

# Biochemical Basis of the Antidiabetic Activity of Oleanolic Acid and Related Pentacyclic Triterpenes

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Oleanolic acid (OA), a natural component of many plant food and medicinal herbs, is endowed with a wide range of pharmacological properties whose therapeutic potential has only partly been exploited until now. Throughout complex and multifactorial mechanisms, OA exerts beneficial effects against diabetes and metabolic syndrome. It improves insulin response, preserves functionality and survival of  $\beta$ -cells, and protects against diabetes complications. OA may directly modulate enzymes connected to insulin biosynthesis, secretion, and signaling. However, its major contributions appear to be derived from the interaction with important transduction pathways, and many of its effects are consistently related to activation of the transcription factor Nrf2. Doing that, OA induces the expression of antioxidant enzymes and phase II response genes, blocks NF- $\kappa$ B, and represses the polyol pathway, AGEs production, and hyperlipidemia. The management of type 2 diabetes requires an integrated approach, which includes the early intervention to prevent or delay the disease progression, and the use of therapies to control glycemia and lipidemia in its late stages. In this sense, the use of functional foods or drugs containing OA is, undoubtedly, an interesting path.

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**T**ype 2 diabetes affects 220 million people worldwide. This number will be doubled by 2030 without urgent action. Diabetes prevalence has burst by the aging of population, socioeconomic disadvantages, and lifestyles that trend toward physical inactivity and overweight/obesity (1). Today, it is clear that insulin resistance plays an early role in diabetes pathogenesis and that failure in insulin secretion by pancreatic  $\beta$ -cells is instrumental in the progression to hyperglycemia.

Diabetes management requires an integrated approach that includes the early intervention to prevent or delay its appearance and the use of combined therapies to control glycemia and lipidemia in its late stages. Although many drugs with different modes of action are available, novel natural antidiabetic agents with insulin-sensitizing effects and preventive actions are highly desirable. The target is not only the reduction of hyperglycemia but also to address the metabolic syndrome as a whole.

Different natural bioactive compounds have antidiabetic potential. Among them are triterpenoids, plant secondary metabolites biosynthesized by the acetate/mevalonate pathway and (3S)-2,3-oxidosqualene cyclization (2). Oleanolic acid (OA) (3 $\beta$ -hydroxy-olean-12-en-28-oic acid) (Fig. 1) is

widely distributed in the plant kingdom as free acid or as aglycone of triterpenoid saponins. More than 120 plant species have been described by their relevant OA contents (3), but few of them are socioeconomically important crops as is olive (*Olea europaea* L.). OA is a component of the cuticle waxes that cover fruit and leaf epidermis. It is especially abundant in the olive leaf, where it represents up to 3.5% of the dry weight (4).

OA and related triterpenes possess interesting pharmacological properties, including the antioxidant, microbicide, antidiabetic, anti-inflammatory, hypolipidemic, and antiatherosclerotic actions (5–7). They interfere in the development of different types of cancer (7) and neurodegenerative disorders (8). OA is therapeutically effective without apparent side effects (9–11). The aim of this review is to summarize the most significant knowledge existing to date on the molecular basis of the OA antidiabetic activity.

## REDUCTION OF POSTPRANDIAL HYPERGLYCEMIA

Reducing postprandial hyperglycemia in diabetic people prevents glucose absorption after food intake. Carbohydrate digestion is facilitated by enteric enzymes, such as  $\alpha$ -glucosidase and  $\alpha$ -amylase, in the brush border of the small intestine cells. Their inhibition permits a better control of postprandial hyperglycemia and originates, at long term, a modest reduction of glycosylated proteins.

OA inhibits  $\alpha$ -glucosidase in vitro in an uncompetitive and dose-dependent fashion (half-maximal inhibitory concentration [IC<sub>50</sub>] 10–15  $\mu$ mol/L) (12–14). OA also inhibits the pancreatic and salivary  $\alpha$ -amylase activity (IC<sub>50</sub> 0.1 mg/mL), producing a hypoglycemic effect in prediabetic individuals (patients having impaired fasting glucose) fed cooked rice. At a dose of 1 mg/kg, OA reduced blood glucose by 23% 30 min after the meal (15). A similar hypoglycemic effect was observed in diabetic *GK/Jcl* rats fed starch (15). Ursolic acid (UA) and lupeol also block  $\alpha$ -amylase, and therefore it has been suggested that inhibition of this enzyme is a feature of the triterpenoid structure (16).

## IMPROVEMENT OF PANCREATIC $\beta$ -CELL FUNCTION AND INTEGRITY

In type 2 diabetes, pancreatic  $\beta$ -cells fail to release insulin enough to compensate for hyperglycemia. This deficit involves morphological and functional  $\beta$ -cell alterations. Accumulated data indicate that OA increases biosynthesis and secretion of insulin and improves glucose tolerance through a multifactorial mechanism (Fig. 2).

