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Quantitative changes in adipocyte plasma membrane in response to nutritional manipulations

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Abstract The effects of changes in adipocyte size and the effects of nutritional manipulations on the quantity of plasma membrane per adipocyte were investigated. A method for estimating the quantity of plasma membrane was developed based on the specific labeling of adipocyte plasma membrane protein with the nonpermeable labeling agent ^{125}I-labeled diazotized diiodosulfanilic acid. By studying rats (ranging in age from 50 to 125 days) fed a standard laboratory chow or a low fat diet or a high fat diet, a wide range of mean fat cell sizes was obtained. It was found that as the volume of the fat cell increased, the amount of plasma membrane increased in a linear fashion and that this linear relationship had the same slope whether the size of the adipocyte increased slowly with age or rapidly in response to a high fat diet. In contrast, fasting for up to 3 days caused a marked decrease in the mean volume of the adipocytes, but either no change or much less change in the amount of plasma membrane per cell than would have been predicted from the linear relationship between adipocyte volume and amount of plasma membrane per cell obtained with fed rats, i.e., adipocytes from fasted rats contain more plasma membrane per cell than do fat cells of the same size from fed rats. Neither feeding a high fat diet nor fasting caused detectable changes in the protein and lipid composition of the adipocyte plasma membrane.—**Lewis, D. S., E. J. Masoro, and B. P. Yu.** Quantitative changes in adipocyte plasma membrane in response to nutritional manipulations. *J. Lipid Res.* 1981. **22:** 1094–1101.

Supplementary key words diazotized diiodosulfanilic acid · adipocyte volume · plasma membrane composition

The response of fat cells to hormones that regulate fat deposition and fat mobilization is markedly influenced by the nutritional state of the animal. For example, in the rat, fasting results in a decreased response of adipocytes to insulin; i.e., the promotion of glucose utilization in adipocytes by insulin is decreased (1). On the other hand, the promotion of adenylate cyclase activity in adipocytes by epinephrine is enhanced by fasting (2). High fat diets also influence the responsiveness of fat cells to hormones; e.g., the ability of epinephrine or glucagon to promote adenylate cyclase activity and lipolysis is depressed (2, 3).

The mechanisms by which nutrition modulates the response to hormones have not been fully delineated. In the instances mentioned above, neither a change in the number of nor the affinity of plasma membrane receptors appears to play an important role (1, 4). However, other changes in the membrane composition or structure may be involved; e.g., the quantity of membrane per adipocyte may modify the coupling of the hormone-receptor complex with intracellular events. Little work has been done on this because the required methodology has not been available.

This study presents a method for determining the amount of plasma membrane per adipocyte. It also reports on the use of this method to determine the effects of cell size and nutritional manipulations on the quantity of plasma membrane per adipocyte.

MATERIALS AND METHODS

Rats and diets

Male Fischer 344 rats, purchased from Charles River Breeding Laboratories, Inc., were fed ad libitum one of the following diets: *a*) standard laboratory chow (Wayne Lab Blox); *b*) the low fat diet of Gorman, Tepperman, and Tepperman (2) prepared by ICN Nutritional Biochemicals; *c*) the high fat diet of Gorman et al. (2) prepared by ICN Nutritional Biochemicals.

Abbreviation: DDISA, diazotized diiodosulfanilic acid.

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Analytical assays

Membrane fractions were assayed for 5′-nucleotidase activity utilizing [^{3}H]adenosine monophosphate as described by Avruch and Wallach (5), for cytochrome C reductase activity by the method of Dallner, Siekevitz, and Palade (6), and for succinate dehydrogenase activity by the procedure of Green, Mii, and Kohout (7). The protein content of fat cell components was determined by the method of Lowry et al. (8). Lipids were isolated from the membrane fractions by the procedure of Sanslone et al. (9). The phospholipid-phosphorus was measured by the method of Bartlett (10) and cholesterol was assayed enzymatically by the method of Allain et al. (11). Cellular proteins were precipitated by adding two volumes of 10% trichloroacetic acid per volume of biological material; this mixture was incubated on ice for 30 min and the precipitate was collected by centrifugation. The ^{125}I content of samples was assayed by means of a gamma counter.

