kinase/Akt by up-regulating insulin-like growth factor-1 receptor signaling in acute myeloid leukemia: rationale for therapeutic inhibition of both pathways, Blood 111 (1) (2008) 379–382.
[33] J.-F. Tanti, J. Jager, Cellular mechanisms of insulin resistance: role of stress-regulated serine kinases and insulin receptor substrates (IRS) serine phosphorylation, Curr. Opin. Pharmacol. 9 (6) (2009) 753–762.
[34] T.P. Patel, K. Rawal, A.K. Bagchi, G. Akolkar, N. Bernardes, D.d.S. Dias, S. Gupta, P. K. Singal, Insulin resistance: an additional risk factor in the pathogenesis of cardiovascular disease in type 2 diabetes, Heart Fail. Rev. (2015) 1–13.
[35] G. Musso, R. Gambino, M. Cassader, Emerging molecular targets for the treatment of nonalcoholic fatty liver disease, Annu. Rev. Med. 61 (2010) 375–392.

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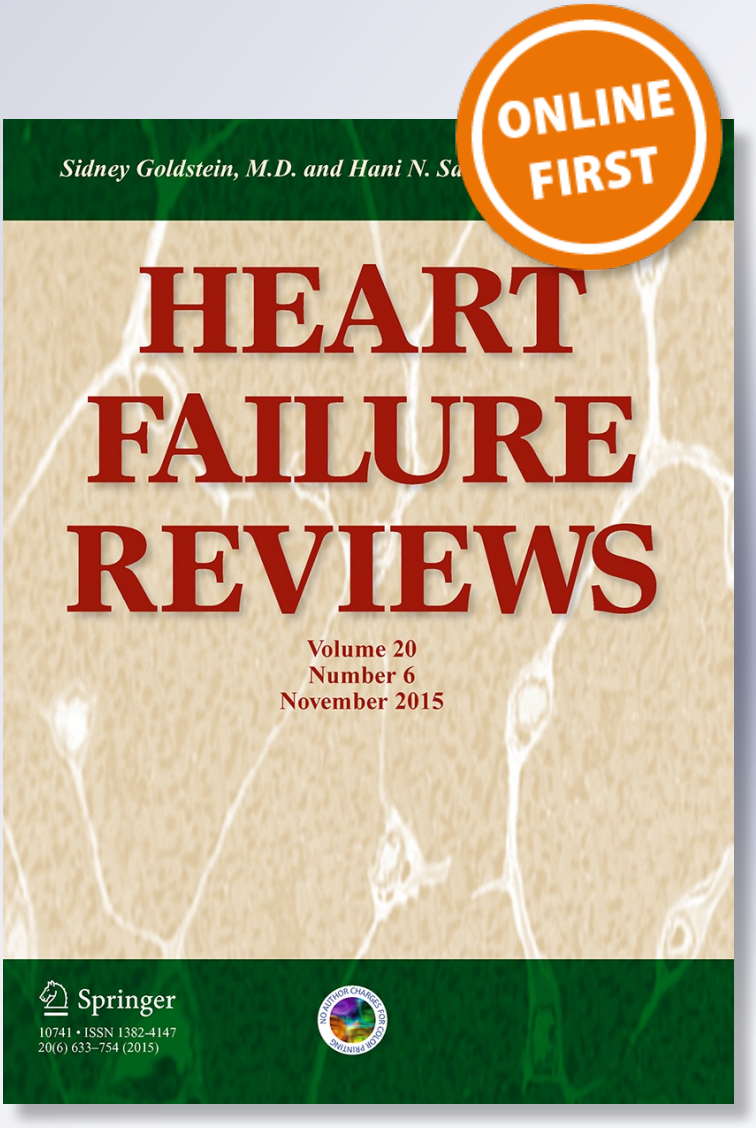
Heart Failure Reviews

ISSN 1382-4147

Heart Fail Rev
DOI 10.1007/s10741-015-9515-6



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Heart Fail Rev
DOI 10.1007/s10741-015-9515-6

Insulin resistance: an additional risk factor in the pathogenesis of cardiovascular disease in type 2 diabetes

Tushar P. Patel[1] · Komal Rawal[1] · Ashim K. Bagchi[2] · Gauri Akolkar[2] · Nathalia Bernardes[2] · Danielle da Silva Dias[2] · Sarita Gupta[1] · Pawan K. Singal[2]

Abstract Sedentary life style and high calorie dietary habits are prominent leading cause of metabolic syndrome in modern world. Obesity plays a central role in occurrence of various diseases like hyperinsulinemia, hyperglycemia and hyperlipidemia, which lead to insulin resistance and metabolic derangements like cardiovascular diseases (CVDs) mediated by oxidative stress. The mortality rate due to CVDs is on the rise in developing countries. Insulin resistance (IR) leads to micro or macro angiopathy, peripheral arterial dysfunction, hampered blood flow, hypertension, as well as the cardiomyocyte and the endothelial cell dysfunctions, thus increasing risk factors for coronary artery blockage, stroke and heart failure suggesting that there is a strong association between IR and CVDs. The plausible linkages between these two pathophysiological conditions are altered levels of insulin signaling proteins such as IR-β, IRS-1, PI3K, Akt, Glut4 and PGC-1α that hamper insulin-mediated glucose uptake as well as other functions of insulin in the cardiomyocytes and the endothelial cells of the heart. Reduced AMPK, PFK-2 and elevated levels of NADP(H)-dependent oxidases produced by activated M1 macrophages of the adipose tissue and elevated levels of circulating angiotensin are also cause of CVD in diabetes mellitus condition. Insulin sensitizers, angiotensin blockers, superoxide scavengers are used as therapeutics in the amelioration of CVD. It evidently becomes important to unravel the mechanisms of the association between IR and CVDs in order to formulate novel efficient drugs to treat patients suffering from insulin resistance-mediated cardiovascular diseases. The possible associations between insulin resistance and cardiovascular diseases are reviewed here.

Keywords Insulin resistance · CVD · Dyslipidemia · Metabolic syndrome · Oxidative stress · Inflammation

Abbreviations

ACE	Angiotensin-converting enzyme
ARBs	Angiotensin receptor blockers
ADA	American Diabetes Association
AGEs	Advanced glycation end-products
AMPK	AMP-activated protein kinase
AT1R	Angiotensin II type I receptor
C/EBP	CCAAT/enhancer binding protein
CAN	Cardiac autonomic neuropathy
CRP	C-reactive protein
CVD	Cardio vascular diseases
DG	Diacyl glycerol
DM	Diabetes mellitus
eNOS	Endothelial nitric oxide synthase
ERR	Estrogen-related nuclear receptors
ET-1	Endothelin-1
FAT/CD36	Fatty acid translocase
FetA	Fetuin-A
FFA	Free fatty acid
Glut4	Glucose transporter 4
HDL	High-density lipoprotein
HO-1	Heme oxygenase-1
ICAM-1	Intracellular adhesion molecule-1
IL-6	Interleukin-6

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Published online: 05 November 2015

IR	Insulin resistance
IR-β	Insulin receptor β
IRS-1	Insulin receptor substrate-1
JNK	Janus kinase
STAT	Signal transducer and activator of transcription
LCFA	Long-chain fatty acid
LDL	Low-density lipoprotein
LPL	Lipoprotein lipase
MAPK	Mitogen-activated protein kinase
MCP-1	Macrophage chemo attractant protein-1
mTOR	Mammalian target of rapamycin
NADP	Nicotinamide adenine dinucleotide phosphate
NEFA	Non-esterified fatty acid
NFAT	Nuclear factor of activated T cells
NFκ-B	Nuclear factor kappa-light-chain-enhancer of activated B cells
NO	Nitric oxide
NOXs	NADPH oxidases
NRF1	Nuclear respiratory factor 1
OXPHO	Oxidative phosphorylation
PAI-1	Plasminogen activator inhibitor-1
PFK2	Phosphofructokinase 2
PGC-1α	PPAR-γ coactivator 1α
PH	Pleckstrin homology
PI3K	Phosphatidylinositol 3-kinase
PKC	Protein kinase C
PKB/Akt	Protein kinase B
PPARs	Peroxisome proliferator-activated receptors
PTEN	Phosphatase and tensin homolog
PTP1B	Protein tyrosine phosphatase 1B
ROS	Reactive oxygen species
SHIP	SH2-containing inositol 5′-phosphatase
SOCS	Suppressors of cytokine signaling
SREBP	Sterol regulatory element binding protein
TAG	Triacyl glycerol
Tfam A	Mitochondrial transcription factor A
TLRs	Toll-like receptors
TNF-α	Tumor necrosis factor-α
UCP	Uncoupling protein
VAT	Visceral adipose tissue
VEGF	Vascular endothelial growth factor
VLDL	Very low-density lipoprotein

Introduction

Cardiovascular diseases (CVDs) are often associated with metabolic diseases and are one of the major cause of early deaths in diabetic population. In diabetic patients, 65 % of the deaths are listed due to CVD [1]. In Framingham studies, it has been reported that diabetes is a precursor of cardiovascular morbidity, mortality and congestive heart failure [2, 3]. Several hypothesis such as hyperglycemia, oxidative stress, inflammation, obesity, sedentary life style, genetic predisposition etc. have emerged to connect the dots between diabetes and CVD. However, none of the hypothesis is able to completely define the underlying pathophysiology. In aggressive conventional therapy for diabetes the risk of CVD is reduced by about 42 %. Thus, a high-quality management of diabetes alone does not explain the high incidence of CVD in these patients. One of the overlooked links that is common in type 2 diabetic patients is the occurrence of insulin resistance (IR). The role of insulin as an atherogenic molecule as well as the significance of insulin signaling in the endothelial cells has been underappreciated even though these cells are the key players involved in the vascular function. Furthermore, the complexity of IR syndrome, arising in the major peripheral insulin-dependent tissues leading to the microvascular and macrovascular complications in diabetes due to systemic IR, is not clearly understood.

