# Quercetin Selectively Inhibits Insulin Receptor Function in Vitro and the Bioresponses of Insulin and Insulinomimetic Agents in Rat Adipocytes†

Assia Shisheva and Yoram Shechter*

*Department of Hormone Research, The Weizmann Institute of Science, Rehovot 76100, Israel*

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ABSTRACT: We report here that quercetin, a naturally occurring bioflavonoid, is an effective blocker of insulin receptor tyrosine kinase-catalyzed phosphorylation of exogenous substrate. The $ID_{50}$ was estimated to be $2 \pm 0.2$ μM in cell-free experiments, using a partially purified insulin receptor and a random copolymer of glutamic acid and tyrosine as a substrate. Insulin-stimulated autophosphorylation of the receptor itself was not blocked by quercetin (up to 500 μM). In intact rat adipocytes, quercetin inhibited insulin-stimulating effects on glucose transport, oxidation, and its incorporation into lipids. Inhibition of lipogenesis (50%) occurred at $47 \pm 4$ μM, whereas full inhibition was evident at $110 \pm 10$ μM quercetin. In contrast, the effect of insulin in inhibiting lipolysis remained unaltered in quercetin-treated adipocytes. The inhibitor was devoid of general adverse cell affects. Basal activities and the ability of lipolytic agents to stimulate lipolysis were not affected. Inhibition by quercetin enabled us to evaluate which insulinomimetic agents are dependent on tyrosine phosphorylation of endogenous substrates for stimulating glucose metabolism. Quercetin blocked lipogenesis mediated by insulin, wheat germ agglutinin, and concanavalin A. The lipogenic effect of $Zn^{2+}$ and $Mn^{2+}$ was partially blocked, whereas that of vanadate was not affected at all. These results suggest that (a) insulin-activated glucose metabolism in rat adipocytes is dependent on receptor activity to phosphorylate endogenous substrates, (b) insulin receptor tyrosine kinase activity is not a prerequisite for insulin's lipolysis inhibiting effect, (c) insulinomimetic lectins need endogenous tyrosine phosphorylation to express their insulin-like effects, and (d) glucose metabolism can be activated in an alternative fashion (with vanadate ions), under conditions in which, presumably, the endogenous insulin receptor tyrosine transphosphorylation is fully arrested.

According to current concepts, insulin alters cell metabolism initially by binding to its plasma membrane receptor. The insulin receptor is a highly insulin-regulated entity and undergoes autophosphorylation upon insulin binding (Czech, 1985; Shechter, 1985; Rosen, 1987; Shoelson & Kahn, 1989). Phosphorylation of the specific region of the receptor's β-subunit (including Tyr-1158, Tyr-1161, and Tyr-1162) correlates with receptor tyrosine kinase activation and propagation of the biological actions of the hormone (Rosen, 1987; Shoelson & Kahn, 1989; McClain & Olefsky, 1989). However, several studies have shown an uncoupling between receptor autophosphorylation, transphosphorylation, and the stimulation of biological responses (Simpson & Hedo, 1984; Zick et al., 1984; Morrison & Pessin, 1987; Forsayeth et al., 1987; Ponzio et al., 1988; Debant et al., 1988, 1989; Rafaeloff et al., 1990). Anti-insulin receptor antibodies that otherwise mimic insulin actions fail to activate receptor β-subunit autophosphorylation and tyrosine kinase (Simpson & Hedo, 1984; Zick et al., 1984; Forsayeth et al., 1987; Ponzio et al., 1988). Mutant receptors with deletion in the C-terminal portion have retained all insulin receptor properties (binding, autophosphorylation, kinase activation, and endocytosis) but yet are ineffective in signaling insulin metabolic effects (McClain & Olefsky, 1989). Cells that express mutant insulin receptors (Tyr-1162 and Tyr-1163 substituted for Phe) devoid of autophosphorylation and tyrosine kinase activity can reinstitute receptor-mediated stimulation of glycogen synthesis upon treatment with anti-insulin receptor antibodies (Debant et al., 1989). Moreover, these mutant receptors can signal several biological functions such as amino acid uptake, S6 kinase activation, cell growth, and proliferation, despite the lack of receptor autophosphorylation and tyrosine kinase activity (Debant et al., 1988; Rafaeloff et al., 1990). Taken together these observations raise the question as to whether insulin receptor (IR)[1] kinase activity is necessary and sufficient for signaling the pleiotropic biological actions of insulin. In fact, since immediate insulin effects include both serine and threonine phosphorylation and dephosphorylation (Czech, 1985; Shechter, 1985; Rosen, 1987), a single mechanism may not underlie all the insulin-dependent events. Therefore, specific inhibition of IR tyrosine phosphorylation might be a valuable tool for further elucidation of receptor kinase–effector coupling. Recently, we have synthesized and identified a family of carboxyl compounds containing a hydroxyphenyl moiety as competitive inhibitors of IR tyrosine kinase (tyrphostins, Shechter et al., 1989). Compared to epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor (PDGFR) these compounds were weak inhibitors of IR-catalyzed phosphorylation of exogenous substrates (Levitzki et al., 1991). In this study we report that the bioflavonoid quercetin is a potent blocker of IR tyrosine kinase activity in vitro without affecting receptor autophosphorylation. The results presented herein provide further evidence for differential involvement of IR tyrosine kinase in

