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Intracellular pH in adipocytes: Effects of free fatty acid diffusion across the plasma membrane, lipolytic agonists, and insulin

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ABSTRACT **The main function of white adipose tissue is to store nutrient energy in the form of triglycerides. The mechanism by which free fatty acids (FFA) move into and out of the adipocyte has not been resolved. We show here that changes in intracellular pH (pH_i) in adipocytes correlate with the movement of FFA across cellular membranes as predicted by the Kamp and Hamilton model of passive diffusion of FFA. Exposure of fat cells to lipolytic agents or external FFA results in a rapid intracellular acidification that is reversed by metabolism of the FFA or its removal by albumin. In contrast, insulin causes an alkalinization of the cell, consistent with its main function to promote esterification. Inhibition of Na^+/H^+ exchange in adipocytes does not prevent the changes in pH_i caused by FFA, lipolytic agents, or insulin. A fatty acid dimer, which diffuses into the cell but is not metabolized, causes an irreversible acidification. Taken together, the data suggest that changes in pH_i occur in adipocytes in response to the passive diffusion of un-ionized FFA (flip-flop) into and out of the cell and in response to their metabolism and production within the cell. These changes in pH_i may, in turn, modulate hormonal signaling and metabolism with significant impact on cell function.**

Several recent studies describe the cloning of membrane "fatty acid transport proteins" in adipocytes (1, 2). Nevertheless, the transport function of these cloned peptides has not been proven, and there is controversy as to how free fatty acids (FFA) enter and exit cells. To design molecular therapies for obesity and diabetes, it is imperative to understand the mechanism of FFA transport in adipose tissue and to know whether proteins are essential for the transmembrane movement of FFA. Methods to show that FFA entry into cells is a direct consequence of unfacilitated diffusion have been lacking, and evidence for either diffusion or transport has been indirect. Recently, a model of passive diffusion has been proposed and tested in model membranes by measuring pH changes in response to FFA movement across protein-free phospholipid bilayers (3–5). Data supporting carrier-independent passive diffusion of FFA across the plasma membrane of pancreatic β-cells has also been obtained with this new fluorescence approach (6).

FFA are stored and released from adipose tissue in response to nutrient need and hormonal stimulation of the tissue. The increase in fuel storage that results from exposure of adipocytes to insulin is mediated by a series of signaling events, which also lead to the regulation of protein synthesis and gene transcription (7). The immediate consequences of some of the transducing mechanisms that have been defined to date are not entirely clear (7). For example, exposure of adipose tissue (8), muscle (9), and liver (10) to insulin results in an increase in intracellular pH (pH_i). It has been suggested that this effect is mediated by activation of Na^+/H^+ exchange (8). Alternatively, the effect of insulin on pH_i also could arise from the removal of cytosolic FFA by esterification. The resulting alkalinization, by whatever mechanism, could function as a vital component of the tissue response to insulin, much as pH changes are involved in the regulation of fertilization, proliferation, and metabolism in other cellular contexts (11).

The purpose of the studies presented here was to determine the mechanism by which FFA move into adipocytes (the major functional goal of insulin action) and out of adipocytes (the major functional goal of lipolysis). Our data indicate a passive diffusion mechanism as opposed to a carrier-mediated process. The observed rise in pH_i caused by insulin stimulation of adipocytes is consistent with the role of insulin in promoting esterification of FFA. Incubation of adipocytes with FFA or lipolytic agents raises the intracellular level of FFA, resulting in a decrease in pH_i. Thus, the physiological response of the fat cell to store and to release FFA is accompanied by changes in pH_i, which could also influence the subsequent signaling or hormonal effects on the cell.