Pathophysiology of cardiovascular diseases in diabetes

Persistent hyperglycemia causes microvascular and macrovascular complications in both type 1 and type 2 diabetes. Microvascular complications include nephropathy, neuropathy and retinopathy, while macrovascular complications include coronary artery disease, peripheral arterial disease and stroke [4]. Some of the main etiological factors for CVD are as follows:

Oxidative stress, inflammatory response and endothelial dysfunction

Much of the enduring pathology of diabetes occurs as a consequence of persistent hyperglycemia leading to increased reactive oxygen species (ROS) production by mitochondria, which is the main source of oxidative stress involving complications of diabetic pathologies including CVD [1, 5, 6]. In type 1 diabetes, endothelial dysfunction is an essential determinant of inflammatory activities and considered as an early CVD marker. The inflammatory response is generated by innate immunity which includes augmentation of cytokine and chemokine release, enhanced leukocyte marginalization and increased superoxide release [7]. It is coupled with the impairment of the endothelial signal transduction and redox-regulated activation of transcription factors [8], and endothelial dysfunction in type 2 diabetes has also been shown to occur [9, 10]. It has been

demonstrated that excess ROS production due to hyperglycemia induces epigenetic changes like: monomethylation of lysine from histone 3 which increases expression of p65 subunit of NFκ-B. These epigenetic reactions can be considered as mediators between diabetes, chronic inflammatory response and CVD [11, 12].

Dyslipidemia, obesity and hypertension

Risk of CVD persists in dyslipidemia, due to atherogenic profile which comprises of increased very low-density lipoprotein (VLDL) cholesterol, triglycerides and low-density lipoprotein (LDL) cholesterol levels whereas decreased high-density lipoprotein (HDL) cholesterol levels. This is also supported by studies showing that diabetic hyperlipidemia or hyperglycemia accelerates atherogenesis [13, 14], also obesity and diabetes are coupled with major increase in morbidity and mortality due to CVD [15, 16]. It is observed that in obesity, visceral fat deposition leads to inflammation which also plays an important role in diabetic complications. The association between hypertension and obesity is also known to cause higher rate of morbidity and mortality associated with CVD [17, 18]. It is reported that approximately 10–30 % type 1 diabetic and 60 % type 2 diabetics suffer from hypertension and thus high risk of CVD.

Hypoglycemia

Insulin and hypoglycemic drugs control glycemic load and may lead to frequent hypoglycemia-related cardiovascular mortality in diabetes patients [19]. Hypoglycemia is found to cause unusual electrical activity in the heart and is thus believed to aggravate sudden death [20, 21]. Inflammation in hypoglycemic condition occurs due to C-reactive protein (CRP), IL-6, vascular endothelial growth factor (VEGF), increased platelet and neutrophil activation [22].

Autonomic neuropathy

Cardiac autonomic neuropathy (CAN) is one of the common complications of type 1 and type 2 diabetes. It prevails in about 20 % and is reported to increase with age as well as duration of diabetes with annual increase of 2 % [23, 24]. EURODIAB study reported that poor glycemic control is strong risk factor for CAN which is the predictor of CVD morbidity and mortality in type 1 diabetes [25].

Apparently, all the above factors play an important role in diabetes related CVD risk. However, association of these factors with hyperinsulinemia leading to IR, hall marks of type 2 diabetes as a causal factor in cardiovascular diseases is not fully appreciated. An effort is made to delineate the relationship of insulin resistance and CVD in the pathogenesis of type 2 diabetes.

Molecular mechanism of insulin resistance

Insulin plays a central role in carbohydrate and lipid metabolism in peripheral system and also has other functions in heart and brain. Obesity is the major contributory factor to systemic interventions like hyperglycemia, hyperinsulinemia, hyperlipidemia etc. which leads to inefficiency or failure of insulin action that leads to systemic insulin resistance condition [26, 27] as shown in Fig. 1.

Muscle, adipose and liver are the most affected organs due to overload of lipid accumulation and thus, lead to peripheral insulin resistance [28]. Free fatty acids (FFA) circulate and deposit into the skeletal muscle myocytes and intramuscular lipid accumulation occurs, which further aggravates the insulin resistance condition by downregulating the expression as well as reducing total insulin receptor number [26].

Excess fat activates the Toll-like receptors (TLRs) on the resident macrophages of adipose tissue that secrete TNF-α. The later, activates NFκB-mediated cellular toxicity by activating various PKCs and downregulating tyrosine phosphorylation of insulin receptors. This compromises insulin signaling and promotes insulin resistance [29, 30]. Activation of various isoforms of PKCs, also activates Ser/Thr kinases which phosphorylates IRS-1 at Ser 307 that hampers the association of IRS-1 and PI3K, ultimately causing proteasomal degradation of IRS-1 and IRS-2. These events inhibit insulin signaling as well as the translocation of glucose transporter 4 (GLUT4) and insulin stimulated glucose uptake [29, 31]. Stimulated SREBP-1c also decreases IRS-2 levels in insulin resistance condition. Downstream in the signaling, altered ratio of PI3K subunits p110/p85 inhibits the dimerization of the enzyme and thus reduces its activity. Suppressors of cytokine signaling (SOCS) proteins, which are induced by inflammatory cytokines, bind to the insulin receptors and block their signaling. Insulin resistance can also be due to an increase in the activity as well as amount of the enzymes that normally reverse insulin action, including the phosphotyrosine phosphatases, e.g., PTP1b, and the PIP phosphatases, e.g., PTEN and SHIP.

All these events lead to a concomitant reduction in insulin stimulated glycogen synthesis and glucose uptake which leads to activation of the phosphoenolpyruvate carboxykinase, the rate limiting enzyme of gluconeogenesis. This increases hepatic glucose production which not only leads to hepatic insulin resistance but overall insulin resistance as well [31, 32].

Mechanism of oxidative stress in diabetes and insulin resistance

Glucose toxicity, being the hallmark of diabetes mellitus is responsible for ROS production leading to oxidative stress [32]. There are several mechanisms by which

