Up-Regulation of Intracellular Signalling Pathways May Play a Central Pathogenic Role in Hypertension, Atherogenesis, Insulin Resistance, and Cancer Promotion – the ‘PKC Syndrome’

M. F. McCARTY

Nutrition 21, 1010 Turquoise Street, Suite 335, San Diego, CA 92109, USA

Abstract — The modern diet is greatly different from that of our paleolithic forebears’ in a number of respects. There is reason to believe that many of these dietary shifts can up-regulate intracellular signalling pathways mediated by free intracellular calcium and protein kinase C, particularly in vascular smooth muscle cells; this disorder of intracellular regulation is given the name ‘PKC syndrome’. PKC syndrome may entail either a constitutive activation of these pathways, or a sensitization to activation by various agonists. The modern dietary perturbations which tend to induce PKC syndrome may include increased dietary fat and sodium, and decreased intakes of omega-3 fats, potassium, calcium, magnesium and chromium. Insulin resistance may be both a cause and effect of PKC syndrome, and weight reduction and aerobic training should act to combat this disorder. PKC syndrome sensitizes vascular smooth muscle cells to both vasoconstrictors and growth factors, and thus promotes both hypertension and atherogenesis. In platelets, it induces hyperaggregability, while in the microvasculature it may be a mediator of diabetic microangiopathy. In vascular endothelium, intimal macrophages, and hepatocytes, increased protein kinase C activity can be expected to increase cardiovascular risk. Up-regulation of protein kinase C in stem cells may also play a role in the promotion of ‘Western’ fat-related cancers. Practical guidelines for combatting PKC syndrome are suggested.

Introduction

It is well known that the transition from a hunter-gatherer lifestyle to modern dietary practices has entailed dramatic shifts in dietary intakes (1). It is equally clear that the age-adjusted incidence of many vascular diseases (coronary and peripheral atherosclerosis, essential hypertension, myocardial infarction), mature-onset diabetes, and various types of cancer (breast, colon, ovarian, and prostate, in addition to the smoking-related cancers) are vastly higher in modern society than they are in hunter-gatherer cultures, as
documented primarily by British physicians serving native populations in the (former) British empire (2–4).

Initial attempts to account for the likely protection afforded by a hunter-gatherer diet have focused primarily on its far higher fiber content (3). However, other aspects of such a diet may be equally germane to its protective merits (1). Wild game is typically far lower in fat than modern livestock, but tends to provide a higher proportion of omega-3 fats. The absence of dairy products or refined oils also reduces the fat content of hunter-gatherer diets and promotes a high relative omega-3 content. Vegetation-rich hunter-gatherer diets, undiluted by refined sugars or fats, are estimated to provide about 4-fold the potassium found in typical American diets, but are very low in sodium (prior to the introduction of salt). Magnesium and – surprisingly, in light of the absence of dairy products – calcium are much more richly supplied by most hunter-gatherer diets in comparison to typical American intakes, and it can be presumed that such diets are also rich in many trace minerals and certain vitamins (carotenes, C and K). Naturally, these vegetation-rich diets are also superior sources of protective phytochemicals.

Since the ancestors of modern-day humans were hunter-gatherers for millions of years prior to the invention of agriculture and animal husbandry about 10,000 years ago, it is reasonable to view the recent dramatic shifts in dietary intake as aberrant, and to expect some adverse consequences for physiological function and disease risk. Indeed, although reduced physical activity and certain modern pollutants (e.g. tobacco smoke) make an important contribution, the rise of ‘Western’ degenerative diseases is doubtless primarily attributable to the aberrant modern diet acting on genetically susceptible individuals – is often of equivalent importance in the pathogenesis of these vascular disorders.

While the pathogenic connections between the aberrant modern diet and modern degenerative diseases are clearly multifactorial in the extreme, I wish to propose that a characteristic derangement of intracellular signalling pathways, induced by a modern diet, plays a fundamental pathogenic role in many of these diseases. More specifically, I propose that, in vascular smooth muscle cells, skeletal muscle, and perhaps other tissues as well, a modern diet tends to induce an up-regulation or increased responsiveness of certain key intracellular signalling pathways – notably those mediated by free intracellular calcium and protein kinase C. I will refer to this disorder of cellular function as ‘the protein kinase C hyper-responsiveness syndrome’, or ‘PKC syndrome’ for short. I further postulate that this PKC syndrome plays a fundamental pathogenic role in atherogenesis, essential hypertension, hypertensive medial hypertrophy, coronary vasospasm, insulin resistance, platelet hyperaggregation, and the microvascular and macrovascular complications of diabetes – and, moreover, may exert a promotional effect in the genesis of certain major ‘Western’ cancers.

Aberrant behavior of vascular smooth muscle cells (VSMCs) clearly is a central pathogenic factor in vascular degenerative disease. Atherosclerotic lesions develop when VSMCs, stimulated by various paracrine factors (platelet derived growth factor, transforming growth factor-β, etc.) released from platelets, macrophages, or endothelial cells, migrate into the arterial intima, undergo a morphological transformation, grow and divide repeatedly, and initiate scarring by secreting collagen and other ground substance factors (5). The increased peripheral resistance that characterizes essential hypertension results both from excessive contractile activity of medial VSMCs as well as the hypertrophy of these cells which develops as the disease progresses. In each of these disorders, the increased contractile, mitogenic, or hypertrophic response of VSMCs is indicative of an increased concentration of vasoconstrictive/growth factors acting on these cells and/or a hyper-responsiveness of these cells to these stimuli.

Attempts to explain the pathogenesis of atherosclerosis and hypertension have usually focused on external agents which influence the production of the paracrine/hormonal factors stimulatory to VSMCs: oxidized LDL, activated platelets or macrophages, angiotensin conversion, sympathetic activity etc. I suggest that an increased sensitivity of VSMCs to these stimulatory factors – induced primarily by the aberrant modern diet acting on genetically susceptible individuals – is often of equivalent importance in the pathogenesis of these vascular disorders.

Role of \([Ca^{2+}]\) and PKC in regulation of vascular muscle

The paracrine/hormonal factors which stimulate contraction or mitosis in VSMCs typically trigger an increase in intracellular free calcium levels (\([Ca^{2+}]_i\)) and/or protein kinase C activity. Many of these stimulatory factors, including angiotensin, vasopressin, norepinephrine, and PDGF, bind to receptors that trigger a G protein-mediated activation of phospholipase C-β (6–9). The preferred substrate for this phospholipase, phosphatidylinositol-4,5-diphosphate, is then cleaved to yield diacylglycerol (DAG) and inositol-1,4,5-triphosphate (IP₃), both of which serve as second messengers (10,11). IP₃ diffuses to the sarcoplasmic reticulum, where it opens calcium channels, allowing the calcium stored in this organelle to stream into the cytoplasm and raise \([Ca^{2+}]_i\). This elevation of cyto-
plasmic free calcium induces a conformational change in soluble protein kinase C (PKC) that increases its affinity for both membrane phospholipids (notably phosphatidyl serine) and DAG. An increased proportion of PKC becomes membrane bound as a result of its increased affinity for phosphatidyl serine. Interaction of this membrane-bound PKC with membrane-associated DAG completes the activation of this enzyme, enabling it to phosphorylate serine and threonine residues in its substrates and thereby triggering a phosphorylation/dephosphorylation regulatory cascade.

The activation of phospholipase C-β is only transient, in part owing to its feedback down-regulation by activated PKC (12). Nevertheless, there is continued generation of DAG in the plasmalemma, apparently owing to delayed activation of a phospholipase D for which phosphatidylcholine is the preferred substrate (13,14), this activation may be mediated by PKC and/or G proteins (13). The continued activation of PKC also requires a new source of cytoplasmic calcium, since IP$_3$ production falls off and membrane calcium pumps (which are stimulated on PKC in another feedback loop) continuously remove calcium from the cytoplasmic compartment. This calcium is supplied by increased calcium influx through voltage-gated dihydropyridine-sensitive (L-type) calcium channels; this activated influx results both from a reduction of membrane potential and a shift in the voltage-dependency of the open probability of the L-type calcium channels (such that a given degree of depolarization leads to a higher open probability) (15). These effects appear to be mediated, at least in part, by PKC activity, which inhibits a calcium-activated potassium channel (thus reducing membrane potential) (16), and also has been reported to increase the open probability of L-type calcium channel (17). (However, it is not clear that PKC activity is the only reason for this increased calcium influx.) This calcium influx is not always sufficiently large to increase the [Ca$^{2+}$]i measured in the bulk cytoplasm, but apparently raises the free calcium concentration immediately beneath the plasmalemma, sufficient to maintain activation of membrane-bound PKC (18). The tonic vasoconstrictive or the mitogenic response of VSMCs to agonists which activate PKC is substantially inhibited by calcium channel blocker drugs, indicating that calcium influx through L-type calcium channels plays an obligate role in these processes (15,18–20).

It should be noted that agents or measures which provoke an increase in [Ca$^{2+}$]i, without concurrently increasing DAG generation, will not in themselves activate PKC; they will, however, sensitize PKC to activation by other agonists which do simulate DAG production. Increased [Ca$^{2+}$]i induces membrane binding of PKC (21,22); only membrane-bound PKC can be activated by DAG, which is of course lipophilic and confined to membranes. Conversely, agents which increase DAG production will sensitize PKC to activation by calcium-releasing agonists.

Vasoconstriction in VSMCs is a complex process involving both an initial and tonic phase, and requiring a transient increase in [Ca$^{2+}$]i; and a sustained activation of PKC (23). The transient increase in [Ca$^{2+}$]i mediated by generation of IP$_3$, leads to a calmodulin-dependent activation of myosin light chain kinase and phosphorylation of myosin light chain, resulting in activation of the myosin ATPase and the initial phase of contraction. However, [Ca$^{2+}$]i, as well as the phosphorylation state of myosin light chain, subside to basal level within a few minutes, but tonic contraction is nevertheless maintained. This tonic phase of contraction is dependent on PKC activity (as it is prevented by specific PKC inhibitors), and apparently results from PKC-mediated phosphorylation of other filament-associated proteins such as desmin and caldesmon; the exact mechanisms involved in tonic contraction still remain to be determined. As noted, calcium influx is required to maintain this tonic contraction (apparently to sustain PKC activity), even though [Ca$^{2+}$]i remains near basal levels.