**Activation of the  $\beta$ -cell M3 muscarinic receptors.** Acetylcholine facilitates the glucose-dependent insulin release by activating the M3-subtype muscarinic receptors in the pancreatic  $\beta$ -cell membrane (17). In Wistar rats, the intraperitoneal injection of OA reduced fasting glycemia in

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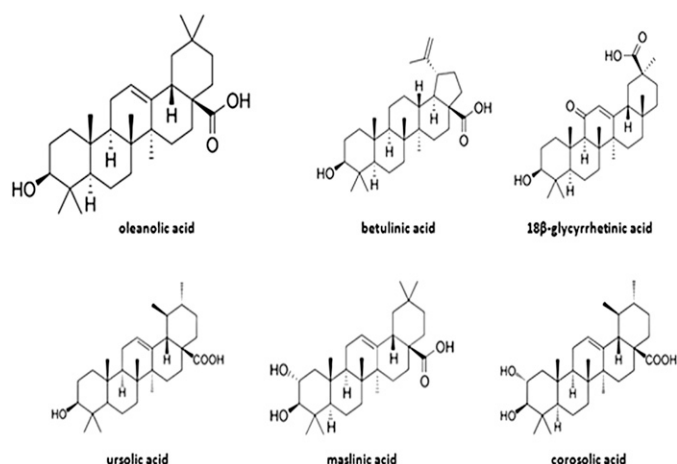


FIG. 1. Chemical structures of OA and related natural triterpenes with antidiabetic effects.

parallel with the increase of plasma insulin (18). These actions were abolished by hemicholinium-3 and vesamicol, inhibitors of the choline uptake and acetylcholine transport, respectively, indicating that OA raises acetylcholine release from nerve terminals.

**Agonist action on the TGR5 receptor.** Bile acids emerged as signaling molecules endowed with systemic endocrine functions. They induce production of glucagon-like peptide-1 by a TGR5-mediated mechanism that increases insulin secretion and  $\beta$ -cell regeneration (19). OA performs as a selective TGR5 agonist ( $EC_{50}$  1.42  $\mu$ mol/L) (20), since a difference of bile acids does not activate the FXR receptor. Betulinic acid (BA) ( $EC_{50}$  1.04  $\mu$ mol/L) and UA ( $EC_{50}$  1.43  $\mu$ mol/L) also exhibit specific activity on

TGR5. They are better TGR5 agonists than lithocholic acid, the most potent natural activator. TGR5 stimulation also increases cAMP production and the thyroid hormone-activating enzyme deiodinase type 2 activity in brown adipose tissue, enhances oxidative phosphorylation in muscle, and stimulates eNOS expression and improves the immune and inflammatory responses in enteroendocrine cells (21).

Different structure/activity studies have revealed the importance of C3-hydroxyl and C28-carboxylate groups in OA molecule for TGR5 activation. It has been postulated that triterpenes bind TGR5 through three binding motifs: 1) a narrow H-bonding site recognizing the hydroxyl, 2) other polar pocket anchoring the carboxylate, and 3) hydrophobic interactions with the pentacyclic skeleton that favor orientation of the polar groups (21).

**Protective action on  $\beta$ -cell under oxidative stress.** Overproduction of mitochondrial reactive oxygen species (ROS) represent a common pathway of injury that ultimately results in  $\beta$ -cell failure.  $\beta$ -Cells have moderate catalytic capacity for conversion of superoxide anion into  $H_2O_2$ . However, their levels of  $H_2O_2$ -inactivating enzymes are extremely low, and therefore they are vulnerable to  $H_2O_2$  accumulation and hydroxyl radical production (22). ROS injure mitochondria by promoting DNA fragmentation, protein cross-linking and membrane phospholipid peroxidation, and by activating stress-signaling pathways. In this regard, OA protects  $\beta$ -cells. It shows moderate free radical-scavenging activity but strongly reinforces cellular defenses. The antioxidant activity of OA will be detailed below.

**Enhancement of the Shp-2 enzyme activity.** The Src-homology phosphotyrosyl phosphatase 2 (Shp-2) is implicated in receptor-activated pathways, including insulin biosynthesis and signaling. Cytoplasmic Shp-2 plays