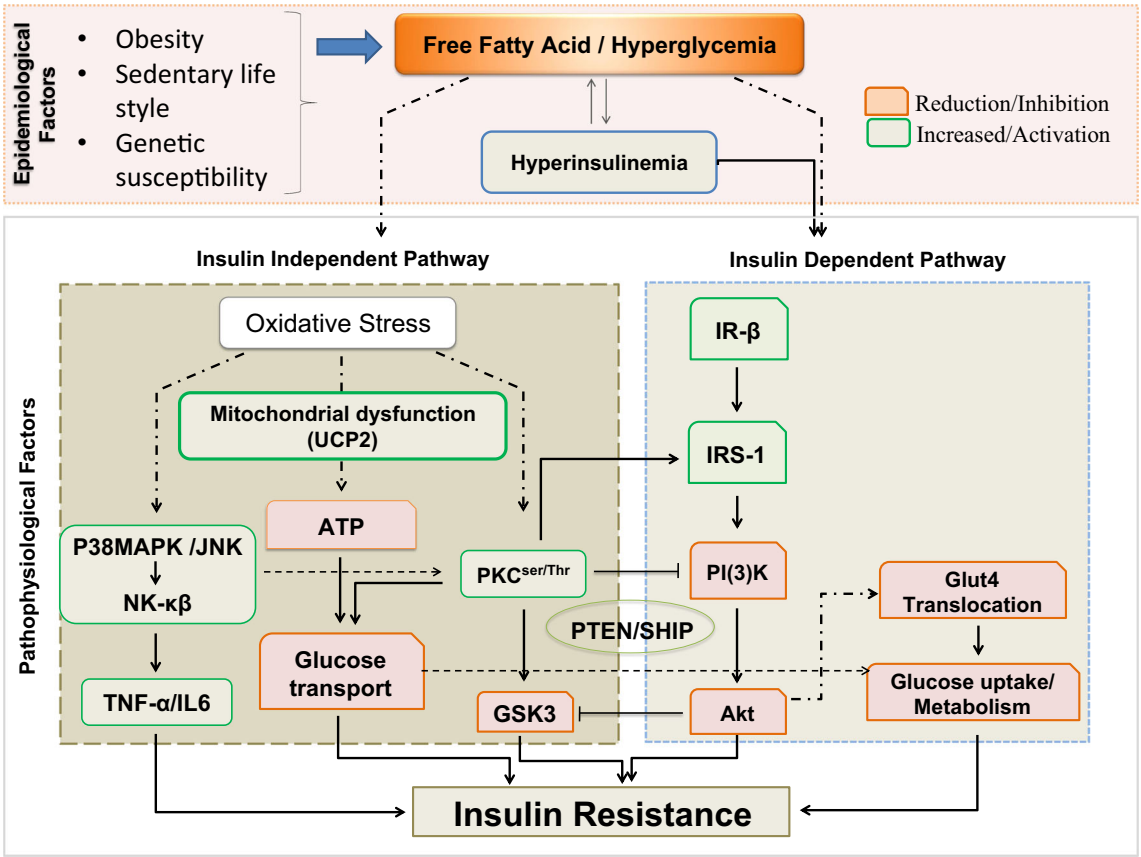

Fig. 1 Etiological factors affecting insulin-dependent and insulin-independent pathways which cumulatively lead to alterations in metabolism and cause insulin resistance (IR). Factors such as obesity and sedentary life style leading to excess free fatty acids (FFA) availability and hyperinsulinemia are associated with IR. Pathophysiological processes involve insulin-independent pathway where oxidative stress leads to the activation of stress kinases such as p38 and JNK MAPKs stimulating secretion of proinflammatory cytokines like TNF-α and IL-6 under the transcriptional activation of NF-κB and induce protein kinase C (PKCs) at serine/threonine residues and GSK-3. Activation of PKC phosphorylates and subsequently activates IkB kinase to promote phosphorylation of insulin receptor substrate-1 (IRS-1) which inhibit the ability of IRS-1 to bind to SH2 domains of the p85 regulatory subunit of phosphatidylinositol 3-kinase (PI3K) and as a result impairs insulin signal transduction. Inhibition of PI3K causes an upregulation of phosphatase and tensin homolog (PTEN) and SH-2 containing inositol 5′-phosphatase (SHIP). On the other hand, oxidative stress also leads to mitochondrial dysfunction by downregulating the uncoupling protein-2 (UCP-2) decreasing ATP production and control glucose transport by the expression and translocation of glucose transporter 4 (GLUT4). Insulin-dependent pathway, involving etiological factors mainly excess FFA, hyperglycemia and hyperinsulinemia, leads to Ser phosphorylation of insulin receptors (IR-β) and its substrate (IRS-1) to desensitize tyrosine phosphorylation of PI3K and Akt. This reduces GLUT4 translocation mainly by the activation of serine/threonine phosphorylation caused by activated PKCs ultimately leading to insulin resistance

hyperglycemia can induce oxidative stress. These include the activation of polyol pathway, glucose auto oxidation, formation of advanced glycation end (AGEs) products, increased FFA, leptin levels and increased mitochondrial ROS generation [6, 33].

Activation of polyol pathway and formation of advanced glycation end (AGE) products

In hyperglycemia, up to 35 % of glucose is metabolized by polyol pathway. NADPH is needed for the production of reduced Glutathione, which is consumed by aldol-reductase (first enzyme of the pathway), thus preventing the regeneration of reduced Glutathione further aggravating oxidative stress. The second enzyme is sorbitol dehydrogenase that converts sorbitol to fructose producing NADH. NAD(P)H oxidases can use NADH to produce more superoxide anion [34].

Ketoaldehyde, protein reactive dicarbonyl sugar, is a product of glucose autoxidation and a member of AGE, which not only yields H_2O_2 but also the other highly reactive oxidants that become an important source of diabetes mellitus (DM)-induced oxidative stress [6]. Glyoxal and arabinose have been reported to be the major dicarbonyl intermediates that cause protein browning observed in DM and aging. Oxidative stress increases with age as well as severity of DM, which stimulates Maillard reaction where these dicarbonyl intermediates attach non-enzymatically to

protein amino-structures generating ROS which can promote oxidation of glycation products leading to AGE formation, AGEs precursors can further bind to AGE receptors on the surface of endothelial cells and macrophages resulting in receptor-mediated production of oxygen free radicals and dysregulating their functions [35, 36].

FFA- and leptin-mediated oxidative stress during hyperglycemia

Free or non-esterified fatty acids (NEFA) are elevated in diabetic patients. Mitochondrial superoxide production increases when excess FFA enters into the citric acid cycle and generates acetyl-CoA to produce excess NADH. This also elevates isoprostanes, which are markers of lipid peroxidation. Leptin is an adipocyte secretory hormone that acts on the central nervous system to abate food intake. It also increases ROS levels when incubated with endothelial cells, vascular smooth muscle cells, monocytes, and macrophages. Plasma levels of leptin are increased in type 2 diabetics and are associated with CVD [37, 38].

Mechanism of hyperglycemia, hyperlipidemia and oxidative stress causing insulin resistance

Glucotoxicity and hyperinsulinemia induced IR is pathologically associated with hyper-Ser/Thr phosphorylation of IRS1 and IRS2 that impairs their interaction with the cytoplasmic domain of insulin receptor, which abolishes the propagation of normal insulin signaling [39]. Under normal conditions, this is an important counterbalancing mechanism that stops insulin's action. However, in the case of DM, the hyperserine phosphorylation of the IRS proteins may lead to a chronic cellular desensitization to insulin [40].

It has been reported that under oxidative stress condition, insulin stimulated serine PKB phosphorylation and the translocation of GLUT4 from internal pool to the plasma membrane were dramatically reduced [41]. Introduction of prolonged oxidative stress to L6 myotubes and 3T3-L1 adipocytes mediates GLUT1 transcriptional activation and insulin-independent glucose uptake. This result in an increase in mitochondrial ROS production due to an increase in the basal glucose uptake and metabolism in various cell types including cardiomyocytes [42, 43]. It is known that cardiomyocytes expresses both GLUT4 and GLUT1 glucose transporters [44]. Binding affinity of C/EBP, i.e., the CCAAT enhancer binding protein to the GLUT4 promoter is affected during oxidative stress. This alteration in C/EBP function plays a role in the down-regulation of GLUT4 expression in the cells under oxidative stress [43].

Pathophysiology of DM is not only about an insulin–glucose axis, but fat derangements are also a major cause of type 2 diabetes. Central obesity is due to an overload of TG in abdominal adipocytes. Subcutaneous fat has a high rate of basal lipolysis. The enlarged visceral adipocytes pour out FFA which is mainly responsible for ectopic fat deposition [45]. This leads to ectopic TG accumulation in muscles, liver, heart and pancreatic β-cells, resulting in IR at the systemic level by interfering with both insulin secretion and insulin signaling [46].

Hyperinsulinemia is also known to enhance hepatic VLDL synthesis thus, leads to the increased plasma triglyceride and LDL cholesterol levels [47]. Resistance to the action of insulin on lipoprotein lipase in peripheral tissues further contributes to the elevated triglyceride and LDL cholesterol levels [48]. IR condition reduces the levels of HDL cholesterol despite enhanced HDL cholesterol synthesis. This decrease in plasma HDL cholesterol was entirely accounted by an increase in the rate of apolipoprotein A1/HDL cholesterol degradation, which exceeds the enhanced rate of its synthesis [49]. It further supports the view that dysregulation of fatty acid metabolism contributes to the pathophysiology of the IR syndrome which relates to the risk of cardiovascular disease [50].

Mitochondrial stress and IR

Superoxide anion production is promoted during hyperglycemia by the proton electrochemical gradient of the mitochondrial electron transport. In culture cells, it is observed that there is inhibition of mitochondrial superoxide formation followed by complete inhibition of PKCs and NF-κB activation. In normal conditions, heme oxygenase (HO)-1 has low expression but gets upregulated in response to oxidants such as heme, H_2O_2 and TNF-α, whereas its activity decreases in the case of hyperglycemia in diabetic rats having increased superoxide anion production. Thus, HO-1 is one of the major defense against oxidative stress which becomes vulnerable and contributes to mitochondrial oxidative stress in diabetes mellitus [51].

Association of IR and CVD

Hyperinsulinemia is a predictor of coronary artery disease (CAD) and has been confirmed by patient studies performed in Finland and Quebec [52, 53]. Other studies have also shown a relationship between carotid wall atherosclerotic lesions, angina, and insulin levels/resistance [54].

IR leading to hyperinsulinemia causes hypertension. It has been observed that the hypertensive patients have higher fasting and postprandial insulin levels than normal subjects [55]. Also, the relationship between insulin and hypertension is mainly seen in first-degree hypertensive patients, which does not occur with secondary hypertension [56, 57]. Accordingly, IR and hyperinsulinemia are not

consequences of hypertension, instead, a genetic predisposition may contribute to both disorders. Activation of the sympathetic nervous system, renal sodium retention, altered transmembrane cation transport, growth-promoting effects of vascular smooth muscle cells, and vascular hyperreactivity are some of the mechanisms for developing hypertension in IR condition [58].

Microalbuminuria represents a significant risk factor for CVD in patients with (or without) clinical diabetes. Several studies have reported elevated systolic blood pressure in the development of microalbuminuria in type 2 diabetic patients. Thus understanding of the risks involved in the insulin-resistant patients becomes paramount as they are more prone toward elevated systolic blood pressures [59]. In patients both lean and obese hypertensive IR is also associated with enhanced salt sensitivity [60].

Obesity contributes significantly to impaired glucose tolerance, hyperinsulinemia, type 2 diabetes, dyslipidemia, and hypertension. All these factors play an important role in the pathophysiology of IR. Alteration in major metabolism of fat leads to obesity and IR-related complications such as atherosclerosis, hypertension and CVDs. IR thus is not simply a problem of deficient glucose uptake in response to insulin, but a multifaceted syndrome that significantly increases the risk for cardiovascular disease. The link between IR and the associated dyslipidemia, hypertension, hypercoagulability, and atherosclerosis are numerous and complex [61]. In Quebec study, 2000 middle aged men were monitored for 5 years. The study revealed that visceral fat, as compared to peripheral fat, is more resistant to the metabolic effects of insulin, more sensitive to lipolytic hormones and more prone to CVD [62]. Visceral obesity has been positively correlated with higher levels of plasminogen activator inhibitor-1 (PAI-1). It complexes with tissue-type plasminogen activator and eliminates its fibrinolytic activity [63]. Hence, CVD can be predicted by comparing low levels of plasminogen activator with PAI-1 levels. Type 2 diabetic patients have been observed to have higher levels of PAI-1 suggesting that hyperinsulinemia itself is a potent stimulator for PAI-1 production [64].