In light of the well-known role of PKC in tumour promotion, it is not surprising that agonists which activate PKC activate PKC in VSMCs tend to induce either mitosis or hypertrophy (9,24–26). The VSMCs in situ in the arterial media are not capable of division, but those that migrate into the arterial intima undergo a morphological transformation in which they lose the ability to contract but gain the ability to multiply and secrete collagen; in effect, they become more similar to fibroblasts (5,19,27). Obviously, this is the form of VSMCs that can be grown in tissue culture. The PKC-activating stimuli which provoke mitosis in intimal VSMCs or VSMC cultures, instead induce hypertrophy in medial smooth muscle (24). (As a clinical correlate of this observation, it should be noted that treatment of hypertensives with angiotensin-converting enzyme inhibitors often leads to a regression of medial hypertrophy; 28.) The morphological transformation of intimal smooth muscle cells also alters their expression of growth factor receptors; they express fewer receptors for angiotensin, but more receptors for PDGF (27); this explains why angiotensin is more crucial to the genesis of hypertension, whereas PDGF plays a greater role in the induction of atherosclerosis. The ability of PDGF to stimulate mitosis or DNA synthesis in VSMCs in culture is apparently dependent on PKC activation, since inhibition of PKC, or its down-regulation by protracted phorbol ester treatment, substantially abrogates the mitotic response to PDGF (9,26).
PKC activation also plays a prominent role in mitotic signalling in many other tissues. Thus, joint administration of calcium ionophores (which increase \([\text{Ca}^{2+}]\)) and phorbol esters (which activate PKC) is sufficient to induce cell division in some cell cultures (9,29). In other cell lines, these measures are insufficient to induce mitosis, but do potentiate the mitogenic response to growth factors that stimulate tyrosine kinase activity (9,30). The cancer-promotional activity of phorbol esters presumably reflects an increased mitotic rate of carcinogen-damaged stem cells.

Since free intracellular calcium plays a crucial role not only in the activation of many isoforms of PKC, but also exerts calmodulin-dependent effects on vasoconstriction and mitogenesis (23,31), its concentration must be tightly regulated. Calcium which flows into the cytoplasm through activated calcium channels in the sarcoplasmic reticulum or plasma membrane, is rapidly extruded from the cytoplasm by two types of membrane pump: a Ca-ATPase (high affinity, low capacity) and a Na/Ca exchanger (low affinity, high capacity) (32). As its name implies, the Ca-ATPase is driven by free energy supplied by the hydrolysis of ATP, whereas the Na/Ca exchanger is driven by the transmembrane sodium gradient (as established by the Na,K-ATPase). These pumps extrude calcium to the extracellular space; a separate isoform of Ca-ATPase pulls calcium into the sarcoplasmic reticulum. These pumps work so efficiently that the transmembrane sodium gradient under basal conditions is about 10,000 fold (e.g. \([\text{Ca}^{2+}]\) \(\approx 10^{-7}\) M, serum calcium \(\approx 10^{-3}\) M).

In light of its relatively low affinity for calcium, it has been suggested that the Na/Ca exchanger in VSMCs is only physiologically important in 'emergency' situations in which \([\text{Ca}^{2+}]\) is unduly increased. However, since the calcium concentration directly beneath the plasmalemma may often be higher than that measured in the bulk cytosol (18,23), this may not necessarily be the case. Blaustein and colleagues have presented evidence that the transmembrane sodium gradient has an important impact on regulation of intracellular calcium; when the sodium gradient is reduced (by incubating cells in low-sodium media or inhibiting the Na,K-ATPase), basal \([\text{Ca}^{2+}]\) increases, and the amplitude and duration of \([\text{Ca}^{2+}]\) transients in agonist-stimulated cells increases as well (33). The findings are consistent with an important physiological role for the Na/Ca exchanger in regulation of \([\text{Ca}^{2+}]\).

This brief overview of VSMC function, as severely oversimplified as it obviously is, nevertheless provides a framework for understanding the potential effects of various nutritional measures and metabolic conditions on PKC activation and intracellular calcium metabolism in VSMCs.

**Insulin resistance – cause and effect of PKC syndrome?**

The modern diet seems virtually 'custom-designed' for the induction of insulin resistance. As contrasted with paleolithic fare, typical modern diets are exceptionally high in fat (though usually low in omega-3), low in fibre but high in fructose and rapidly assimilated carbohydrates, a poor source of minerals such as potassium, magnesium, and bioavailable chromium, but very high in sodium. Clinical and/or animal studies indicate that each of these dietary perturbations can exert an adverse effect on insulin sensitivity or glucose tolerance (34–41) – though undoubtedly the most important factor is the high fat intake. In addition, the fatty refined modern diet – in conjunction with a more sedentary lifestyle, which of course promotes insulin resistance in and of itself – is ideal for the induction of abdominal obesity, another major cause of insulin resistance (42,43). In light of these considerations, it is not surprising that about a quarter of American adults display a degree of insulin resistance comparable to that seen in type II diabetics (44); most of these individuals avoid diabetes only by a substantial upregulation of insulin secretion, but nevertheless tend to show mildly impaired glucose tolerance. Unfortunately, an increasing proportion become overtly diabetic with increasing age as the efficiency of insulin secretion declines.

The association between insulin resistance, essential hypertension, and cardiovascular disease is now well known, and there is considerable reason to believe that this association is causative in nature (45,46). In light of the facts that insulin can promote sodium retention and activate the sympathetic nervous system, and has stimulated growth of VSMCs in certain cell culture studies, many experts have concluded that hyperinsulinemia mediates the association between insulin resistance and vascular disorders (46,47). However, recent studies cast considerable doubt on this thesis (48–51). Indeed, it is now clear that insulin has a direct vasodilatory effect on resistance vessels supplying insulin-sensitive tissues (such as skeletal muscle), and that this mechanism plays a physiological role in expediting the postprandial storage of glucose (52,53); this mechanism works less effectively in insulin-resistant subjects, contributing to their glucose intolerance. Moreover, the stimulatory effect of insulin on VSMCs in culture has been traced to activation of IGF-1 receptors by supraphysiological concentrations of insulin (54) – an effect which might be mean-
ingful in vivo (if at all) only in the most extreme cases of hyperinsulinaemia. In addition, in light of increasing evidence that hypertension reflects decreased insulin activity on VSMCs in resistance vessels, it would seem paradoxical to insist that atherogenesis nevertheless reflects excessive insulin action on VSMCs in conduit vessels. Finally, the lipid abnormalities typically associated with insulin resistance (high triglycerides, low HDL cholesterol) are likely to result from a net deficit of insulin activity (55). Thus, it appears increasingly likely that insulin resistance per se, leading to subnormal insulin activity in VSMCs, is a causative factor in vascular disease.

The lipid and hemostatic perturbations associated with diabetes and insulin resistance, while no doubt pathogenically significant, nevertheless appear insufficient to explain the greatly increased cardiovascular risk associated with these conditions (51,56). Thus, it is reasonable to look for direct effects of insulin resistance on the arterial wall.

The effects of insulin on PKC activity and intracellular calcium metabolism are complex, and no doubt are tissue-specific. In some cell cultures – notably the BC3H-1 myocytes studied by Farese and co-workers – insulin treatment increases PKC activity, apparently owing to stimulated de novo synthesis of DAG (57). Also, there is evidence that some effects of insulin receptor activation may be mediated by a G protein-linked stimulation of an isoform of phospholipase C that cleaves specific phosphatidylinositolglycans, liberating phosphoinositolglycan as a second messenger (58,59); this process will of course concurrently generate DAG, which presumably would participate in the activation of PKC.

However, a number of investigators have failed to observe PKC activation in insulin-treated cells (60). Blackshear’s group, assessing PKC activity in intact cells by measuring phosphorylation of the MARCKS protein, found that insulin increased PKC activity slightly in only one of five fibroblast cell lines examined (61). Furthermore, these investigators showed that insulin exerted its full range of effects on cells in which PKC activity had been abolished by phorbol ester down-regulation; this shows that – contrary to some speculation – PKC is not likely to be an obligate mediator of any effects of insulin.

With respect to VSMCs, there is intriguing recent evidence that insulin-pretreatment dampens the [Ca\textsuperscript{2+}]\textsubscript{i} response to various hormones which activate PKC by stimulating phospholipase C-\(\beta\); this effect has so far been demonstrated for the agonists angiotensin II, arginine vasopressin (48,62–65), and serotonin. For example, Sowers and colleagues found that, in VSMCs pretreated with insulin for 30 minutes, the [Ca\textsuperscript{2+}]\textsubscript{i} transients elicited by vasopressin were smaller and the inward calcium current was decreased; this latter effect reflected a rightward shift in the voltage-dependency of the open probability of L-type calcium channels (i.e. open probability was less at a given degree of depolarization), as well as reduced influx through a receptor-operated channel (62). These data suggest that, in VSMCs, insulin activity may induce a conformational change in the L-type calcium channels – in effect, mimicking the action of dihydropyridine calcium channel blocker drugs. In addition, insulin may impede the ability of IP\textsubscript{3} to release calcium from the sarcoplasmic reticulum, as demonstrated by Saito et al (63). Further studies show that insulin pretreatment accelerates [Ca\textsuperscript{2+}], recovery to baseline in either rat or human VSMC cultures stimulated with either angiotensin II or vasopressin (64), and that such pretreatment also attenuates the contractile response of VSMCs (measured by photomicroscopy) to angiotensin II or serotonin (66).

More recently, Zemel’s group has reported that insulin stimulates calcium efflux in phenylephrine-contracted VSMCs by increasing activity of the plasmalemma Ca-ATPase (65), and that relatively prolonged insulin pretreatment induces increased synthesis of the Ca-ATPase (as indicated by increased levels of the mRNA for this protein) (48). Whereas the acute effects of insulin noted above might be responsible for the postprandial vasodilatory action of insulin, the inductive effect on Ca-ATPase might be expected to influence vasal tone during the postabsorptive phase. It thus appears that, in VSMCs, insulin induces a coordinate response that both impedes calcium influx and promotes calcium efflux during agonist stimulation.

In skeletal muscle, insulin is known to have a rapid stimulatory effect on the Na,K-ATPase (67); I am not aware of data in this regard relating to VSMCs. If in fact insulin does exert this effect on VSMCs, then it would be expected to hyperpolarize the cells – reducing calcium influx through voltage-gated channels – and to promote Na/Ca exchange by lowering [Na\textsuperscript{+}]. (The impact of Na,K-ATPase activity on vasoregulation will be discussed below in greater detail in the context of sodium/potassium nutrition.)

The reduced [Ca\textsuperscript{2+}]\textsubscript{i} response to vasopressor/growth factor agonists in insulin-treated VSMCs presumably should limit the concurrent activation of PKC, but these investigators did not measure PKC activity. However, yet unpublished studies by Zemel indicate that insulin pretreatment impedes the induction of c-fos by angiotensin II; since this inductive effect of angiotensin appears to be mediated by PKC (68), this constitutes suggestive evidence that insulin may indeed limit PKC activation by angiotensin. The reduced contractile response in insulin-treated VSMCs
is also suggestive of reduced PKC activation (66,69,70). Clearly, further studies are needed to provide direct confirmation that PKC activation by phospholipase C-β-linked agonists can indeed be impeded by insulin in VSMCs.

These findings in cell culture investigations have correlates in in vivo studies. Norepinephrine, a well-known physiological vasopressor, activates PKC via α1 receptors in VSMCs and other tissues. A number of years ago, Alexander and Oake presciently reported that insulin pretreatment attenuates the vasoconstrictive response to norepinephrine in rat tails (71). More recently, it has been demonstrated that the contractile response to norepinephrine of aortas or mesenteric arteries from streptozotocin-diabetic rats is markedly enhanced (72,73); pretreatment with either calcium channel blockers or the PKC inhibitor staurosporine abolishes the difference in response between control and diabetic animals. These findings suggest that lack of insulin activity enhances the activation of PKC by norepinephrine, in large part owing to increased calcium influx through L-type calcium channels. Increased pressor responsiveness has also been reported in insulin-resistant Zucker rats (74) and in type I diabetics (75).