Preparation of ^{125}I-labeled diazotized diiodosulfanilic acid

The membrane protein labeling agent, iodinated diazotized diiodosulfanilic acid ([^{125}I]DDISA), was prepared by the method of Helmkamp and Sears (12) utilizing 5 mCi of carrier-free ^{125}I (ICN Radiochemicals) and 80 μmoles diiodosulfanilic acid (a generous gift from Dr. David Sears). The [^{125}I]DDISA was stored in the dark at -20°C in the form of a wet precipitate and used within 6 weeks. Immediately before use, the [^{125}I]DDISA was suspended in 1.0 ml of distilled water at 4°C and centrifuged at 900 g for 5 min. The resulting supernatant, containing approximately 1 μmole DDISA per ml, was immediately used to label adipose tissue and protein.

Preparation of [^{125}I]DDISA-labeled albumin and collagenase

Bovine serum albumin (0.5 g) was incubated for 1 min at 37°C in 1.0 ml of [^{125}I]DDISA solution and then separated from free [^{125}I]DDISA on a Biogel P-2 column. The specific activity of ^{125}I-labeled albumin was 1.36×10^{7} cpm/g albumin. Collagenase (225 mg) was incubated in 5 ml of Krebs bicarbonate buffer and 1.0 ml of [^{125}I]DDISA for 10 min at 37°C. This resulted in 1.9×10^{6} cpm [^{125}I]DDISA incorporated per g collagenase. Most of the [^{125}I]DDISA had reacted by this time and was therefore not removed. The labeled proteins were then added to intact adipose tissue and collagenase-freed fat cells were isolated as previously described.

Procedure for estimation of mean adipocyte plasma membrane mass

The mean amount of plasma membrane per adipocyte was estimated by the following procedure.

The perirenal and epididymal fat depots from 20 rats were excised and the tissue was minced with sharp scissors. All procedures utilizing the minced tissue were carried out at 37°C. Two grams of the minced tissue were transferred to a 35-ml disposable syringe and washed twice with 35 ml of saline and once with 35 ml of Krebs bicarbonate buffer, pH 7.4. The washed, minced adipose tissue was transferred to a plastic beaker containing 1 ml of [^{125}I]DDISA (1 μmole, $1-5 \times 10^{8}$ cpm) and incubated for 1 min with constant swirling. This labeled tissue was then washed twice with 35 ml of Krebs bicarbonate buffer.

The labeled tissue mince was combined with the portion of tissue that was not labeled and free adipocytes were prepared by the collagenase digestion procedure of Rodbell (13) as modified by Bertrand, Masoro, and Yu (14). This digest was filtered through 105 μm nylon mesh into a plastic separatory funnel and washed free of collagenase as described previously (14).

The adipocyte suspension (0.5-ml aliquots in triplicate) was analyzed for number of cells per ml and for mean adipocyte volume by the method of Stiles, Francendese, and Masoro (15).

In order to measure the amount of ^{125}I bound to membrane protein per ml of fat cell suspension, the following method was used: 4 ml of mineral oil was layered on top of 1 ml of the adipocyte suspension in a centrifuge tube which was then centrifuged for 1 hr at 40,000 rpm at room temperature in a SW 50.1 rotor. For best results, the rotor was allowed to reach 40,000 rpm with the slowest acceleration possible. This procedure totally lysed the cells and yielded a membrane pellet containing more than 95% of the adipocyte plasma membrane which was then resuspended in Tris buffer (10 mM, pH 7.4) containing 1 mM EDTA. Trichloroacetic acid-precipitated protein was then assayed for ^{125}I content. Approximately 89% of adipocyte ^{125}I was recovered in this pellet.

The amount of ^{125}I incorporated into the plasma membrane per adipocyte was calculated by dividing the amount of membrane-bound ^{125}I per ml of adipocyte suspension by the number of adipocytes per ml of adipocyte suspension.