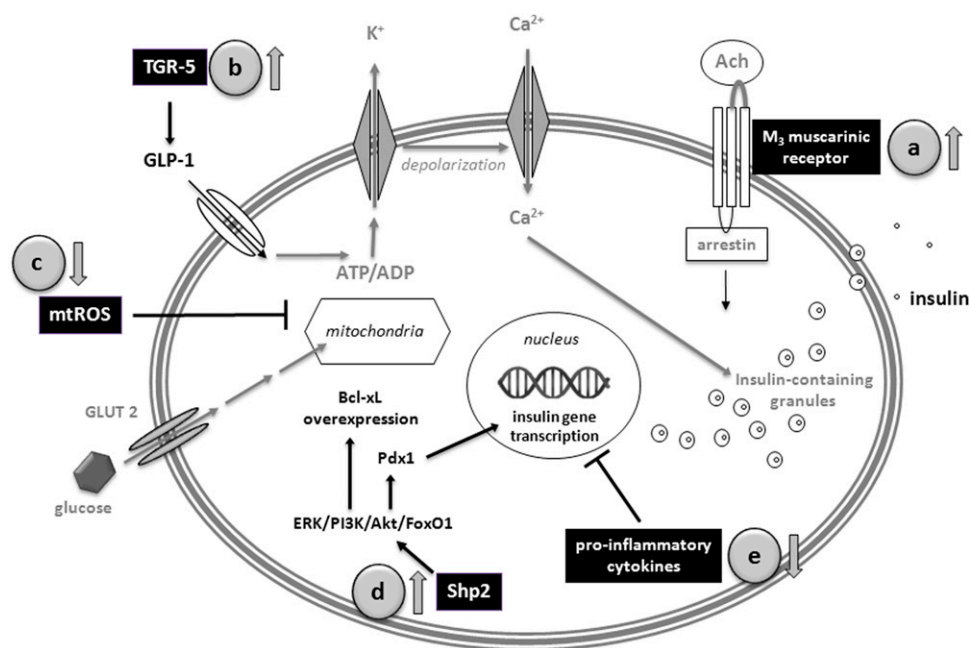


FIG. 2. OA increases insulin biosynthesis and secretion and improves glucose tolerance. It also promotes  $\beta$ -cell survival and proliferation. Actions of OA on pancreatic  $\beta$ -cells involve multitargeted mechanisms. *a*: Increase of acetylcholine release and activation of M<sub>3</sub> muscarinic receptors. *b*: Enhancement of the glucagon-like peptide-1 (GLP)-1 release by agonist action on the TGR-5 receptors. *c*: Alleviation of the oxidative stress. *d*: Stimulation of the Src-homology phosphotyrosyl phosphatase 2 activity and PKB/Akt pathway. *e*: Improvement of the  $\beta$ -cell survival and proliferation.

a critical role in insulin gene transcription, modulating signals that flow through PI3K/Akt/FoxO1 and extracellular signal-related kinase (ERK) pathways and culminate in control of the pancreatic and duodenal homeobox 1 (*Pdx1*) gene expression and activity on *Ins1* and *Ins2* promoters. OA acts as enhancer of the *Shp-2* causing a hypoglycemic effect in streptozocin (STZ) diabetic mice (23). Its action is dose dependent and selective, since OA does not activate other phosphotyrosyl phosphatases such as *Shp1*, *Vhr*, and *HePTP*. The chronic administration of OA (30–50  $\mu\text{mol/L}$ ) to *INS-1* rat  $\beta$ -cells stimulated insulin biosynthesis at the transcriptional level (24), enhancing the insulin protein level (~25%), with a parallel increase in proinsulin and a twofold rise in the insulin-2 mRNA. In addition, OA enhanced mitochondrial *Shp-2* activity and repressed the caspase-3 apoptotic cascade (25,26).

**Promotion of  $\beta$ -cell survival and proliferation.**  $\beta$ -Cells are extremely sensitive to cytokines, which lead to islet degeneration and cell death (27). In STZ diabetic mice, OA prolongs survival of transplanted islets by inhibiting the cytokine production by macrophages and antigen-presenting and other infiltrating cells (28). OA markedly reduced IP-10 and interleukin (IL)-4 cytokines in serum and declined the frequency of  $\gamma$ -interferon-, IL-4-, IL-7-, and IL-2-producing T cells. Likewise, asiatic acid, a natural OA analog, preserved functional  $\beta$ -cells in STZ diabetic rats as a consequence of both the improvement of  $\beta$ -cells survival and promotion of their proliferation (29). UA also preserved  $\beta$ -cell functionality and enhanced the immune system in diabetic mice. Triggering of protein kinase B (PKB)/Akt pathway was proposed as the mechanism that leads to Bcl-XL overexpression (30).

#### IMPROVEMENT OF THE INSULIN RESPONSE

At the molecular level, insulin resistance, the pathogenic feature of type 2 diabetes, means impaired insulin signaling. Consistent evidence indicates that OA has beneficial effects on the insulin receptor (IR) and downstream signaling pathway (Fig. 3).

**Insulin-mimetic effect as IR activator.** Small non-peptide molecules known as IR activators are useful for treating type 2 diabetes because they restore IR autophosphorylation in insulin-resistant cells. OA and analogs act as activators that synergistically enhance the low-dose (1 nmol/L) insulin-mediated IR autophosphorylation in hamster ovary cells expressing the human receptor (31). In the absence of insulin, triterpenoids at 1  $\mu\text{g/mL}$  could not activate IR, although at concentrations higher than 50  $\mu\text{g/mL}$  they duplicated autophosphorylated receptors. These results suggest that triterpenes bind IR not at the insulin site but rather on the  $\beta$ -subunits.

**Inhibition of protein-tyrosine phosphatases PTP1B and TCPTP.** Tyrosine phosphatases PTP1B and TCPTP negatively regulate insulin signaling in vivo. Inhibition of these proteins improves insulin sensitivity and stimulates glucose uptake (32). Oleanane-type triterpenes inhibit PTP1B in a potent, selective, and reversible way. The mechanism could be referred to as linear-mixed type (33). UA shows the greatest inhibition ( $\text{IC}_{50}$  3.1  $\mu\text{mol/L}$ ), followed by OA ( $\text{IC}_{50}$  3.4  $\mu\text{mol/L}$ ) and maslinic acid (MA) ( $\text{IC}_{50}$  5.93  $\mu\text{mol/L}$ ) (34). Molecular docking studies indicate that triterpenes bind not in the PTP1B catalytic site but in a secondary aryl phosphate binding site (35), where C28-carboxylate forms an extensive hydrogen bonds network with the enzyme. Other protein residues interact through Van

der Waals contacts with the triterpene. The  $\beta$ -configuration of C3-OH also seems relevant because of the lower inhibitory potency of the  $\alpha$ -epimer.

OA and UA discriminate other phosphatases involved in the insulin pathway but do not exhibit obvious selectivity among PTP1B and TCPTP. Since MA shows the highest selectivity (3.3-fold), a series of synthetic derivatives with modifications at C2 and C3 was assayed; all of them were stronger and more selective PTP1B inhibitors (36).

