Cholesterol Depletion Disrupts Caveolae and Insulin Receptor Signaling for Metabolic Control via Insulin Receptor Substrate-1, but Not for Mitogen-activated Protein Kinase Control*

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Insulin exerts its cellular control through receptor binding in caveolae in plasmalemma of target cells (Gustavsson, J., Parpal, S., Karlsson, M., Ramsing, C., Thorn, H., Borg, M., Lindroth, M., Peterson, K. H., Magnusson, K.-E., and Strålfors, P. (1999) FASEB. J. 13, 1961–1971). We now report that a progressive cholesterol depletion of 3T3-L1 adipocytes with β-cyclodextrin gradually destroyed caveolae structures and concomitantly attenuated insulin stimulation of glucose transport, in effect making cells insulin-resistant. Insulin access to or affinity for the insulin receptor on rat adipocytes was not affected as determined by 125I-insulin binding. By immunoblotting of plasma membranes, total amount of insulin receptor and of cavelolin remained unchanged. Receptor autophosphorylation in response to insulin was not affected by cholesterol depletion. Insulin treatment of isolated caveolae preparations increased autophosphorylation of receptor before and following cholesterol depletion. Insulin-increased tyrosine phosphorylation of an immediate downstream signal transducer, insulin receptor substrate-1, and activation of the further downstream protein kinase B were inhibited. In contrast, insulin signaling to mitogenic control as determined by control of the extracellular signal-related kinases 1/2, mitogen-activated protein kinase pathway was not affected. Insulin did not control Shc phosphorylation, and Shc did not control extracellular signal-related kinases 1/2, whereas cholesterol depletion constitutively phosphorylated Shc. In conclusion, caveolae are critical for propagating the insulin receptor signal to downstream targets and have the potential for sorting signal transduction for metabolic and mitogenic effects.

Insulin controls target cells by binding to its cell surface receptor. The further intracellular transmission of the insulin signal involves phosphorylation of the receptor as well as other proteins, in particular the insulin receptor substrate (IRS), on specific tyrosine residues. After tyrosine phosphorylation IRS is recognized by Src homology 2 domain-containing proteins for metabolic and glucose transport control, or activation of the mitogen-activated protein kinase (MAP kinase) pathway and mitogenic control (1–4). In type 2 diabetes target cells of the hormone are not fully responsive, which is compensated temporarily by enhanced insulin secretion. The pathogenic mechanisms for this insulin resistance are not known, but an important common feature appears to be a reduced activation/takyne phosphorylation of IRS-1 (5).

The insulin receptors are sequestered in the caveolae microdomains of the plasma membrane in adipocytes, and caveolae appear to be critical for insulin control (6). By thin-section electron microscopy, caveolae appear as omega-shaped invaginations of 50–100 nm diameter in the plasma membrane (7). Caveolae invaginations are found in the plasma membrane of many cell types, but are particularly abundant in adipocytes where they increase in number in conjunction with the differentiation of 3T3-L1 fibroblasts to mature adipocytes (8–10). Caveolae are involved in receptor-mediated uptake of solutes into the cytosol (11) and in transcytosis (12). A number of proteins, in addition to the insulin receptor, involved in signal transduction are localized to caveolae, which suggests that they may be involved in cellular signaling and control (reviewed in Refs. 13–16).

Caveolae are rich in cholesterol and sphingolipids. Caveolae may indeed form from cholesterol- and sphingolipid-rich rafts in the membrane in a process requiring the caveolae-specific structural protein caveolin. Caveolin is found in the plasma membrane and intracellularly, but in the plasma membrane is confined to caveolae; it is therefore used as a marker for these structures. The function of caveolae is dependent on a sufficient level of cholesterol in the plasma membrane and caveolae (12, 17). We have also demonstrated a critical dependence of the insulin receptor signal transduction on cholesterol; depletion of cholesterol from the plasma membrane of rat adipocytes reversibly inhibited insulin stimulation of glucose transport and metabolic protein phosphorylation control (6). The importance of caveolae for insulin receptor signaling is further indicated by a consensus binding site for interaction with caveolin (18), and coprecipitation of the receptor with caveolin (4) indicates that the interaction may be physiological. Moreover, the insulin receptor appears to phosphorylate caveolin (19), whereas caveolin was shown to activate the isolated receptor, although the physiological relevance of this is not known (20).

Herein we examine in detail the dependence of the insulin receptor on caveolae for signal transduction: the effects of cholesterol depletion on plasma membrane and caveolae morphology, on the insulin receptor and on the downstream propagation of the insulin signal. In short, the insulin receptor is unaffected, but interaction with the immediate downstream mediator molecule IRS-1 and metabolic control is inhibited by
cholesterol depletion and caveole destruction, while insulin signaling via the MAP kinase pathway remains intact. We also determined that adipocytes become insulin resistant in the presence of 25 μM cytochalasin B.