The amount of ^{125}I per mg of plasma membrane protein was determined in the following way. The remaining adipocyte suspension was homogenized and a plasma membrane fraction was highly purified

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TABLE 1. Distribution of [^{125}I]DDISA in subcellular fractions of adipocytes isolated from [^{125}I]DDISA-labeled adipose tissue mince

Fraction	[^{125}I]DDISA	
	Intact Tissue	Lysed Tissue
	cpm/mg protein × 10^{-3}	
Plasma membrane	4.8	0.6
Mitochondria	2.5	0.4
Endoplasmic reticulum	0.6	1.2
Nuclear pellet	0.6	0.4
100,000 *g* supernatant	0.1	3.8

Adipocyte subcellular fractions were isolated as described by Lewis et al. (16) from fat cells isolated from [^{125}I]DDISA-labeled minced adipose tissue. The data presented are from a typical experiment utilizing 20 rats. Approximately 50% of the ^{125}I was recovered in the plasma membrane fraction.

as previously described (16). This membrane fraction was then assayed for protein content and for trichloroacetic acid-precipitable ^{125}I. The amount of ^{125}I incorporated per mg of plasma membrane protein was calculated.

The mean amount of plasma membrane protein per adipocyte was calculated by dividing the cpm ^{125}I per adipocyte by cpm ^{125}I per mg plasma membrane protein. Since the plasma membrane lipid:protein weight ratio was also determined, it was possible to calculate mean mass for plasma membrane per adipocyte assuming that protein and lipid are the sole membrane components.

RESULTS

[^{125}I]DDISA as a nonpermeant labeling agent of plasma membrane protein

The method just described for the estimation of the mean amount of plasma membrane per adipocyte requires that [^{125}I]DDISA label the plasma membrane proteins but not permeate the membrane and enter the cell. In an earlier study (16) in which only fed rats were studied, [^{125}I]DDISA was shown not to permeate the plasma membranes of isolated adipocytes. However, when isolated fat cells from fasted rats were labeled with [^{125}I]DDISA in pilot work for the present study, the plasma membrane was found to be permeable to this labeling agent.

It seemed possible that collagenase treatment of adipose tissue from fasted rats might be responsible for DDISA permeating the plasma membrane. It was therefore decided to determine if the adipocyte plasma membrane of minced adipose tissue could be effectively labeled with DDISA. The results of this study are reported in **Table 1.** The labeling pattern obtained with minced adipose tissue from fed rats is similar to that previously reported with isolated fat cells from fed rats (16). The plasma membrane proteins have the highest ^{125}I specific activity and the proteins of 100,000 *g* supernatant have the lowest specific activity.

Although the protein in the supernatant fraction had a low ^{125}I specific activity, the total amount of label in the fraction was considerable (~10% of the ^{125}I taken up by the cells). However, for the following reasons, this probably does not indicate permeation of the fat cell by DDISA. *a*) When lysed cells were labeled, the supernatant proteins had the highest ^{125}I specific activity, while the specific activity of the supernatant proteins with intact cells was about 2% of that of the plasma membrane proteins (Table 1). *b*) When lysed cells were treated with [^{125}I]DDISA, many protein components of the mitochondrial and plasma membrane fractions were labeled (**Fig. 1A**), while the labeling of intact cells resulted in a limited labeling of protein components (Fig. 1B) with the mitochondria and plasma membrane patterns being almost identical. This is consistent with the label in the mitochondria being derived from plasma membrane contamination. The radioactivity in the supernatant fraction probably relates to ^{125}I-labeled protein loosely associated with the exterior of the cell (e.g., albumin and/or collagenase) since experiments attempting to wash ^{125}I away from the isolated adipocytes revealed a slow removal through many washings (**Table 2**). Further evidence that this is so was obtained by using [^{125}I]DDISA-labeled albumin and collagenase and determining the association of these proteins with fat cells. Our procedure for isolating adipocyte plasma membranes removed almost all of these proteins. Specifically, the plasma membranes contained no detectable ^{125}I-labeled albumin, and less than 1.5% of the plasma membrane protein was derived from collagenase. These data establish the labeling of minced adipose tissue of fed rats with [^{125}I]DDISA to be sufficiently selective for the plasma membrane for our purposes.