Since chromium's physiological role is to support the insulin responsiveness of various tissues (41), and in light of recent studies by Evans demonstrating that pre-incubation with chromium picolinate can markedly potentiate insulin action on rat myoblasts (76,77), Zemel has initiated studies to define the effects of chromium picolinate pre-incubation on calcium regulation in VSMCs. His findings confirm previous observations that insulin dampens the angiotensin-induced [Ca++]i transients and induces the Ca-ATPase, and moreover demonstrate that these effects are larger in VSMCs pre-treated with chromium picolinate (78). However, he was surprised to find that chromium picolinate alone, in the absence of insulin, also exerts these effects. Indeed, it is not yet clear from the data whether the substantial response to the joint application of insulin and chromium represents a potentiation of insulin, or rather reflects an additive response to the two agents. In any case, these data suggest an important role for chromium picolinate in control of PKC syndrome – with the caveat that the studies so far reported by Zemel have used supraphysiological (1 μM) concentrations of chromium. Clinical studies to define the impact of various doses of chromium picolinate on essential hypertension, and on insulin sensitivity and glycemic control in subjects with type II diabetes or insulin resistance, are currently in progress.

The studies cited above make it reasonable to postulate that physiological concentrations of insulin should reduce the mitogenic response of VSMCs to growth factors such as PDGF or angiotensin whose effects are mediated largely by PKC activation; if this prediction can be confirmed experimentally, it may provide important insight into the link between insulin resistance and atherogenesis. Zemel's demonstration that insulin can block angiotensin's induction of c-fos, appears consistent with this prediction. Inasmuch as insulin appears to mimic the action of calcium channel blockers on L-type calcium channels in VSMCs (62), it should also be noted that nifedipine treatment of rat VSMCs incubated with either serum or PDGF, inhibits both DNA synthesis and the morphological transformation of VSMCs to a more fibroblast-like phenotype (19); calcium channel blockers also inhibit the mitotic response to angiotensin (20). In many animal models of atherogenesis, calcium channel blocker drugs reduce the severity of arterial lesions (79–81); clinically, administration of these drugs has been shown to retard the progression of stenotic arterial lesions (82). It is probably not coincidental that Abrahams and colleagues have repeatedly noted that chromium administration can retard the development and accelerate the regression of arterial plaques in cholesterol-fed rabbits (83–85) (a finding which could not be confirmed by one group) (86). There is also suggestive epidemiological evidence that good chromium nutrition or increased serum chromium may be associated with reduced risk for atherosclerosis (87–89).

The foregoing suggests that insulin resistance or insulinopenia, by impeding control of [Ca++]i, may be one possible cause of PKC syndrome, at least in VSMCs. However, there is reason to believe that the converse may also be the case – namely that increased activation of PKC and/or increased [Ca++]i, may be clinically important causes of insulin resistance.

**PKC syndrome may induce insulin resistance**

Activity of the insulin receptor – both with respect to its tyrosine kinase activity and other putative actions – is regulated not only by insulin binding, but also by the phosphorylation status of the cytoplasmic portion of the receptor's β-subunit (90). As is well known, autophosphorylation of three key tyrosine residues on the β chain is necessary for optimal tyrosine kinase activity (91). However, a number of serines and threonines in the β chain are also susceptible to phosphorylation, and their phosphorylation status can have an important impact on the ability of the insulin-bound receptor to autophosphorylate and ex-
press tyrosine kinase activity (92–95). Clinical studies indicate that insulin receptors isolated from various tissues of type II diabetics or obese subjects, have an impaired capacity to autophosphorylate or express tyrosine kinase activity when exposed to insulin - despite the fact that receptor affinity for insulin has usually been found to be normal (96–102). Since genetic mutations of the insulin receptor are found in only a very small proportion of diabetics (who typically experience severe insulin resistance from birth), the most reasonable explanation for this phenomenon is an altered phosphorylation state of the β chain. Not surprisingly, PKC activity can have an important impact on this phosphorylation state.

PKC appears to increase phosphorylation at some sites on the β chain (perhaps by a direct action) and reduce phosphorylation at other sites (presumably by activating a serine phosphatase) (92–95,103). Whether in studies with isolated receptors, or in intact cells, activation of PKC has been shown to ‘turn off’ the insulin receptor. This effect has been attributed to phosphorylation of a specific threonine residue near the carboxyl end of the β chain (95). However, since PKC can also ‘turn off’ mutant insulin receptors which lack this residue (104), this is clearly not the only means by which PKC deactivates the receptor. An important study by Siddle’s group shows that PKC has constitutive activity in the suppression of insulin receptor function; down-regulation of PKC by chronic exposure to phorbol esters in a cell line that over-expresses the human insulin receptor leads to a 2-fold enhancement of the tyrosine kinase activity expressed by the insulin-stimulated receptors (103). Surprisingly, these hyper-reactive receptors show a net enhancement of phosphorylation state, suggesting that PKC-mediated activation of a phosphatase (or deactivation of a kinase) plays a physiological role in restraining insulin activity.

It should be noted that PKC may also antagonize insulin activity at a post-receptor level. For example, it somehow inhibits activation of the phosphatidylinositol glycan-specific phospholipase C mentioned above (59).

Thus, the key question becomes: is increased PKC activity a significant, common cause of clinical insulin resistance? This cannot readily be answered, since specific inhibitors of PKC are not available for clinical use. Possibly relevant is a recent clinical demonstration that mega-dose vitamin E treatment improves insulin sensitivity in diabetics (105); in vitro, d-α-tocopherol can inhibit PKC activity (106), though it is not known whether clinically feasible doses of this vitamin can achieve this effect in vivo. In several animal models of insulin resistance (Zucker rats, starvation diabetes, denervated muscle), PKC activity and/or DAG levels have been reported to be elevated, consistent with a role for PKC activation in the observed insulin resistance (107–109).

Poor glycemic control in type I diabetics can promote insulin resistance. In insulinopenic animal models of diabetes, elevated PKC activity and/or increased DAG levels have been reported in many tissues (110–116). This has been attributed to increased de novo DAG synthesis induced by hyperglycaemia (112,113). Haring and colleagues have proposed that hyperglycaemia-induced insulin resistance is mediated by activation of PKC, and have shown that the inhibitory effect of high glucose levels on insulin tyrosine kinase activity in cultured adipocytes, is eliminated by inhibitors of PKC (117). Several investigators have suggested that the elevation of PKC activity observed in diabetic tissues may be a significant mediator of both the microvascular and macrovascular complications of diabetes (112–115).

If high levels of glucose can stimulate de novo DAG synthesis, is it not reasonable to suspect that, by mass action, increased levels of free fatty acids will have a similar effect? Increased levels or flux of free fatty acids, in serum or tissues, can be expected in individuals with abdominal obesity (43), or who consume high-fat diets (118) – conditions which are clearly associated with insulin resistance. It is pertinent to note that feeding high-fat diets reduces the tyrosine kinase activity of insulin receptors in rats (119,120) – an effect which conceivably could be mediated by PKC activation. In rat studies attempting to define the mechanism of the cancer-promotional activity of high-fat diets, Birt and colleagues have measured increased DAG levels as well as elevated PKC activity in skin epithelial cells of high-fat-fed rats (121,122). Activation of PKC has also been reported in the colonic epithelium of high-fat-fed rats (123,124); in this circumstance, colonic bacteria may contribute to the increased production of DAG (125).

A stimulatory effect of increased fatty acid availability on DAG synthesis might thus play a role in the insulin resistance and increased cancer risks associated with both fatty modern diets and obesity. This fascinating possibility clearly merits much further evaluation. (The more traditional view – that increased FFA oxidation mediates the reduction in insulin sensitivity – is not tenable; 126.)

In circumstances in which DAG synthesis – and thus presumably phospholipid synthesis – is enhanced, it seems reasonable to expect an accompanying up-regulation of phospholipase activity; otherwise, steady-state phospholipid levels could not be maintained. Such an up-regulation would seem likely to increase...
production of DAG and possibly other second messengers from membrane phospholipids. Indeed, there are two reports that unsaturated free fatty acids increase phospholipase C-β activity in cell cultures (127,128).

Whether or not increased PKC activity is a clinically important cause of insulin resistance – the data certainly appear consistent with this possibility – Siddle’s data, indicating that PKC is a constitutive regulator of the insulin receptor (103), suggest that circumstances which elevate PKC activity in a tissue will concurrently impair the insulin responsivity of that tissue. In conjunction with data cited previously, this suggests a vicious circle mechanism in VSMCs: insulin resistance will render PKC more susceptible to activation by agonists linked to phospholipase C-β; increased PKC activity, in turn, will exacerbate the insulin resistance. Presumably, a similar effect would obtain in any other tissue in which insulin restrains the activation of PKC.

The other salient features of PKC syndrome is elevation of [Ca^{2+}]. In adipocytes, increased [Ca^{2+}] has been shown to impair insulin responsivity; whether PKC activation plays any role in this is not clear (129,130). Whether a similar effect can occur in VSMCs deserves evaluation. If so, elevated [Ca^{2+}] could also play a role in the vicious circle mechanism hypothesized above.

The foregoing invites speculation regarding the epidemiological link between insulin resistance and hypertensive/atherogenic disease. It is unlikely that hyperinsulinemia or excess insulin activity mediates this connection; conversely, a net decrement of insulin activity and/or insulin resistance per se may not be the most central factor. I propose that insulin resistance is typically associated with and induced by characteristic alterations of intracellular signalling pathways – including, but not necessarily limited to, PKC syndrome – and that, operating at the level of VSMCs, this disorder of intracellular regulation not only induces insulin resistance, but pari passu increases sensitivity to vasoconstrictor/growth factor agonists, and is thereby primarily responsible for the increased risk of vasculopathy associated with insulin resistance. In the treatment of patients with insulin resistance or type II diabetes, measures which correct insulin resistance are likely to be protective, insofar as this correction reflects a normalized function of the regulatory pathways which mediate insulin resistance. Thus, agents which directly promote insulin sensitivity (e.g. biguanides, chromium) are likely to prove more protective than are measures which increase insulin levels (sulfonylureas, injectible insulin). Nevertheless, increased net insulin activity, however it is achieved, may have a beneficial impact on VSMC function, either directly – by promoting better control of [Ca^{2+}], or indirectly – by improving glycemic control and thus limiting de novo synthesis of DAG.

Protective effects of omega-3 fats

Whereas high-fat diets in general appear likely to increase PKC activation, with adverse consequences for both vascular and cancer risk, the long-chain omega-3 polyunsaturates from fish oil (notably EPA/DHA) may indeed act to limit PKC activation under certain circumstances.