3T3-L1 Cell Culture and Differentiation—3T3-L1 fibroblasts were grown on 13 mm (diam.) glass coverslips in DMEM with 25 mM 2-gluconic acid, 10% newborn calf serum, 50 μM penicillin, and 0.1 mg/ml streptomycin in 10% CO2-humidified atmosphere at 37 °C. The medium was changed every 2–3 days. Two days after the fibroblasts reached confluence, differentiation was induced with a medium change to DMEM containing 10% fetal bovine serum, 5 μg/ml insulin, 0.25 mM dexamethasone, and 0.1 mM 3-isobutyl-1-methylxanthine. The cells were then incubated for an additional 2 days in the same medium excluding dexamethasone and 3-isobutyl-1-methylxanthine. Cells were maintained for 8–10 days in DMEM with 10% fetal bovine serum to attain maximal differentiation. More than 95% of the cells expressed the adipocyte phenotype, as determined from accumulation of triacylglycerol droplets.

Immunogold Transmission Electron Microscopy—3T3-L1 cells were grown on Formvar-coated grids (300-mesh) and differentiated to adipocytes. They were kept for 2 h in serum-free DMEM supplemented with 0.5% bovine serum albumin (fatty acid-free) and then washed and incubated in 120 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl2, 1.2 mM MgSO4, 1.2 mM KH2PO4 containing 1% bovine serum albumin, and 20 mM Hepes, pH 7.5, with additions as indicated. Plasma membranes attached to grids were prepared by initial incubation for 30 s in 1 M poly-L-lysine, 137 mM NaCl, 2.7 mM KCl, 10 mM NaPO4, 1.8 mM KH2PO4, pH 7.5, followed by 20 s in hypotonic solution A (solution A diluted to 1/3). The grids were then placed in solution A (70 mM KCl, 5 mM MgCl2, 3 mM EGTA, 30 mM Hepes, pH 7.5), with 1 mM dithioerythritol and 0.1 mM phenylmethylsulfonyl fluoride and probe-sonicated for 2 s (22).

Membranes were then fixed in 3% paraformaldehyde, 0.05% glutaraldehyde, 1% sodium pyrophosphate, 2 mM Na3VO4, 0.5 mM phenylmethylsulfonyl fluoride, 1 μM aprotinin, 10 μM leupeptin, 1% Nonidet P-40 with or without 100 μg/ml genistein (Calbiochem). The solubilized cell lysate was filtered through a 0.22-μm pore size filter to remove fat. Equal amounts of protein were incubated with antibodies for 14 h at 4 °C, when Sepharose G-Plus (Santa Cruz Biotechnology) was added for 1 h at room temperature. Non-specific binding was blocked with 1% bovine serum albumin and 0.1% gelatin before incubation with primary antibodies: mouse anti-insulin receptor β-chain monoclonal antibodies (20 μg/ml) and rabbit anti-caveolin polyclonal antibodies (20 μg/ml) for 90 min at 37 °C. These were detected with gold(15 nm)-conjugated anti-mouse antibody and gold (6 nm)-conjugated anti-rabbit antibody for 15 h at 4 °C. The membrane preparations were finally fixed in 2% glutaraldehyde for 10 min and 1% OsO4 for 30 min at room temperature. After rinsing, the grids with membranes were frozen, lyophilized, and covered with 2-nm tungsten. Transmission electron microscopy was done with a Jeol JEM1200 TEM-Scan (Tokyo, Japan), equipped with a Gatan Biscan CCD camera; images were obtained with Gatan Digital Micrograph software.

Isolation and Incubation of Rat Adipocytes—Adipocytes were isolated by collagenase digestion from epididymal fat pads of Harlan Sprague-Dawley rats (160–200 g) (23). Cells, at a final concentration of 106 cells/ml, were centrifuged through dinonylphthalate. 125I in cell cake and medium standards (Sigma) that were identified by ninhydrin staining. Glucose transport was determined in 3T3-L1 adipocytes as uptake of 2-deoxy-[2-14C]-D-glucose (21). Cells were grown on 13-mm plastic coverslips (ThermoVitro, Copenhagen) in 24-well culture dishes. 2-Deoxy-D-[1-14C]glucose was added to a final concentration of 50 μM (0.3 μCi/ml), and the cells were incubated for 9 min. Glucose uptake was stopped by rinsing the coverslips in three successive solutions of ice-cold buffer. Nonspecific uptake was determined in the presence of 25 μM cytochalasin B. Coverslips were transferred to scintillation vials and the cells were dissolved in 1% SDS. Radioactivity

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the amount of plasma membrane cholesterol by treatment of the cells with 10 mM β-cyclodextrin (Fig. 2c), perhaps reflecting flattened caveolar patches or "rafts" remaining in the membrane after the extensive but partial cholesterol depletion (34).

