Direct regulation of ion channels by fatty acids

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A variety of fatty acids regulate the activity of specific ion channels by mechanisms not involving the enzymatic pathways that convert arachidonic acid to oxygenated metabolites. Furthermore, these actions of fatty acids occur in patches of membrane excised from the cell and are not mediated by cellular signal transduction pathways that require soluble factors such as nucleotides and calcium. Thus, fatty acids themselves appear to regulate the action of channels directly, much as they regulate the action of several purified enzymes, and might constitute a new class of first or second messengers acting on ion channels.

Fatty acids are well positioned to act as messenger molecules that regulate ion channels. In the plasma membrane, they are covalently linked to other molecules to form the phospholipids that comprise a major portion of the lipid bilayer. These fatty acids can be liberated by cellular phospholipases, producing non-esterified or 'free' fatty acids. Thus, agonists that activate phospholipases release fatty acids, allowing them to act as second messengers. Fatty acids can also be delivered to cells from extracellular sources by albumin and lipoproteins. A number of studies have now shown that the non-esterified or 'free' form regulates ion channels.

The actions of fatty acids on ion channels can be grouped into two broad categories – direct and indirect (Fig. 1). Indirect effects arise from the metabolic conversion of one fatty acid, arachidonic acid, to active oxygenated metabolites, which then affect certain channels[2–5]. In contrast, direct effects do not depend on such metabolic conversion, but appear instead to result from the interaction of fatty acids themselves with the ion channel, or some associated site within the plasma membrane. In this article, we will concentrate on the direct actions of fatty acids.

It is a straightforward matter to demonstrate the direct effects of fatty acids on ion channels[6] using patch-clamp techniques. A glass micropipette is used to form a high-resistance electrical seal with the plasma membrane. A patch of membrane is then excised from the surface of the cell and fatty acids are applied to the patch, as illustrated in Fig. 2. In this instance an 'outside-out' patch from a smooth muscle cell was used so that the fatty acid could be applied to the extracellular surface. Upon application, a potassium (K^+)-selective channel was promptly activated.

The direct nature of the action of the fatty acids was demonstrated in two ways. (1) The fatty acids that were used included those that are not substrates for the cyclo-oxygenase or lipoxygenase enzymes that convert arachidonic acid to active, oxygenated metabolites. One such fatty acid is myristic acid, which is fully saturated and hence lacks the *cis* double bonds required of substrates (Fig. 3 and legend). As seen in Fig. 2, both myristic acid and arachidonic acid activated the same channel in excised, cell-free patches of membrane. (2) Experiments such as the ones illustrated in Fig. 2 were carried out in the absence of all nucleotides (i.e. ATP, GTP, cyclic nucleotides and NADPH) and calcium (Ca^{2+}) (5 mM EGTA). Thus, events involving phosphorylation, GTP-dependent proteins, Ca^{2+} and the NADPH-dependent, cytochrome P450 metabolic pathway (see Fig. 1)[9] cannot mediate the fatty acid action. So fatty acids appear to activate ion channels directly by interacting with the channel protein itself or with some other component of the membrane. In this regard, direct effects of fatty acids are similar to direct effects of other agents, such as G proteins[10] and nucleotides[11], on ion channels.

In the study described above[6] that used gastric smooth muscle cells, fatty acids were effective when applied to either the extracellular surface of the membrane (for example, in whole-cell or outside-out patch-recording configurations) or the cytosolic surface (for example, in the inside-out patch configuration). Furthermore, they activated K^+ channels in cell-attached patches when applied to the cell membrane outside the patch. In other preparations where fatty acids regulated ion channels (see below), they were always effective when applied at the cytosolic membrane surface[6,12–19], and often effective when applied extracellularly[6,14–16,20–23]. However, in two instances they were reported to be effective only when applied at the cytosolic, and not the extracellular, membrane surface[12,18].