Several reports indicate that fish-oil-rich diets tend to reduce elevated blood pressure (131,132). More recently, it has been demonstrated that fish-oil-feeding tends to reduce the vasoconstrictive response to agonists such as angiotensin II and norepinephrine in humans (133,134). In an effort to explain these findings, Falardeau and colleagues have studied the effects of vasopressin on rat VSMCs pre-incubated with various fatty acids, including eicosapentaenoic acid (EPA) (135). After 24-hour exposure to 30 µM EPA (and fat-free albumin), which significantly increased the EPA content of all classes of membrane phospholipids, the cells were washed, resuspended in a fatty acid-free medium, and treated with vasopressin. As compared with cells pre-exposed to other fatty acids or with untreated control cells, the EPA pretreatment virtually abolished the ability of vasopressin to increase membrane content of DAG. This suggests that cell membranes enriched in EPA somehow block the ability of the vasopressin receptor to activate phospholipase C-β.

An analogous effect is suggested by a previous study in which rat VSMCs were pre-incubated for at least 2 weeks with various concentrations of fish oil or EPA (136). After washing, the cells were exposed to either angiotensin II or LDL. The fish oil/EPA pre-treatment was found to significantly decrease agonist-stimulated IP₃ release – again suggesting a fish oil-mediated suppression of phospholipase C-β activation. Thus, this effect does not appear to be specific to the vasopressin receptor. The general anti-vasopressor activity of fish oil feeding suggests that this may be a general effect that could be elicited with other agonists such as norepinephrine and PDGF – more studies along these lines are warranted.

Not surprisingly, fish oil-fed animals appear to be relatively resistant to cholesterol-induced atherogenesis (137). Aboriginal Eskimos have been reported to have low risk for cardiovascular disease, and some epidemiological studies suggest that increased fish consumption is protective in other populations as well (137). In coronary bypass patients, fish oil supplements have retarded restenosis of the graft in some
Na,K-ATPase acts in kidney tubules to foster the linolenic acid (DHLA) of membrane phospholipids; the generation of certain arachidonate-derived second effects (154). It is quite conceivable that, by impeding bition of the transforming enzymes. For example, the models (152,153). EPA tends to act as an arachidonic acid antagonist - both by reducing the arachidonic acid content of membranes, and by competitive inhibition of the transforming enzymes. For example, the ability of fish oil to inhibit leukotriene production may be partially responsible for its anti-inflammatory effects (154). It is quite conceivable that, by impeding the generation of certain arachidonate-derived second messengers, omega-3s may help to restrain the inappropriate cellular proliferation involved in both atherogenesis and cancer promotion.

Although there does not appear to be any evidence that paleolithic diets were unusually rich in gamma-linolenic acid (GLA), it is of interest to note that GLA-enriched diets have exerted anti-promotional or growth retarding effects analogous to those of fish oil in certain animal cancer models (149,155-157). GLA supplementation increases the dihomo-gamma-linolenic acid (DHLA) of membrane phospholipids; conceivably in a manner analogous to EPA, DHLA limits the production of certain arachidonate-derived second messengers. It would be interesting to determine whether membrane enrichment with DHLA exerts any inhibitory effect on PKC activation.

The sodium/potassium ratio – influence on ion transport

In addition to its role in maintaining membrane potential and appropriate intracellular cation levels, the Na,K-ATPase acts in kidney tubules to foster the retention of sodium and the excretion of potassium. In land animals eating natural diets, this is an extremely important action, since ample amounts of potassium are ingested, but very little sodium. The adrenal cortical hormone aldosterone functions to increase the expression of this enzyme in kidney tubules; appropriately, aldosterone secretion is stimulated directly by increased serum potassium, and indirectly by decreased serum sodium (via renal recruitment of the renin/angiotensin systems). However, in the presence of vascular volume expansion and/or increased sodium intake, the activity of the renal Na,K-ATPase can become counterproductive. Recent studies show that these circumstances provoke adrenal secretion of a steroid-like hormone – known as the endogenous digitalis-like hormone, or EDLS – which may be structurally identical to digitalis, and, like digitalis, inhibit the Na,K-ATPase in kidney tubules, helping to prevent volume overload and hyponatremia (158-162).

The actions of EDLS and aldosterone are not confined to the renal tubules, however. EDLS is equally effective in inhibiting the Na,K-ATPase of other tissues. Additionally, there is recent evidence that aldosterone promotes the synthesis and/or the activity of the Na,K-ATPase in certain other tissues, including vascular muscle cells (163-166).

The typical paleolithic diet provided about 16 times as much potassium as sodium (1). Among modern hunter-gatherers who do not salt their food, such as Brazil’s Yanomamo Indians, a similar ratio is seen (167). In contrast, the typical salted modern diet is prodigiously higher in sodium, while potassium intakes have plunged about 4-fold, as calorically-dense fatty refined foods have largely supplanted the vegetation prominent in paleolithic diets. As a result, sodium and potassium contents are roughly equal in most modern diets, and in some groups sodium intake far exceeds potassium intake.

This extraordinary perturbation of electrolyte intakes has profound implications for aldosterone and EDLS production. The Yanomamos secrete approximately 20-fold as much aldosterone as the ‘civilized’ scientists who have studied them (167); looking at this another way, humans are now producing about 5% as much aldosterone as their paleolithic forbears. In individuals who have a genetically-linked tendency to sodium retention, the modern diet also promotes a substantial increase in EDLS production (162).

The implications for the Na,K-ATPase activity of VSMCs – particularly in individuals who overproduce EDLS – are grim. With less aldosterone to induce this enzyme, and more EDLS to inhibit it, this pump naturally functions less efficiently, as reflected by the increased intracellular sodium content regularly observed in essential hypertension (168-170). The lower serum potassium associated with a reduced-
beyond its antihypertensive action. However, the benefits of a high-potassium diet extend - and an increased production of aldosterone. VSMCs by stimulating the electrogenic sodium pump levels - which increases membrane polarization in reflecting elevated blood pressure (175,176). The hypotensive effect of increased potassium intake presumably implies that the open probability of the voltage-gated calcium channels of the plasma membrane will be greater at baseline, and will be increased more substantially by agonists that reduce membrane potential further. However, the cells will be less prepared to cope with the expected increased influx of calcium, since the reduced transmembrane sodium gradient will inhibit the activity of the Na/Ca exchanger (33). This should result in increased [Ca²⁺]i, under both basal and stimulated conditions, most likely coupled with increased activation of PKC. This naturally translates into increased peripheral resistance and increased sensitivity to vasopressor agonists - as is typical of essential hypertension. Not surprisingly, serum EDLS levels correlate positively with blood pressure (159,162).

It has been observed that essential hypertension is virtually absent even in elderly members of societies which do not salt their food (172-174). Furthermore, the typical age-related rise of blood pressure - even in non-hypertensives - seen in modern cultures, is absent in these no-salt societies. Owing presumably to large individual genetic differences in susceptibility, and relatively narrow differences in sodium intake, intra-cultural studies have not always been able to correlate 24-hour sodium excretion with blood pressure or risk for hypertension. However, the largest multi-cultural study - the so-called INTERSALT study - shows that in within-population analyses involving over 10,000 persons, sodium excretion correlates directly and potassium excretion correlates inversely with systolic blood pressure (174).

While dietary sodium restriction is commonly recommended for blood pressure control, potassium supplementation is virtually equally effective for lowering elevated blood pressure (175,176). The hypotensive effect of increased potassium intake presumably reflects both a modest increase in serum potassium levels - which increases membrane polarization in VSMCs by stimulating the electrogenic sodium pump (171) - and an increased production of aldosterone. However, the benefits of a high-potassium diet extend beyond its antihypertensive action.

Tobian, working with sodium-sensitive strains of rats that develop not only hypertension, but also medial hypertrophy, cardiomegaly, and stroke on high-sodium diets, has shown that increased potassium intakes tend to prevent all of these sequelae of hypertension, even when blood pressure is not reduced (177). This is paralleled by findings from the Rancho Bernardo epidemiological study; when individuals with equivalent blood pressures were matched, increased potassium intake equivalent to one banana per day was associated with a 40% reduction in stroke risk (178)! Tobian has also reported that, in salt-fed salt-sensitive rats, increased potassium intakes act to preserve healthful function of vascular endothelium (179), and, in the context of fat feeding, reduce LDL uptake by the arterial wall (180). (In light of these findings, it is very unfortunate that the new US government-mandated food labels do not require a listing of potassium content. Whereas individuals can readily assure - if they are so inclined - ample intakes of most micronutrients by using appropriate supplements, they are constrained to obtain most of their potassium from foods. Choosing potassium-rich foods would obviously be much easier if potassium contents were listed.)

One feasible means of increasing the potassium/sodium ratio of modern diets is to replace ordinary salt with modified salts in which sodium chloride is partially replaced by potassium and magnesium salts. One of these products, commercially available in a few countries under the name `Pansalt', is said to have flavor virtually indistinguishable from ordinary salt, and, when substituted wholesale for ordinary salt in the diets of institutionalized elderly subjects, not only lowers blood pressure, but also improves glucose tolerance (39,181-183). (Perhaps this improvement in glucose tolerance reflects a more effective vasodilatory action of postprandial insulin on resistance vessels in skeletal muscle.) Unfortunately, since the great majority of sodium in modern diets comes from pre-packaged convenience foods and restaurants rather than the kitchen salt shaker, this protective potential will have little practical import until most food manufacturers can be persuaded to use a slightly higher-priced salt substitute in place of dirt-cheap sodium chloride - which is unlikely without government mandate. (Since the US government apparently is only dimly aware that potassium exists, I won't hold my breath.) In the meantime, concerned individuals can try to avoid overtly salty foods and include more fruits and vegetables in their diet.

In passing, I should comment further on the seemingly paradoxical notion that aldosterone may help to keep blood pressure low. As is well known, inappropriately increased aldosterone secretion (hyperaldosteronism), in the context of a heavily salted
modern diet, increases blood pressure. This is because the sodium-retaining action of aldosterone causes volume expansion, and also promotes vasopressin production by inducing hypernatraemia; vasopressin is a potent vasoconstrictor (184). However, the direct effect of aldosterone on arteries – an enhanced expression of Na,K-ATPase – is vasodilatory (163,164). In the context of a very-low-sodium paleolithic diet, high aldosterone secretion does not promote volume overload or vasopressin secretion, and moreover is mandatory for survival.

**Anti-hypertensive actions of calcium and magnesium**

As noted, the paleolithic diet, despite the absence of dairy products, was a rich source of calcium (1). This calcium was supplied primarily by green vegetation and animal bones. Modern adults who don’t drink milk may be getting less than half as much calcium as their paleolithic forbears.

McCarron is primarily responsible for initiating interest in calcium as an antihypertensive factor (185). Starting with the hypothesis that dietary calcium might increase hypertensive risk (since intracellular calcium mediates vasoconstriction), he was surprised when his research findings led to the diametrically opposite conclusion. In within-population epidemiological surveys, dietary calcium intakes have often proved to be more predictive of blood pressure than sodium intakes. The relationship is inverse – low calcium intakes typically correlate with increased blood pressure and increased risk for hypertension. These findings have induced many investigators to examine the effects of supplementary calcium in hypertensives. Most of these studies verify that supplemental calcium can indeed produce a modest reduction in blood pressure. Benefit may be most regularly achieved in those with low-renin hypertension (186,187), although the data are not unanimous on this point.

