Temperature optimum of insulin-stimulated 2-deoxy-D-glucose uptake in rat adipocytes

Correlation of cellular transport with membrane spin-label and fluorescence-label data

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The effects of temperature alterations between 22°C and 48°C on basal and insulin-stimulated 2-deoxy-D-[1-¹⁴C]glucose uptake were examined in isolated rat adipocytes. A distinct optimum was found near physiological temperature for uptake in the presence of maximally effective insulin concentrations where insulin stimulation and hexose uptake were both conducted at each given assay temperature. Basal uptake was only subtly affected. Control and maximally insulin-stimulated cells incubated at 35°C subsequently exhibited minimal temperature-sensitivity of uptake measured between 30 and 43°C. The data are mostly consistent with the concept that insulin-sensitive glucose transporters are, after stimulation by insulin, functionally similar to basal transporters. Adipocyte plasma membranes were labelled with various spin- and fluorescence-label probes in lipid structural studies. The temperature-dependence of the order parameter *S* calculated from membranes labelled with 5-nitroxide stearate indicated the presence of a lipid phase change at approx. 33°C. Membranes labelled with the fluorescence label 1,6-diphenylhexa-1,3,5-triene, or the cholesterol-like spin label nitroxide cholestane, reveal sharp transitions at lower temperatures. We suggest that a thermotropic lipid phase separation occurs in the adipocyte membrane that may be correlated with the temperature-dependence of hexose transport and insulin action in the intact cells.

The metabolic actions of insulin occur after the interaction of this hormone with cell-surface binding sites. Although the mechanism by which insulin influences membrane functions is not understood in detail, clues to this process have been provided by examining the effects of membrane-perturbing treatments on insulin action.

Studies of the effects of temperature on adipocyte hexose transport/uptake, conducted in several laboratories (Crofford & Renold, 1965; Czech, 1976; Kono *et al.*, 1977; Olefsky, 1978; Vinten, 1978; Vega & Kono, 1979; Amatruda & Finch, 1979; Sauerheber *et al.*, 1984), have revealed important features of the glucose-transport system

and the mechanism by which insulin stimulates the cellular uptake of glucose. In most of the above work, the temperature was varied from approx. 15°C to physiological temperature.

We here investigate adipocyte basal and insulin-stimulated uptake of 2-deoxy-D-glucose over a wide temperature range (up to 48°C). In parallel studies, the temperature-dependence of the lipid structure of adipocyte plasma membranes was studied with exogenous spin and fluorescence labels. E.s.r. and fluorescence spectroscopy have proved to be useful experimental methods for examining the relative order or 'fluidity' of model and biological membranes and for assessing the presence of thermotropic membrane lipid 'phase separations' or transitions in model lipid components. Possible structural features of the membrane that may mediate the effects of temperature on glucose transport are discussed in the light of our findings.

Abbreviations used: I(12,3), 5-nitroxide stearate spin label, 2-(3-carboxypropyl)-4,4-dimethyl-2-tridecyl-3-oxazolidinyloxyl; DPH, 1,6-diphenylhexa-1,3,5-triene (fluorescence label); CSL, nitroxide cholestane spin label, 4',4'-dimethylspiro[5 α -cholestan-3,2'-oxazolidin]-3'-yloxy.

Materials and methods

Preparation of cells and membranes

Adipocytes were isolated from male Sprague-Dawley rats (160–180 g weight range) by digesting minced epididymal adipose tissue in a 305 mosm digestion buffer containing 110 mM-NaCl, 5 mM-KCl, 2.5 mM-MgCl₂, 1 mM-CaCl₂, 1 mM-KH₂PO₄, 25 mM-Mops (4-morpholinepropanesulphonic acid) (Sigma), 4% (w/v) bovine serum albumin (RIA grade, Sigma), pH 7.4 at 37°C, and 1 mg of collagenase (Worthington lot no. CLS F2B261)/ml. Mops was chosen to minimize temperature-induced changes in buffer pH ($\Delta pK_a/^\circ\text{C} = -0.006$). The cells were filtered, washed in Mops/1% albumin, counted by microscopic examination of 10-fold diluted samples, and resuspended at 10⁶ cells/ml.

Rat adipocyte plasma membranes for fluorescence and spin-label studies were prepared as described by Jarett (1974). Membrane protein was assessed by the method of Lowry *et al.* (1951). Membrane purity was assessed by the measurement of 5'-nucleotidase (Giacobino & Chmelar, 1975), similarly to that described by Michell & Hawthorne (1965). The purification of the plasma-membrane marker was 10.8-fold compared with the homogenate.