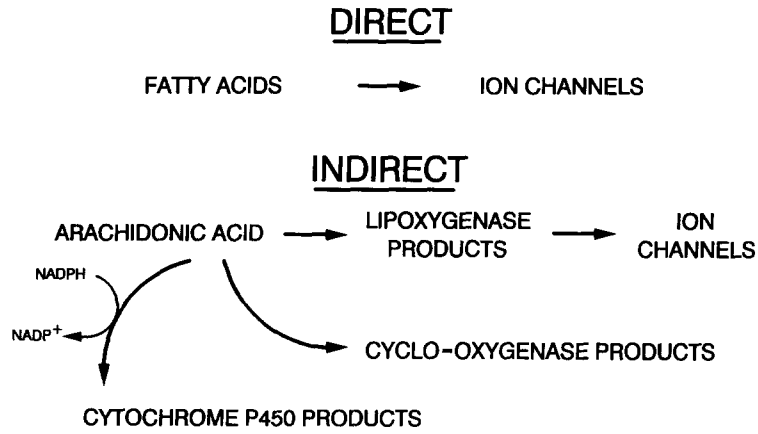

Fig. 1. *Mechanisms of ion channel regulation by fatty acids. The metabolic pathways for conversion of arachidonic acid to a variety of oxygenated metabolites, e.g. prostaglandins (cyclo-oxygenase pathway), leukotrienes (lipoxygenase pathway), and epoxides (cytochrome P450 pathway), are reviewed in Ref. 1.*

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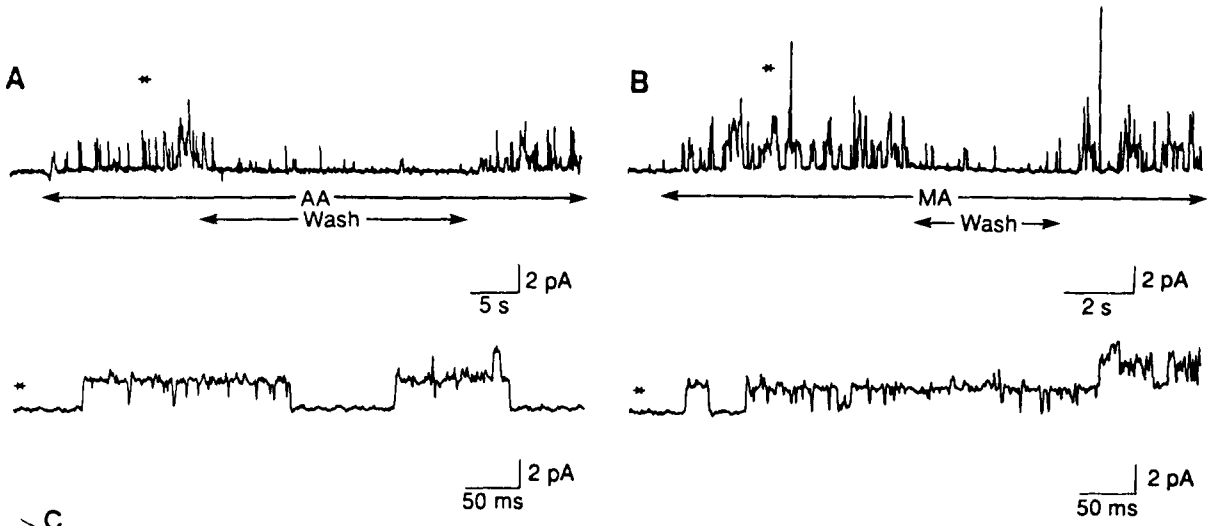

Fig. 2. *Fatty acids activate K^+ channels in excised, outside-out, membrane patches from smooth-muscle cells isolated from the stomach of the toad* Bufo marinus. **(A)** *Arachidonic acid (AA) (40 μM) and* **(B)** *myristic acid (MA) (40 μM) were applied by pressure ejection from a micropipette, as shown in* **(C)**. *Both of these fatty acids activated K^+ channels rapidly (seen as upward deflections from the baseline). A more powerful stream of bath solution simultaneously applied from a second pipette – 'wash' in (C) – prevented access of fatty acids to the patch, resulting in a marked decline in channel activity. When this second stream was turned off, allowing access of fatty acids to the patch again, channel activity returned. The regions of the upper traces marked with asterisks are shown in the lower traces on an expanded timescale. Similar results were also obtained in excised, inside-out patches and cell-attached patches. However, in these recording configurations, onset and recovery of the response were slower.* (Taken, with permission, from Ref. 6.)