**Insulin-stimulated Glucose Uptake after Cholesterol Depletion**—The cholesterol depletion concomitantly also inhibited progressively the ability of insulin to enhance glucose uptake (Fig. 2a). The extent of insulin stimulation of glucose transport was correlated to the amount of cholesterol, and hence to degree of caveolar intactness, in the plasma membrane of the cells (Fig. 2b).

**Effect of Cholesterol Depletion on Insulin Receptors in the Plasma Membrane**—By immunogold labeling and electron microscopy examination, it appeared that the insulin receptors remained associated with the caveolin clusters and hence with the underlying caveolar remains or rafts after the depletion of cholesterol (Fig. 2). This was affirmed by immunoprecipitation of the insulin receptor, which coprecipitated caveolin to the same extent with or without prior cholesterol depletion (data not shown). We used competitive 125I-insulin binding to isolated rat adipocytes to determine the effect of cholesterol depletion on the accessibility of insulin receptors at the cell surface (Fig. 4). Cholesterol depletion (with 10 mM β-cyclodextrin, which reduced plasma membrane cholesterol concentration by ~60% (Ref. 6)) did not affect the time taken for steady state binding of insulin (Fig. 4a). We subsequently incubated the cells with 125I-insulin for 30 min when near-maximal binding was obtained. Specific insulin binding was not affected by prior cholesterol depletion (Fig. 4b). We used nonlinear regression to fit a one-site binding equation (GraphPad Software, Inc.) to the data in Fig. 4b, to determine the effect on number of insulin receptors and on the dissociation constant for insulin binding. Neither the number of receptors nor the binding affinity was significantly affected by the cholesterol depletion.

After subcellular fractionation of rat adipocytes and isolation of the plasma membranes, SDS-PAGE and immunoblotting with antibodies against the insulin receptor indicated that the total amount of receptor protein in the plasma membrane fraction was not affected by the 10 mM β-cyclodextrin treatment (Fig. 5a). This agrees well with the electron microscopy and 125I-insulin binding results. Similarly, the amount of caveolin in the plasma membrane fraction was not affected by treatment with up to 10 mM β-cyclodextrin (Fig. 5b), also in agreement with the electron microscopic findings.

**Effect of Cholesterol Depletion on Downstream IRS-1 and Protein Kinase B**—We concluded that cholesterol depletion does not adversely affect the number of insulin receptors at the cell surface, the insulin receptor's interaction with or activation by insulin, or its ability to autophosphorylate. We therefore next examined the ability of the receptor to tyrosine phosphorylate an immediate downstream target for insulin's metabolic control, IRS-1. After cholesterol depletion of rat adipocytes, the tyrosine-specific phosphorylation of IRS-1 in response to insulin treatment was severely reduced (Fig. 7). The depletion of cholesterol inhibited the 1 nM insulin-stimulated increase in IRS-1 phosphorylation by 71 ± 6% (mean ± S.E., n = 10). Cholesterol or intact caveolae are apparently required for signal transfer from the insulin receptor to IRS-1. As an expected
consequence of the inhibition of IRS-1 phosphorylation, cholesterol depletion also completely blocked insulin stimulation to phosphorylation/activation of the further downstream protein kinase B (Akt) (Fig. 8).

It has been suggested that serine phosphorylation of IRS-1 acts to inhibit the insulin receptor (35, 36), and serine/threonine phosphorylation of IRS-1 has been found to inhibit IRS-1 binding to the insulin receptor and hence IRS-1 tyrosine phosphorylation (37, 38). Similarly, an unknown serine kinase activity against IRS-1 was detected in insulin-resistant cells (39), and it has been suggested that caveolin acts as a general serine/threonine protein kinase inhibitor (40). Cholesterol depletion had, however, no effect on the total phosphorylation of IRS-1 or on the distribution of phosphorylation between serine, threonine and tyrosine (Fig. 9). An enhanced degradation and lowered levels of IRS-1 in states of insulin resistance have been reported (41, 42). We found no effect of cholesterol depletion on the total cellular levels of IRS-1; the amount of IRS-1 protein in cholesterol-depleted rat adipocytes determined by immunoblotting after immunoprecipitation of IRS-1 and SDS-PAGE was not significantly different from control cells (107 ± 6%, mean ± S.E., n = 3). To directly determine the effect of cholesterol depletion on IRS-1 binding to the insulin receptor, we immunoprecipitated the insulin receptor and looked for IRS-1. The effect of insulin to increase the amount of IRS-1 and tyrosine-phosphorylated IRS-1 that coimmunoprecipitated with the in-
sulin receptor was much reduced in cholesterol-depleted cells (Fig. 10), which suggests that IRS-1 binding to the insulin receptor is inhibited in cholesterol/caveolae-depleted cells.