EXPERIMENTAL PROCEDURES

Cell Preparation and Measurement of pH. Adipocytes from male Sprague–Dawley rats weighing 150–250 g were prepared by the collagenase digestion method of Rodbell (12), using a modified Krebs buffer containing 118 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, 1.1 mM KH_2PO_4, 1.1 mM $MgSO_4$, 3% BSA, and 20 mM Mops (pH 7.4), as described (13). Cells were loaded with 1 μM 2′7′-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF)/acetoxymethyl ester (AM) for 30 min at 37°C with continuous shaking. For fluorescence recordings, cells ($\approx 10^6$ cells per ml; 0.6 mg protein per ml) were maintained in suspension by continuous stirring in modified Krebs buffer at 30°C in a computer-controlled Hitachi F-2000 spectrofluorometer. Unless otherwise indicated, albumin was absent from the incubation media to facilitate comparison of fatty acids with fatty acid analogues that bind poorly to albumin. On the other hand, in the presence of albumin, even higher concentrations of FFA may be needed to achieve partitioning of FFA to cells because of the strong binding of FFA to albumin (4, 6).

A limitation of this protocol is that a significant portion of the FFA (and analogs) may bind to the walls of the cuvette (14) or precipitate before binding to cells because of the very low solubility in aqueous buffer at pH 7.4 (15). Thus, the amount of FFA (or analog) incorporated into cells is expected to be lower than the amount of FFA added.

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Abbreviations: FFA, free fatty acids; pH_i, intracellular pH; EIPA, 5-(*N*-ethyl-*N*-isopropyl) amiloride.

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BCECF fluorescence was monitored at excitation wavelengths of 440 and 495 nm and an emission wavelength of 535 nm. The average basal pH_i was 7.0 ± 0.1 (n = 14). The pH_i was calibrated by measuring the fluorescence of the dye at different pH values between 6 and 8 as determined with a pH electrode after the cells were permeabilized to equilibrate pH_i with extracellular pH (6). Traces shown are those obtained at an excitation wavelength of 495 nm. The scale is indicated by the bar showing a pH_i change of 0.2 pH unit. NH_4Cl (10 mM final concentration) was added at the end of each experiment to confirm cell viability (6); the observed increase in pH_i, followed by a restoration of the basal pH_i, indicated viable cells and an intact plasma membrane.

Measurement of Extracellular FFA. The fluorescent engineered intestinal fatty acid binding protein, ADIFAB, was used to measure release of FFA into the media at an excitation wavelength of 390 nm and emission wavelengths of 432 and 505 nm (16, 17). The K_d of ADIFAB for oleic acid is 0.39 μM (15) and the final concentration of ADIFAB in the buffer was 0.2 μM.

Materials. BCECF/AM and ADIFAB were purchased from Molecular Probes. BSA (type V) was purchased from United States Biochemicals. FFA, 5-(*N*-ethyl-*N*-isopropyl) amiloride (EIPA), hormones, and other chemicals were from Boehringer Mannheim, Calbiochem, or Sigma. The fatty acid dimer comprised of two 18-carbon chains with no unsaturation (6) was obtained from Unichema Chemicals (Chicago). The tetradecylamine was from Aldrich.

RESULTS AND DISCUSSION

FFA Movement Alters pH_i. The use of the fluorescent dye, BCECF, to monitor pH_i is based on the expectation that diffusion of un-ionized FFA across the plasma membrane would cause acidification, whereas movement of the anion would cause alkalinization. Fig. 1 shows that addition of FFA to the medium in which isolated rat adipocytes were suspended caused a decrease in pH_i. The extent of acidification was dependent on the amount of added FFA (compare traces A and B) and was reversed over time (trace A); lower amounts of FFA produced smaller pH_i changes, and higher amounts produced larger pH_i changes. The pH_i decrease began immediately after addition of FFA and showed a rapid component ($t_{0.5} \ll 1$ min) followed by a slower component. The rapid component is more evident in the experiment with higher FFA (trace B). It is not clear whether the biphasic behavior reflects a biphasic response of most cells or different populations of cells, some of which respond rapidly and others of which respond slowly. After a maximal decrease in pH_i was reached, there was a slower return toward basal pH_i. This process could represent H^+ leakage or transport from the cell, or metabolism of FFA.