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* To whom correspondence should be addressed.

[1] Abbreviations: IR, insulin receptor; EGFR, epidermal growth factor receptor; PDGFR, platelet-derived growth factor receptor; $PolyGlu_4$-Tyr, poly[(GluNa:Tyr)4:1]; KRB, Krebs–Ringer bicarbonate; KRBH, Krebs–Ringer bicarbonate HEPES; BSA, bovine serum albumin; WGA, wheat germ agglutinin; Con A, concanavalin A; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis.

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insulin-stimulated effects on glucose and lipid metabolism in rat adipocytes. In addition, we tested which insulinomimetic agents could bypass IR tyrosine kinase inhibition.

## MATERIALS AND METHODS

*Materials.* D-[U-$^{14}$C]Glucose, D-3-*O*-[methyl-$^{14}$C]glucose and [$\gamma$-$^{32}$P]ATP were purchased from New England Nuclear (Boston, MA). Collagenase type I (134 unit/mg) was obtained from Worthington Biochemicals (Freehold, NJ). Porcine insulin was purchased from Eli Lilly Co. (Indianapolis, IN). Phloretin, 3-*O*-methyl-D-glucose, D,L-isoproterenol hydrochloride, quercetin, and poly[(GluNa:Tyr)4:1] (PolyGlu$_4$Tyr) were purchased from Sigma Chemical Co. (St. Louis, MO). Affinity-purified monoclonal antibodies to phosphotyrosine were kindly donated by Mono-Yeda (Rehovot, Israel). Krebs–Ringer bicarbonate (KRB) buffer, pH 7.4, contained NaCl, 110 mM; $NaHCO_3$, 25 mM; KCl, 5 mM; $KH_2PO_4$, 1.2 mM; $CaCl_2$, 1.3 mM; and $MgSO_4$, 1.3 mM. Krebs–Ringer bicarbonate HEPES (KRBH) buffer, pH 7.4, consisted of NaCl, 117 mM; $NaHCO_3$, 10 mM; $CaCl_2$, 1 mM; $MgSO_4$, 1 mM; $KH_2PO_4$, 4 mM; and HEPES, 30 mM. All other chemicals and reagents used in this study were of analytical grade.

*Cell Preparation and Bioassays.* Rat adipocytes were prepared from fat pads of male Wistar rats (100–200 g) by collagenase digestion (Rodbell, 1964). Cell preparations showed more than 95% viability by Trypan blue exclusion, at least 3 h after digestion. All bioassays were performed as described in the figure legends. *Glucose* transport was carried out using 3-*O*-[methyl-$^{14}$C]glucose (Whitesell & Glieman, 1979), *glucose* oxidation was measured by conversion of [U-$^{14}$C]glucose to $^{14}CO_2$ (Rodbell, 1964) and *lipogenesis* by the incorporation of [U-$^{14}$C]glucose into lipids according to Moody et al. (1974). *Lipolysis* was evaluated by measuring glycerol released as described previously (Shechter, 1982).