Which fatty acids are capable of activating channels directly? In the case of the K^+ channel described above, a variety of *cis*-polyunsaturated, *trans*-polyunsaturated, *cis*-monounsaturated and saturated fatty acids proved to be effective at micromolar concentrations (Fig. 3)[6]. However, not all fatty acids in each of these categories activated the channel; for example, a saturated fatty acid of intermediate chain length, myristic acid (14 carbons), was active, but neither the shorter chain, more hydrophilic fatty acid, caprylic acid (8 carbons), nor the longer chain, more hydrophobic fatty acid, palmitic acid (16 carbons) was effective. Thus, among saturated fatty acids, those of intermediate chain length were most effective in the concentration range employed (10^{-5} to 10^{-4} M).

The effectiveness of saturated fatty acids of intermediate chain length is a common feature among preparations where fatty acids elicit responses[16,24–30]. One possible explanation for this is related to their hydrophobicity. Since the hydrophobicity of fatty acids is increased with increasing saturation and chain length[31], long chain saturates are the least soluble in water. Thus, it is possible that active fatty acids must be sufficiently water soluble to reach an effective concentration in the aqueous solution, and yet sufficiently hydrophobic to interact with a hydrophobic protein or lipid binding site[15,16,24,25].

Direct regulation of ion channel activity by fatty acids has now been demonstrated for a number of channel types in a variety of preparations (Table 1). Gap junction channels in lacrimal gland cells[20] were closed by an array of fatty acids similar to those that activated K^+ channels in gastric smooth muscle. A number of K^+ channels, in addition to the one discussed above, were also directly activated by fatty acids. The large-conductance, Ca^{2+}-activated K^+ (K_{Ca}) channel in pulmonary artery smooth muscle cells was activated by a similar set of fatty acids[13]. The same type of K_{Ca} channel was activated by one type of fatty acid in human aortic smooth muscle cells[14]. In cardiac atrial cells, fatty acids activated a large-conductance K^+ channel (not Ca^{2+}-activated)[12]. In this case only *cis*-polyunsaturated, but not monounsaturated or saturated, fatty acids were effective. In squid giant axons, Na^+ current was decreased by a variety of fatty acids, although at concentrations

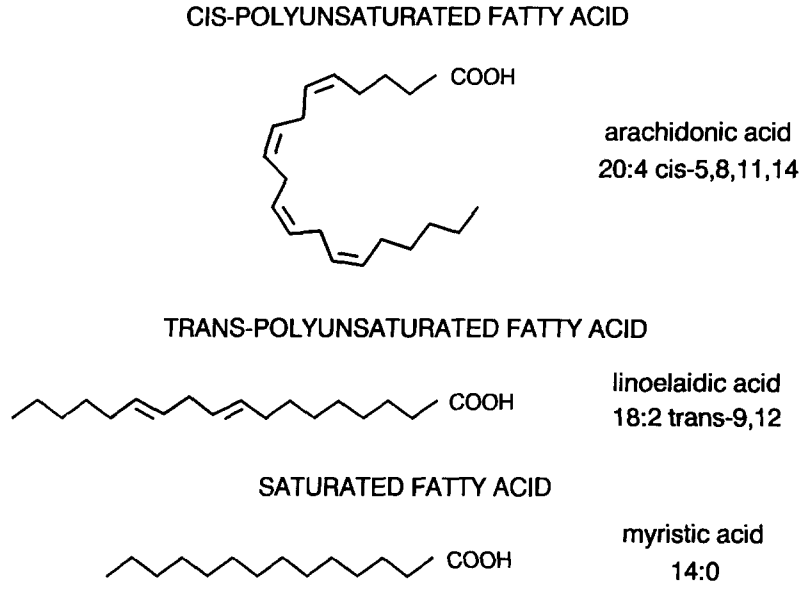

Fig. 3. *Fatty acid structures (schematically represented with all carbon atoms in one plane). Structures are designated as in the following example: 20:4* cis-*5,8,11,14 represents arachidonic acid, a 20-carbon fatty acid with four* cis *double bonds at carbons 5,8,11,14. Fatty acids that lack a* cis-*1,4-pentadiene unit (–C=C–C–C=C–) are not substrates for cyclo-oxygenase[7] and lipoxygenase[8] enzymes. Thus, of those depicted above, only arachidonic acid will be converted to oxygenated metabolites by these enzymes.*