To evaluate the direct role of FFA in the acidification, BSA was added to the cells after the addition of FFA, but before the pH_i had returned to the initial value (trace B). This caused rapid alkalinization and restoration toward the initial pH_i. The extent or magnitude of the pH_i increase following BSA addition depended upon the interval after its addition and the concentration of added FFA (compare traces A and B). When BSA was added after pH_i had returned to equilibrium little effect on pH_i was observed (trace A). The effects of BSA on pH_i strengthen the argument that FFA are directly involved in the observed pH_i changes. After addition of 65 nmol of FFA, 1 μM of BSA (1.3 nmol total) resulted in complete reversal of the decrease in pH_i. After addition of 130 nmol of FFA, most but not all of the pH_i decrease was reversed. In the latter case, the amount of BSA was likely insufficient for complete extraction of FFA. The observation that low amounts of BSA completely or nearly completely reversed the pH_i change suggests that much lower amounts of FFA than added to the suspension actually entered the cells, as the partitioning of FFA from membranes to BSA decreases markedly after 4–5 mol of FFA are bound per mol of BSA (4). The return of pH_i to equilibrium with time, together with the inability of BSA to induce pH_i, increases after a long exposure to FFA (trace A), suggesting (*i*) that FFA are metabolized within the 10-min period required for pH_i equilibration and (*ii*) that BSA removes only that portion that has not yet been metabolized.

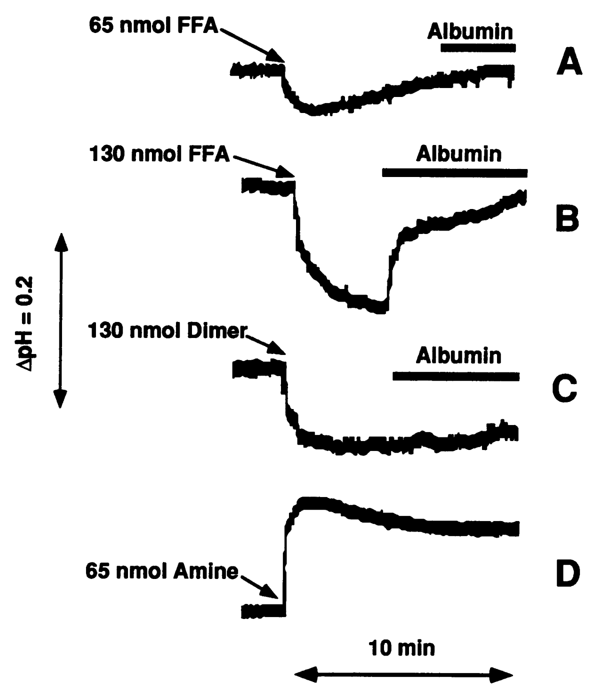

FIG. 1. Illustration of the time course of change in pH_i following addition of FFA to suspensions of isolated rat adipocytes. Rat adipocytes (0.6 mg/ml) loaded with the pH indicator BCECF were suspended in 1.3 ml of Krebs buffer containing no albumin (pH 7.4) at 30°C. Traces A and B show the acidification caused by addition of 65 or 130 nmol of oleic acid. The ability of 1 μM BSA addition to reverse the effect of FFA addition is compared at different times after addition of FFA (traces A and B). Trace C shows acidification by 130 nmol of a fatty acid dimer. Trace D shows alkalinization by 65 nmol of tetradecylamine. No reversal of the pH_i change occurred upon addition of BSA in traces C or D. The vertical bar represents a change in pH_i of 0.2 pH unit. The illustrations shown are representative of experiments repeated at least six times.

The mechanism by which FFA move into adipocytes was explored further by using FFA analogs that should not bind to putative FFA transport proteins and are not appreciably metabolized. We first studied a dimer consisting of two 18-carbon fatty acid chains, covalently bound at the middle, which retain their terminal carboxyl groups and are thus able, like single-chain FFA, to liberate protons upon ionization. This molecule was previously shown to exhibit rapid transbilayer movement in model membranes and to bind poorly to BSA (6). Fig. 1*C* shows that the fatty acid dimer caused a rapid and sustained acidification that was not reversed by BSA (compare traces C and A). A similar experiment was performed with a 14-carbon fatty acid analog (tetradecylamine), whose ionizable group is an amine instead of a carboxyl group. Diffusion of the un-ionized species of the amine through the plasma membrane followed by its protonation on the inner leaflet would result in removal of H^+ from the cytosolic compartment and thus a rise in pH_i (3). Trace D shows that addition of the amine caused sustained elevation of pH_i, which was not prevented or reversed by BSA (data not shown). Neither the FFA dimer nor the amine are rapidly metabolized, and the sustained changes in pH_i with these analogs suggest that the cells cannot quickly return to the initial pH by H^+ pumping or leakage. Similar pH_i

changes for the dimer and amine were previously observed in pancreatic β-cells (6). It is important to note that in all experiments of Fig. 1 addition of NH_4Cl (see *Experimental Procedures*) showed that cell membranes remained intact and cells remained viable and able to maintain a pH gradient after addition of FFA or analogs.