The mechanism of dietary calcium’s influence on blood pressure may be analogous to the impact of sodium and potassium. Serum calcium levels must be tightly controlled, in the face of greatly varying dietary calcium intakes. For this reason, calcium ingestion modulates the production of hormones – parathyroid hormone (PTH), calcitriol (1,25 dihydroxycholecalciferol), and calcitonin – which influence calcium transport at the level of the intestinal mucosa, bone, and renal tubules. However, as in the case of the hormones which regulate sodium and potassium transport, the influence of these hormones apparently extends to other tissues, including vascular smooth muscle.

Calcitriol appears to be the best candidate for mediating the impact of low dietary calcium on hypertensive risk. PTH, which tends to rise on low-calcium diets, actually has a direct vasodilatory effect on resistance vessels (188) – as do other agonists which increase intracellular cAMP levels. As for calcitonin, its physiological role is still in doubt, and in any case there do not appear to be any data relating it to blood pressure control. However, studies from a number of groups provide suggestive evidence that the increased calcitriol levels evoked by low-calcium diets may indeed exert a pressor effect.

Epidemiological data appear to be consistent with this possibility. Thus, calcitriol levels are elevated in individuals with low-renin hypertension – the subgroup which may be most responsive to supplemental calcium (189). In another study, both PTH and calcitriol were significantly elevated in the hypertensive group as a whole in comparison to normotensive controls (190). When blood pressure and calcitriol were measured in 373 women aged 20–80, calcitriol levels showed a highly significant correlation with both systolic and diastolic blood pressure; in multiple regression analyses, calcitriol explained as much of the variability in blood pressure as did Quetelet’s index (a measure of obesity; (191)). When normotensive offspring of hypertensive Japanese were placed on a high-sodium diet, their calcitriol levels increased significantly, and these increases correlated with the observed increases in blood pressure (192). (Note that sodium exerts a calciretic effect, and thus reduces calcium availability.)

At physiological concentrations (~1 nM), calcitriol has been shown to produce a rapid influx of calcium through L-type calcium channels in rat osteosarcoma cultures (193). The effect of calcitriol is analogous to that of the calcium channel agonist BAY K8644, and leads to a shift in the voltage-dependency of the open probability of the channels such that a lesser degree of depolarization can initiate calcium influx. Calcitriol exerts a similar effect on calcium channels in the basolateral membrane of the duodenal epithelium; this mechanism appears to be crucial to calcium absorption (194). (Calcium influx causes calcium-containing vesicles to fuse with the basolateral membrane and extrude their contents on the anti-luminal side; thus, paradoxically, increased calcium influx causes a net calcium efflux.)

Several groups have reported that physiological levels of calcitriol also increase calcium influx in VSMC cultures, but that this effect is only seen after a number of hours and requires protein synthesis (195,196). (One reasonable but speculative possibility is that calcitriol, via a nuclear receptor mechanism, induces the synthesis of the calcium channels that are
calcitriol responsive.) Regardless of the mechanism, the increased influx of calcium has obvious implications for blood pressure regulation. The magnitude of this effect is more dramatic in VSMCs derived from spontaneously hypertensive rats.

Bukoski et al studied the effect of calcitriol in vivo on arterial contractility in rats (197). After 3 days of i.p. administration in a dose sufficient to double baseline serum levels, the mesenteric resistance arteries were excised and their sensitivity to norepinephrine and serotonin assessed; the calcitriol pre-treatment increased maximal stress generation by 30–40%. These investigations then studied arterial contractility in rats fed either 0.5% or 2% calcium; contractility in response to norepinephrine was greater on the lower calcium diet. Clinical blood pressure response to oral calcitriol administration has also been assessed; in subjects with low renin hypertension, 0.25 mcg daily led to a 5% increase in diastolic blood pressure (198).

In rat aortic VSMC cultures, including 10% fetal calf serum, physiological concentrations of calcitriol significantly enhanced proliferation rate (199). Whether this effect solely reflects increased calcium influx, or is dependent on other effects mediated by calcitriol's interaction with its nuclear receptor, is not clear. This study demonstrated a specific high-affinity receptor for calcitriol in the cytosol of the VSMCs.

The data are thus consistent with the hypothesis that, in susceptible individuals, a low-calcium diet, by evoking an adaptive increase in calcitriol production, alters the voltage-dependency of L-type calcium channels in VSMCs, thereby promoting greater calcium influx and increasing their sensitivity to vasoconstrictor/growth factor agonists. It is of course possible that calcium nutrition influences VSMC function in other ways, some of which might be mediated by calcitriol nuclear receptors (200). Since PTH is the chief stimulus to calcitriol synthesis, the increased PTH production associated with low-calcium diets may be indirectly responsible for the calcitriol effect; as noted, the direct effect of PTH on arteries is vasodilatory.

Magnesium is the other major mineral which plays a key role in vasoregulation. Extracellular magnesium concentrations regulate calcium entry; hypermagnesemia acutely reduces calcium influx and desensitizes resistance vessels to vasoconstrictors, whereas hypomagnesemia has the opposite effect (201,202). Thus, magnesium has been described as 'nature's physiologic calcium blocker' (203). It is not yet clear which calcium channels magnesium inhibits — they may not be limited to the L-type calcium channels.

Magnesium also functions intracellularly to support the activity of ATP-dependent enzymes, including notably the membrane pumps Na,K-ATPase and Ca-ATPase (201,204). This may explain why magnesium deficiency tends to reduce cellular potassium while increasing intracellular sodium and calcium (204–207). Relatively modest intracellular levels are required to support full activity of these enzymes, and supranormal intracellular magnesium levels do not increase their activity further; the effect of magnesium in this regard is permissive rather than stimulatory. There is little evidence that increased intracellular magnesium can also act as a calcium blocker, although it may competitively inhibit a calcium receptor that triggers calcium release from the sarcoplasmic reticulum. In any case, it is clear that adequate levels of magnesium, both intracellularly and in the serum, are required for proper control of [Ca$^{2+}$].

In light of these considerations, it is not surprising that rats fed magnesium-deficient diets develop hypertension with increased peripheral resistance and vasospasm of contractile arteries (208,209). Human magnesium deficiency resulting in hypomagnesemia is also typically associated with increased blood pressure (210,211).

However, clinical studies to date suggest that the clinical utility of oral magnesium supplements for lowering elevated blood pressure, may be confined to individuals who are significantly deficient. An uncontrolled study in long-term diuretic users reported a rather substantial reduction of blood pressure in long-term diuretic users (mean reduction 12/8 mmHg; (212)), and a controlled Japanese study in subjects consuming very-high-sodium diets also reported a significant blood pressure reduction with oral magnesium (213); both diuretic use and high-sodium-diets promote renal magnesium loss. In contrast, three other controlled studies failed to observe any effect of supplemental magnesium on the blood pressure of hypertensives (214–216). Apparently, even though modern diets are far lower in magnesium than those of hunter-gatherers, they are usually sufficient to maintain adequate intracellular and extracellular magnesium levels unless other circumstances intervene. Theoretically, oral magnesium could lower the blood pressure of magnesium-replete subjects by raising plasma magnesium levels. But, unfortunately, it is difficult to produce sustained, clinically significant increases in magnesium with feasible oral doses of magnesium, except in individuals who are overtly deficient. (The feasible oral dose is limited by osmotic diarrhea.) Thus, magnesium supplementation is unlikely to be of general utility for control of hypertension, though it may be useful in selected individuals in which special circumstances – long-term diuretic use (217), very-high-sodium diet (218), alcoholism, anorexia, and diabetes – have compromised magnesium availability. (With respect to diabetes, it should be
noted that insulin promotes intracellular magnesium uptake in many tissues, that glycosuria impedes renal magnesium retention, and that reduced magnesium levels are typically seen in diabetics; 219–222.) However, these considerations do not detract from the well-documented clinical efficacy of parenteral magnesium for control of hypertensive crises such as eclampsia (223); hypermagnesemia is readily achieved and maintained with an intravenous magnesium drip.

Analogously, there appears to be little evidence that ambient variations in magnesium intake are likely to have a major impact on atherogenesis, or that high magnesium diets are likely to be notably protective in this respect. The limited efficacy of magnesium in these respects may be traceable to the fact that, unlike sodium, potassium, calcium, or chromium, normal variations in magnesium intake do not appear to have major impact on the production or action of hormones. (At normal serum levels, the ability of magnesium to suppress PTH production is of minimal significance; 224.)

On the other hand, there is considerable evidence that ambient variations in magnesium do have a considerable impact on cardiac function – in particular, on arrhythmic risk (201,225–228). Many investigations establish an increased risk for non-occlusive sudden-death arrhythmias in areas with low-magnesium soils or low-magnesium soft water. Victims of sudden-death arrhythmias typically are found to have a reduced myocardial magnesium content; this is associated with decreased intracellular levels of potassium and increased sodium/calcium levels – possibly indicative of impaired activity of membrane pumps. Myocardial cells maintain a higher intracellular magnesium level than most other tissues – presumably for a reason (229). Ischaemia impairs the ability of myocardial cells to maintain this high magnesium content (201,230). Magnesium loss, in turn, may exacerbate the adverse effect of ischaemia on the function of membrane pumps, leading to membrane depolarization and increased [Ca²⁺], – conditions which favour automaticity, arrhythmias, and cell death. (It is notable that rather modest degrees of magnesium deficiency impair the cardiac Na,K-ATPase activity of rats; 204.)

The epidemiological data regarding increased sudden-death risk in low-magnesium areas, suggest that relatively modest variations in magnesium intake may have a significant impact on the magnesium content and electrical function of the ischaemic myocardium. The massive clinical studies required to provide direct proof that magnesium supplementation can be protective in this regard, have not been done. However, parenteral magnesium administration for treatment of acute myocardial infarction, does appear to reduce the incidence of subsequent arrhythmias and promote increased survival (207,231,232); since i.v. administration achieves hypermagnesemia, these findings may or may not be relevant to utility of oral magnesium as an arrhythmia-preventive strategy.

Also worthy of mention are recent studies demonstrating that magnesium supplementation can improve insulin sensitivity in diabetics (233–235). It is not clear whether this effect is related to better control of [Ca²⁺], or PKC activity. Since intracellular magnesium is typically decreased in diabetics, more study of the clinical impact of supplemental magnesium in diabetics is warranted. Two studies report that diabetic retinopathy is associated with a further decrease in magnesium levels (236,237); whether this is indicative of a pathogenic role for magnesium deficiency in diabetic retinopathy, or merely serves as a marker for poor glycaemic control, is not clear. In light of suggestive evidence that increased PKC activity may be a mediator of diabetic microangiopathy (112–115), it should be noted that magnesium deficiency would likely increase [Ca²⁺], and thus cause a further activation of PKC. A role for magnesium deficiency in the genesis of diabetic microangiopathy is a credible possibility.