The following data indicate that such is also the case for the other nutritional states studied. The ratio of ^{125}I specific activity in plasma membrane protein to that of the 100,000 *g* supernatant protein is reported in **Table 3** for fed rats, fasted rats, and rats fed low and high fat diets. For each of these nutritional states, the ratio is about 40. When disrupted tissues composed of broken cells are labeled, the ratio is about 0.2. These data strongly indicate that when minced adipose tissue is used [^{125}I]DDISA is a nonpermeant labeling agent of the adipocyte plasma membrane in

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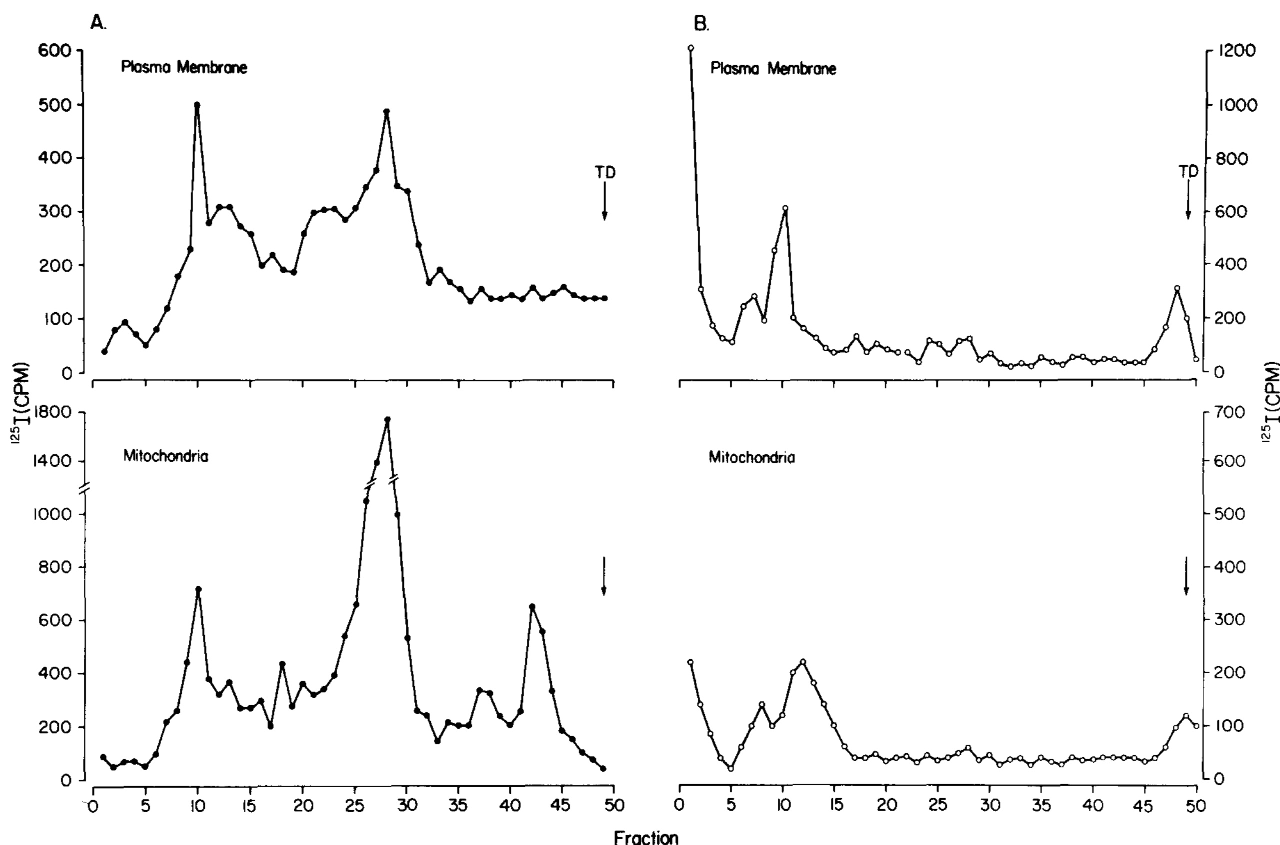