*Purification of Insulin Receptor.* Partially purified insulin receptor was obtained from rat liver membranes as described elsewhere (Meyerovitch et al., 1990). Briefly, the liver was homogenized in the presence of proteinase inhibitors, solubilized with 1% Triton X-100, and centrifuged. The supernatant was allowed to pass through a wheat germ agglutinin (WGA)-agarose column, and the bound insulin receptor portion was eluted with 0.3 M *N*-acetyl-D-glucosamine in 50 mM HEPES, pH 7.4, containing 0.1% Triton X-100, 10% glycerol, and 0.15 M NaCl.

*Phosphorylation of PolyGlu$_4$Tyr.* WGA-purified IR aliquots (1 $\mu$g of protein) were diluted to a final volume of 50 $\mu$L in a solution, containing 50 mM HEPES (pH 7.4), 0.1% Triton X-100, 2 mM $MnCl_2$, and 10 mM $MgCl_2$. These mixtures were preincubated with the indicated concentrations of quercetin at 22 °C for 30 min in the absence or presence of insulin ($10^{-7}$ M) and 80 $\mu$M ATP to allow receptor autophosphorylation. PolyGlu$_4$Tyr was then added, and the incubation was continued for 20 min at 22 °C. Phosphotyrosine content in PolyGlu$_4$Tyr was quantitated in aliquots of the incubation mixture by radioimmunoassay procedure as described elsewhere (Shisheva et al., 1991). This assay included specific monoclonal antibodies to phosphotyrosine (final dilution 1:100 000) and [$^{125}$I]BSA–phosphotyrosine conjugate. In an alternative procedure, the enzyme assay was carried out similarly but using [$\gamma$-$^{32}$P]ATP (500 cpm/pmol). In this case the reaction was terminated by spotting aliquots (50 $\mu$L) onto Whatman 3 MM filter paper followed by several washings with 10% trichloroacetic acid, containing 10 mM sodium pyrophosphate (Corbin & Reimann, 1983).

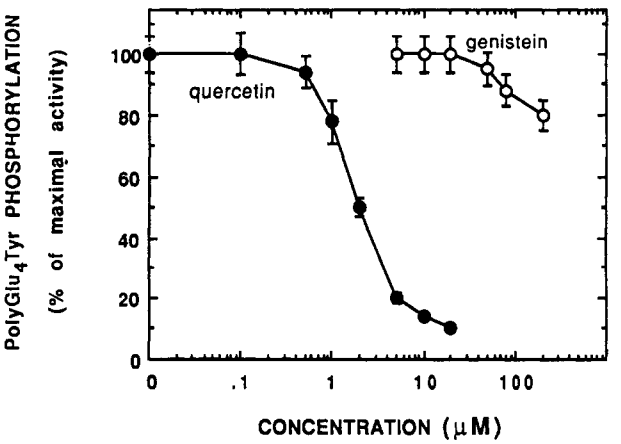


FIGURE 1: Inhibition of IR phosphotransferase activity by quercetin. The assay was run for 30 min at 22 °C in 60 $\mu$L of 50 mM HEPES (pH 7.4)–0.1% Triton X-100, containing WGA-purified rat liver IR (1 $\mu$g); $MgCl_2$, 10 mM; $MnCl_2$, 2 mM; ATP, 80 $\mu$M; insulin, 0.1 $\mu$M; the indicated concentrations of quercetin or genistein. PolyGlu$_4$Tyr was then added (final concentration 0.35 mg/mL), and the reaction proceeded for an additional 20 min at 22 °C. Aliquots (50 $\mu$L) were withdrawn for determining phosphotyrosine content as described under Materials and Methods.

The paper strips were then dried and analyzed in a $\beta$ spectrometer.