**Activation of PI3K/Akt.** PKB/Akt is essential for insulin-stimulated events such as glucose uptake and glycogen synthesis. OA and  $3\beta$ -taraxerol have been reported for stimulating Akt in vascular smooth muscle cells and 3T3-L1 adipocytes, respectively. Since wortmannin blocked these events, the requirement of PI3K activation seems obligatory (37,38). Recently, it was described that OA exerts a potent glucose-lowering effect in diabetic mice that is sustained well beyond the treatment period (39). The downregulation of glucose-6-phosphatase, the gate-keeping gluconeogenesis enzyme, induced by AKT and FoxO1, was proposed as the likely mechanism.

**Activation of LKB1/AMPK.** The AMP kinase (AMPK) participates in many metabolic processes, including glucose uptake and fatty acid oxidation in muscle and fatty acid synthesis and gluconeogenesis in liver. AMPK is activated when ATP-to-AMP ratio decreases in response to nutrient deprivation and pathological stresses. OA, UA, and BA stimulate AMPK in human HepG2 hepatoma cells (40). Similarly, MA activates AMPK and improves insulin sensitization in *KK-A<sup>y</sup>* mice (41). By using the synthetic OA derivative 2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid methyl ester (CDDO-Me), we know that these events were mediated by phosphorylation of both liver kinase B1 (LKB1) and AMPK. Although the exact step that triggers LKB1/AMPK remains unclear, it seems that CDDO-Me targets the upstream kinase ERK1/2 (42).

**Inhibition of glycogen synthase kinase-3 $\beta$ .** Glycogen synthase kinase (GSK)3 $\beta$  acts as negative modulator of insulin signal in absence of stimulus. OA, UA, and  $3\beta$ -taraxerol inhibit GSK3 $\beta$  by phosphorylation (38,43,44), and molecular docking studies have indicated that triterpenes, like other GSK3 $\beta$  inhibitors, link to the ATP-binding site by hydrogen bonds (45).

**Other effects on the glycogen pool.** Diabetes reduces glycogenesis and enhances glycogenolysis. UA has the ability to increase hepatic glycogen in STZ diabetic mice by stimulating glucokinase and inhibiting glucose-6-phosphatase (46). Inhibition of glycogen phosphorylase, the rate-limiting step of glycogenolysis, is another good target for treating diabetes. OA represses this activity in lung cancer A549 cells ( $\text{IC}_{50}$  5.98  $\mu\text{mol/L}$ ) (47) and rabbit muscle ( $\text{IC}_{50}$  14  $\mu\text{mol/L}$ ) (48)—very similar to the behavior of other natural OA analogs (49,50). Triterpenes bind at the AMP allosteric site (51), their C3 and C28 positions being important for the quaternary structure changes that cause enzyme inhibition (49,52).

**Alleviation of the oxidative stress-induced insulin resistance.** ROS play a causal role in several types of insulin resistance. As we mentioned, OA exerts antioxidant activity through direct chemical response but mainly by indirect biological effects. OA moderately scavenges hydroxyl and superoxide radicals, although this effect is stronger than those of known antioxidants such as BHT or LD- $\alpha$ -lipoic acid (52). The presence of the phenolic OH at C3 is speculated to be responsible for this action (53). By contrast, OA yields solid protection against oxidative