These data support two important conclusions. First, the observed transport is not likely to be protein-mediated, since there is no precedent for a protein carrier that could bind both a double-chain negatively charged amphiphile (dimer) and a single-chain positively charged amphiphile (amine). Second, the sustained pH changes induced by both analogs indicate that the restoration of pH after FFA is added (Fig. 1*A*) is probably due to metabolism of FFA, since either leakage or H^+ pumping should be independent of the lipid transported. Taken together, the results of Fig. 1 show that FFA reaches the cytosolic leaflet of the plasma membrane at a rate that is much faster than rates of intracellular utilization of FFA.

The possible role of the Na^+/H^+ exchanger in FFA-induced acidification in adipocytes was evaluated by replacing the Na^+ in the medium with Mg^{2+}. This change in buffer did not appreciably modify the pH change caused by addition of FFA (data not shown), indicating that the exchanger does not play a major role in the observed FFA-mediated acidification. The effect of the FFA analogs on pH_i (Fig. 1 *A* or *B*) also are inconsistent with a role for the Na^+/H^+ exchanger under our experimental conditions.

Taken together, these data support a passive diffusion mechanism ("flip-flop") in which FFA added to adipocytes first bind to the outer leaflet of the plasma membrane and become about 50% ionized at the membrane interface, assuming the apparent pK_a of the bound FFA is similar to that in a phospholipid bilayer (18, 19). Un-ionized FFA passively diffuse across the membrane and reach ionization equilibrium on the cytosolic interface, with the liberation of H^+ into the cytosol (3). The equilibrium distribution of FFA among cellular compartments will be governed by their solubility in the plasma membrane and intracellular membranes and the affinities of various proteins for FFA. Prior to the consequences of metabolism, the total quantity of long-chain FFA and the total binding sites in membrane phospholipids and cytosolic fatty acid-binding proteins will determine the uptake of FFA into cells. Because of such complexities, the decrease in pH_i in cells cannot be correlated in a simple manner with the amount of added FFA, as can be done with phospholipid vesicles (4). In addition, some of the added FFA may precipitate or bind to the surface of the cuvette.

It is unlikely that a protein carrier in the plasma membrane plays an important role in the observed pH_i changes, since such a carrier would likely bind the FFA anion and cause intracellular alkalinization by attracting H^+ in the cytosol. (The hypothesis that such a protein is acting as a cotransporter of H^+ is unlikely because of the observed pH_i effects of analogs.) However, intracellular binding proteins, such as fatty acid-binding protein or acyl-CoA synthetase, might increase the net transport of FFA and hence the observed acidification by providing a sink to increase the partitioning of FFA into cells. Likewise, the presence of an external binding protein, such as albumin, would diminish apparent transport and acidification by decreasing partitioning of FFA into cells, as illustrated by our experiments with the addition of albumin (Fig. 1, trace B).

Lipolytic Agents Cause Intracellular Acidification. To evaluate whether the passive diffusion mechanism of FFA movement into adipocytes described above is of physiological or pharmacological relevance, we investigated the influence of hormones that modulate FFA storage or breakdown in adipocytes. Lipolytic agonists liberate FFA from triglycerides where they are stored, and the ionization of such liberated FFA could decrease pH_i. Hydrolysis of the triglyceride contained within intracellular droplets occurs at the interface, and the FFA produced will initially be present primarily in the droplet interface with an apparent pK_a of 7.4 (20). Thus, at this step, about 50% of the FFA will contribute H^+ that could acidify the cytosol. Fig. 2 shows that decreases in the pH_i were observed following additions to the extracellular medium of maximally stimulatory concentrations of three lipolytic agents: norepinephrine (trace A), which has a broad adrenergic specificity; forskolin (trace B), which directly activates adenyl cyclase and bypasses the receptor; and isoproterenol (traces C and D), a specific β-agonist. The observed decreases in pH_i began immediately after addition of the lipolytic agonists and the time dependencies show mainly a slow phase, probably reflecting the finite rate of triglyceride hydrolysis.