*In Vitro Autophosphorylation Assay.* The autophosphorylation and immunoprecipitation of the insulin receptor was accomplished by a modification of the procedure of White et al. (1988). WGA-purified IR (~50 $\mu$g of protein) was incubated at 22 °C for 50 min with the indicated concentrations of quercetin in a solution, containing 50 mM HEPES, pH 7.4, 2 mM $MnCl_2$, and 10 mM $MgCl_2$, either in the absence or presence of 0.1 $\mu$M insulin. Phosphorylation was initiated by the addition of 25 $\mu$M [$\gamma$-$^{32}$P]ATP (4 $\mu$Ci). After 10 min the reaction was terminated by adding a 0.5-mL portion of 50 mM HEPES, pH 7.4, containing 0.1% Triton X-100, 100 mM NaF, 10 mM sodium pyrophosphate, 5 mM EDTA, and 2 mM $NaVO_3$. The phosphorylated IR was immunoprecipitated with anti-phosphotyrosine antibodies (affinity purified, 2 $\mu$g/tube) for 2 h at 4 °C. The antibody was immobilized on Pansorbin (10%, 50 $\mu$L) and washed three times with 1 mL of 50 mM HEPES, pH 7.4, containing 1% Triton X-100, 0.1% sodium dodecyl sulfate (SDS), 150 mM NaCl, 100 mM NaF, and 2 mM $NaVO_3$. The phosphoproteins were eluted from Pansorbin with sample buffer (Laemmli, 1970), containing 100 mM dithiothreitol, resolved by 7.5% SDS–polyacrylamide gel electrophoresis (PAGE), and identified by autoradiography.

Protein concentration was determined by the method of Bradford (1976). All assays were performed either in duplicate or triplicate. All data are presented as means ± SE ($n \geq 3$).

## RESULTS

*Inhibition of PolyGlu$_4$Tyr Phosphorylation by Quercetin, but Not by Genistein.* Addition of increasing concentrations of quercetin to WGA-purified insulin receptors inhibited IR-catalyzed phosphorylation of PolyGlu$_4$Tyr in a dose-dependent manner (Figure 1). Half-maximal inhibition was obtained at 2 ± 0.2 $\mu$M and near full inhibition was evident at 10 $\mu$M (Figure 1). In contrast, another bioflavonoid, the isoflavone genistein (1–100 $\mu$M), which inhibits EGFR and PDGFR autophosphorylation and substrate phosphorylation (Hill et al., 1990; Akiyama & Ogawara, 1991), was not effective in inhibiting insulin receptor phosphotransferase activity with PolyGlu$_4$Tyr as a substrate.

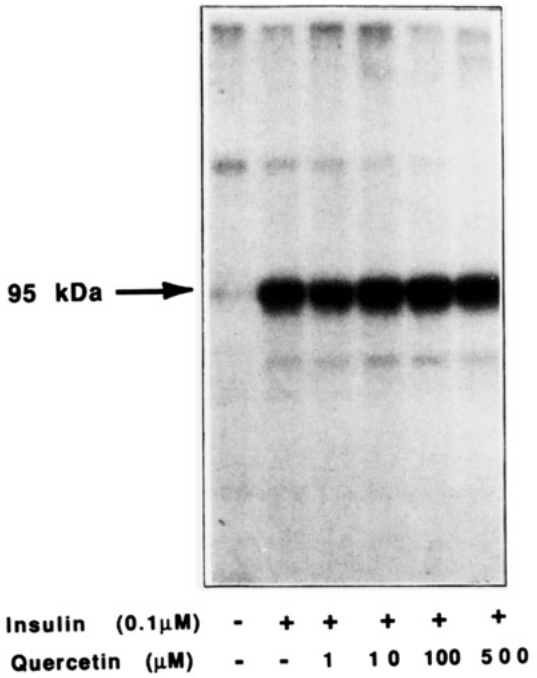


FIGURE 2: Lack of inhibition of IR autophosphorylation by quercetin. WGA-purified IR (~50 μg of protein) was incubated with the indicated concentrations of quercetin at 22 °C for 50 min in a mixture, containing 50 mM HEPES (pH 7.4)–0.1% Triton X-100, 2 mM $MnCl_2$, and 10 mM $MgCl_2$, either in the absence or presence of insulin. Autophosphorylation was initiated by adding 25 μM [γ-$^{32}$P]ATP (4 μCi). The assay was terminated after 10 min, and IR was immunoprecipitated as described under Materials and Methods. The proteins were then separated by 7.5% SDS–PAGE, under reducing conditions. The gel was dried and autoradiographed. Arrow indicates IR β-subunit.