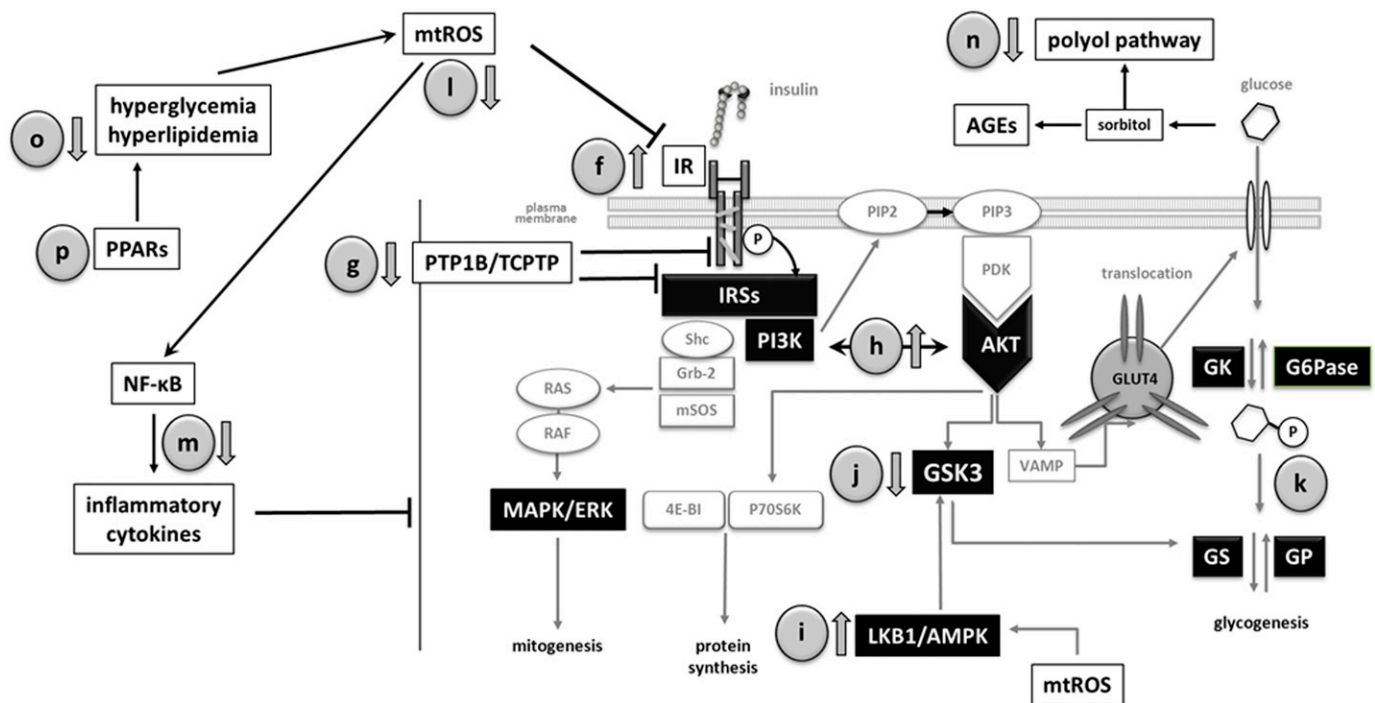


FIG. 3. OA and related triterpenoids improve insulin sensitivity in peripheral tissues through a multiple mechanism. *f*: Stimulation of IR auto-phosphorylation. *g*: Inhibition of PTP1B/TCPTP activities. *h*: Stimulation of the PI3K/AKT pathway. *i*: Activation of LKB1/AMPK. *j*: Inhibition of GSK3. *k*: Stimulation of glycogenesis and inhibition of gluconeogenesis. *l*: Improvement of the antioxidant defenses. *m*: Inhibition of the pro-inflammatory cytokine production. *n*: Repression of polyol pathway and AGE formation. *o*: Amelioration of hyperlipidemia. GP, glycogen phosphorylase; GS, glycogen synthase; mtROS, mitochondrial ROS; VAMP, vesicle-associated membrane protein.

damage by indirect means. OA upregulated the expression of glutathione peroxidase (GSHPx) and superoxide dismutase (SOD) in hepatocytes treated with *tert*-butyl hydroperoxide (52). In addition, OA and UA improved the viability of PC12 cells treated with H<sub>2</sub>O<sub>2</sub> or 1-methyl-4-phenylpyridinium by increasing glutathione content and catalase and SOD activities. They also attenuated the lactate dehydrogenase release and malondialdehyde formation (54). Likewise, OA protected rat heart against myocardial ischemia reperfusion injury by enhancing the glutathione-mediated mitochondrial antioxidant mechanism (55). OA also increased GSHPx and SOD activities and diminished malondialdehyde level in liver and kidney of alloxan diabetic rats (56).

These OA effects appear mediated, to a great extent, through the activation of the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2), which is a pivotal regulator of endogenous antioxidants and phase II genes (53). **Effects on inflammatory mediators.** Many inflammatory cytokines that adversely affect insulin signaling are regulated by the transcription factor nuclear factor (NF)- $\kappa$ B. NF- $\kappa$ B is activated by endogenous and exogenous stimuli via phosphorylation of the inhibitory subunit I $\kappa$ B by IKK. Several natural triterpenes inhibit IKK and block NF- $\kappa$ B activation. In this way, OA and UA reduce tumor necrosis factor- $\alpha$ -induced E-selectin expression in human endothelial cells (57), inhibit IL-6 release in lipopolysaccharide-activated Mono Mac 6 cells (58), and suppress the endothelin-1 pathway in Zucker diabetic rats (59). BA also represses NF- $\kappa$ B in human colorectal carcinoma HCT116, colon carcinoma Caco-2, and lung carcinoma H1299 cells, with these effects being correlated with its ability to inhibit IKK (60). Suppression of the NF- $\kappa$ B pathway is also the mechanism

by which BA plays an antifibrotic role in ethanol-activated hepatic stellate cells (61).

Lastly, OA is also reported as an irreversible inhibitor of the inflammatory enzyme phospholipase A<sub>2</sub> at micromolar concentrations (62).

**Inhibition of polyol pathway and AGEs.** Chronic hyperglycemia significantly increases the polyol pathway in tissues that are not insulin sensitive. Aldose reductase (AR), the rate-limiting enzyme in this pathway, reduces glucose to sorbitol, which is further metabolized to fructose by sorbitol dehydrogenase (SDH). High levels of sorbitol and fructose promote the synthesis of advanced glycation end products (AGEs) and stimulate stress-sensitive signaling pathways. OA and UA inhibit both AR and sorbitol dehydrogenase in kidney and liver of STZ diabetic mice (46). In addition, OA enhances glyoxalase-I (G1) and reduces methylglyoxal concentration. In vitro, OA dose dependently inhibits the formation of pentosidine and N<sup>ε</sup>-(carboxymethyl)lysine (CML), whereas UA suppressed CML but not pentosidine (63). In alloxan diabetic mice, OA and UA decreased the levels of plasma HbA<sub>1c</sub>, the renal pentosidine and CML, and urinary glycated albumin, with the inhibitory potency of OA greater than that of UA at equal concentration (56). With these data considered together, it seems that methyl position in the E ring that differentiates OA from UA determines their antiglycative strength.

**Hypolipidemic effects.** Hypolipidemic and antiatherosclerotic abilities of OA are well-known since the nineties (5). In Sprague-Dawley rats fed a high-cholesterol diet, OA and MA reduced plasma concentrations of total cholesterol, triglyceride, HDL cholesterol, and LDL cholesterol and downregulated the expression of lipogenic genes

(acetyl-CoA carboxylase [ACC], stearoyl-CoA desaturase 2 [SCD2], glycerol-3-phosphate acyltransferase [Gpat], and acyl-CoA cholesterol acyltransferase [ACAT]) (64). Likewise, OA and BA significantly reduced visceral fat in obese Swiss mice, with an increase of leptin and a decline of plasma lipids and ghrelin. The histological study of liver indicated that triterpenes markedly reduced microvesicular steatosis and lipid droplets caused by the diet (65). 18 $\beta$ -Glycyrrhetic acid also decreased total cholesterol, triglyceride, free fatty acids, phospholipids, LDL cholesterol, and VLDL cholesterol in plasma, kidney, liver, and heart of STZ diabetic rats (66).