Thus, even though magnesium supplementation may not be beneficial to the majority of people from the standpoint of controlling elevated blood pressure or atherogenesis, its likely cardioprotective activity, as well as its ability to correct or prevent overt magnesium deficiency in susceptible individuals, make it prudent to include ample amounts of magnesium in daily supplementation regimens. In this regard, it should be noted that high calcium intakes – which are now widely recommended – can competitively inhibit magnesium absorption, particularly when diets are relatively low in magnesium (206); thus 'balanced' supplementation with calcium and magnesium appears advisable.

Platelet hyperaggregability and PKC syndrome

Platelet aggregation in response to various agonists is frequently elevated in untreated hypertensives (238–240). This may not be coincidental – the intracellular signalling pathways which mediate platelet aggregation and the release reaction are analogous to those which mediate vasoconstriction (241). In platelets, increased [Ca²⁺], PKC activation, as well as some unexplained action of activated Gs (linked to thrombin and epinephrine receptors), each produce characteristic alterations of the phosphorylation status of contractile proteins, and act synergistically to induce aggregation. However – in contrast to the physiological regulation of VSMCs – membrane potential and
Dietary modulation of endothelial function

It is appropriate to at least cursorily consider the impact of the modern diet on function of the vascular endothelium. This tissue is responsive to a wide range of agonists, and produces factors which promote vasodilation (EDRF, EDHF), vasoconstriction and smooth muscle growth (endothelin, superoxide), and platelet stabilization (prostacyclin, EDRF) (252–254). Remarkably, many stimuli provoke the release of both EDRF and endothelin.

In some respects, intracellular calcium and PKC activity work at cross purposes in endothelial cells. An increase in \([\text{Ca}^{2+}]_i\) triggers the production of prostacyclin and EDRF (255,256); (owing to the fact that both phospholipase A\(_2\) and nitric oxide synthetase are activated by calmodulin). PKC, on the other hand, promotes synthesis of endothelin and superoxide (257–261), while providing feedback inhibition of the signalling mechanisms which increase \([\text{Ca}^{2+}]_i\) (262–264); this latter mechanism may reflect PKC-mediated phosphorylation of G proteins that activate receptor-operated calcium channels in the plasma membrane (265–267). Thus, an up-regulation of PKC would be expected to increase endothelin production, while suppressing release of EDRF and prostacyclin. In hypertension, diabetes, and atherosclerosis, the stimulated endothelial loses its vasodilatory capacity and may even promote vasoconstriction (252,253); it is tempting to speculate that a constitutive increase in PKC activity is largely responsible for this phenomenon.

It can be anticipated that various aspects of the modern diet and lifestyle – high-fat diet, low omega-3 intake, abdominal obesity, enhanced adipocyte lipolysis secondary to insulin resistance, diabetic hyperglycaemia – will tend to increase DAG production in endothelial cells and thus enhance the activity of PKC.

Concurrently, a high dietary sodium/potassium ratio can be expected to reduce calcium influx, thereby suppressing EDRF production. This conclusion may seem paradoxical in light of the fact that, in VSMCs, the depolarizing action of a high sodium/potassium ratio tends to increase calcium influx through voltage-gated calcium channels. However, endothelial cells lack voltage-gated calcium channels; their calcium influx passes through receptor-operator channels, and the rate of this influx increases with membrane hyperpolarization, since this maximizes the electrochemical gradient for calcium (255,268). By reducing membrane polarization, a high sodium/potassium ratio will thus slow the rate of calcium influx; EDRF synthesis unlike that of prostacyclin, is determined primarily by this rate of calcium influx (268–270). PKC over-activity will compound the problem by impeding the agonist-stimulated opening of receptor-operated calcium channels.

It is unlikely that calcium nutrition or calcitriol will influence endothelial cells, owing to their lack of voltage-gated calcium channels. The impact of magnesium is equivocal (271–273); adequate intracellular magnesium is required to activate the Na,K-ATPase and thus promote membrane polarization, but increased extracellular magnesium will tend to block calcium influx. How chromium influences endothelial cells remains to be determined; it has recently been reported that physiological concentrations of insulin stimulate endothelin production by endothelial cell cultures (274).

Low-density lipoprotein, either native or oxidized, blocks the release of EDRF by cultured endothelial cells, and suppresses the vasorelaxant response to acetylcholine in intact arteries (252). The mechanism of this effect is not clear; it apparently is not mediated by high-affinity LDL receptors. Thus, fatty diets which raise LDL levels can further perturb endothelial activity by an LDL-mediated effect.

It thus appears that a modern diet will tend to favor production of the potent vasoconstrictor/growth-factor endothelin, and suppress that of protective vasodilator/platelet-stabilizer EDRF. This is a significant consideration in light of evidence that each of these agonists is a major determinant of basal vascular...
The favorable effects of dietary potassium and fish oil on endothelial function, as cited previously (141-143,179), are consistent with this view.

**Role of PKC in vascular superoxide generation**

All major cell types in atherosclerotic intima — endothelium, VSMCs, macrophages/foam cells, and fibroblasts — are capable of producing superoxide (O₂⁻). This superoxide has vasoconstrictive activity (276), and moreover deactivates EDRF (nitric oxide), impeding its vasodilatory activity (277). Superoxide is also a key mediator of the oxidative modification of LDL (278-280) — a process which appears crucial for foam cell generation (281,282). Cell culture studies suggest that LDL oxidation in the presence of macrophages or VSMCs involves O₂⁻ as an obligate intermediate (278,279), whereas LDL oxidation by endothelial cells may depend on either O₂⁻ or cell-associated lipoxygenase activity (280,283).

Superoxide generation and the availability of free transition metals (copper or iron) appear to be necessary and sufficient conditions for the production of destructive free radicals in biological systems (284). In the vascular wall, free radicals not only oxidize LDL but also inhibit prostacyclin synthetase (285). The ability of various antioxidants to inhibit atherogenesis in animal models (282), well as epidemiological studies demonstrating decreased cardiovascular risk in those using high-potency vitamin E supplement (286,287), suggest that free radical mechanisms do indeed play an important role in atherogenesis. This in turn implicates vascular O₂⁻ generation as crucial to this process.

In most cells, O₂⁻ is a by-product of normal metabolic activity, but in phagocytes — including presumably the macrophages/foam cells of the arterial intima; O₂⁻ is essential for bactericidal activity, and is generated ‘intentionally’ by a membrane-associated NADPH oxidase (288). This NADPH oxidase can be activated by PKC, and it appears that most agents which stimulate a respiratory burst (rapid O₂⁻ generation) in phagocytes, do so via activation of PKC (289,290). The ability of phorbol esters to stimulate O₂⁻ production in endothelial cell cultures (261), suggests that PKC also promotes O₂⁻ generation in vascular endothelium. A recent study shows that lysophosphatidylcholine is a potent stimulant of O₂⁻ production in rabbit aorta, and that this effect is mediated by activation of PKC (291).

Thus, up-regulation of PKC in intimal macrophages/foam cells (as well as endothelial cells) can be expected to promote the free radical-mediated aspects of atherogenesis. Conversely, down-regulation of PKC should be protective in this regard. It is relevant to note that fish oil supplementation decreases the ex vivo production of O₂⁻ by stimulated human neutrophils (292). It is likely that omega-3 fats would exert an analogous effect on intimal macrophages in vivo.

The impact of dietary factors on the PKC activity of phagocytes may thus have important implications for atherogenesis, and should be addressed in future research. In light of the foregoing discussion, it is reasonable to postulate that certain aspects of the modern diet can enhance vascular O₂⁻ production by up-regulating PKC in intimal macrophages (as well as endothelial cells). This possibility is heightened by recent evidence that voltage-gated calcium channels may play a role in macrophage activation (293); since macrophages are also insulin-responsive (294), their regulatory pathways may be somewhat analogous to those of VSMCs.

**PKC, hepatocytes and hyperfibrinogenemia**

The importance of elevated fibrinogen as a strong and independent cardiovascular risk factor is well documented (295); this presumably reflects its multiple roles as the chief determinant of plasma viscosity, a promoter of platelet aggregation, and precursor of fibrin. Fibrinogen is an acute phase reactant; although hepatocytes synthesize it constitutively, its synthesis can be substantially enhanced by interleukin 6 (IL-6) (296,297). Indeed, hyperfibrinogenemia is likely to reflect amplification of an interleukin 6-mediated signal.

The inductive effect of IL-6 on hepatocytes is mediated, at least in part, by increased synthesis and activation of a ‘leucine zipper’ transcriptional factor, NF-IL6, which binds to characteristic response elements in the promoter regions of genes coding for acute phase proteins (298,299). NF-IL6 is susceptible to phosphorylation on several residues by various protein kinases (300-302); these phosphorylations modulate its ability to bind to response elements and/or activate transcription. Recent evidence indicates that activation of PKC promotes phosphorylation of a serine residue in NF-IL6 (Ser 105), thereby substantially enhancing its ability to stimulate transcription of acute phase proteins (302). In several studies, short-term treatment of hepatocytes with phorbol esters has been found to increase fibrinogen transcription, or to potentiate the inductive activity of IL-6 (303,304).

Pickart has adduced considerable evidence that elevated FFA levels – as typically seen in abdominal obesity complicated by impaired insulin function or nicotine addiction – are usually associated with
elevated fibrinogen levels, and may indeed play a causative role in hyperfibrinogenemia (305,306). This might account for correlations between plasma fibrinogen, triglycerides, and various other cardiovascular risk factors (307–312). It is reasonable to postulate that elevated portal FFA levels, by increasing de novo synthesis of DAG (an intermediate in triglyceride synthesis), will increase hepatocyte PKC activity and thereby promote NF-IL6-induced transcription of acute phase proteins, including fibrinogen. Thus, measures which decrease the influx of FFAs to the liver, promote hepatic lipid oxidation, or otherwise block triglyceride synthesis, can be expected to reduce plasma viscosity and thrombotic risk.

The hypotriglyceridemic action of dietary fish oil reflects a reduction of hepatic triglyceride synthesis, possibly owing to increased hepatic FFA oxidation (313,314). Although previous short-term studies have yielded inconsistent conclusions, a recent report by Saynor and Gillott indicates that long-term fish oil supplementation can exert a profound suppressant effect on fibrinogen levels; after 4 years of supplementation in 109 subjects, average reduction of plasma fibrinogen from baseline was 0.9 g/l (315). (Although reduced hepatocyte PKC activity may play a role in this effect, an additional interesting possibility is that the anti-inflammatory action of fish oil limits hepatocyte exposure to IL-6. Although the impact of fish oil supplements on IL-6 production has received little if any study, such supplements can reduce monocyte production of both IL-1 and TNFα (316), which in turn are the chief stimulants of IL-6 production in many tissues; 296.)

Fish oil may thus be remarkably versatile in its ability to impede PKC activation — inhibiting phosphatidylinositol-mediated activation, and, at least in hepatocytes, reducing the de novo synthesis of DAG.