TABLE I. Regulation of ion channel activity by fatty acids

Preparation	Conductance (pS)	Applied FAs	Effective FAs	Concentration (μM)	Ref.
K^+ Channel activation					
Gastric smooth muscle cells	50	*Cis*-PUFA *Trans*-PUFA *Cis*-MUFA SFA	*Cis*-PUFA *Trans*-PUFA *Cis*-MUFA SFA	10–100	6
Cardiac atrial cells	160	*Cis*-PUFA *Cis*-MUFA SFA	*Cis*-PUFA	10–50	12
Pulmonary artery smooth muscle cells	260	*Cis*-PUFA *Trans*-PUFA SFA	*Cis*-PUFA *Trans*-PUFA SFA	20–40	13
Aortic smooth muscle cells	260	*Cis*-MUFA	*Cis*-MUFA	100–900	14
Channel inhibition					
Lacrimal cell gap junctions	100	*Cis*-PUFA SFA	*Cis*-PUFA SFA	20–100	20
Airway epithelial cells (Cl^- channels)	30	*Cis*-PUFA *Cis*-MUFA *Trans*-MUFA SFA	*Cis*-PUFA *Cis*-MUFA	1–25	17
Airway epithelial cells (Cl^- channels)	30	*Cis*-PUFA *Trans*-PUFA *Cis*-MUFA SFA	*Cis*-PUFA *Trans*-PUFA *Cis*-MUFA	5–100	18
Squid giant axon (Na^+ current)		*Cis*-PUFA *Cis*-MUFA SFA	*Cis*-PUFA *Cis*-MUFA SFA	100–30 000*	15,16

Abbreviations: PUFA, polyunsaturated fatty acid (FA) (containing multiple double bonds); MUFA, monounsaturated fatty acid (containing a single double bond); SFA, saturated fatty acid (containing no double bonds).
Cis and *trans* indicate the configuration of the double bonds (see also Fig. 3).
In all cases, K^+ channel conductances were measured with 130 or 140 mM K^+ on both sides of the membrane. Cl^- channel conductances were measured as the slope conductance at 0 mV with 140 or 150 mM Cl^- on both sides of the membrane.
* In the case of arachidonic acid, a small effect was seen at 30 μM.

higher than those used in other studies[15,16]. In cardiac cells, doxyl stearic acids closed gap junctions, but stearic acid without the doxyl group had no effect[21]. An inhibitory effect of millimolar concentrations of doxyl stearic acid, as well as other fatty acids, was seen on $^{22}Na^+$ flux through nicotinic acetylcholine receptor channels[32]. Most recently, micromolar concentrations of fatty acids were found to block[17] or inactivate[18] Cl^- channels of epithelial cells in the airway; these channels are implicated in the pathogenesis of cystic fibrosis. In both of these studies, unsaturated fatty acids were effective and saturated fatty acids appeared to have little or no effect. Finally, fatty acid-induced decreases in Ca^{2+} and Na^+ currents[22,23], and an additional example of fatty acid activation of K_{Ca} channels[19] have been reported.

Mechanisms of direct fatty acid action

How do fatty acids regulate ion channels directly? They might elicit their effects by interacting with the channel itself (or an accessory protein), or by altering the lipid bilayer. Perhaps because of the hydrophobic nature of fatty acids, a mechanism involving the lipid bilayer is often assumed without considering the numerous examples of direct interactions between fatty acids and proteins.

Biochemical studies demonstrating that fatty acids act directly on a variety of enzymes provide ample precedent for a similar action on ion channels. Micromolar concentrations of fatty acids can activate enzymes found in membranes, such as particulate guanylate cyclase[24], as well as purified, water soluble enzymes, such as soluble guanylate cyclase[33–35] and protein kinase C[28,36–38]. These enzymes are activated by an array of fatty acids similar to that which activated gastric smooth muscle K^+ channels. The effect on the purified, water soluble enzymes is of special interest since it demonstrates that fatty acids can activate proteins without the involvement of a lipid bilayer. An additional similarity between the K^+ channels and these purified, soluble enzymes is that they can all be directly activated by eicosatetraynoic acid (ETYA)[6,28,34], a commonly used inhibitor of arachidonic acid metabolic pathways[39,40].