Lack of Effect of Cholesterol Depletion on MAP Kinase Pathway—IRS-1 is believed to transmit insulin signaling for metabolic as well as mitogenic control. However, there is a redundancy among insulin receptor substrates and mitogenic control by insulin has in some cell-types been described via, e.g., the protein Shc. It has also been shown that cholesterol depletion of fibroblasts activates the MAP kinase pathway and epidermal growth factor causes its hyperactivation (43). We therefore examined the effect of cholesterol depletion of rat adipocytes on the extracellular signal-related kinases (ERK1 and ERK2) of the MAP kinase pathway. Extensive cholesterol depletion (60% reduction) did not by itself have an effect on ERK1/2 phosphorylation (Fig. 11). Moreover, insulin enhanced the phosphorylation/activation of ERK1/2 to the same extent whether cholesterol was depleted or not (Fig. 11). It was verified in the same experiments that insulin-stimulated tyrosine-specific phosphorylation of IRS-1 was inhibited.

In contrast to ERK1/2, the cholesterol depletion strongly enhanced the state of Shc 52-kDa protein tyrosine phosphorylation (Fig. 12). Inclusion of the protein tyrosine kinase inhibitor genistein in the lysis buffer and during immunoprecipitation did not affect the increase in Shc phosphorylation, making a postlysis artifact unlikely. Insulin on the other hand had no effect on Shc phosphorylation, neither before nor after cholesterol depletion, even at a supraphysiological insulin concentration (Fig. 12), in accordance with recent findings in rat adipocytes (44).

**DISCUSSION**

β-Cyclodextrin is a widely used tool for control of cellular levels of cholesterol through its ability to extract cholesterol from the plasma membrane of intact cells. Without itself incorporating into the membrane, β-cyclodextrin selectively extracts cholesterol from the surface of the cells (45, 46). We have shown previously that the inhibition of insulin signaling by cholesterol extraction using β-cyclodextrin was reproduced by cholesterol extraction using β-cyclodextrin. Without itself incorporating into the membrane, β-cyclodextrin selectively extracts cholesterol from the surface of the cells (45, 46). We have shown previously that the inhibition of insulin signaling by cholesterol extraction using β-cyclodextrin.
oxidation using cholesterol oxidase (6). Moreover, cholesterol-loaded β-cyclodextrin did not inhibit insulin signaling (6), attesting against cholesterol-unrelated effects on insulin signaling by the β-cyclodextrin treatment. We now demonstrate that a reduction of adipocyte plasma membrane cholesterol concentration with β-cyclodextrin in a dose-dependent manner affected the structural integrity of caveolae in the plasma membrane. Eventually, the caveolar structure was lost by cholesterol depletion as determined by transmission electron microscopy of plasma membranes. Likewise, insulin signaling to enhanced glucose uptake was progressively inhibited in an apparently linear manner by cholesterol depletion. Hence, there was a correlation between the level of plasma membrane cholesterol, caveolae structural integrity, and insulin stimulation of glucose transport. Earlier studies have indicated that experimental modulation of membrane physical properties affects insulin signaling (47–49), but caveolae or cholesterol levels were not examined.

Cholesterol depletion and loss of the caveolae structures in the plasma membrane did not reduce the amount of insulin receptors in the membrane, or their affinity for insulin, as determined by ¹²⁵I-insulin binding to intact adipocytes, by immunogold-electron microscopy, or by immunoblotting for the receptor in isolated plasma membranes after SDS-PAGE. Nor was insulin receptor interaction with caveolin affected as...

**Fig. 8.** Effects of cholesterol depletion on insulin stimulation of protein kinase B phosphorylation. Isolated rat adipocytes were incubated without or with 10 mM β-cyclodextrin for 50 min when the cells were incubated with or without 1 nM insulin for another 10 min. After SDS-PAGE the state of protein kinase Bα phosphorylation was determined by immunoblotting with antibodies against the phosphorylated form of the protein. The experiment has been repeated three times with the same result.

**Fig. 9.** Effects of cholesterol depletion on IRS-1 serine/threonine phosphorylation. Isolated rat adipocytes were incubated with [³²P]phosphate and then with 10 mM β-cyclodextrin for 50 min, when cells were lysed and immunoprecipitated with antibodies against IRS-1. After SDS-PAGE and transfer to PVDF-membrane (a), total ³²P-phosphorylation was analyzed by phosphorimaging and IRS-1 protein by immunoblotting with antibodies against IRS-1. Indicated are the molecular masses of reference proteins (Rainbow, Amersham Pharmacia Biotech). b, the ³²P-phosphorylated IRS-1 protein band was cut out and the protein partially hydrolyzed in HCl. Phosphoamino acids were separated by silica thin-layer electrophoresis and analyzed by phosphorimaging. Lane 1, control incubation; lane 2, β-cyclodextrin-treated; lane 3, ninhydrin stain of internal standards.