To investigate the relationship between internal acidification and release of FFA from intracellular stores, the engineered fluorescent fatty acid-binding protein ADIFAB was added to the external buffer and the experiment of Fig. 2*C* repeated (trace D). ADIFAB fluorescence showed a continuous increase, which was the inverse of the decrease in pH_i. The extent of the decrease in pH_i was reduced compared with Fig. 2*C* because ADIFAB contributed additional external binding sites for FFA. Thus, the increase in ADIFAB fluorescence reflects binding of FFA that has moved from the cytosol to the external medium, and this movement occurs as rapidly as FFA are generated. The FFA that desorb from the cell are expected to remove H^+ from the cytosol if they passively diffuse out of the cell, partially offsetting the pH_i decrease from newly generated FFA. This result with ADIFAB also provides evidence that FFA were released from intracellular sites following isoproterenol administration. Further evidence for a role for

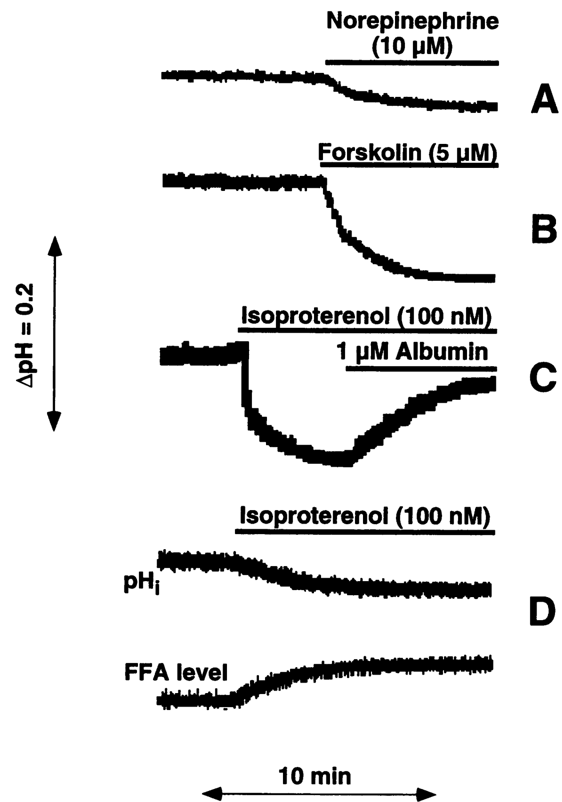

FIG. 2. Effect on pH_i of the lipolytic agonists norepinephrine (*A*), forskolin (*B*), or isoproterenol (*C* and *D*) and of isoproterenol on FFA release (*D*). Experiments were performed and adipocytes incubated as described in Fig. 1. In the experiment of Fig. 1*D*, relative FFA concentration in the medium was monitored with 0.3 μM of ADIFAB; an increase in fluorescence indicates an increase in medium FFA. The illustrations shown are representative of experiments repeated at least six times.

FFA in the isoproterenol-mediated fall in pH_i comes from the demonstration in trace C of the ability of BSA to reverse the decrease in pH_i.

The results of Fig. 2 show, first, that lipolytic agents caused release of similar concentrations of H^+ into the cytosol as did addition of exogenous FFA (e.g., Fig. 1 *A* and *B*). This comparison suggests that the amounts of FFA entering the adipocytes after addition of FFA to the buffer are "physiologically relevant." Second, Fig. 2 shows that the pH_i decrease induced by lipolytic agents can be attributed to FFA. These newly generated FFA were shown to move rapidly across the plasma membrane to binding sites on albumin or ADIFAB in the external medium. *In vivo*, the effect of albumin in plasma might be to diminish the pH_i changes considerably, though this would depend on the prior degree of saturation of the FFA binding sites.