As stated above, patients with hypertension and IR are more prone to disturbances of the fibrinolytic system. Deficiency of clotting inhibitors such as endogenous antithrombotic factors (i.e., factors C and S and antithrombin III) has been associated with the insulin levels. Also, hyperfibrinogenemia is a powerful independent risk factor for CVD caused by elevated levels of fibrinogen, which have also been observed in the insulin-resistant state [65, 66].

Administration of inhibitors of Nitric oxide (NO) synthase abolishes peripheral vasodilatation in response to insulin, suggesting a crucial role for NO in the normal vasodilatory response to insulin. This response is lost in insulin-resistant/obese individuals suggesting resistance to the action of insulin to induce vascular NO production [66]. Further, abatement of insulin-mediated glucose uptake and decrease in insulin stimulated blood flow has been observed in insulin-resistant obese patients. In more specific vascular studies, it was observed that in insulin-resistant obese patients the ability of insulin to decrease aortic wave reflection was severely blunted, as determined from augmentation index. Also, this defect was a consequence of impaired insulin action, as it was not observed in the basal state suggesting that IR extends to large conduit vessels as well as to vessels regulating peripheral blood flow thereby increasing the risk toward cardiovascular diseases [60, 67, 68].

Inflammatory responses in obesity, IR and CVD

IR in hyperinsulinemia, hypertension, hyperlipidemia leads to type 2 DM, and an increased risk of atherosclerotic CVDs are the adverse effects of central obesity [69]. Apart from adults, obese children are also found to be on the alarming edge of IR, hypertension and abnormal lipid profiles [70]. Various bioactive compounds released by adipose tissue provoke IR, alterations in lipids, coagulation, fibrinolysis, inflammation that leads to endothelial dysfunction and atherosclerosis [71].

Adipose tissue is comprised of different cells such as adipose derived stem cells, adipocytes, endothelial cells and the immune cells (macrophages—M1 and M2). The M1 phenotype is involved in inflammatory processes and the M2 are associated with tissue remodeling. Excess of adipose tissue expansion and hypoxic conditions activate inflammatory responses that secrete various pro-inflammatory cytokines. Infiltrated pro-inflammatory leukocytes differentiate into M1 macrophages that clear the adipocytes that comprise large lipids and hence they resemble foam like cells (crown-like), structures characteristic of dysfunctional adipocytes [72]. Neuroimmune guidance cue netrin-1's expression is high in adipose tissue of obese human and mice but not in lean. Netrin-1 is responsible for retention of macrophages into adipose tissue via Unc5b receptor and hence leads to increased IR in obesity [73].

Infiltration of macrophages into adipose tissue leads to expansion of the tissue causing chronic low-grade inflammation. Phenotypic switching of macrophages is governed by cells of innate and adaptive immunity which further leads to inflammation. The phenotypic switch involves recruitment of B cells and T cells by changes in the phenotype of T cells [74]. With concomitant increase in the subcutaneous fat tissue, the fat gets deposited in the visceral fat which releases more pro-inflammatory cytokines. Elevated central obesity is associated with worsened cardiovascular risk profiles, IR, hyperlipidemia, increasing the

influx of FFA into cardiomyocytes. The common pathological pathways between obesity and CVDs are IR and low-grade inflammation. Presence of NEFA causes lipotoxicity and hence impairs endothelium-dependent vasodilation, increases oxidative stress and has cardio toxic effect [71]. Various NADPH oxidases (NOXs) present in macrophages are responsible for generation of ROS in adipose tissue which aids in cardiovascular diseases [75]. Increased expression levels of β_3-adrenoreceptors make visceral adipose tissue (VAT) more sensitive to catecholamine-induced lipolysis that makes it eventually less sensitive to α2 effects and to anti-lipolytic activity of insulin. Excess influx of FFA into myocytes hampers the oxidative capabilities of substrate switching, leading to the deposition of the FFAs, and hence causing lipotoxicity. As a result cardiac dysfunction is promoted by ROS generation and ceramide production that further impairs insulin signaling, decreases sarcoplasmic reticular Ca_2^+ stores, and causes mitochondrial dysfunction. Due to FFA deposition, heart relies more on it for energy supply. Myocardial IR hampers insulin signaling proteins [72]. FFAs induce inflammation through TLR4 and the liver secretory protein fetuin-A (FetA), acts as an adaptor protein [76].

There is a strong link between leptin and cardiovascular functions and its remodeling. Hyperleptinemia, central leptin resistance, and leptin deficiency are all associated with impaired post-receptor leptin signaling and contractile response. Alterations in the pathways regulated by leptin in cardiomyocytes are associated with the pathology of these cells in obesity. Negative inotropic and hypertrophic responses are found due to the alterations in JAK/STAT, MAPK, NO, and β-adrenergic pathways [77].

Interplay of fat cells macrophages, endothelial cells in IR and CVD

As explained in the earlier section, adipose tissue harbors two types of macrophages of which M1-macrophages is predominant in obesity, secreting TNF-α and IL-6 thereby enhancing inflammation [78]. Both macrophages and adipocytes are capable of accumulating lipids and secreting cytokines. Adipocyte hypertrophy during obesity leads to release of more FFAs which can bind to TLR-4 resulting in NF-κB activation leading to augmentation of TNF-α levels [79]. In turn, macrophage-derived TNF-α activates adipocytes, thereby further inducing lipolysis and enhancing the expression of various genes [intracellular adhesion molecule-1 (ICAM-1), IL-6, macrophage chemo attractant protein-1 (MCP-1)]. FFA and TNF-α in turn also lead to the activation of serine threonine kinases and promoted IR condition [80].

A key step in the initiation of CVD is the reduction of NO bioavailability [81, 82]. The bioavailability of NO is dependent on the balance between its production by eNOS and its inactivation by ROS. A feature action of insulin in the endothelial cells is the regulation of eNOS for nitric oxide production [83, 84]. Thus endothelial cells are crucial target during diabetes and whole body IR because of obesity and adipocyte inflammation which leads to a reduction in NO synthesis. Plasminogen activator inhibitor 1 (PAI-1) is a marker for risk of premature CVD [85, 86], and the link between elevated PAI-1 and IR has been studied extensively where endothelial cells respond to increased levels of insulin by synthesizing and secreting more PAI-1 [87]. Since there is a putative VLDL response element in the gene for PAI-1 in endothelial cells, VLDL (increased during diabetes) also increases PAI-1 synthesis and secretion [50]. The fat cell macrophages that are hyperactivated during obesity in addition to inflammation initiate the whole body IR. Hyperinsulinemia and IR consecutively affect normal functions of endothelial cells increasing the risks of CVD (Fig. 2).

Cross-talk of signaling pathways in development of IR in the heart

Discussion thus far makes a strong case that there is an association between IR and endothelial cell dysfunction with the cardiovascular diseases-including coronary artery disease, hypertension, heart failure, and stroke. Further in the regulation of various aspects of cardiovascular metabolism and function such as glucose and long-chain fatty acid (LCFA) metabolism, protein translation, and vascular tone, insulin plays a key role [88]. As the heart is an energy-consuming organ, it constantly requires supply of fuel and oxygen in order to maintain its intracellular ATP level. The heart gets the same from mitochondria and this ATP is essential for the uninterrupted myocardial contraction/relaxation cycle. Under physiological conditions, the heart produces ATP from the mitochondrial oxidation of different substrates, LCFAs (60–70 %) being predominant over glucose (20 %) and lactate (10 %). In pathological conditions such as starvation or chronic heart failure, ketone bodies become a major substrate and when glucose and insulin concentrations rise, glucose becomes the favored oxidized substrate of the heart [89].

Insulin signaling is a complex cascade wherein, the effector signaling proteins like IRS-(1/2)/PI3K/Akt have various downstream substrates, potentially activated by insulin thus, depicting varied biological roles of insulin.

Glucose uptake

Insulin favors the use of glucose in cardiomyocytes by activating cardiac 6-phosphofructo-2-kinase (PFK-2) isoform.