Fig. 1. SDS-polyacrylamide gel profile of plasma membranes and mitochondria from [^{125}I]DDISA-labeled adipose tissue. Disrupted adipose tissue (panel A) and intact tissue (panel B) were labeled with [^{125}I]DDISA as described in Materials and Methods. Plasma membranes (75 μg protein) and mitochondria (150 μg protein) were isolated as described previously (16) and submitted to 1% SDS–9% polyacrylamide gel electrophoresis for 4 hr at 6 mamps per gel. Each gel was then fractionated into approximately 1-mm slices with each slice being counted in a Beckman gamma spectrometer.

all nutritional states studied. Thus the labeling of minced adipose tissue became the standard procedure in all studies presented below.

In addition to a nonpermeant labeling agent, our method of estimating the amount of plasma membrane per cell requires highly purified plasma membranes. A method accomplishing this was recently published for fat cells from fed rats (16).

However, fasting and high fat feeding are known to cause considerable morphological alterations in adipocytes. Such alterations may influence the purification of plasma membranes. Therefore, the marker enzyme characteristics were determined for fat cells isolated from fasted rats and from rats fed high fat diets. The 5′-nucleotidase activity and the extent of labeling with DDISA per mg membrane protein were virtually identical in plasma membranes from rats fed either the low fat diet, the high fat diet, or the high fat/fasted diet (**Table 4**). In addition, the extent of contamination of the plasma membrane with microsomal and mitochondrial marker enzymes was not affected by nutritional state. Thus the procedure for isolating purified plasma membranes appears to be adequate for all nutritional states studied.

This conclusion is supported by the analysis of the composition of the plasma membranes. Fasting and high fat feeding did not influence the lipid:protein weight ratio, the phospholipid:protein weight ratio, and the cholesterol:phospholipid mole ratio of the fat cell plasma membrane (**Table 5**). Moreover, on the basis of thin-layer chromatography for phospholipids and SDS-gel electrophoresis for the proteins, no evidence was found to indicate that the relative abundance of phospholipid subclasses (i.e., phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, etc.), or the protein profile (Coomassie Blue staining), or the glycoprotein profile (staining with acid-Schiff reagent) were changed by any of the nutritional manipulations used (data not shown).

Effect of fasting on amount of plasma membrane per adipocyte

Male Fischer 344 rats, 10 weeks of age, previously maintained on standard laboratory chow were fasted

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TABLE 2. Study determining efficiency of removal of [^{125}I]DDISA not bound to cells

Fraction		Total cpm ^{125}I $\times 10^{-5}$
[^{125}I]DDISA added		985
Initial washes plus collagenase media	75 ml	670
Wash #1	35 ml	106
Wash #2	35 ml	32
Wash #3	35 ml	9
Wash #4	35 ml	6
Wash #10[a]	35 ml	2

[a] Washes 5 through 9 revealed a slowly diminishing removal of ^{125}I from the cells.

for 0, 24, or 48 hr prior to being killed. The mean adipocyte volume and the mean amount of plasma membrane per adipocyte of the combined perirenal and epididymal depots were measured. Fasting for 24 and 48 hr significantly reduced the mean cell volume by 30 and 60% respectively, $P < 0.01$ (panel A of **Fig. 2**). Surprisingly, the mean amount of plasma membrane per adipocyte was not significantly changed by either a 24- or 48-hr fast (panel B of Fig. 2). It should be noted, however, that in the case of rats fed the high fat diet, which resulted in very large adipocytes, fasting for 72 hr did result in some loss in amount of plasma membrane per adipocyte (**Table 6**).