Insulin Causes Intracellular Alkalinization. An important action of insulin is to promote the conversion of FFA to triglycerides. To determine whether the resulting conversion of FFA, which bear a net negative charge, to a neutral molecule could remove H^+ from the cytosol and raise the pH, the influence of insulin on pH_i was studied. Fig. 3*A* shows that insulin addition resulted in a sustained and concentration-dependent increase in pH_i. The slower time course of the pH_i change compared with the diffusion of FFA in and out of adipocytes is consistent with synthesis of triglyceride over a time period of minutes, and the delay of onset of the pH_i change indicates that there are time-dependent steps in the signaling pathway. This contrasts with the immediate pH changes seen with the addition of the amine (Fig. 1*D*) or with exogenous or endogenous elevation of FFA. The ability of insulin to raise pH_i was observed in response to physiologically relevant concentrations of the hormone, with a half-maximal increase in pH_i obtained at about 0.2 nM (30 microunits/ml).

To test whether insulin could also antagonize the pH_i effect resulting from lipolysis by promoting reesterification of newly formed FFA, the experiment of Fig. 2*D* was repeated with and without insulin. Consistent with its established anti-lipolytic effect, insulin diminished the ability of isoproterenol to acidify pH_i (Fig. 3*B*). Release of FFA into the medium as measured by ADIFAB was also diminished. The time course of binding of FFA to ADIFAB was generally similar to the time course of acidification following addition of isoproterenol, with or without insulin.

To explore further the use of pH_i to monitor changes in intracellular FFA pools, the time course of pH_i changes in adipocytes, following two protocols, was monitored (Fig. 3*C*): first, addition of FFA followed by insulin (left trace) and second, addition of insulin followed by the same amount of FFA (right trace). The magnitude of the drop in pH_i after addition of FFA and the time period required to regain the initial pH_i are presumably a reflection of the rate of FFA entry less the rate of FFA metabolism. Addition of FFA caused a rapid decrease in pH_i, which returned to equilibrium within 10 min (Fig. 3*C*, left trace), as shown also in Fig. 1*A*. Addition of insulin then caused an increase in pH_i similar to that observed for the addition of insulin without prior addition of FFA (Fig. 3*A*). When FFA were added after insulin, the FFA-induced acidification and its duration were greatly attenuated (Fig. 3*C*, right trace). These effects may reflect a primary role of insulin in FFA metabolism, the stimulation of esterification to triglyceride, and suggest that incoming FFA are more rapidly esterified and removed in the presence than in the absence of insulin.

Finally, we demonstrated that high levels of BSA in the medium markedly diminished the alkalinization induced by insulin (Fig. 3*D*). This result suggests that a major component of this effect was due to reutilization of endogenous FFA. The large pH_i effect of insulin may not be completely attributed to FFA.