**Fig. 10.** Effect of cholesterol depletion on IRS-1 binding to the insulin receptor. Isolated rat adipocytes were incubated without or with or without 10 mM β-cyclodextrin for 50 min when the cells were incubated with or without 1 nM insulin for another 10 min. Cells were homogenized in lysis buffer (see “Experimental Procedures”), without detergent, containing 100 µM genistein, centrifuged at 4000 × g for 10 min to remove fat, and immunoprecipitated with polyclonal anti-insulin receptor antibodies as under “Experimental Procedures.” Immunoblotting against IRS-1 (upper panel) or against phosphotyrosine (PY) (lower panel). Indicated is the molecular mass of reference protein (Rainbow, Amersham Pharmacia Biotech).
shown by coimmunoprecipitation and by electron microscopy. Insulin-stimulated autophosphorylation of the insulin receptor was not affected by cholesterol depletion either of intact cells or of isolated caveolae. This is compatible with the demonstrated insulin-responsiveness of the detergent-solubilized receptor (50). In contrast, cholesterol has been found to be necessary for ligand binding (51) or receptor activity (52), with GalaninR2 and the nicotinic acetylcholine receptor, respectively.

Hence, intact caveolae are necessary not for the insulin receptor to bind insulin or to signal per se, but for the further downstream propagation of the signal. Activation of the immediate downstream protein IRS-1 was clearly abrogated, since after cholesterol depletion its insulin-stimulated tyrosine phosphorylation was inhibited. As a consequence, the further downstream propagation of the signal to protein kinase B phosphorylation was also blocked. There is a redundancy of IRS proteins in mediating metabolic control by insulin; IRS-2, -3, and -4 have also to varying degrees been implicated, although IRS-4 appears to have a very restricted tissue expression. The fact that cholesterol depletion inhibits the end effects of metabolic signal transduction suggests that insulin receptor interaction with all involved IRS proteins was inhibited.

Insulin-resistant states and reduced insulin-stimulated tyrosine phosphorylation of IRS-1 have been associated with reduced levels of IRS-1 (41, 42) or with serine-phosphorylation of IRS-1 (37, 38). We have ruled out these possibilities for the effect on IRS-1 of caveolae destruction/cholesterol depletion. A pleckstrin homology and phosphotyrosine-binding domain of IRS-1 have been shown to direct binding to the insulin receptor (53, 54). We assessed if IRS-1 binding to the insulin receptor was affected by cholesterol/caveolae depletion. The reduced amount of IRS-1 that coprecipitated with anti-insulin receptor antibodies suggests that cholesterol/caveolae are required for IRS-1 binding to the receptor. The exact function of caveolae/cholesterol in insulin receptor IRS-1 interaction in intact cells will require further investigation.

Interestingly, our findings demonstrate that, although intact caveolae/caveolar cholesterol was necessary for metabolic control, it was not required for insulin's mitogenic control via ERK1 and ERK2 and the MAP kinase pathway. Shc has been suggested to dominate over IRS-1 in mediating insulin's control via the MAP kinase pathway in some cell types (55–58), but apparently not in, e.g., skeletal muscle (59). Our findings demonstrate that, although Shc is present, insulin does not affect Shc in isolated adipocytes and Shc does not control ERK and the MAP kinase pathway in adipocytes. Lack of insulin effect on Shc phosphorylation in adipocytes was recently described (44). The differential effects of caveolae destruction on insulin IRS/metabolic and ERK/mitogenic control indicate that caveolae may have a role in insulin signal sorting for metabolic...
versus mitogenic control. Hence, in cell types with no or little caveola in insulin signaling may be mainly via the MAP kinase pathway, at the expense of IRS-1-mediated metabolic signal- ing. It remains to identify the protein(s) which transmits insulin receptor activation of the MAP kinase pathway and is immune to caveolar destruction (e.g., the insulin receptor substrates Gab1 [Ref. 60], p62 [Ref. 61], c-Cbl [Ref. 62], or Tub (Ref. 63)).