Fatty acid-binding sites on a number of proteins have been well defined. The best-studied sites are those of albumin, a water-soluble protein that binds structurally diverse fatty acids with K_d values that in many instances are in the micromolar range[41]. It is possible that analogous binding sites exist on ion channel proteins, either within the lipid bilayer or in the aqueous environment.

Fatty acids might act by altering the interaction of channels with the lipid bilayer. Reconstitution of integral membrane proteins, such as the purified glucose transporter[42], the Ca^{2+}/Mg^{2+}-dependent ATPase[43], the nicotinic acetylcholine receptor channel[44] and K_{Ca} channels[45], in defined lipids has

established that the composition of the lipid bilayer can dramatically affect protein behavior. For example, the activity of the purified glucose transporter is increased as the phospholipid headgroup (which resides at the surface of the bilayer) is changed to a more negatively charged species. Such findings suggest that specific chemical interactions with membrane lipids are important for protein function. Furthermore, such reconstitution methods have determined that some membrane proteins appear to associate preferentially with certain lipids[46]. Fatty acids might act by altering such specific interactions between lipids and ion channels, but at present there is no evidence indicating that they affect membrane proteins in this way.

Another possible mechanism of fatty acid action, in addition to a modification of specific protein–lipid interactions, is an effect on the bulk properties of the membrane lipid. One such property is commonly referred to as 'membrane fluidity'. As described in a critical review by Stubbs[47], membrane fluidity is a broad and somewhat poorly defined term that refers to a variety of characteristics of motion within the membrane lipid bilayer. It has been proposed that fatty acid regulation of squid giant axon Na^+ channels[15,16] and aortic smooth muscle K^+ channels[14] is mediated by an effect of fatty acids on membrane fluidity. However, correlations between fatty acid effects on membrane fluidity and ion channels remain problematical[14–16,48,49]. Furthermore, several investigators have now questioned the usefulness of relating bulk membrane fluidity measurements to the behavior of membrane proteins[42,43,47].

In conclusion, the mechanism by which fatty acids affect ion channels remains unclear. However, in light of the existence of fatty acid-binding sites and the activation of purified proteins by fatty acids, the most likely mechanism at present appears to be the interaction of fatty acids with ion channel proteins themselves. Such a mechanism would not be unique among hydrophobic second messengers, since diacylglycerol, the best-studied example, interacts directly with an enzyme: protein kinase C[50].

The role of endogenous fatty acids

So far, direct actions of fatty acids on ion channels have been shown only for exogenously applied fatty acids, and the presence of endogenous counterparts to these effects awaits demonstration. However, several features of cellular metabolism that govern the level of non-esterified or 'free' fatty acids in cells suggest that endogenous fatty acids might function as biological signals (Fig. 4).

Agonist-stimulated liberation of one fatty acid, arachidonic acid, and the subsequent generation of active metabolites are established components of several signal transduction pathways[1], including pathways that regulate ion channels. It has been shown that agonists activate K^+ channels in *Aplysia* neurons[2] and cardiac atrial cells[5] by stimulating release of arachidonic acid and the production of lipoxygenase metabolites, the latter of which act on the channels (Fig. 1). Although these are clearly indirect effects of arachidonic acid, it is worthwhile comparing them to direct fatty acid actions. The concentrations of arachidonic acid that produce sufficient metabolites to mimic the activation of K^+ channels by agonists (20–50 μM) are similar to the concentrations of fatty acids that directly affect ion channels. Thus, stimulation by agonists might liberate sufficient arachidonic acid to act directly as a second messenger. Moreover, there might be parallel direct and indirect pathways for the regulation of ion channels by arachidonic acid. Such a dual system would be analogous to isoproterenol modulation of the Ca^{2+} current in cardiac myocytes[52], which appears to be mediated by parallel indirect (cAMP-mediated) and direct effects of G proteins.