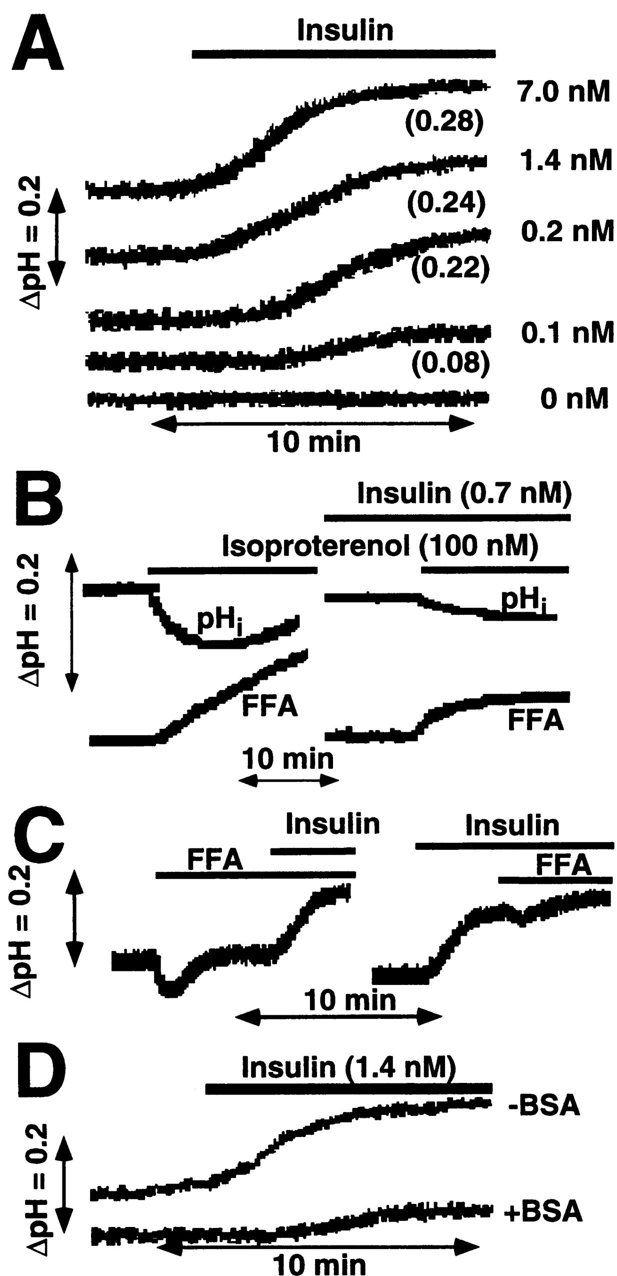

FIG. 3. Effects of insulin on pH_i in isolated adipocytes. (*A*) Concentration dependence of insulin-induced alkalinization. Values in parentheses represent the maximal increases in pH_i at the designated insulin concentration. (*B*) Interaction of insulin and isoproterenol effects on pH_i and FFA production (note: in the experiment on the right insulin was added 10 min before the beginning of the trace). (*C*) Effect of prior treatment with insulin on FFA-induced pH_i changes. (*D*) Alkalinization induced by insulin (1.4 nM) in the absence and presence of 500 μM BSA. Experiments were performed and adipocytes incubated as described in Fig. 2. The illustrations shown are representative of experiments repeated at least six times.

Other mechanisms that could also contribute to the insulin-induced pH_i include insulin activation of the Na^+/H^+ exchange (21). However, pretreatment with 50 μM of 5-(*N*-ethyl-*N*-isopropyl)amiloride (EIPA), an analog of amiloride that inhibits the exchanger, caused only a small decrease in the ability of insulin to alkalinize pH_i (Fig. 4, trace A). EIPA alone caused a slow decrease in the pH_i (trace B), which is expected, if Na^+/H^+ exchange is required to maintain basal pH_i. This decrease is much slower than that observed with FFA or with activators of intracellular triglyceride hydrolysis, suggesting

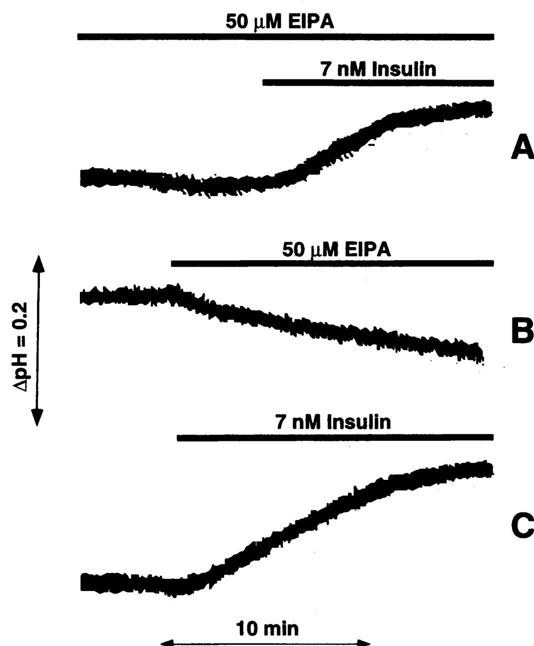

FIG. 4. Effect on insulin-induced pH_i changes of inhibition of Na^+/H^+ exchange by EIPA (trace A) or by replacement of extracellular Na^+ with Mg^{2+} (trace C). Adipocytes were incubated as described in Fig. 1. The effect of EIPA alone is shown in trace B. The addition of EIPA caused continuous acidification, which was subtracted from trace A to permit the effect of insulin to be seen clearly. The illustrations shown are representative of experiments repeated at least six times.

that Na^+/H^+ exchange activation by insulin does not occur in the same time frame as FFA movement. Likewise, replacement of Na^+ in the medium with Mg^{2+} did not prevent the alkalinization induced by insulin (trace C).