*Quercetin Does Not Inhibit IR Autophosphorylation.* In contrast to the high potency of quercetin to inhibit $PolyGlu_4$-Tyr phosphorylation, the bioflavonoid did not inhibit insulin-mediated autophosphorylation of the receptor itself at concentrations up to 500 μM (Figure 2). This result provided us with an opportunity to assess the relevance of receptor kinase activity on a variety of insulin bioresponses, independent of receptor autophosphorylation.

*Effects of Quercetin in Rat Adipocytes.* (*a*) *Quercetin Inhibits Glucose Utilization.* Subsequent to a 30–40-min preincubation of rat adipocytes with quercetin, the insulin-dependent [U-$^{14}$C]glucose incorporation into fat was inhibited in a dose-dependent manner (Figure 3). $IC_{50}$ value amounted to 47 ± 4 μM, whereas full inhibition was evident at 110 ± 10 μM quercetin. Quercetin did not alter the basal level of lipogenesis at any of the concentrations studied (Figure 3). The shift in sensitivity of the concentration-effect curve in vitro versus the intact cells probably reflects a lower concentration of quercetin within the cell interior. We have also observed that the quercetin inhibition is blunted upon increasing BSA concentration (3%) during cell incubation (not shown).

The effect of insulin in stimulating 3-*O*-methylglucose uptake was inhibited by quercetin as well (Figure 4). The basal glucose uptake was only weakly affected. The insulin-dependent stimulation of glucose oxidation to $^{14}CO_2$ was also completely inhibited in quercetin-treated adipocytes (Figure 5).

(*b*) *Quercetin Does Not Affect Antilipolysis.* Quercetin did not affect lipolysis at any dose studied (Table I), indicating that the long cascade constituting activation of adenylate cyclase, cAMP-dependent activation of protein kinase, and phosphorylation and activation of hormone sensitive lipase (Belfrage et al., 1986) is not altered. In contrast to the inhibitory effect of quercetin on insulin actions related to glucose metabolism, the agent (at 110 μM) failed to reverse insulin-dependent inhibition of lipolysis (Table I). Even upon doubling the highest effective concentration of quercetin (at 220 μM) the effect of insulin in inhibiting lipolysis was practically unaltered (Table I). This result seems to indicate that (a) receptor signaling upon insulin binding is not altered in the intact cell system and (b) insulin receptor phosphotransferase activity is not an essential entity for the expression of the antilipolytic action of insulin.

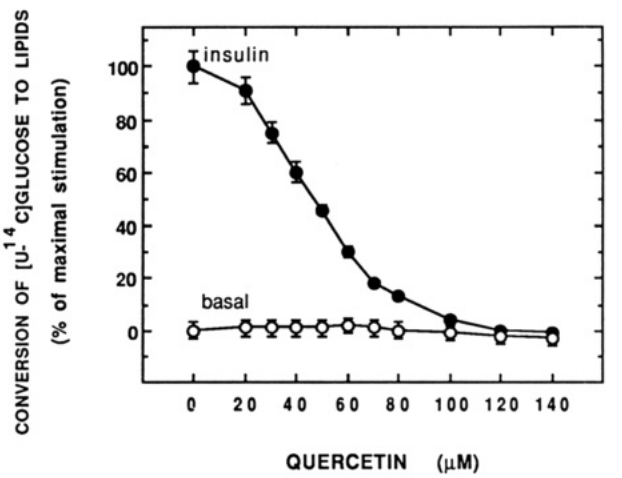


FIGURE 3: Inhibition of insulin-stimulated lipogenesis in rat adipocytes by quercetin. Adipocytes (2 × $10^4$ cells/mL) suspended in KRB buffer, containing 0.7% BSA, were subjected to the indicated concentrations of quercetin for 40 min at 37 °C. The cells were then supplemented with 0.2 mM [U-$^{14}$C]glucose in either the presence or absence of insulin (17 nM), and lipogenesis was carried out for 1 h at 37 °C. The reaction was terminated by adding toluene-based scintillation fluid. The radioactivity of the extracted lipids was determined. In all experiments, insulin-stimulated lipogenesis was between 3- and 5-fold higher than basal.