**Transactivation of peroxisome proliferator-activated receptors.** The peroxisome proliferator-activated receptors (PPARs) are transcriptional regulators of genes involved in lipid metabolism and glucose homeostasis. Accumulated evidence indicates that OA may modulate PPAR activity. Acting as a PPAR- $\alpha$  agonist, OA improves cardiac lipid metabolism in the ZDF diabetic rat (59) and stimulates differentiation in keratinocytes HaCaT and African green monkey kidney fibroblast (CV-1) cells (67). In parallel, transactivation of PPAR- $\gamma$  by OA produces a hypoglycemic effect in diabetic KK-A<sup>y</sup> mice (68). By contrast, the attenuated lipid accumulation and decreased visfatin levels in differentiated 3T3-L1 adipocytes resulted from the OA-induced PPAR- $\gamma$  downregulation (69). Two glycosylated OA derivatives from *Kalopanax pictus* achieve the transactivation of the three PPAR subtypes (70). Conceptually, these pan-PPAR activators are very interesting for the treatment of metabolic diseases because they could target simultaneously insulin resistance, atherogenic dyslipidemia, and obesity/overweight.

#### UNIFYING HYPOTHESIS OF THE OA ANTIDIABETIC ACTIVITY

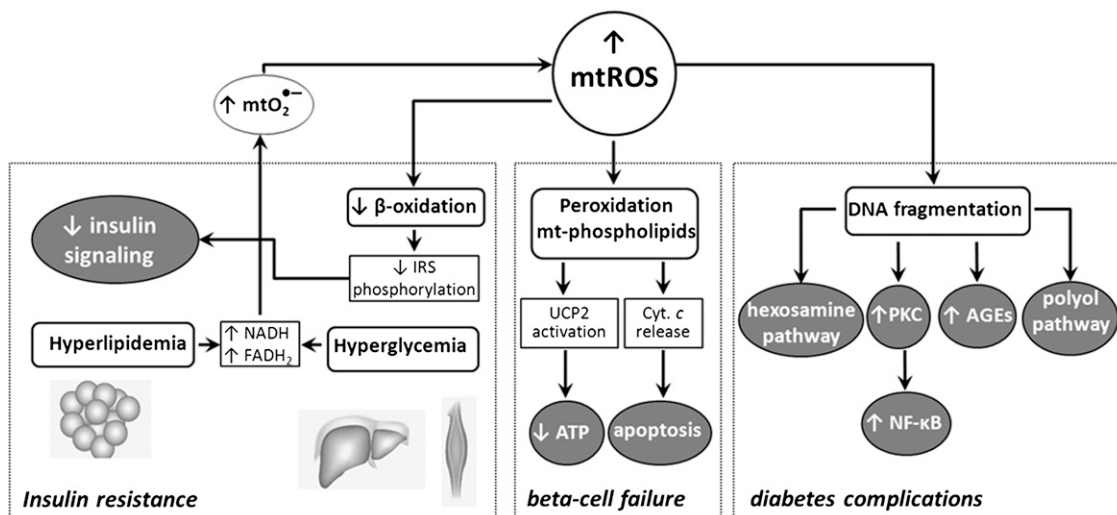
Hyperglycemia, hyperlipidemia, AGEs, and inflammatory cytokines all contribute to the progression of diabetes. Although many mechanisms have been proposed to underlie their effects, a unifying theme is that supraphysiological levels of ROS represent a common pathway of injury (22) (Fig. 4). Type 2 diabetes is now recognized as

a phenotype of mitochondrial dysfunction, where impaired oxidative activity accumulates superoxide and other ROS (22,71). These radical species injure mitochondria by promoting DNA fragmentation, protein cross-linking, membrane phospholipid peroxidation, and activation of stress pathways, which result in insulin resistance,  $\beta$ -cell failure, and diabetes complications (71). Regarding this, much of the OA antidiabetic potential could be better explained if we understood the molecular basis of its protection against mitochondrial ROS overproduction and oxidative stress.

In vitro and in silico studies have revealed that OA is a versatile molecule. Its stereochemical structure, a hydrophobic pentacyclic skeleton with a  $\beta$ -phenolic hydroxyl at C3 and a carboxylate at C28, is appropriated to interact directly with single proteins involved in carbohydrate digestion, insulin secretion, and signaling. However, the major contributions of OA against diabetes seem to be derived from its interaction with transduction pathways modulating the expression of key defensive genes (Fig. 5). A significant number of OA effects might be consistently related to activation of Nrf2 (52). Nrf2 binds antioxidant response elements (AREs) in the gene promoter regions and stimulates transcription of cytoprotective genes in response to oxidative/electrophilic stresses. Nrf2 increases transcription of antioxidant enzymes (SOD, CAT, Heme Oxygenase 1 [HO-1], GSHPx, or peroxiredoxin [prdx]), as well as of genes involved in GSH biosynthesis (glutamate-cysteine ligase [*Gclc*] and glutathione synthase [*GSS*]) and regeneration (glutathione reductase [GSHR]). Nrf2 is also involved in the expression of NADPH-producing genes, such as malic enzyme and many of the pentose phosphate pathway ones (72).