The dependence of the insulin receptor on intact caveola/cholesterol for transmission of its metabolic signals stands in contrast to other tyrosine kinase receptors, which are inhibited by cholesterol/caveolae/caveolin: Cholesterol depletion (using cycloheximide) or caveolin-1 depletion (using caveolin-1 antisense expression) therefore constitutively activated the p42/p44 MAP kinase cascade (43, 64), and epidermal growth factor caused hyperactivation of ERK in cholesterol-depleted cells (43). The adipocyte p42/p44 ERK1 and ERK2 were however, not phosphorylated/activated by cholesterol depletion. Cholesterol depletion of adipocytes did not significantly reduce the amount of caveolin in the plasma membrane (herein), nor the amount of caveolin associated with the insulin receptor (herein), whereas depletion or oxidation of cholesterol was found to reduce plasma membrane-localized caveolin in fibroblasts (43, 65) and in MDCK cells (66). It can be argued that, in our experiments, the adipocyte plasma membrane concentration of cholesterol and caveole were reduced, but not caveolin, and hence we do not see constitutive activation or hyperactivation of ERK in cholesterol-depleted adipocytes. However, this does not explain the reduced ability of active insulin receptors to transduce the insulin molecule (20). The strong increase in Shc phosphorylation after cholesterol depletion may be explained by activation of a Shc kinase other than the insulin receptor (an interpretation supported by the lack of insulin effect on Shc phosphorylation in adipocytes) or by cholesterol depletion making Shc accessible as a substrate for kinases.

A pronounced threshold effect with caveola structures van- ishing after more than 30% reduction of the normal cellular cholesterol concentration was described in MDCK cells (66). In MA104 epithelial cells, the number of caveolae was reduced to 10% of normal after lowering total cellular cholesterol by 55% through inhibition of endogenous cholesterol synthesis (67). In the 3T3-L1 adipocytes, we found that the structure of caveola was not affected after 20% reduction, was partially destroyed after 40%, and was almost completely destroyed after 50% reduction of the plasma membrane cholesterol. In MDCK cells, caveolin expression was correlated to the cellular cholesterol concentration (66), but was in rat or 3T3-L1 adipocytes apparently not affected by a 50% reduction of the plasma membrane concentration of cholesterol. This discrepancy may be due to the different cell types and/or to the acute effects studied herein and the long term (overnight to several days) effects of cholesterol reduction examined in the MDCK cells. Ilangumaran and Hoessli (68) found that methyl-β-cyclodextrin treatment of lymphocytes caused a release of apparently raft-associated proteins and lipids, but not caveolin, from the cells. The adipocytes, however, remained intact after β-cyclodextrin extraction of up to 50% (3T3-L1 adipocytes [herein]) or 60% (rat adipocytes [Ref. 6]) of the plasma membrane cholesterol, when the cells were able to regain insulin control upon cholesterol replenishment (6). The insulin receptor, moreover, was quantita- tively retained in the plasma membrane after cholesterol depletion. However, extraction of more than 60% of the plasma membrane cholesterol caused the adipocytes to easily rupture (data not shown). Apparently the cholesterol extraction made the adipocyte membranes brittle.

A uniform finding in insulin resistance has been that IRS-1 tyrosine phosphorylation in response to insulin is decreased in adipocytes and skeletal muscle in obesity and type 2 diabetes (5). Indeed, our findings demonstrate that cholesterol depletion induces insulin resistance with decreased insulin-stimulated IRS-1 tyrosine phosphorylation. It is also interesting to note that in skeletal muscle of type 2 diabetic patients insulin stimulation to phosphorylation of MAP kinase was normal, while insulin-stimulated tyrosine phosphorylation of IRS-1 was severely impaired (69, 70). Similarly, in the vasculature of insulin-resistant obese Zucker fa/fa rats, insulin-stimulated tyrosine phosphorylation of IRS-1/2 were reduced, while the phosphorylation of ERK-1/2 were equal to that in normal rats (71). Insulin-resistant state induced by β2-adrenergic stimulation blocked insulin-stimulated phosphorylation of IRS1/2 without affecting MAP kinase (72). Severely insulin-resistant patients with pseudocromegaly had impaired insulin activation of IRS-1-associated phosphatidylinositol 3-kinase, with intact activation/phosphorylation of MAP kinase (73). Furthermore, glucocorticoid-induced insulin resistance blocked protein synthesis-stimulation by insulin without affecting the MAP kinase pathway (74). These findings are similar to the insulin resistance incurred on adipocytes by cholesterol depletion.

In conclusion, our findings point to the caveola as forming a hub, which is critical for insulin’s cellular control, apparently with the potential to sort for metabolic versus mitogenic control. Our findings, moreover, suggest that caveola dysfunction can be involved in the pathogenesis of insulin resistance.