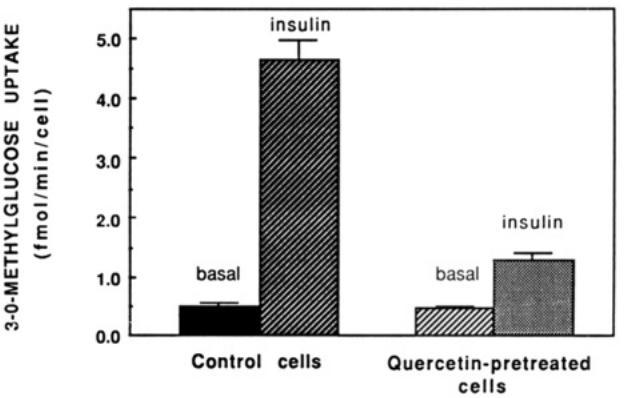


FIGURE 4: Inhibition of insulin-activated 3-*O*-methylglucose uptake in rat adipocytes by quercetin. Adipocytes (2 × $10^6$ cells/mL) suspended in KRBH buffer, containing 1.2% BSA, were preincubated for 45 min at 37 °C without or with 115 μM quercetin, and then for an additional 30 min with or without insulin (14 nM). Aliquots were then transferred to prewarmed (37 °C) tubes containing 3-*O*-[methyl-$^{14}$C]glucose (47 000 cpm/nmol, final concentration 0.1 mM). Basal uptake was measured for 15 s, and insulin activated uptake for 3 s. Termination was achieved by adding phloretin (final concentration 0.1 μM) followed by centrifuging an aliquot through the silicone oil layer.

(*c*) *Vanadate Can Bypass Quercetin-Inhibited Receptor Kinase.* The ability of several insulinomimetic agents (Dubyak & Kleinzeller, 1980; Shechter, 1983, 1990) to bypass quercetin-inhibited IR phosphotransferase activity was also studied in intact rat adipocytes. Like in the case of

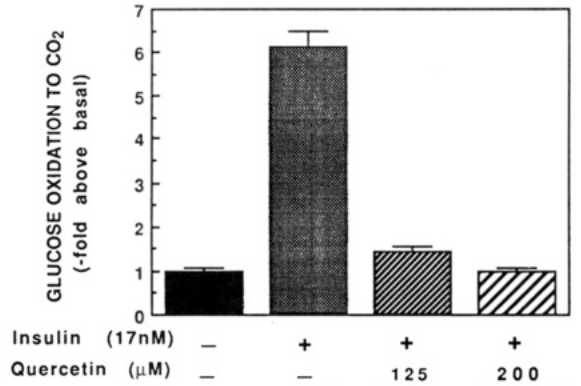


FIGURE 5: Inhibition of insulin-stimulated glucose oxidation in rat adipocytes by quercetin. Adipocytes ($2 \times 10^4$ cells/mL) suspended in KRB buffer, containing 0.7% BSA, were preincubated with the indicated concentrations of quercetin for 40 min at 37 °C. Glucose oxidation was measured by conversion of D-[U-$^{14}$C]glucose to $^{14}CO_2$ over a period of 1 h at 37 °C.

Table I: Lack of Quercetin Effect on β-Adrenoreceptor-Evoked Lipolysis and on Antilipolytic Activity of Insulin[a]

| addition | glycerol released, nmol ($3 \times 10^5$ cells)$^{-1}$ h$^{-1}$ | % inhbn of lipolysis |
|---|---|---|
| basal | 10 ± 0.5 | |
| quercetin (115 μM) | 9 ± 0.4 | |
| isoproterenol (32 μM) | 160 ± 7.0 | |
| + quercetin (110 μM) | 154 ± 5.0 | |
| isoproterenol (0.08 μM) | 85 ± 3.0 | 0 |
| + insulin (17 nM) | 19 ± 1.0 | 88 |
| + insulin (17 nM) + quercetin (110 μM) | 21 ± 1.4 | 85 |
| + insulin (17 nM) + quercetin (220 μM) | 24 ± 1.6 | 81 |