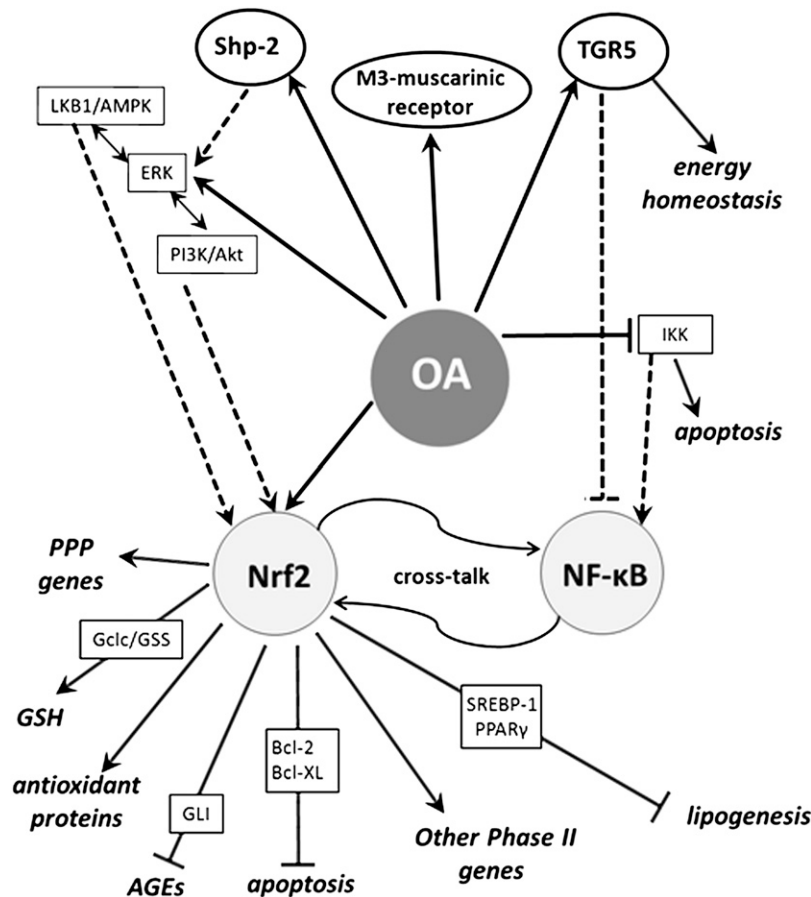
OA-type triterpenes are extremely potent enhancers of phase 2 responses, with potential to activate Nrf2 at protein level via Michael addition reaction with Keap1, the primary sensor that retains Nrf2 for ubiquitin-dependent degradation (73). In addition, OA may activate Nrf2 through stress-induced signaling pathways.

ERK is thought to be part of the defensive mechanisms against H<sub>2</sub>O<sub>2</sub>. OA stimulates ERK and Jun NH<sub>2</sub>-terminal kinase phosphorylation in response to oxidative stress and



**FIG. 4.** Diabetes is now considered a phenotype of mitochondrial dysfunction. Hyperglycemia and hyperlipidemia provoke overproduction of superoxide and ROS, which contribute to impair insulin signaling,  $\beta$ -cell failure, and other pathologies associated with diabetes.





**FIG. 5.** A unifying hypothesis of the OA antidiabetic action. OA exerts its activity mainly through indirect mechanisms, stimulating stress-induced pathways in which the transcription factor Nrf2 plays a protagonist role. In this scheme, OA is a potent inducer of antioxidant enzymes and other phase II response genes, as well as a repressor of NF- $\kappa$ B.

inhibits the disruption of the mitochondrial membrane potential (74). However, these mitogen-activated protein kinases (MAPKs) appear to operate indirectly, since significant evidence demonstrates that direct phosphorylation by MAPKs has a limited contribution in modulating Nrf2 *in vivo* (74).

AMPK is activated by elevated mitochondrial ROS or a decline in the ATP-to-AMP ratio. AMPK stimulates HO-1 expression in human endothelial cells through activation of Nrf2 (75). As we aforementioned, OA induces phosphorylation of LKB1 and AMPK (40), which is consistent with activation of the upstream kinase ERK1/2 (42). AMPK could operate on Nrf2 through phosphatidylinositol 3-kinase (PI3K) (35), which is a necessary condition for events signaled by the PKB-Akt pathway, such as repression of  $\beta$ -cell apoptosis (30) and glycogen metabolism (36–38). We have also shown that OA is a potent and selective activator of Shp-2, which participates in PI3K activation in the  $\beta$ -cell (23) (Fig. 2). By triggering cytoplasmic Shp-2, OA stimulates insulin biosynthesis at the transcriptional level (24), whereas by enhancing mitochondrial Shp-2 it contributes to the preservation of  $\beta$ -cell mass (29,38).

On the other hand, oxidative stress in diabetes excites damaging pathways, such as NF- $\kappa$ B, leading to cell death. Since several anti-inflammatory agents that suppress NF- $\kappa$ B signaling also activate the Nrf2-ARE cascade, the existence of a cross-talk with each other was postulated (60). OA upregulates cytoprotective genes through Nrf2 activation, but it also blocks NF- $\kappa$ B via IKK inhibition

(57–59). Hence, it seems clear that OA damps both oxidative stress and inflammatory response acting on these two opposite pathways.