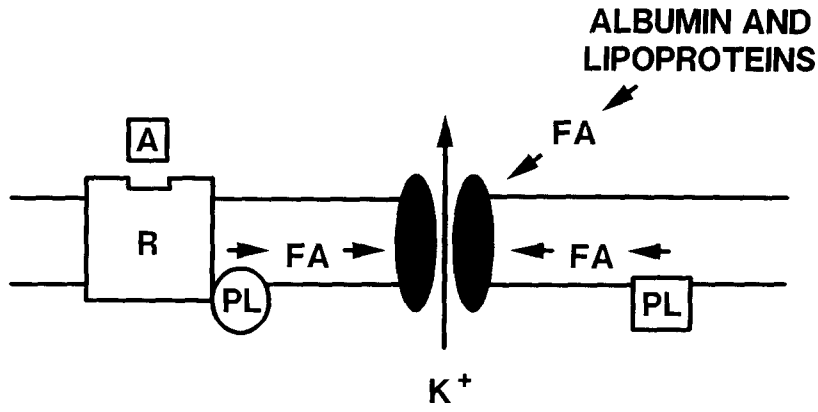

Fig. 4. *Sources of fatty acids for the regulation of ion channels. Sources of fatty acids include those released from phospholipid by agonist-stimulated and basal phospholipase activities (see text) as well as those delivered to cells via the circulation. Sources of circulating fatty acids include those bound to serum albumin[41] and those incorporated into serum lipoproteins[51]. The bulk of the fatty acid derived from lipoproteins is transported in the form of triglyceride, and is delivered to cells after lipolysis and release of fatty acids at the endothelial surface[51]. Abbreviations: A, agonist; FA, fatty acid; R, receptor; PL, agonist-activated (circle) and basal (square) phospholipase activities.*

In addition to arachidonic acid, monounsaturated and saturated fatty acids that are not converted to active metabolites can also be liberated from phospholipids upon cell stimulation. This has been seen for thrombin stimulation of platelets[53] and caerulein stimulation of exocrine pancreas[54], as well as for stimulation of fibroblasts by a Ca^{2+} ionophore[55]. Thus, these fatty acids might serve as second messengers as well. Additionally, basal phospholipase activity[56] and influx of fatty acids from the circulation might affect ion channels by providing a basal pool of cellular fatty acids (see Fig. 4).

Fatty acids might also be important modulators under pathological conditions, such as tissue ischemia[57,58] or diabetes[59], where levels of non-esterified fatty acids in the circulation are elevated. Interestingly, fatty acid activation of K^+ channels in the heart is enhanced by acidification, a condition that occurs during cardiac ischemia[12].

It is intriguing that fatty acids have recently been found to block[17] or inactivate[18] the Cl^- channels implicated in the pathogenesis of cystic fibrosis. Since these Cl^- channels are known to be inhibited in cystic fibrosis, it will be of considerable interest to find out whether fatty acids play a role in the origin of this disease[17,18].

Physiological implications

So far, the physiological effect of fatty acids on excitable cells appears to be antagonism of electrical excitation: K^+ channels are activated and Na^+ and

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Ca^{2+} channels are inhibited. These effects would tend to decrease the amplitude and duration of action potentials and/or hyperpolarize the membrane, and thereby inhibit excitation. In this way fatty acids might function as endogenous counterparts to K^+ channel opener[60] and Ca^{2+} channel antagonist[22] drugs that promote relaxation in smooth muscle.

In addition to their direct effects on purified proteins and ion channels, fatty acids have been found to regulate a variety of cell processes[25,26,30,48,61–65], including synaptic transmission[61,63], neurotransmitter uptake[62] and suppression of Na^+ and Ca^{2+} currents[48], by mechanisms that do not appear to involve active, oxygenated metabolites. In these instances, actions of fatty acids might involve more complex interactions with cell signaling and metabolic pathways than the direct effects described above. For example, fatty acids decrease whole-cell Na^+ and Ca^{2+} currents in rat neuroblastoma cells[48], apparently by directly activating protein kinase C, which then affects ion channels via phosphorylation. This is in contrast to the mechanisms of fatty acid action on channels in smooth muscle[6,13,14], lacrimal gland[20], cardiac atrial cells[12], and airway epithelial cells[17,18], where fatty acids directly regulate ion channels, independent of phosphorylation.

The actions of fatty acids on cell processes, purified proteins and ion channels suggest that changes in the cellular content of non-esterified or 'free' fatty acids might have a profound effect on cell function. The widespread nature of these fatty acid effects, found in numerous preparations, has made clear that, in addition to the known function of arachidonic acid as a precursor to metabolites, possible signaling roles for fatty acids themselves must also be considered.

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