[a] Adipocytes were preincubated for 30 min at 37 °C in KRB buffer, containing 0.7% BSA with the indicated concentrations of quercetin. Lipolysis and antilipolysis was performed for 1 h at 37 °C. Aliquots (0.3 mL) were withdrawn for glycerol determination. Isoproterenol was used at submaximal concentrations since insulin does not antagonize high concentrations of the lipolytic agent (Shechter, 1982).

insulin, quercetin (110 μM) blocked the stimulating effects of WGA and concanavalin A (Con A) on lipogenesis, suggesting that these lectins propagate their activating effect via the phosphorylating cascade initiated by IR tyrosine kinase. In contrast to insulin, WGA, and Con A, the activating effect of vanadate on lipogenesis was not inhibited at all. This was evident under experimental conditions in which the insulin effect was blocked by more than 95% (Table II). Thus, the effects of vanadate and insulin were dissociated from each other by quercetin. The stimulating effects of zinc and manganese ions (Veda et al., 1984; Ezaki, 1989) reflected an intermediate condition; they were 50–75% inhibited by quercetin (Table II).

As with lipogenesis, quercetin at concentration which blocked completely the insulin effect did not inhibit vanadate in stimulating 3-*O*-methylglucose uptake in rat adipocytes (Figure 6).

## DISCUSSION

Previous attempts of several laboratories to find low molecular weight blockers which have good cellular permeation and high potency for inhibiting insulin receptor-catalyzed tyrosine phosphorylation in intact cells were relatively unsatisfactory (Saperstein et al., 1989; Shechter et al., 1989). In the present study we report that the bioflavonoid quercetin demonstrates both of these properties.

Quercetin was found to be a powerful blocker of IR-catalyzed tyrosine phosphorylation of the exogenous substrate

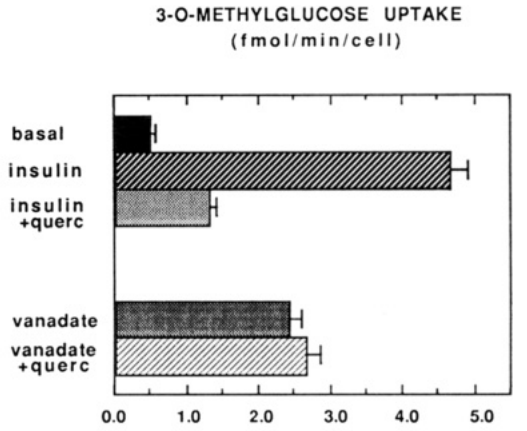


FIGURE 6: Lack of inhibition of vanadate-activated hexose uptake in rat adipocytes by quercetin. Adipocytes were treated with quercetin under similar experimental conditions described in the legend to Figure 4, followed by stimulation with either insulin or sodium orthovanadate (final concentration 0.8 mM).

Table II: Effect of Quercetin on Activation of Lipogenesis by Insulinomimetic Agents[a]

| addition | [U-$^{14}$C]glucose conversion into lipids, cpm ($10^4$ cells)$^{-1}$ h$^{-1}$ | % maximal stimulation |
|---|---|---|
| basal | 2700 ± 150 | 0 |
| insulin (17 nM) | 8400 ± 560 | 100 |
| + quercetin | 2900 ± 220 | 3.5 |
| WGA (5 μg/mL) | 8100 ± 500 | 95 |
| + quercetin | 2750 ± 120 | 1 |
| Con A (20 μg/mL) | 7800 ± 420 | 90 |
| + quercetin | 2650 ± 100 | 0 |
| vanadate (0.3 mM) | 6800 ± 300 | 72 |
| + quercetin | 7200 ± 280 | 79 |
| $Mn^{2+}$ (10 mM) | 7770 ± 400 | 89 |
| + quercetin | 4200 ± 220 | 26 |
| $Zn^{2+}$ (0.6 mM) | 5700 ± 250 | 53 |
| + quercetin | 3400 ± 300 | 12 |

[a] Adipocytes ($2 \times 10^4$ cells/mL) suspended in KRB buffer, containing 0.7% BSA, were preincubated in the absence or presence of quercetin (110 μM) for 40 min at 37 °C. Lipogenesis was measured over a period of 1 h in the presence of indicated concentrations of insulin or insulinomimetic agents as described under legend to Figure 3.