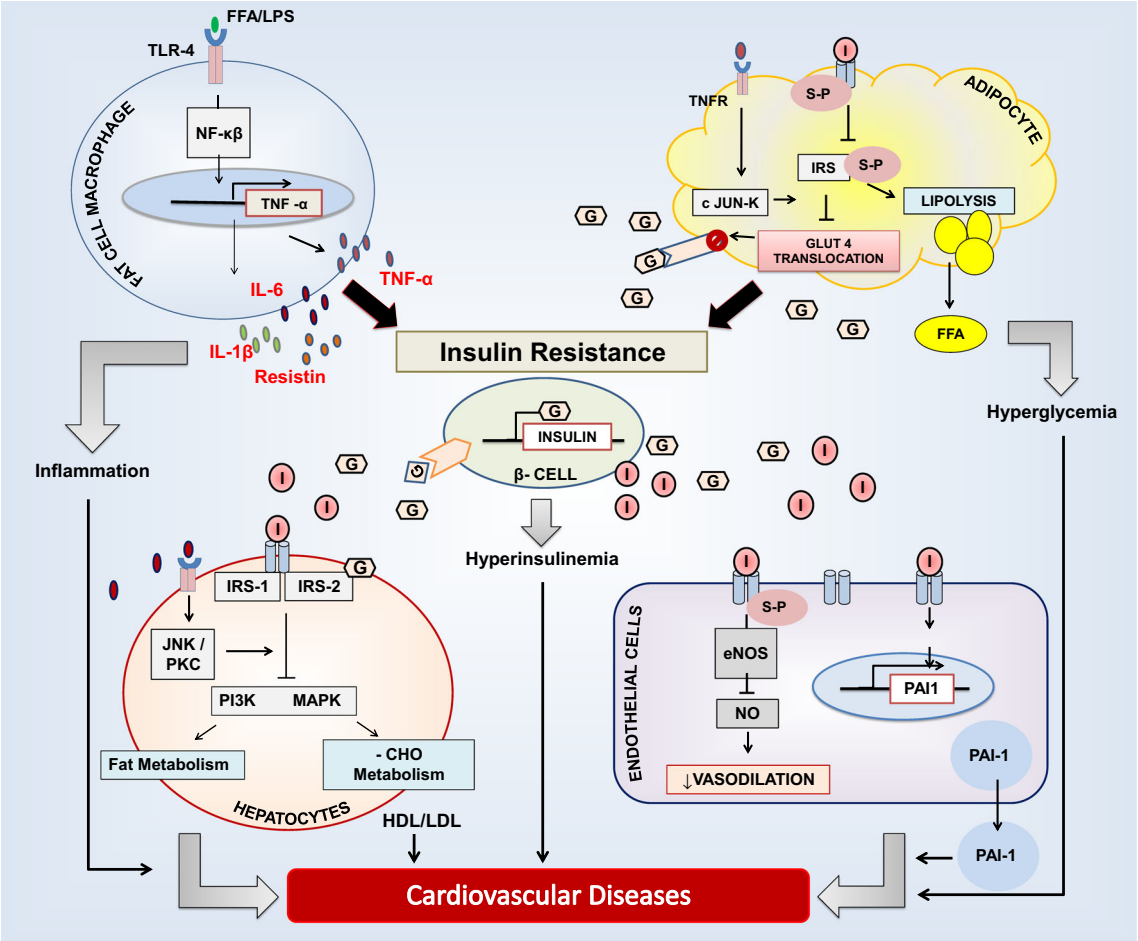

Fig. 2 Representation of interplay between fat cell macrophages, adipocytes, β-cells, hepatocytes and endothelial cells in insulin resistance (IR) and cardiovascular disease (CVD) complications. Excess of free fatty acids (FFA) and lipopolysaccharide (LPS) also activate TLR4 present on the fat cell macrophages (M1). This produces pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6 and resistin through the activation of NF-κ-B which hamper insulin signaling proteins causing IR. The secreted FFAs are responsible for the ectopic fat deposition in muscle myocytes and hepatocytes thus causing peripheral IR due to increased lipolysis and decreased Glut4 translocation further elevating FFA and blood glucose (G) level. This in turn triggers insulin (I) secretion by the β-cells and results in hyperinsulinemia. The later also results in elevated PAI-1 promoter activity in the endothelial cells which is a marker for CVD. IR also results in decreased NO synthesis in endothelial cells resulting in decreased vasodilation, thus increasing the CVD complications. Furthermore, insulin binds to its receptor on hepatocytes and activates substrate to inhibit phosphorylation of tyrosine kinase (e.g., PI3K) and MAPK activity and controls fat and carbohydrate (CHO) metabolism thus decreasing HDL/LDL and promotes CVD problems including atherosclerosis. Thus cumulatively, an interplay of fat cell macrophages, adipocytes, β-cells, hepatocytes and endothelial cells as well as insulin resistance leads to cardiovascular diseases

Simultaneously, LCFA uptake in cardiomyocytes occurs through insulin stimulated activation of PI3K and then translocation of the LCFA transporter FAT/CD36 to the plasma membrane [88].

Protein translation

Insulin regulates protein synthesis in cardiomyocytes through the regulation of PKB/Akt/TSC2/mTOR and their downstream targets 4E-BP1, p70S6K/S6 and eEF2K/eEF2. Apart from these, insulin by inhibiting GSK-3 activates eIF2B and stimulates the initiation of protein synthesis [90]. GSK-3 participates in the negative regulation of cardiac hypertrophy by phosphorylating and inactivating the nuclear factor of activated T (NFAT) cells responsible for the pro-hypertrophic gene expression [91].

Vasculature

Insulin functions as an important vasodilator by stimulating increased production of the potent vasodilator NO from vascular endothelium through the activation of eNOS by PI3K/PKB/Akt pathway [92]. PI3-kinase increases the trafficking and translocation of NO synthase and cation

pump units as well as glucose transporters, which mediates an increase in NO, Na^+ pump, K^+ channel, and calcium (Ca^{2+}) myofilament sensitivity [93]. These effects are blunted in IR states, predisposing to the development and the progression of cardiovascular diseases. Angiotensin II, a component of renin-angiotensin system (RAS) via stimulation of the angiotensin II, type I receptor (AT1R) activates JNK and MAP-kinase pathways, leading to increased serine phosphorylation of IRS-1 and 2 proteins ultimately inhibiting PI3K signaling and further causing deleterious effects [94].

Mitochondrial biogenesis in IR: a secondary cause of cardiovascular disorder

The myocardium maintains a high energy demand to keep the heart viable, and this energy is supplied by mitochondria. Cardiac muscle of IR individuals generally contains 30 % less mitochondria than that of insulin sensitive individuals [95]. High-fat diet tends to promote an increase in mitochondria in order to oxidize fat which is concomitant with the development of tissue-specific IR [96, 97]. Thus, increase in FFA, induced by high-fat diet can be correlated with mitochondrial biogenesis [98, 99]. Impaired mitochondrial oxidative phosphorylation (OXPHO) and mitochondrial biogenesis contributes to an inhibition of insulin metabolic signaling [100]. The mechanism by which mitochondrial dysfunction is directly involved, an impairment of IR signaling occurs by controlling the PGC-1 α, nuclear respiratory factors 1 (NRF1) and NRF2 genes [101], which therefore regulate mitochondrial ATP production [102–104]. It is also suggested that transcriptional activation of PGC1α promotes mitochondrial proliferation and its associated markers that are required for mitochondrial biogenesis in the myocardium [104, 105]. Studies on cardiac-specific deletion of NRF1 and ERRα suggest that PGC1α activates estrogen-related nuclear receptors-α and γ (ERRα and ERRγ) to induce genes participating in glucose and fatty acid uptakes. This results in upregulation of ATP transport via NRF1/2-mediated stimulation of mitochondrial transcription factor A (Tfam A) [106–108] and OXPHOS genes [109]. Thus, there is sufficient evidence that supports the role of mitochondrial biogenesis in CVD associated with IR.

IR related to CVDs and potential therapeutic approaches

Accumulation of fatty acid metabolites, DG and LCFA-CoA because of mitochondrial dysfunction by atypical PKCs activation results in IR [110, 111]. Mitochondrial function and insulin sensitivity can be improved by increased expression of UCP2/3 or decrease in ROS production by antioxidants [111]. Modulation of glucose/LCFA metabolism could be an approach for establishing a new equilibrium favoring glucose uptake and oxidation in opposition to LCFA oxidation. Adjustment of insulin signaling can also be done by using thiazolidinediones and metformin–insulin sensitizers, which are reported to reduce ROS production, increase expression of PGC-1α, and stimulate AMPK, thus improving mitochondrial function by reducing oxidative stress and stimulating mitochondrial biogenesis [111]. AMPK can also directly stimulate glucose uptake by phosphorylating and inactivating AS160 (converging point between insulin and AMPK-signaling pathways), enhancing PKB/Akt overactivation and decreasing serine phosphorylation of IRS-1 in cardiomyocytes [112]. Under ischemic conditions, the activated AMPK counteracts the PKB/Akt-mediated activation of p70S6K, phosphorylation of eEF2, and stimulation of protein synthesis in cardiomyocytes. In the cardiac system, expression of a kinase-dead phosphofructokinase 2 (PFK2) decreases glycolytic flux, induces hypertrophy and fibrosis, and reduces cardiomyocyte function thus explaining the importance of PFK2 in the regulation of cardiac function.

Under normal conditions, insulin stimulates the production of NO from endothelium, leading to vasodilation, increased blood flow, augments glucose disposal in skeletal muscle. Under IR condition, hyperinsulinemia overdrives unaffected MAPK-dependent pathways leading to secretion of the vasoconstrictor endothelin-1 (ET-1) from vascular endothelium. This imbalance between vasoconstrictor and vasodilator actions of insulin under IR condition is an important factor in the vascular pathophysiology of IR and endothelial dysfunction. Pharmacological blockage of ET-1 receptors (ET-A isoform) improves endothelial function in cardiovascular disorders [113]. Intraarterial vitamin C improves endothelial-dependent vasodilation in type 2 diabetes mellitus associated CVD [114]. Adiponectin directly stimulates the production of NO from vascular endothelium using a PI3-kinase-dependent signaling mechanism similar to that of insulin, thus opposing atherogenesis and improve endothelial function [115]. The development of IR along with cardiometabolic syndrome is associated with increased tissue renin–angiotensin system activity [111].