Effect of increasing adipocyte size on the amount of plasma membrane per adipocyte

To obtain adipocytes of a markedly larger size than the cells of rats fed the standard laboratory chow, rats were fed a high fat diet for 7 days. Adipocytes from the rats fed this high fat diet had a mean volume almost twice that of rats of the same age fed a low fat diet for the same length of time (Table 6). In addition, the adipocytes from the rats fed the high fat diet had more than twice the amount of plasma membrane per adipocyte than did the cells from rats fed the low fat diet. Fasting the rats fed the high fat diet for 3 days resulted in a mean adipocyte volume almost identical to that of the rats fed the low fat diet, but with 1.5 times the amount of plasma membrane per adipocyte as cells from rats fed the low fat diet.

Relationship between adipocyte size and the amount of plasma membrane

By studying rats of various ages (50 to 125 days) fed standard laboratory chow or the low fat or high fat diets, a wide range of mean fat cell sizes was obtained. The relationship between mean fat cell volume and mean amount of plasma membrane per adipocyte is presented in **Fig. 3.** In the case of fed rats, the size of the fat cells and the amount of plasma membrane are linearly related; i.e., as the fat cell volume increases, the amount of plasma membrane per cell correspondingly increases. Surprisingly, when data for the fasted rats are plotted on this graph, they do not fall on the line obtained with adipocytes from fed rats. Rather, another line can be drawn for the adipocytes from fasted rats with a different intercept on the y axis ($P < 0.01$). These data show that for a given adipocyte volume, adipocytes from rats fasted 1, 2, or 3 days have more plasma membrane per adipocyte than do adipocytes from fed rats.

As a first approximation, adipocytes might be viewed as being spheres. If so, the increase in adipocyte plasma membrane that occurs with increasing fat cell size should correspond to the increase in the surface area of a sphere. This relationship was analyzed and the data revealed that, as an adipocyte enlarges, the increase in amount of plasma membrane per cell is greater than would be predicted for corresponding increase in the surface area of a sphere model.

TABLE 3. Effect of diet on [^{125}I]DDISA plasma membrane: soluble protein specific activity ratio

Diet	cpm ^{125}I/mg Plasma Membrane Protein / cpm ^{125}I/mg Soluble Protein
Commercial	
0-hr fast	43
24-hr fast	39
48-hr fast	43
Low fat	43
High fat	40
High fat/fasted	41

Low fat and high fat diets were fed to rats ad libitum for 7 days. The high fat/fasted rats were fed the high fat diet for 7 days followed by 3 days fasting. Adipose tissue was labeled as described in Table 1. The data presented are from a typical experiment utilizing 20 rats.

DISCUSSION

A reliable method for measuring the amount of adipocyte plasma membrane has been lacking. Such a method has been reported in this paper. The accuracy of this method is limited by *a*) the extent to which the plasma membrane can be isolated free of contaminating structures and *b*) the extent of labeling of cellular components other than the plasma membrane by [^{125}I]DDISA. The method used to isolate adipocyte plasma membranes was developed in our laboratory and has been shown to yield a highly purified preparation (16) with less than 6% mitochondrial contamination and less than 8% microsomal contamination. That [^{125}I]DDISA almost exclusively

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TABLE 4. Comparison of plasma membrane preparations from rats fed low fat, high fat, and high fat/fasting diets

Property	Diet		
	Low Fat	High Fat	High Fat/Fast
5′-Nucleotidase activity[a]	2.6 ± 0.14	2.4 ± 0.20	2.3 ± 0.20
cpm[^{125}I]DDISA/mg protein	2100 ± 250	2036 ± 116	2251 ± 261
Cytochrome c reductase[b] activity	20 ± 3	21 ± 2.5	23 ± 2
Succinate dehydrogenase[c] activity	0.08 ± 0.01	0.07 ± 0.01	0.08 ± 0.01

[a] Activity expressed as μmoles adenosine released per mg protein per hour at 37°C.
[b] Activity expressed as micromoles cytochrome c reduced per mg protein per minute at room temperature.
[c] Activity expressed as nanomoles 2,6-dichlorophenol-indophenol reduced per mg protein per minute at room temperature.
Plasma membranes were purified from adipocytes containing the [^{125}I]DDISA label as previously mentioned in Materials and Methods. Rats were fed each diet as described in Table 6. The data from the low fat diet and the high fat diet are average values from three experiments, each utilizing 20 rats. Data from the high fat/fast experiment are average values from two experiments consisting of 20 rats each.