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REFERENCES

1. White, M. F., and Kahn, C. R. (1994) J. Biol. Chem. 269, 1–4
2. Yenush, L., and White, M. F. (1997) BioEssays 19, 491–500
3. Cohen, P. (1999) Philos. Trans. R. Soc. Biol. Sci. 354, 485–495
4. Nystrom, F. H., Chen, H., Cong, L.-N., Li, Y., and Quon, M. J. (1999) Mol. Endocrinol. 13, 2013–2024
5. Virkamaki, A., Ueki, K., and Kahn, C. R. (1999) J. Clin. Invest. 103, 931–943
6. Gustavsson, J., Parpal, S., Karlsson, M., Ransmeier, C., Thorn, H., Berg, M., Lindroth, M., Peterson, K. H., Magnusson, K.-E., and Strålfors, P. (1999) FASEB J. 13, 1961–1971
7. Westermann, M., Leutbecher, H., and Meyer, H. W. (1999) J. Histochem. Cytochem. 111, 71–81
8. Fan, J. Y., Carpentier, J.-L., Obbergen, E. V., Grunfeld, C., Gorden, P., and Orci, L. (1983) J. Cell Sci. 61, 219–230
9. Kandror, K. V., Stephens, J. M., and Pflüg, P. F. (1995) J. Cell Biol. 129, 999–1006
10. Scherer, P. E., Lisanti, M. P., Baldini, G., Sargiacomo, M., Corley, Mastick, C., and Lodish, H. F. (1994) J. Cell Biol. 127, 1223–1243
11. Anderson, R. G. W., Kamen, B. A., Rothberg, K. G., and Lacey, S. W. (1992) Science 255, 410–411
12. Schnitzer, J. E., Oh, P., Finney, E., and Allard, J. (1994) J. Cell Biol. 127, 1217–1222
13. Anderson, R. G. W. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 10909–10913
14. Lisanti, M. P., Scherer, P. E., Tang, Z., and Sargiacomo, M. (1994) Trends Cell Biol. 4, 231–235
15. Simons, K., and Ikonen, E. (1997) Nature 387, 569–572
16. Smart, E. J., Graf, G. A., McNiven, M. A., Sessa, W. C., Engelman, J. A., Scherer, P. E., Okamoto, T., and Lisanti, M. P. (1999) Mol. Cell. Biol. 19, 7289–7294
17. Chang, W.-J., Rothberg, K. G., Kamen, B. A., and Anderson, R. G. (1992) J. Cell Biol. 118, 63–69
18. Courtois, J., Li, S., Okamoto, T., Ihezu, T., and Lisanti, M. P. (1997) J. Biol. Chem. 272, 6525–6533
19. Mastick, C. C., and Saltiel, A. R. (1997) J. Biol. Chem. 272, 20706–20714
20. Yamamoto, M., Toya, Y., Schwemke, C., Lisanti, M. P., Myers, M. G., and Ishikawa, Y. (1988) J. Biol. Chem. 273, 29962–39968
21. Frost, S. C., Kohnaki, R. A., and Lane, M. D. (1987) J. Biol. Chem. 262, 9872–9876
22. Robinson, L. J., Pang, S., Harris, D. S., Heuser, J., and James, D. E. (1992) J. Cell Biol. 117, 1181–1196
23. Strålfors, P., Honnor, R. C. (1989) Eur. J. Biochem. 182, 379–385
24. Oka, Y., and Czech, M. P. (1984) J. Biol. Chem. 259, 8125–8133
25. Song, K. S., Li, S., Okamoto, T., Quilliam, L. A., Sargiacomo, M., and Lisanti, M. P. (1996) J. Biol. Chem. 271, 9690–9697
26. Laemmli, U. K. (1970) Nature 227, 680–685
27. Gustavsson, J., Parpal, S., and Strålfors, P. (1996) Mol. Med. 2, 367–372
28. Alemany, S., Mato, J. M., and Strålfors, P. (1987) Nature 330, 77–79
29. Strålfors, P. (1988) Eur. J. Biochem. 171, 199–204
30. Heider, J. G., and Boyett, R. L. (1978) J. Lipid Res. 19, 515–518
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31. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
32. Rothberg, K. G., Heuser, J. E., Donzell, W. C., Ying, Y.-S., Glenney, J. R., and Anderson, R. G. W. (1992) Cell 68, 673–682
33. Fujiimoto, T., and Fujiimoto, K. (1997) J. Histochem. Cytochem. 45, 595–598
34. Anderson, R. G. W. (1998) Annu. Rev. Biochem. 67, 199–225
35. Hotamisligil, G. S., Peraldi, P., Budavari, A., Ellis, R., and White, M. F. (1996) Science 271, 665–670
36. Fujimoto, T., and Fujimoto, K. (1995) J. Biol. Chem. 270, 23780–23784
37. Paz, K., Hemi, R., LeRoith, D., Karasik, A., Elhanany, E., Kanety, H., and Zick, Y. (1997) J. Biol. Chem. 272, 29911–29918