These considerations also raise the prospect that, in so-called ‘carbohydrate-sensitive’ individuals, the ability of high dietary fructose to promote triglyceride synthesis may be coupled with increased synthesis of fibrinogen. Fructose has a more substantial effect on hepatocyte glyceral-3-phosphate levels than other sugars, owing to the fact that the fructokinase of hepatocytes feeds fructose directly into the glycolytic pathway (317); glyceral-3-phosphate is of course an obligate precursor for triglyceride synthesis. I am unable to find any studies addressing the impact of dietary fructose on the fibrinogen levels of carbohydrate-sensitive subjects. However, Ernst refers to epidemiological evidence that ‘high-carbohydrate’ diets tend to be associated with increased fibrinogen levels (295). ‘High-carbohydrate’ in this instance is likely to refer, not to Pritikin-type fiber-rich regimens, but to diets high in refined sugars. The impact of fructose on fibrinogen synthesis merits study.

Ancillary strategies for control of PKC syndrome

Although the crass dietary perturbations that have occurred since paleolithic times may be primarily responsible for the current prevalence of PKC syndrome, it does not follow that mimicking the paleolithic diet is the only feasible approach to correcting this syndrome. Nor would I wish to imply that the paleolithic diet was in every respect ideal for maximizing human health and life span, or that our modern situation represents a ‘fall from grace’, a lapse from a formerly Edenic existence. The fact that humans evolved under a given set of circumstances does not imply that those circumstances were ideal for promoting maximum human health and longevity; natural selection only assures that a reasonable number of fertile individuals will survive to procreative age — it is indifferent to maximum longevity. Regimens to promote maximum healthful survival will undoubtedly require certain artifices — vaccination, water purification, nutritional supplements, aging-retardant drugs, trauma management — not available to our paleolithic forebears. However, given the fact that our human ancestors evolved for millions of years dependent on a characteristic type of diet, we should not be surprised when gross perturbations of that diet have various physiological consequences — some of which are inimical to long-term health.

Although this article has focused on diet, it is clear that regular aerobic exercise can play a prominent role in the correction of PKC syndrome. Such exercise promotes insulin sensitivity (318–320) — for reasons which remain obscure — and, in the longer term, helps to correct or prevent abdominal obesity. These effects appear to be primarily responsible for the beneficial impact of exercise training on elevated blood pressure; the reduction of blood pressure correlates with reduction in hyperinsulinemia, suggesting that improved insulin function — or perhaps correction of the intracellular dysfunctions which induce insulin resistance — may mediate the reduction in pressure (318). Whether exercise training can ameliorate PKC syndrome through other mechanisms, is not clear. Epidemiological studies indicate that habitual exercise — especially life-long exercise — as well as increased cardiovascular fitness, are associated with greater longevity as well as reduced risk for various types of cancer (321,322).

As noted above, d-alpha-tocopherol is a direct inhibitor of PKC (106). Whether this inhibition can be
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achieved in vivo with feasible oral doses, and whether it plays any role in the ability of mega-dose E to improve insulin sensitivity (105) or reduce cardiovascular risk (as suggested by recent epidemiological studies; 286,287) is not clear. Oxidant conditions can activate PKC in cell culture, and, moreover, some of the downstream effects of PKC (such as ornithine decarboxylase activation) appear to be dependent on free radical-mediated mechanisms (323–325). Thus, it is conceivable that certain nutritional and pharmaceutical antioxidants may be found useful for control of PKC syndrome.

Another agent which may prove useful for control of PKC syndrome is the non-protein amino acid taurine. Vertebrates maintain high intracellular levels of taurine by active transport, particularly in electrically active or secretory tissues (326). This intracellular taurine interacts electrostatically with cell membranes, thereby influencing the function of membranes and of membrane-associated proteins. In cardiac cells, high taurine levels promote the activity of both the plasmalemma Ca-ATPase and the Na/Ca-exchanger (327,328). Although analogous data is not yet available for VSMCs, taurine perfusion has been shown to promote relaxation in vessels preconstricted with norepinephrine or high extracellular potassium, consistent with the possibility that taurine aids control of [Ca\(^{2+}\)] in VSMC (329,330). In vivo, taurine administration prevents or retards the induction of hypertension in various rodent models of this disease (331–333), and also lessens the extent of atheroma produced by high-cholesterol diets in rabbits (334). Arterial calcinosis, induced in rats by high-dose vitamin D plus nicotine, is prevented by taurine in a manner analogous to the benefit of calcium channel blocker drugs (335). Clinically, several reports suggest that fairly high supplemental taurine intakes (~6g daily) can lower elevated blood pressure (336,337). These findings are consistent with the possibility that supplemental taurine may help to ameliorate PKC syndrome. Of related interest is the ability of supplemental taurine to stabilize platelets and promote more efficient cardiac function in congestive failure (242–244,338–340). The vascular-protective potential of taurine is discussed in more detail in a companion article (341).

The hyperglycaemia associated with poorly-controlled diabetes can, as previously noted, increase PKC activity by stimulating de novo DAG production (110–116). Thus, in addition to protein glycation, free radical generation, and sorbitol accumulation, PKC activation may be an important mediator of the hyperglycaemia-induced secondary complication of diabetes. This provides further rationale for maintain-

ing tight glycaemic control in the management of diabetes.

Many of the protective measures cited above function ultimately to impede calcium influx through calcium channels. These nutritional measures are quite appropriate for lifelong use by healthy people. However, in individuals who have clinically symptomatic vascular disease, it would seem prudent to complement these nutritional measures with appropriate calcium channel blocker drug therapy. These drugs are often protective in animal models of atherogenesis, reduce the proliferative response of VSMCs to vasoconstrictor/growth factor agonists in vitro, and, clinically, have been found to retard the progression of arterial stenoses (19,20,31,80–82). Their utility for treating hypertension and controlling cardiac arrhythmias is well known.

Pharmaceutical correction of insulin resistance may also be beneficial in PKC syndrome; metformin (342) and the newly-available thiazolidinedione drugs (343,344) may be worthwhile in this regard. The well-tolerated antilipolytic agent acipimox, which achieves sustained reduction of free fatty acids with fewer side effects than mega-dose niacin, also should be evaluated in PKC syndrome (345–347).

PKC syndrome in perspective

In overview, it should be noted that the various mechanisms which engender PKC syndrome do so by either increasing [Ca\(^{2+}\)], and/or by increasing DAG levels; these increases may be present constitutively, or may represent an increased response to various agonists. Increased [Ca\(^{2+}\)], will reflect increased flux of calcium into the cytoplasm (resulting from decreased membrane potential, altered conformation of L-type calcium channels or receptor-operated channels, increased production of IP\(_3\), or increased sensitivity of IP\(_3\)-activated channels) and/or diminished function of the calcium transporters that extrude calcium (Ca-ATPase, Na/Ca-ATPase). Increased DAG may reflect increased activation of phospholipase C or D, and/or increased de novo synthesis.

These effects, in turn, will either activate PKC or increase its responsiveness to activating agonists. Depending on the tissue affected, a joint increase in [Ca\(^{2+}\)] and PKC activity may promote vasoconstriction, hyperplasia/hypertrophy (as in atherogenesis, hypertensive medial hypertrophy, retinal neovascularization, and cancer promotion), or platelet aggregation, and may also induce insulin resistance.

The concept of PKC syndrome brings a surprising structural unity to a disparate array of nutritional mea-
sures which, on empirical grounds, have long been suspected to promote vascular health. This in itself serves as strong evidence that the concept is sound, and that PKC syndrome is indeed an important pathogenic factor in vascular disease. However, it is by no means an alternative to other current theories of vascular pathogenesis, but rather a complement to them. Whereas most other theories focus on 'external' influences impinging on vascular tissue, the concept of PKC syndrome addresses the inherent susceptibility of vascular tissue to these influences. It may be assumed that, in many if not most instances, the genesis of vascular disease will require both instigating external factors, and an enhanced responsiveness of VSMCs to these factors. PKC syndrome will render VSMCs more susceptible to the effects of vasoconstrictor/growth factors, or to the loss of natural vasodilators – but unless excessive (or deficient) levels of these agonists are present, overt disease will be unlikely to develop.

For example, though the high sodium content of a traditional Japanese diet leads to a high incidence of hypertension and stroke, atherosclerotic coronary disease has been relatively rare in the Japanese. Presumably production of PDGF, oxidized LDL, and other mediators is relatively low in the arterial intima of these individuals, owing to other aspects of their diet (low-fat, omega-3-rich).

It is encouraging to observe that many of the nutritional measures typical of the paleolithic diet, and/or recommended above for control of PKC syndrome, are likely to protect vascular health in ways that are independent of their direct beneficial effects on VSMC function. High intakes of potassium and of fish oil have been reported to preserve healthful function of vascular endothelium in hyperlipidemic animals (141–143,179). Fish oil has a platelet-stabilizing action, which should limit production of PDGF and reduce thrombotic risk; it also reduces arrhythmic risk in animals, and can markedly decrease plasma fibrinogen (139,140,146,296). Insulin-sensitizing measures, such as bioactive chromium, metformin, and exercise training, may increase HDL cholesterol, lower triglycerides, and limit the production of certain proinflammatory factors (51,348–352). A low-fat diet should limit LDL production; oxidized LDL damages vascular endothelium and stimulates macrophages to release vasoactive agonists such as PDGF (5). Recent evidence indicates that high-dose vitamin E may inhibit LDL oxidation (353,354); it also helps to sustain prostacyclin synthesis by preventing lipid peroxide-mediated deactivation of prostacyclin synthetase (355,356), and is therapeutically beneficial in intermittent claudication (357). As noted above, taurine can stabilize platelets and treat congestive failure. Moreover, in animal models, taurine prevents arrhythmias, lessens reperfusion damage to ischemic hearts, and enhances insulin sensitivity (358–362).

However, one potential adverse effect merits mention. The secretion of insulin is triggered by calcium influx through L-type channels in response to a reduction in membrane potential (363). It is therefore reasonable to expect that certain measures which correct PKC syndrome may impede insulin secretion. Indeed, both fish oil (364) and calcium channel blockers (365) can impair glucose control in type II diabetics by reducing insulin output, and taurine can decrease insulin secretion by β cells in vitro (362). When it is desired to use these measures in the treatment of overt or borderline (type II) diabetes, concurrent treatment with sulfonylureas may be indicated (366). These drugs in turn raise some concern, since they work by inhibiting ATP-sensitive potassium channels (363), thereby reducing membrane potential in β cells as well as other tissues (367,368); theoretically, this could exacerbate PKC syndrome, and might cast some light on the failure of tolbutamide to reduce mortality in long-term prospective or retrospective studies (369–371). In vascular smooth muscle, the vasorelaxant response to hypoxia or endogenous vasodilators such as EDHF is mediated primarily by an opening of ATP-sensitive potassium channels – precisely those which sulfonylureas inhibit (372,373). However, the second-generation sulfonylureas such as glyburide have a substantially higher affinity for the potassium channels in beta cells than those in vascular or cardiac muscle (374,375); thus, in the lowest doses that are clinically effective, they are unlikely to have any significant adverse effect on vascular or cardiac function (376), and in fact may aid control of PKC syndrome by reducing glycaemia and promoting more effective insulin activity on VSMCs.