labels plasma membranes and no other cellular component of adipocytes has been clearly shown by data in an earlier report (16) and by the data presented in this study. However, both the extent to which the isolated plasma membranes are contaminated with other cellular components and the extent to which [^{125}I]DDISA labels structures other than plasma membrane will cause the amount of plasma membrane per adipocyte to be overestimated. Therefore, the estimates of the amount of plasma membrane per adipocyte reported in this study are probably higher than the true value. However, on the basis of the data presented in this and our previous report (16), it seems likely that the extent of this overestimation is small (less than 15%) and the accuracy is not appreciably affected by the nutritional regimens utilized.

The effects of fasting on the plasma membrane of adipocytes has been explored previously by electron microscopy. It has been reported that loops, folds, and invaginations of the plasma membrane occur as the adipocyte decreases in volume during a fast (17, 18). These findings have led to the suggestion that the amount of plasma membrane per cell may not decrease during fasting. However, others have claimed that the number of invaginations of the plasma membrane per cell does not change during fasting but, because the cell has a reduced size, the number of such invaginations per μm^2 of cell surface increases (19). It has also been suggested that these morphologic changes relate to changes in the ratio of lipid to protein in the membrane (19). Our studies involving the

TABLE 5. Effect of diet on plasma membrane lipid composition

	g Phospholipid / g Protein	mole Cholesterol / mole Phospholipid	g Lipid[a] / g Protein
Commercial lab chow	0.92 ± 0.02[b]	0.60 ± 0.03	1.2 ± 0.1
Commercial lab chow			
fasted 24 hr	0.90 ± 0.03	0.63 ± 0.02	1.1 ± 0.1
fasted 48 hr	0.89 ± 0.03	0.55 ± 0.04	1.1 ± 0.1
Low fat	0.83 ± 0.10	0.61 ± 0.03	1.1 ± 0.2
High fat	0.93 ± 0.04	0.61 ± 0.04	1.2 ± 0.1
High fat/fasted 72 hr	0.91 ± 0.04	0.60 ± 0.03	1.1 ± 0.1

[a] Lipid is equal to total phospholipid plus total cholesterol.
[b] Data presented as mean ± SEM for four experiments of 20 rats each.

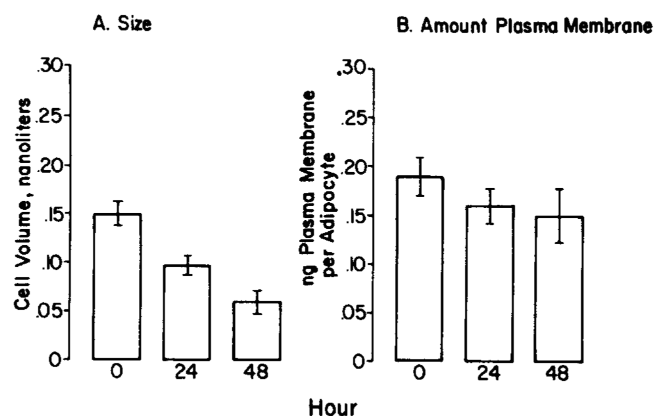

Fig. 2. Effect of fasting on adipocyte size (panel A) and amount of plasma membrane (panel B) of rats fed a commercial chow. Each experiment utilized 60 rats divided equally into three groups; 0-hr fast, 24-hr fast, and 48-hr fast. Starting on day 1, 20 rats were killed and the remaining 40 rats were placed into cages without food. At each following 24-hr interval, 20 rats were killed (day 2 and day 3). For each group, epididymal and perirenal fat depots were excised, labeled with [^{125}I]DDISA and digested with collagenase as described in Materials and Methods. Freed adipocytes were sized according to Stiles et al. (15) and mean plasma membrane amount was estimated as described in Materials and Methods. Data are expressed as mean ± SEM for four experiments.