38. Eldar-Finkelman, H., and Krebs, E. G. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 9660–9664
39. Qiao, L., Goldberg, J. L., Russell, J. C., and Sun, X. J. (1999) J. Biol. Chem. 274, 10625–10632
40. Couet, J., Sargiacomo, M., and Lisanti, M. P. (1997) J. Biol. Chem. 272, 30429–30435
41. Egawa, K., Nakashima, N., Sharma, P. M., Maegawa, H., Nagai, Y., Kashiwagi, A., Kikkawa, R., and Olefsky, J. M. (2000) Endocrinology 141, 1930–1935
42. Sun, X. J., Goldberg, J. L., Qiao, L. Y., and Mitchell, J. J. (1999) Diabetes 48, 1359–1364
43. Furuchi, T., and Anderson, R. G. W. (1998) J. Biol. Chem. 273, 21099–21104
44. Liu, H., Kublaoui, B., Pilch, P. F., and Lee, J. (2000) Biochem. Biophys. Res. Commun. 274, 845–851
45. Christian, A. E., Haynes, M. P., Philips, M. C., and Rothblat, G. H. (1997) J. Lipid Res. 38, 2264–2272
46. Yancey, P. G., Rodriguez, W. V., Kilsdonk, E. P. C., Stoudt, G. W., Johnson, W. J., Philips, M. C., and Rothblat, G. H. (1996) J. Biol. Chem. 271, 19403–19408
47. Bruneau, C., Hubert, P., Waksman, A., Beck, J.-P., and Staedel-Flagg, C. (1987) Biochim. Biophys. Acta 925, 297–304
48. Amatruda, J. M., and Finch, E. D. (1979) J. Biol. Chem. 254, 2619–2625
49. McCallum, C. D., and Epand, R. M. (1995) Biochemistry 34, 1815–1824
50. Kasuga, M., Zick, Y., Blithe, D. L., Crettaz, M., and Kahn, C. R. (1982) J. Biol. Chem. 257, 16026–16034
51. Pang, L., Graziano, M., and Wang, S. (1999) Biochemistry 38, 12003–12011
52. Rankin, S. E., Addinna, G. H., Kluczewski, M. A., Bugge, B., and Miller, K. W. (1997) Biophys. J. 73, 2446–2455
53. Yasuda, K., Makati, K. J., Smith-Hall, J., Ishibashi, O., Myers, M. G., and White, M. F. (1996) J. Biol. Chem. 271, 24900–24906
54. O'Neill, T. J., Craparo, A., and Gustafson, T. A. (1994) Mol. Cell. Biol. 14, 6433–6442
55. Sasaoka, T., Drayzin, B., Leitner, J. W., Langlois, W. J., and Olefsky, J. M. (1994) J. Biol. Chem. 269, 10734–10738
56. Harada, S., Smith, R. M., Smith, J. A., White, M. F., and Jarett, L. (1996) J. Biol. Chem. 271, 30222–30226
57. Yamauchi, K., and Pessin, J. E. (1994) J. Biol. Chem. 269, 31107–31114
58. Pronk, G. J., de Vries-Smits, A. M., Buday, L., Downward, J., Maassen, J. A., Medema, R. H., and Bos, J. L. (1994) Mol. Cell. Biol. 14, 1575–1581
59. Yamauchi, T., Yamauchi, T., Tobe, K., Tatemoto, H., Ueki, K., Kaburagi, Y., Yamamoto-Honda, R., F Takahashi, Y., Y., Aizawa, S., Akasaka, Y., Sonenberg, N., Yazaki, Y., and Kadowaki, T. (1996) Mol. Cell. Biol. 16, 3074–3084
60. Holgado-Madruga, M., Emlet, D. R., Moscatello, D. K., Godwin, A. K., and Wong, A. J. (1996) Nature 379, 560–564
61. Carpio, N., Wisniewski, D., Strife, A., Marshak, D., Kobayashi, R., Stillman, B., and Clarkson, B. (1997) Cell 86, 197–204
62. Liu, Y. C., and Altman, A. (1998) Cell Signal. 10, 377–385
63. Kapeller, R., Moriaty, A., Strauss, A., Stubdal, H., Theriault, K., Siebert, E., Chickering, T., Morgenstern, J. P., Tartaglia, L. A., and Lillie, J. (1999) J. Biol. Chem. 274, 24880–24886
64. Galbiati, F., Volonté, D., Engelman, J. A., Watanabe, G., Burkh, R., Pestell, R. G., and Lisanti, M. P. (1998) EMBO J. 17, 6633–6648
65. Smart, E. J., Ying, Y.-S., Conrad, P. A., and Anderson, R. G. W. (1994) J. Cell Biol. 127, 1165–1177
66. Hailstones, D., Sleer, L. S., Parton, R. G., and Stanley, K. K. (1998) J. Biol. Chem. 273, 665–670
67. Takahata, D., Sornet, C., and Grizard, J. (1999) J. Endocrinol. 162, 77–85
68. Dardevet, D., Sorret, C., and Grizard, J. (1999) J. Endocrinol. 162, 77–85
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