While this article has focused on vascular disease, the possibility that PKC syndrome may play a role in the induction of various cancers – notably the ‘Western’ cancers associated with high-fat diets or abdominal obesity – deserves further evaluation. The observations of increased PKC activity and increased DAG content in epithelia of animals ingesting high-fat diets, are fascinating and potentially important. However, the reader should bear in mind that many of the nutritional effects cited above pertain specifically to VSMCs; measures which correct (or promote) PKC syndrome in VSMCs may or may not exert analogous effects on the stem cell populations which give rise to cancers.

In this regard, it has been reported that elevated
blood pressure is a risk factor for the subsequent development of cancer (377). Could increased PKC activity be the common pathogenic factor?

A role for PKC in aging?

Finally, it is pertinent to note an intriguing observation by Birt and colleagues: caloric restriction reduces the turnover of phosphatidylinositol in rat epidermis, presumably implying a corresponding reduction in PKC activity and IP₃-mediated calcium release (378). Since caloric restriction entails a reduced availability of both glucose and free fatty acids as precursors for phospholipid synthesis, a reduction in phospholipid turnover is an appropriate homeostatic adaptation. If caloric restriction down-regulates PKC/[Ca²⁺] activity in all tissues, could this be a crucial mediator of its aging-retardant action?

Aging presumably reflects a continuing evolution of the epigenetic mechanisms regulating gene transcription – a continuation of the epigenetic evolution which mediates organogenesis and growth in young animals. Is it not reasonable to expect that crucial and ubiquitous intracellular regulators – such as PKC and [Ca²⁺] – can impact the rate at which this evolution proceeds? As a credible but highly speculative hypothesis, I propose that down-regulation of PKC tends to reduce the rate at which tissues age, and that caloric restriction has an especially profound aging-retardant effect because, unlike many of the other measures recommended here, its effect on PKC activity is not tissue-specific but global, influencing the aging of brain regulatory centers which determine the body’s neurohormonal milieu (and perhaps influencing the rate of spontaneous neuron death in these centers).

Also, in light of evidence that chromium has an inductive effect on Ca-ATPase in VSMCs (78), it is pertinent to note a recent report that dietary supplementation with chromium picolinate increases both median and maximal lifespan in rats (379). Could down-regulation of [Ca²⁺]-responsive signalling pathways also retard the age-related shifts in epigenetic regulation?

Genetic changes (mutations) may also play a role in the aging process. The progressive accumulation of heritable mutations in stem cells is undoubtedly the chief reason for the greatly increased risk for cancer in the elderly (380). PKC activity, by promoting mitogenesis and the generation of superoxide, can be expected to increase the rate at which mutations occur, and might also accelerate epigenetic aspects of cancer development; the cancer-promotional activity of phorbol esters illustrates this principle. Recent reviews have highlighted the roles of increased mitogenesis and of oxygen-derived radicals in cancer induction (381–383). Conversely, global or local down-regulation of PKC activity could be expected to reduce mitotic rates and slow the accumulation of mutations. Caloric restriction has been shown to slow mitotic rates in various rodent tissues (384,385) – an effect which is probably germane to its well-known ability to retard cancer onset, and which might be mediated, at least in part, by down-regulation of PKC.

Practical guidelines for prevention or treatment of PKC syndrome

Although it is obviously impractical to expect people to return to a ‘roots-and-berry’ paleolithic existence, it should be possible to reproduce many of the benefits of a paleolithic diet with less drastic dietary measures and the use of appropriate nutritional supplements.

With respect to dietary choices, avoidance of dietary fat may be the most important guideline. Clinical studies show that a dietary fat content of 10–15% produces substantial improvements in insulin sensitivity (34); such a diet will also aid weight control and reduce cancer risk. By avoiding fatty meats, full-fat dairy products, and the unnecessary use of oils, and by availing oneself of the proliferating variety of tasty reduced-fat convenience foods, it is reasonably feasible for dedicated individuals to achieve this low level of dietary fat intake, without feeling unduly deprived. (The 30% fat dietary guideline currently promulgated by the US government has no apparent basis in scientific fact, and reflects a political compromise with meat and dairy interests, or the belief that the American people are incapable of doing better. There is no reason to think that a 30% fat diet will improve insulin sensitivity or provide any other discernible health benefit. This situation might be analogized to that of an observer who, seeing people jumping lemming-like off the 13th floor of a building, helpfully advises them to jump off the 5th floor instead.)

With respect to sodium, it is virtually impossible to achieve the very low levels found in unsalted paleolithic diets unless a person is willing to cook all of his food himself from scratch. The good news is that moderate levels of dietary sodium may pose little risk to people who don’t have a family history of hypertension and are not currently hypertensive. (Recall that excessive production of EDLF is seen primarily in individuals who have a genetic tendency to renal sodium retention.) Thus, avoidance of foods that are overtly salty may be an adequate guideline for many people. For those who are currently hypertensive or
do have a family history of the disorder, careful attention to the sodium content on food labels is warranted. When salting is a culinary necessity, it is preferable to use a potassium-enriched salt alternative (such as ‘Pansalt’ cited above).

An ample potassium intake can be assured by emphasizing fruits and vegetables in the diet, and by reducing intake of ‘empty calories’ from refined sugars and oils. Unsalted vegetable juice can be especially recommended, as it is low in calories (and thus does not displace other potassium-containing foods from the diet), is loaded with potassium and other protective phytochemicals (the juice of cruciferous vegetables may be especially beneficial in this regard). Also, it is simply more convenient to consume large amounts of juice than the equivalent amount of whole vegetables. The avid devotees of ‘juicing’, despite their habit of justifying this practice with folkloric rationales of dubious validity (‘live enzymes’), seem to be barking up the right tree. There are probably few practices that can confer greater health benefits than the regular and promiscuous consumption of unsalted vegetable juices.

Ample intakes of calcium, magnesium, chromium, vitamin E, and other essential micronutrients can be insured by the regular ingestion of a well-designed ‘nutritional insurance formula’ (386). It is hard enough to minimize dietary fat and avoid excess sodium, without also having to worry about assuring adequate (let alone optimal) intakes of over twenty essential micronutrients through food choices alone. Furthermore, the optimally protective doses of certain nutrients – such as chromium and vitamin E – may be beyond the levels that can be provided by natural foods. Insurance formulas can also provide an ample amount of taurine, through the expedient of using magnesium taurate as the chief magnesium source.

(However, it may be desirable for men and post-menopausal women to choose an insurance formula which lacks iron. Free iron encourages LDL oxidation, and is an obligate mediator of hydroxyl radical-mediated mutagenesis (387); some recent epidemiological studies suggest that increased body iron stores pose a risk for heart disease and cancer (388–391). Also, since optimal insurance supplementation should be directed toward prevention of as many diseases as possible – not just PKC syndrome – it is prudent to choose a supplement that is also rich in folic acid and selenium, which likely can reduce risk for birth defects and cancer; 392–398.)

Good omega-3 nutrition can best be insured through regular consumption of fish oil supplements. A supplement enriched in omega-3s is desirable (e.g. 50–80% omega-3), since these products enable one to achieve an ample intake of omega-3 with relatively few capsules per day, while minimizing the ingestion of extraneous fats. It is likely that a given dose of omega-3 will be more effective within the context of a low fat intake, as recommended above. Concurrent consumption of modest amounts of a GLA-rich oil (evening primrose, borage) may also be appropriate. Of course, the frequent consumption of (uncontaminated) fish – as an alternative to fatty meats – can be recommended as well.

In addition to these nutritional measures, a regular programme of aerobic exercise should aid control of PKC syndrome. In conjunction with low-fat eating and possibly chromium, it should help prevent or correct abdominal obesity – also crucial for control of PKC syndrome – and may also have a favourable effect on mood and perceived well-being.

In individuals with pre-existing disease, selected drug therapies (as discussed above) may provide additional aid in control of PKC syndrome.

In summary: minimize dietary fat and emphasize potassium-rich natural foods, get regular aerobic exercise, and use an optimally-designed insurance formula and enriched fish-oil supplement.

Since the measures suggested above should down-regulate [Ca\(^{2+}\)]/PKC-mediated signalling pathways by diverse mechanisms, it is reasonable to anticipate that joint application of these measures should exert complementary or synergistic effects on PKC syndrome. Low-fat diets appear to reduce baseline PKC activity by decreasing membrane DAG levels. Fish oil reduces agonist-induced DAG and IP\(_3\) generation, decreases de novo synthesis of DAG in hepatocytes, and may also inhibit arachidonate-dependent mechanisms which promote mitogenesis. Efficient insulin activity – as promoted by chromium picolinate, weight control, and exercise training – reduces calcium influx, impedes IP\(_3\)-mediated calcium release and induces Ca-ATPase. Vitamin E may exert a direct inhibitory effect on PKC and help prevent oxidant-mediated PKC activation. Ample intakes of potassium may help control [Ca\(^{2+}\)], in vascular muscle cells by promoting membrane polarization and Na/Ca-counter-transport; magnesium supports these actions, and in addition acts directly as an inhibitor of calcium channels. Calcium channel blocker drugs reduce calcium influx by inhibiting voltage-gated calcium channels, whereas good calcium nutrition limits the activation of these channels by calcitriol. Taurine may aid the activity of transporters that extrude calcium.

Inasmuch as low-fat diet with exercise (399), as well as treatment with calcium channel blockers (82), have been reported to occasionally induce regression of pre-existing atheroma, it may not be unreasonable to hope that joint application of the complementary
molecular biology, and very little of the mammoth re-
nutritionist who wants to make a real contribution to medical
has helped to partially alleviate my severe ignorance of recent
emphasizes nutrition-induced intracellular regulatory dysfunction -
High Blood Pressure Solution
deserving thanks is Dr Richard Moore, whose excellent book
Michael Zemel, to whom I owe a profound debt of gratitude. Also
conversation, helpful suggestions, and pioneering research of Dr
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found implications for human health.

I would like to conclude with a plea for greater
integration of nutritional and molecular biological re-
search. There is surprisingly little nutritional research
that takes cognizance of the recent developments in
molecular biology, and very little of the mammoth re-
cent research literature in molecular biology explores
the influence of nutritional factors. Nutritionists need
to recognize that the intracellular regulatory pathways
clarified over the last two decades are every bit as
crucial as the enzymatic pathways with which they are
more familiar. Molecular biologists need to recognize
that nutritional factors are the chief environmental
modulators of the regulatory mechanisms they are
unraveling. An integration of these two disciplines
should lead to breakthroughs that could have pro-
found implications for human health.

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emphasizes nutrition-induced intracellular regulatory dysfunction –
has been very helpful in the formulation of my own thoughts.
Finally, I wish to commend the Third Edition of Molecular Biology
of the Cell, by Dr Bruce Alberts and colleagues (402). This is
undoubtedly one of the greatest and most fascinating books ever
written on any topic, and is moreover a superb educational tool; it
has helped to partially alleviate my severe ignorance of recent
advances in this field. Reading it should be de rigueur for any
nutritionist who wants to make a real contribution to medical
science.

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