Mitochondrial membrane phospholipid oxidation by ROS generates a mixture of oxidized lipid derivatives, including 4-hydroxynonenal (4-HNE). 4-HNE triggers  $\beta$ -cell apoptosis by disrupting the cytosolic Bcl-2/Bax protein interaction and translocation of Bax to mitochondrial membrane. This process involves Bcl-2 phosphorylation by IKK, giving a novel activity for this kinase (76), and is inhibited by substrate excess. Very recently (77), it was reported that Nrf2 controls cell apoptosis via regulation of Bcl-2 transcription. Therefore, the OA competence to inhibit IKK and stimulate PI3K/PKB/Akt pathway and Bcl-XL overexpression (34) supports both antioxidant and anti-inflammatory effects and endorses the existence of the Nrf2/NF- $\kappa$ B cross-talk. In consequence, the OA-induced improvement of  $\beta$ -cell functionality and enhancement of the immune system in diabetic mice could progress, at least partially, via Nrf2 transactivation.

Hyperglycemia increases glucose flux through the polyol pathway, where AR produces sorbitol with consumption of NADPH. Because NADPH is a critical cofactor for GSH regeneration, this pathway increases susceptibility to oxidative damage. Consequently, the inhibition of AR expression by OA (46) contributes to maintain the mitochondrial redox status and limits generation of AGEs. Nevertheless, AR has been postulated as an antioxidant enzyme that detoxifies cytotoxic aldehydes, including 4-HNE. In different

cancerous cells, AR is upregulated by ROS and antioxidants, with putative participation of Nrf2 (78). Therefore, further investigation of regulation of AR expression is required to clarify its physiological role.

Accumulation of methylglyoxal and lipid peroxidation products also provokes DNA fragmentation and mitochondrial dysfunction. These effects are countered by GLI. Since OA upregulates GLI mRNA (46), which carries a functional ARE in exon 1 with the ability to bind Nrf2 (79), this action looks to be coherent with the OA-induced Nrf2 activation.

OA reduces plasma lipids (65,66) and downregulates lipogenic genes (64). The repression of transcriptional regulators SREBP-1 (72) and PPAR- $\gamma$  (74) was proposed as the underlying mechanism. These actions may be now observed as mediated by Nrf2, since both SREBP-1 (64) and PPAR- $\gamma$  (69) are direct targets of the transcription factor. In fact, most of lipogenic genes are downregulated in Nrf2-null mutant mice (72).

In summary, we have shown that many endogenous antioxidant enzymes, most of pentose phosphate pathway genes and those for glutathione biosynthesis and regeneration, are upregulated by Nrf-2, whereas lipogenic enzymes consuming NADPH are downregulated. Thus, in a scenario of ROS overproduction, the physiological consideration of the OA-induced Nrf2 activation is very interesting, since it would prime the use of NADPH to sustain the level of reduced glutathione and to assure the intracellular redox status.

## CONCLUSIONS AND PERSPECTIVES

OA, a natural component of many plant food and medicinal herbs, is endowed with a wide range of pharmacological properties. Throughout complex and multifactorial mechanisms, OA exerts beneficial effects against diabetes and the metabolic syndrome. Although it directly modulates enzymes connected to carbohydrate metabolism and insulin signaling, the main OA contributions appear to be derived from its interaction with critical transduction pathways. Many effects are consistently related to activation of the transcription factor Nrf2. OA induces the expression of genes regulating the intracellular redox status, blocks NF- $\kappa$ B, and represses the polyol pathway, AGE production, and hyperlipidemia.

In spite of the well-contrasted scientific evidence, very few clinical trials using triterpenoids as antidiabetic agents have been developed up to today. Animal and human assays have demonstrated that OA and natural analogs are therapeutically effective without apparent side effects (9–11). OA is under commercialization in the People's Republic of China as a drug against acute and chronic liver diseases (9,10), and its use is authorized in Japanese hair tonics and sport drinks. By contrast, a significant number of trials have used the synthetic derivative CDDO-Me (Bardoxolone) in the treatment of different types of cancer and chronic renal diseases in diabetic individuals ([clinicaltrials.gov/show/NCT00811889](http://clinicaltrials.gov/show/NCT00811889) or [www.clinicaltrials.gov/ct2/show/NCT01563562](http://www.clinicaltrials.gov/ct2/show/NCT01563562)). Unfortunately, this deeply modified molecule is under serious debate as a result of findings of adverse events and mortality.

The prevalence of type 2 diabetes has burst by the aging of the population and lifestyles that trends toward physical inactivity and overweight/obesity. Therefore, it is of capital importance for the National Health Systems to introduce urgent preventive measures that delay or avoid the

appearance of the disease. The use of functional foods containing natural triterpenoids is, undoubtedly, an interesting alternative. In this vein, our research group designed the PREDIABOLE Study ([www.controlled-trials.com/ISRCTN003372660](http://www.controlled-trials.com/ISRCTN003372660)), a 2009–2013 phase 2 trial that pretends to demonstrate that a dietary intervention based in the regular consumption of olive oil enriched in OA may prevent the development of type 2 diabetes in prediabetic patients with impaired fasting glucose and impaired glucose tolerance. Further investigations like this will be necessary to extend the use of natural triterpenes in the design of new drugs and foods, which allow personalized diets and nutrigenomic approaches for the prevention of high-prevalence chronic disorders such as neurodegenerative and cardiovascular diseases, cancer, or diabetes.

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J.M.C. researched data and wrote the manuscript. A.G. and T.D. contributed to discussion and edited the manuscript. M.R. and J.A.C. participated in the critical reading of the text. J.M.C. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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