Angiotensin II via its type I receptors stimulates the production of ROS via NADPH oxidase, increases expression of ICAM-1 and increases ET-1 release from endothelium [111]. Pharmacological inhibitors such as angiotensin-converting enzyme (ACE) inhibitors reduce circulating angiotensin II levels, and angiotensin receptor blockers (ARBs) block the actions of angiotensin II ultimately helping in lowering of blood pressure, improving endothelial function and reduce circulating markers of

inflammation, augmentation of insulin stimulated glucose uptake. ACE inhibitors (ramipril) and ARBs (losartan) support the existence of reciprocal relationships between endothelial dysfunction and insulin. Tempol, a superoxide scavenger, is able to ameliorate cardiac and vascular dysfunction, normalize angiotensin II–induced IR [116]. Fibrates are synthetic PPAR-α ligands that improve the circulating lipoprotein profile, resulting in improved endothelial function, reduced vascular inflammation, and reduction in cardiovascular events through increased adiponectin levels [117].

Conclusions

The complex interplay of IR and CVD has been studied extensively to elucidate the mechanistic pathway underlying the pathogenesis of the disease. The major causes of IR are hyperglycemia, oxidative stress and dyslipidemia which present as risk factors for CVD. The hampered insulin signaling abrogates insulin stimulated glucose uptake, endothelial functions, vasodilation and blood flow which in turn compromise functioning of cardiomyocytes and leads to hypertrophy, fibrosis and atherosclerosis. During severity of the disease anti-oxidants, anti-inflammatory and insulin sensitizers also enhance activities of various molecules such as NO, PI3K, Akt and GLUT4 receptors. Nonetheless, drugs regulating type 2 DM and hyperlipidemia have side effects associated with CVD and associated complications suggesting potential dual links between IR, type 2 DM and CVD at the molecular level. Thus treatment strategies for CVD would depend on the metabolic status of the individual. Given the serious consequences of the global CVD epidemic, understanding of the mechanisms that link IR with the development of CVD and comorbidities should be considered as a high priority in further research.

Acknowledgments Prof. Sarita Gupta was a visiting scientist in Institute of Cardiovascular Sciences. Nathalia Bernardes and Danielle da Silva Dias were exchange students, under the Canada-Brazil Training program. Dr. Pawan Singal is the holder of the Dr. Naranjan S. Dhalla Chair in Cardiovascular Research supported by St. Boniface Hospital and Research Foundation.

Compliance with ethical standards

Conflict of interest None.

References

1. Ceriello A (2005) Postprandial hyperglycemia and diabetes complications is it time to treat? Diabetes 54(1):1–7
2. Kannel WB, Hjortland M, Castelli WP (1974) Role of diabetes in congestive heart failure: the Framingham study. Am J Cardiol 34(1):29–34
3. Garcia M, McNamara P, Gordon T, Kannel W (1972) Cardiovascular complications in diabetics. Adv Metab Disord 2(Suppl 2):493–499
4. Fowler MJ (2008) Microvascular and macrovascular complications of diabetes. Clin Diabetes 26(2):77–82
5. Ceriello A (2003) New insights on oxidative stress and diabetic complications may lead to a "causal" antioxidant therapy. Diabetes Care 26(5):1589–1596
6. Farahmand F, Lou H, Singal PK (2003) Oxidative stress in cardiovascular complications of diabetes. In: Pierce GN, Nagano M, Zahradka P, Dhalla NS (eds) Atherosclerosis, hypertension and diabetes. Kluwer Academic Publications, Boston, pp 427–437
7. Gyurko R, Siqueira CC, Caldon N, Gao L, Kantarci A, Van Dyke TE (2006) Chronic hyperglycemia predisposes to exaggerated inflammatory response and leukocyte dysfunction in Akita mice. J Immunol 177(10):7250–7256
8. Lum H, Roebuck KA (2001) Oxidant stress and endothelial cell dysfunction. Am J Physiol Cell Physiol 280(4):C719–C741
9. Versari D, Daghini E, Virdis A, Ghiadoni L, Taddei S (2009) Endothelial dysfunction as a target for prevention of cardiovascular disease. Diabetes Care 32(suppl 2):S314–S321
10. Schram MT, Chaturvedi N, Schalkwijk C, Giorgino F, Ebeling P, Fuller JH, Stehouwer CD (2003) Vascular risk factors and markers of endothelial function as determinants of inflammatory markers in type 1 diabetes the EURODIAB Prospective complications study. Diabetes Care 26(7):2165–2173
11. El-Osta A, Brasacchio D, Yao D, Pocai A, Jones PL, Roeder RG, Cooper ME, Brownlee M (2008) Transient high glucose causes persistent epigenetic changes and altered gene expression during subsequent normoglycemia. J Exp Med 205(10):2409–2417
12. Hink U, Li H, Mollnau H, Oelze M, Matheis E, Hartmann M, Skatchkov M, Thaiss F, Stahl RA, Warnholtz A (2001) Mechanisms underlying endothelial dysfunction in diabetes mellitus. Circ Res 88(2):e14–e22
13. Watts G, Playford D (1998) Dyslipoproteinaemia and hyperoxidative stress in the pathogenesis of endothelial dysfunction in non-insulin dependent diabetes mellitus: an hypothesis. Atherosclerosis 141(1):17–30
14. Renard CB, Kramer F, Johansson F, Lamharzi N, Tannock LR, von Herrath MG, Chait A, Bornfeldt KE (2004) Diabetes and diabetes-associated lipid abnormalities have distinct effects on initiation and progression of atherosclerotic lesions. J Clin Investig 114(5):659
15. Poirier P, Giles TD, Bray GA, Hong Y, Stern JS, Pi-Sunyer FX, Eckel RH (2006) Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss an update of the 1997 American Heart Association Scientific statement on obesity and heart disease from the obesity committee of the council on nutrition, physical activity, and metabolism. Circulation 113(6):898–918
16. Duncan BB, Schmidt MI, Pankow JS, Ballantyne CM, Couper D, Vigo A, Hoogeveen R, Folsom AR, Heiss G (2003) Low-grade systemic inflammation and the development of type 2 diabetes the atherosclerosis risk in communities study. Diabetes 52(7):1799–1805
17. Turner R, Holman R, Matthews D, Bassett P, Coster R, Stratton I, Cull C, Peto R, Frighi V, Kennedy I (1993) Hypertension in diabetes study (Hds). 1. Prevalence of hypertension in newly presenting type-2 diabetic-patients and the association with risk-factors for cardiovascular and diabetic complications. J Hypertens 11(3):309–317
18. ADA (1993) Treatment of hypertension in diabetes. Diabetes Care 16:1394–1401
19. Goto A, Arah OA, Goto M, Terauchi Y, Noda M (2013) Severe hypoglycaemia and cardiovascular disease: systematic review and meta-analysis with bias analysis. BMJ 347:F4533