PolyGlu$_4$Tyr with an $IC_{50}$ value equal to 2 ± 0.2 μM (Figure 1). The other flavonoids, which supposedly inhibit tyrosine phosphorylation (Hill et al., 1990; Akiyama & Ogawara, 1991) such as genistein (Figure 1) and apigenin (not shown) were found to be ineffective. As such, among the low molecular weight compounds described so far (Saperstein et al., 1989; Shechter et al., 1989), quercetin is perhaps the most powerful cell permeable blocker of IR phosphotransferase activity. While quercetin inhibited substrate phosphorylation, it had no effect on insulin receptor β-subunit autophosphorylation (Figure 2). This allowed us to determine solely the role of IR phosphotransferase activity in the propagation of the various biological responses. It should be emphasized that our results are restricted to general phosphorylating activity of IR kinase; in particular to the in vitro inhibition of PolyGlu$_4$Tyr phosphorylation. Further studies will reveal how quercetin affects the ability of the receptor to phosphorylate endogenous protein substrates (White et al., 1987; Bernier et al., 1988). The link between a putative receptor kinase endogenous substrate and any known targets of insulin action has not yet been established.

Quercetin treatment of intact rat adipocytes blocked insulin-stimulated effects on glucose metabolism, including glucose transport, oxidation, and its incorporation into lipids, sug-

gesting an active receptor tyrosine kinase is necessary for mediating these bioeffects. In contrast, quercetin, even at high concentrations, did not reverse insulin's ability to inhibit isoproterenol-induced lipolysis, hinting that there is no role for IR-catalyzed tyrosine phosphorylation in this insulin-dependent event. It is tempting to speculate that insulin effects mediated by dephosphorylation such as inhibition of lipolysis are not linked to the tyrosine phosphorylation cascade initiated by the IR tyrosine kinase. In this regard Gotchalk has recently reported that insulin action on dephosphorylation and activation of pyruvate dehydrogenase is independent of receptor tyrosine kinase activity (Gotchalk, 1991).

Abler et al. (1992) recently observed that the isoflavone genistein inhibits several actions of insulin in rat adipocytes including glucose oxidation and antilipolysis. Genistein has no inhibitory effect on IR autophosphorylation and substrate phosphorylation, both in vivo and in vitro (Abler et al., 1992; Figure 1 in this study). Thus, quercetin differs from genistein in its ability to inhibit substrate phosphorylation, and its site of action seems to precede that of genistein.

The following experimental evidence supports the contention that quercetin is devoid of general adverse cell effects not related to inhibiting tyrosine phosphorylation: (i) quercetin (at the concentration studied) did not affect basal activities of lipogenesis, glucose transport, and its oxidation; (ii) quercetin did not alter isoproterenol-mediated lipolysis, and (iii) vanadate activated lipogenesis to its maximal extent in quercetin-treated cells. These results indicate that the overall enzyme system for glucose utilization remains unaltered. Quercetin does not inhibit protein kinase A (Graziani et al., 1983; Table I). Previous reports claimed no (Junco et al., 1990) or weak (Akiyama & Ogawara, 1991) inhibiting effect of the agent on protein kinase C. We have confirmed here that the effects of quercetin observed are not related to protein kinase C inhibition. This was concluded because staurosporine, a powerful inhibitor of protein kinase C (Tamaoki et al., 1986), does not block the action of insulin in mediating lipogenesis (not shown) as does quercetin.

Among a variety of agents having insulinomimetic activity, including WGA, Con A, vanadate ions, $Zn^{2+}$, and $Mn^{2+}$ (Dubyak & Kleinzeller, 1980; Shechter, 1983, 1990; Veda et al., 1984; Ezaki, 1989), only vanadate was able to bypass the inhibitory action of quercetin and to fully stimulate glucose transport and lipogenesis. This is important evidence which further supports the notion that the site of action of vanadate is downstream to the insulin receptor itself (Shechter, 1990). In this regard, our study raises the possible existence of an alternative (insulin-independent) pathway for metabolizing glucose. Such a secondary pathway is of significant basic and clinical interest since it may be activated under conditions of insulin resistance when the primary insulin-dependent pathway is faulty.

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