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TABLE 6. Effect of high fat diet and high fat diet/fasting regimes on rat adipocytes

Group	Age of Rats when Killed	Diet	Weight when Killed	Mean Cell Volume	Plasma Membrane per Adipocyte
	(days)		(grams)	(nanoliters)	(nanograms)
I	66	Low fat for 7 days	196 ± 3[a]	0.17 ± 0.01	0.29 ± 0.03
II	66	High fat for 7 days	208 ± 4	0.31 ± 0.03[b]	0.59 ± 0.04[b]
III	69	High fat for 7 days, followed by fasting for 3 days	199 ± 3	0.16 ± 0.02	0.43 ± 0.04[b]

[a] Values expressed as mean ± SEM from four experiments each utilizing 20 rats.
[b] Significant difference at $P < 0.05$ compared to Group I.

direct measurement of the amount of plasma membrane per adipocyte show that a loss of plasma membrane during fasting, for as long as 3 days in the case of rats fed the high fat diet, does occur but the extent of this loss is less than would be expected based on the relationship between cell volume and amount of plasma membrane in adipocytes of fed rats. Moreover, in the case of rats fed the standard chow diet, fasting for 24 or 48 hr did not significantly change the amount of plasma membrane per cell. In addition, no change in the amount of lipid relative to the amount of protein was found to occur in the plasma membrane during fasting.

In contrast, acute increases in the volume of the fat cell by feeding high fat diets caused a marked increase in the amount of plasma membrane per cell. The new membrane appears to be of the same composition as the plasma membrane present in the cell prior to its enlargement. It is particularly interesting that much of the membrane added to the cell when its size is acutely expanded by fat feeding is not lost when the size of the cell is acutely reduced by fasting. The little or no change in amount of adipocyte plasma membrane during fasting could be due to a slow turnover rate of membrane constituents. To our knowledge, there are no data directly addressing plasma membrane turnover in adipocytes. Data on liver plasma membranes indicate that the mean half-life of the protein components is about 2 days (20, 21).

The role, if any, that the changes in plasma membrane reported in this paper play in the changes in hormonal responsiveness caused by these nutritional manipulations has not been defined by this study. However, the fundamental methodology and fundamental changes in plasma membrane have been described upon which to base further experimental explorations in this area.

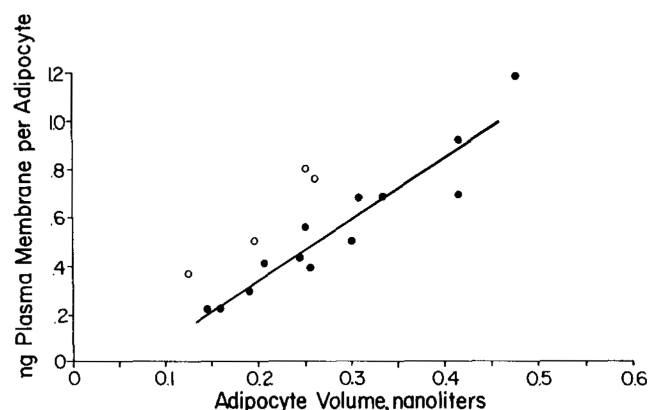

Fig. 3. Relationship between amount of plasma membrane per adipocyte and adipocyte size. Adipocyte suspensions isolated from either fasted rats or rats fed ad libitum a low fat diet or a high fat diet or commercial chow were assayed for mean adipocyte size and mean amount of plasma membrane per adipocyte as previously described in Materials and Methods. The open circles represent data obtained from adipocytes isolated from fasted rats. Closed circles represent data obtained from adipocytes isolated from ad libitum fed rats. Each circle is an average value from cells derived from 20 rats. Test of parallel lines was done by comparison of y-intercept using F ratio test ($P < 0.01$). Regression coefficient for fed rats (closed circles) is 0.95 and slope is 0.9. The regression coefficient and slope for fasted rats (open circles) are 0.96 and 3.4, respectively.

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