20. Sommerfield AJ, Wilkinson IB, Webb DJ, Frier BM (2007) Vessel wall stiffness in type 1 diabetes and the central hemodynamic effects of acute hypoglycemia. Am J Physiol Endocrinol Metab 293(5):E1274–E1279
21. Frier BM, Schernthaner G, Heller SR (2011) Hypoglycemia and cardiovascular risks. Diabetes Care 34(Supplement 2):S132–S137
22. Desouza CV, Bolli GB, Fonseca V (2010) Hypoglycemia, diabetes, and cardiovascular events. Diabetes Care 33(6):1389–1394
23. Spallone V, Ziegler D, Freeman R, Bernardi L, Frontoni S, Pop-Busui R, Stevens M, Kempler P, Hilsted J, Tesfaye S (2011) Cardiovascular autonomic neuropathy in diabetes: clinical impact, assessment, diagnosis, and management. Diabetes Metab Res Rev 27(7):639–653
24. Witte D, Tesfaye S, Chaturvedi N, Eaton S, Kempler P, Fuller J, Group EPCS (2005) Risk factors for cardiac autonomic neuropathy in type 1 diabetes mellitus. Diabetologia 48(1):164–171
25. Astrup AS, Tarnow L, Rossing P, Hansen BV, Hilsted J, Parving H-H (2006) Cardiac autonomic neuropathy predicts cardiovascular morbidity and mortality in type 1 diabetic patients with diabetic nephropathy. Diabetes Care 29(2):334–339
26. DeFronzo RA, Tripathy D (2009) Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. Diabetes Care 32(suppl 2):S157–S163
27. Groop L, Bonadonna R, Del Prato S, Ratheiser K, Zyck K, DeFronzo R (1989) Effect of insulin on oxidative and non-oxidative pathways of glucose and FFA metabolism in NIDDM. Evidence for multiple sites of insulin resistance. J Clin Invest 84:205–213
28. Morino K, Petersen KF, Shulman GI (2006) Molecular mechanisms of insulin resistance in humans and their potential links with mitochondrial dysfunction. Diabetes 55(Supplement 2):S9–S15
29. Langin D, Dicker A, Tavernier G, Hoffstedt J, Mairal A, Rydén M, Arner E, Sicard A, Jenkins CM, Viguerie N (2005) Adipocyte lipases and defect of lipolysis in human obesity. Diabetes 54(11):3190–3197
30. Kern PA (1997) Potential role of TNFα and lipoprotein lipase as candidate genes for obesity. J Nutr 127(9):1917S–1922S
31. Bugianesi E, McCullough AJ, Marchesini G (2005) Insulin resistance: a metabolic pathway to chronic liver disease. Hepatology 42(5):987–1000
32. Dröge W (2002) Free radicals in the physiological control of cell function. Physiol Rev 82(1):47–95
33. Brownlee M (2001) Biochemistry and molecular cell biology of diabetic complications. Nature 414(6865):813–820
34. Greene DA, Stevens MJ, Obrosova I, Feldman EL (1999) Glucose-induced oxidative stress and programmed cell death in diabetic neuropathy. Eur J Pharmacol 375(1):217–223
35. Wolff SP, Dean R (1987) Glucose autoxidation and protein modification. The potential role of 'autoxidative glycosylation' in diabetes. Biochem J 245:243–250
36. Yan S, Stern D, Schmidt A (1997) What's the RAGE? The receptor for advanced glycation end products (RAGE) and the dark side of glucose. Eur J Clin Invest 27(3):179–181
37. Stojiljkovic MP, Lopes HF, Zhang D, Morrow JD, Goodfriend TL, Egan BM (2002) Increasing plasma fatty acids elevates F2-isoprostanes in humans: implications for the cardiovascular risk factor cluster. J Hypertens 20(6):1215–1221
38. S-i Yamagishi, Edelstein D, X-l Du, Kaneda Y, Guzmán M, Brownlee M (2001) Leptin induces mitochondrial superoxide production and monocyte chemoattractant protein-1 expression in aortic endothelial cells by increasing fatty acid oxidation via protein kinase A. J Biol Chem 276(27):25096–25100
39. Paz K, Hemi R, LeRoith D, Karasik A, Elhanany E, Kanety H, Zick Y (1997) A Molecular Basis for Insulin Resistance elevated serine/threonine phosphorylation of irs-1 and irs-2 inhibits their binding to the juxtamembrane region of the insulin receptor and impairs their ability to undergo insulin-induced tyrosine phosphorylation. J Biol Chem 272(47):29911–29918
40. Potashnik R, Bloch-Damti A, Bashan N, Rudich A (2003) IRS1 degradation and increased serine phosphorylation cannot predict the degree of metabolic insulin resistance induced by oxidative stress. Diabetologia 46(5):639–648
41. Ogihara T, Asano T, Katagiri H, Sakoda H, Anai M, Shojima N, Ono H, Fujishiro M, Kushiyama A, Fukushima Y (2004) Oxidative stress induces insulin resistance by activating the nuclear factor-κB pathway and disrupting normal subcellular distribution of phosphatidylinositol 3-kinase. Diabetologia 47(5):794–805
42. Khamaisi M, Potashnik R, Tirosh A, Demshchak E, Rudich A, Trischler H, Wessel K, Bashan N (1997) Lipoic acid reduces glycemia and increases muscle GLUT4 content in streptozotocin-diabetic rats. Metabolism 46(7):763–768
43. Pessler D, Rudich A, Bashan N (2001) Oxidative stress impairs nuclear proteins binding to the insulin responsive element in the GLUT4 promoter. Diabetologia 44(12):2156–2164
44. Castelló A, Rodríguez-Manzaneque JC, Camps M, Perez-Castillo A, Testar X, Palacin M, Santos A, Zorzano A (1994) Perinatal hypothyroidism impairs the normal transition of GLUT4 and GLUT1 glucose transporters from fetal to neonatal levels in heart and brown adipose tissue. Evidence for tissue-specific regulation of GLUT4 expression by thyroid hormone. J Biol Chem 269(8):5905–5912
45. Randle PJ, Kerbey AL, Espinal J (1988) Mechanisms decreasing glucose oxidation in diabetes and starvation: role of lipid fuels and hormones. Diabetes Metab Rev 4(7):623–638
46. Shulman GI (2000) Cellular mechanisms of insulin resistance. J Clin Investig 106(2):171
47. Stalder M, Pometta D, Suenram A (1981) Relationship between plasma insulin levels and high density lipoprotein cholesterol levels in healthy men. Diabetologia 21(6):544–548
48. Sadur UN, Yost TJ, Eckel RH (1984) Insulin responsiveness of adipose tissue lipoprotein lipase is delayed but preserved in obesity*. J Clin Endocrinol Metab 59(6):1176–1182
49. Golay A, Zech L, Shi M-Z, Chiou Y-A, Reaven G, Chen Y-D (1987) High density lipoprotein (HDL) metabolism in noninsulin-dependent diabetes mellitus: measurement of HDL turnover using tritiated HDL*. J Clin Endocrinol Metab 65(3):512–518
50. Eriksson P, Nilsson L, Karpe F, Hamsten A (1998) Very-low-density lipoprotein response element in the promoter region of the human plasminogen activator inhibitor-1 gene implicated in the impaired fibrinolysis of hypertriglyceridemia. Arterioscler Thromb Vasc Biol 18(1):20–26
51. Nishikawa T, Edelstein D, Du XL, S-i Yamagishi, Matsumura T, Kaneda Y, Yorek MA, Beebe D, Oates PJ, Hammes H-P (2000) Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. Nature 404(6779):787–790
52. Pyörälä K (1979) Relationship of glucose tolerance and plasma insulin to the incidence of coronary heart disease: results from two population studies in Finland. Diabetes Care 2(2):131–141
53. Després J-P, Lamarche B, Mauriège P, Cantin B, Dagenais GR, Moorjani S, Lupien P-J (1996) Hyperinsulinemia as an independent risk factor for ischemic heart disease. N Engl J Med 334(15):952–958
54. Suzuki M, Shinozaki K, Kanazawa A, Hara Y, Hattori Y, Tsushima M, Harano Y (1996) Insulin resistance as an independent risk factor for carotid wall thickening. Hypertension 28(4):593–598
55. Shen D-C, Shieh S-M, Fuh M-T, Wu D-A, Chen Y-D, Reaven G (1988) Resistance to insulin-stimulated-glucose uptake in

patients with hypertension*. J Clin Endocrinol Metab 66(3):580–583
56. Reaven GM, Chang H (1991) Relationship between blood pressure, plasma insulin ana triglyceride concentration, and insulin action in spontaneous hypertensive and Wistar-Kyoto rats. Am J Hypertens 4(1 Pt 1):34–38
57. Sechi LA, Melis A, Tedde R (1992) Insulin hypersecretion: a distinctive feature between essential and secondary hypertension. Metabolism 41(11):1261–1266
58. Reaven G (1996) Hypertension and associated metabolic abnormalities—the role of insulin resistance and the sympathoadrenal system. N Engl J Med 334:374–381
59. Mitchell TH, Nolan B, Henry M, Cronin C, Baker H, Greely G (1997) Microalbuminuria in patients with non-insulin-dependent diabetes mellitus relates to nocturnal systolic blood pressure. Am J Med 102(6):531–535
60. Laine H, Yki-Jarvinen H, Kirvela O, Tolvanen T, Raitakari M, Solin O, Haaparanta M, Knuuti J, Nuutila P (1998) Insulin resistance of glucose uptake in skeletal muscle cannot be ameliorated by enhancing endothelium-dependent blood flow in obesity. J Clin Investig 101(5):1156
61. Ginsberg HN (2000) Insulin resistance and cardiovascular disease. J Clin Investig 106(4):453
62. Seidell JC, Pérusse L, Després J-P, Bouchard C (2001) Waist and hip circumferences have independent and opposite effects on cardiovascular disease risk factors: the Quebec Family Study. Am J Clin Nutr 74(3):315–321
63. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH (1997) Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. N Engl J Med 336(14): 973–979
64. Brown NJ, Kim K-S, Chen Y-Q, Blevins LS, Nadeau JH, Meranze SG, Vaughan DE (2000) Synergistic effect of adrenal steroids and angiotensin II on plasminogen activator inhibitor-1 production 1. J Clin Endocrinol Metab 85(1):336–344
65. Sowers JR, Sowers PS, Peuler JD (1994) Role of insulin resistance and hyperinsulinemia in development of hypertension and atherosclerosis. J Lab Clin Med 123(5):647–652
66. Chen Y-Q, Su M, Walia RR, Hao Q, Covington JW, Vaughan DE (1998) Sp1 sites mediate activation of the plasminogen activator inhibitor-1 promoter by glucose in vascular smooth muscle cells. J Biol Chem 273(14):8225–8231
67. Westerbacka J, Vehkavaara S, Bergholm R, Wilkinson I, Cockcroft J, Yki-Järvinen H (1999) Marked resistance of the ability of insulin to decrease arterial stiffness characterizes human obesity. Diabetes 48(4):821–827
68. Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G, Baron AD (1996) Obesity/insulin resistance is associated with endothelial dysfunction. Implications for the syndrome of insulin resistance. J Clin Investig 97(11):2601
69. Després J-P (2006) Abdominal obesity: the most prevalent cause of the metabolic syndrome and related cardiometabolic risk. Eur Heart J Suppl 8(suppl B):B4–B12
70. Steinberger J, Daniels SR (2003) Obesity, insulin resistance, diabetes, and cardiovascular risk in children an American Heart Association scientific statement from the atherosclerosis, hypertension, and obesity in the Young Committee (Council on Cardiovascular Disease in the Young) and the Diabetes Committee (Council on Nutrition, Physical Activity, and Metabolism). Circulation 107(10):1448–1453
71. Van Gaal LF, Mertens IL, Christophe E (2006) Mechanisms linking obesity with cardiovascular disease. Nature 444(7121):875–880
72. Turer AT, Hill JA, Elmquist JK, Scherer PE (2012) Adipose tissue biology and cardiomyopathy translational implications. Circ Res 111(12):1565–1577
73. Ramkhelawon B, Hennessy EJ, Ménager M, Ray TD, Sheedy FJ, Hutchison S, Wanschel A, Oldebeken S, Geoffrion M, Spiro W (2014) Netrin-1 promotes adipose tissue macrophage retention and insulin resistance in obesity. Nat Med 20(4):377–384
74. Sell H, Habich C, Eckel J (2012) Adaptive immunity in obesity and insulin resistance. Nat Rev Endocrinol 8(12):709–716
75. De Marchi E, Faldassari B, Bononi A, Wieckowski M, Pinton P (2013) Oxidative stress in cardiovascular diseases and obesity: role of p66Shc and protein kinase C. Oxid Med Cell Longev 2013:564961. doi:10.1155/2013/564961
76. Borén J, Taskinen MR, Olofsson SO, Levin M (2013) Ectopic lipid storage and insulin resistance: a harmful relationship. J Intern Med 274(1):25–40
77. Yang R, Barouch LA (2007) Leptin signaling and obesity cardiovascular consequences. Circ Res 101(6):545–559
78. Lumeng CN, Bodzin JL, Saltiel AR (2007) Obesity induces a phenotypic switch in adipose tissue macrophage polarization. J Clin Investig 117(1):175
79. Ruan H, Hacohen N, Golub TR, Van Parijs L, Lodish HF (2002) Tumor necrosis factor-α suppresses adipocyte-specific genes and activates expression of preadipocyte genes in 3T3-L1 adipocytes nuclear factor-κB activation by TNF-α is obligatory. Diabetes 51(5):1319–1336
80. Permana PA, Menge C, Reaven PD (2006) Macrophage-secreted factors induce adipocyte inflammation and insulin resistance. Biochem Biophys Res Commun 341(2):507–514
81. Schächinger V, Britten MB, Zeiher AM (2000) Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. Circulation 101(16):1899–1906
82. Bugiardini R, Manfrini O, Pizzi C, Fontana F, Morgagni G (2004) Endothelial function predicts future development of coronary artery disease a study of women with chest pain and normal coronary angiograms. Circulation 109(21):2518–2523
83. Han S, Liang C-P, DeVries-Seimon T, Ranalletta M, Welch CL, Collins-Fletcher K, Accili D, Tabas I, Tall AR (2006) Macrophage insulin receptor deficiency increases ER stress-induced apoptosis and necrotic core formation in advanced atherosclerotic lesions. Cell Metab 3(4):257–266
84. Kuboki K, Jiang ZY, Takahara N, Ha SW, Igarashi M, Yamauchi T, Feener EP, Herbert TP, Rhodes CJ, King GL (2000) Regulation of endothelial constitutive nitric oxide synthase gene expression in endothelial cells and in vivo a specific vascular action of insulin. Circulation 101(6):676–681
85. Hamsten A, Wiman B, de Faire U, Blombäck M (1985) Increased plasma levels of a rapid inhibitor of tissue plasminogen activator in young survivors of myocardial infarction. N Engl J Med 313(25):1557–1563
86. Wiman B, Andersson T, Hallqvist J, Reuterwall C, Ahlbom A (2000) Plasma levels of tissue plasminogen activator/plasminogen activator inhibitor-1 complex and von Willebrand factor are significant risk markers for recurrent myocardial infarction in the Stockholm Heart Epidemiology Program (SHEEP) study. Arterioscler Thromb Vasc Biol 20(8):2019–2023
87. Calles-Escandon J, Mirza SA, Sobel BE, Schneider DJ (1998) Induction of hyperinsulinemia combined with hyperglycemia and hypertriglyceridemia increases plasminogen activator inhibitor 1 in blood in normal human subjects. Diabetes 47(2):290–293
88. Bertrand L, Horman S, Beauloye C, Vanoverschelde J-L (2008) Insulin signalling in the heart. Cardiovasc Res 79(2):238–248
89. Randle P, Garland P, Hales C, Newsholme E (1963) The glucose fatty-acid cycle its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet 281(7285):785–789
90. Proud C (2007) Signalling to translation: how signal transduction pathways control the protein synthetic machinery. Biochem J 403:217–234