Conclusions. Three major conclusions can be made from these studies: (*i*) fatty acids can pass through the plasma membrane rapidly and in a bidirectional manner by passive diffusion (flip-flop), and pH_i is affected by this mechanism; (*ii*) intracellular hydrolysis of triglycerides results in decreases in pH_i; and (*iii*) insulin-induced increases in pH_i may be explained, at least in part, by insulin stimulation of triglyceride synthesis and the consequent decreases in the cellular pool of FFA. All three perturbations cause pH_i changes of similar magnitude, suggesting that levels of FFA produced or esterified intracellularly could be similar to the levels of FFA incorporated in adipocytes from that which is added exogenously. Such FFA-induced changes in cells that are in close contact with capillaries may be buffered by the presence of circulating albumin; the strength of the buffering would be dependent on the relative saturation of albumin-binding sites with FFA and thus on the prior nutritional–metabolic–hormonal status.

The first conclusion merits additional comment because of the general controversy concerning how FFA enter cells, by passive diffusion or by protein-facilitated transport, and the central role of the adipocyte in this controversy (22, 23). Two possibilities need to be considered: first, that nonionic transport of FFA represents the major transport mechanism for FFA or alternatively, that nonionic transport represents a trivial percentage compared with that transported by a protein carrier. Previous investigators have presented evidence for transport by proteins (1, 2, 24), including an extensively glycosylated 88-kDa protein (2) and a newly cloned 63-kDa protein (1). Other investigators have found a glycosylated 85-kDa protein that could be the same protein as the 88-kDa protein and is a substrate for palmitoylation in rat adipocytes (25). However, the transport function of these proteins remains to be established by the classical criteria of reconstitution of the putative transporter into phospholipid vesicles.

Our study establishes the mechanism of passive, nonionic diffusion of FFA into adipocytes and demonstrates that levels of FFA entering cells by this mechanism are comparable to levels produced by stimulation of lipolysis of intracellular stores of triglyceride. Therefore, passive diffusion provides higher than basal levels of FFA to the cytosol. According to our hypothesis, we are measuring only FFA entering by passive diffusion since other modes of transport would not involve increased intracellular H^+ concentration. As discussed above, the quantitative relationship between binding of FFA to the plasma membrane and pH_i has not yet been calibrated because of the complexity of the system. Therefore, our measurements do not eliminate other mechanisms. Because the phospholipid component of cell membranes has a high capacity for binding FFA (14), there is no *a priori* reason to believe that the passive diffusion mechanism cannot provide levels of FFA required for metabolism. Our finding that the pH decrease in adipocytes is sustained for minutes before returning to equilibrium supports this view. At least, our studies make it clear that further studies of putative transport proteins in adipose tissue must account for the physical characteristics of FFA and their rapid movement through lipid bilayers. At most, our results imply that diffusion could be a major, if not the sole, pathway for entry and exit of FFA in adipocytes.

In this study we have explained pH_i changes in terms of changes in intracellular levels of FFA. These occur as a result of FFA movement across the plasma membrane, enzymatic production of FFA from triglycerides, or biosynthesis of triglycerides from FFA. This minimalist hypothesis has testable corollaries and is intended as a working hypothesis rather than a complete explanation of pH_i changes. The quantitative aspects of the observed pH changes remain to be investigated and are likely to involve additional mechanisms. A role for intracellular fatty acid-binding proteins and FFA metabolizing enzymes on the rate and extent of pH_i changes is expected and requires quantitative evaluation of FFA transport in cells where expression of these proteins has been altered.

The data presented here indicate that monitoring pH_i may provide a novel and useful way to assess insulin action and lipolysis in cell suspensions, and for the first time provide a real-time probe for studying hormone-mediated changes in FFA.

Note Added in Proof. After submission of this manuscript Trigatti and Gerber (26) showed that by manipulation of pH_i adipocytes, FFA entered cells in a manner consistent with passive diffusion of unionized FFA.

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