91. Heineke J, Molkentin JD (2006) Regulation of cardiac hypertrophy by intracellular signalling pathways. Nat Rev Mol Cell Biol 7(8):589–600
92. Dimmeler S, Fleming I, Fisslthaler B, Hermann C, Busse R, Zeiher AM (1999) Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. Nature 399(6736):601–605
93. McFarlane SI, Banerji M, Sowers JR (2001) Insulin resistance and cardiovascular disease. J Clin Endocrinol Metab 86(2): 713–718
94. Mehta PK, Griendling KK (2007) Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system. Am J Physiol Cell Physiol 292(1):C82–C97
95. Lowell BB, Shulman GI (2005) Mitochondrial dysfunction and type 2 diabetes. Science 307(5708):384–387
96. Kim YJ, Park T (2008) Genes are differentially expressed in the epididymal fat of rats rendered obese by a high-fat diet. Nutr Res 28(6):414–422
97. Dong F, Li Q, Sreejayan N, Nunn JM, Ren J (2007) Metallothionein prevents high-fat diet-induced cardiac contractile dysfunction role of peroxisome proliferator-activated receptor γ coactivator 1α and mitochondrial biogenesis. Diabetes 56(9):2201–2212
98. Garcia-Roves P, Huss JM, Han D-H, Hancock CR, Iglesias-Gutierrez E, Chen M, Holloszy JO (2007) Raising plasma fatty acid concentration induces increased biogenesis of mitochondria in skeletal muscle. Proc Natl Acad Sci 104(25): 10709–10713
99. Hancock CR, Han D-H, Chen M, Terada S, Yasuda T, Wright DC, Holloszy JO (2008) High-fat diets cause insulin resistance despite an increase in muscle mitochondria. Proc Natl Acad Sci 105(22):7815–7820
100. Petersen KF, Dufour S, Befroy D, Garcia R, Shulman GI (2004) Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. N Engl J Med 350(7):664–671
101. Huo L, Scarpulla RC (2001) Mitochondrial DNA instability and peri-implantation lethality associated with targeted disruption of nuclear respiratory factor 1 in mice. Mol Cell Biol 21(2): 644–654
102. Patti ME, Butte AJ, Crunkhorn S, Cusi K, Berria R, Kashyap S, Miyazaki Y, Kohane I, Costello M, Saccone R (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 100(14):8466–8471
103. Mootha VK, Lindgren CM, Eriksson K-F, Subramanian A, Sihag S, Lehar J, Puigserver P, Carlsson E, Ridderstråle M, Laurila E (2003) PGC-1α-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nat Genet 34(3):267–273
104. Russell LK, Mansfield CM, Lehman JJ, Kovacs A, Courtois M, Saffitz JE, Medeiros DM, Valencik ML, McDonald JA, Kelly DP (2004) Cardiac-specific induction of the transcriptional coactivator peroxisome proliferator-activated receptor γ coactivator-1α promotes mitochondrial biogenesis and reversible cardiomyopathy in a developmental stage-dependent manner. Circ Res 94(4):525–533
105. Lehman JJ, Barger PM, Kovacs A, Saffitz JE, Medeiros DM, Kelly DP (2000) Peroxisome proliferator–activated receptor γ coactivator-1 promotes cardiac mitochondrial biogenesis. J Clin Investig 106(7):847
106. Huss JM, Kelly DP (2004) Nuclear receptor signaling and cardiac energetics. Circ Res 95(6):568–578
107. Kelly DP, Scarpulla RC (2004) Transcriptional regulatory circuits controlling mitochondrial biogenesis and function. Genes Dev 18(4):357–368
108. Garnier A, Fortin D, Delomenie C, Momken I, Veksler V, Ventura-Clapier R (2003) Depressed mitochondrial transcription factors and oxidative capacity in rat failing cardiac and skeletal muscles. J Physiol 551(2):491–501
109. Ritz P, Berrut G (2005) Mitochondrial function, energy expenditure, aging and insulin resistance. Diabetes Metab 31:5S67–65S73
110. Savage DB, Petersen KF, Shulman GI (2005) Mechanisms of insulin resistance in humans and possible links with inflammation. Hypertension 45(5):828–833
111. J-a Kim, Wei Y, Sowers JR (2008) Role of mitochondrial dysfunction in insulin resistance. Circ Res 102(4):401–414
112. Zick Y (2005) Ser/Thr phosphorylation of IRS proteins: a molecular basis for insulin resistance. Sci STKE 2005(268):pe4. doi:10.1126/stke.2682005pe4
113. Mather KJ, Lteif A, Steinberg HO, Baron AD (2004) Interactions between endothelin and nitric oxide in the regulation of vascular tone in obesity and diabetes. Diabetes 53(8):2060–2066
114. Ting HH, Timimi FK, Boles KS, Creager SJ, Ganz P, Creager MA (1996) Vitamin C improves endothelium-dependent vasodilation in patients with non-insulin-dependent diabetes mellitus. J Clin Investig 97(1):22
115. Chen H, Montagnani M, Funahashi T, Shimomura I, Quon MJ (2003) Adiponectin stimulates production of nitric oxide in vascular endothelial cells. J Biol Chem 278(45):45021–45026
116. Whaley-Connell A, Govindarajan G, Habibi J, Hayden MR, Cooper SA, Wei Y, Ma L, Qazi M, Link D, Karuparthi PR (2007) Angiotensin II-mediated oxidative stress promotes myocardial tissue remodeling in the transgenic (mRen2) 27 Ren2 rat. Am J Physiol Endocrinol Metab 293(1):E355–E363
117. Koh KK, Quon MJ, Han SH, Chung W-J, Ahn JY, Seo Y-H, Choi IS, Shin EK (2005) Additive beneficial effects of fenofibrate combined with atorvastatin in the treatment of combined hyperlipidemia. J Am Coll Cardiol 45(10):1649–1653