Measurement of initial rates of 2-deoxy-D-glucose transport into adipocytes

The initial rate of 2-deoxy-D-[1-¹⁴C]glucose (New England Nuclear) transport was determined essentially by established methodology (Gliemann *et al.*, 1972; Olefsky, 1978). Samples (170 μ l) of adipocyte suspension were incubated in 0.25 ml total volume [with or without insulin (Sigma)] for 10 min. 2-Deoxy-D-[1-¹⁴C]glucose was added so that the final hexose concentration was 0.1 mM (sp. radioactivity 2.0 Ci/mol), and the assay was performed for 10 min, unless otherwise indicated. Cells were separated from the buffer by centrifugation (Olefsky, 1978), and the cell layers were counted for ¹⁴C radioactivity. Non-carrier-mediated inward diffusion of the labelled hexose into the cells was assessed by the inclusion of cytochalasin B in the incubation buffer at all temperatures studied (Amatruda & Finch, 1979). After subtraction of passive diffusional uptake from total uptake, hexose transport was linear as a function of time up to 20 min at 37°C (and to 15 min at temperatures above 40°C) under all incubation conditions, and always extrapolated to zero uptake at zero incubation time (results not shown).

The effects of temperature on the uptake of hexose were determined by conducting incubations in a temperature-gradient device (Houslay & Palmer, 1978), an aluminium alloy 6061 block with holes drilled to accommodate sample tubes, suspended

between two constant (high and low)-temperature shaking water baths. Sample temperatures were continuously monitored with a thermistor probe (Markson) and were within $\pm 1^\circ\text{C}$ during a given run. Unless indicated otherwise, cells were not exposed to high temperatures (above 35°C) for longer than 20 min total. Photomicrographs (magnification $\times 100$) of adipocytes taken after long exposure to high temperature indicated no detectable cell breakage above that noted at lower temperatures (results not shown).

The time-course of the ability of insulin to activate hexose transport was also assessed over the temperature range utilized in these studies. Insulin activation of hexose transport was complete after 1 min at temperatures above 40°C, and after 10 min at 22°C under our assay conditions. We therefore consider these measurements to approximate closely to initial rates of hexose transport, over the temperature range studied. The dose-response curves indicate that the uptake of hexose (0.1 mM) is saturated at 170 pM-insulin (1 ng/ml), with half-maximal increases occurring at 24 pM (0.14 ng/ml). The increase in uptake caused by maximally stimulating insulin ranges from 1.7- to 3-fold in several preparations when assayed at 37°C.

E.s.r. spectral measurements

The 5-nitroxide stearate spin label I(12,3), where 'nitroxide' refers to the 4',4'-dimethyl-N-oxylazolidine ring, was obtained from Syva Co. (Palo Alto, CA, U.S.A.). The label was incorporated into adipocyte plasma membranes at 21°C (Sauerheber *et al.*, 1980, 1982) with, where indicated, experimentally determined low and high I(12,3)/membrane-protein ratios, as defined by Sauerheber *et al.* (1977). In studies with the nitroxide cholestane spin label (Syva), samples of label dissolved in ethanol, at 1 mM, were mixed with 25 μ l of Percoll (polyvinylpyrrolidone-coated silica) (Sigma) and were freeze-dried under reduced pressure before addition of membrane samples. The label/membrane mixture was vortex-mixed for 1 min. E.s.r. spectra were recorded with a Varian E-104A Century Series e.s.r. spectrometer, equipped with a Varian variable-temperature accessory, after 5 min waits for temperature equilibration.

The order parameters S , $S(T_{\parallel})$ and $S(T_{\perp})$ (Gordon & Sauerheber, 1977) are sensitive to the flexibility of membrane-incorporated spin labels undergoing rapid anisotropic rotational motion about a unique symmetry axis. Each may be measured from the outer ($2T_{\parallel}$) and inner ($2T_{\perp} - 1.6$) hyperfine splittings observed in the e.s.r. spectra of the I(12,3)-labelled membranes as described previously (Sauerheber *et al.*, 1980, 1982).

As indicated by Hubbell & McConnell (1969), biological membrane dispersions labelled with steroid spin probes exhibit e.s.r. spectra that indicate that the labels undergo rapid rotational motion about an axis essentially parallel to the fused ring of the steroid (the long molecular axis). Thus, for steroid spin labels, the outer (maximum) splitting in the e.s.r. spectra of labelled membrane dispersions ($2T_{\perp}'$) corresponds roughly to the time-average splitting ($2T_{\perp}$) observed when the membrane surface is aligned parallel to the applied magnetic field $\vec{H}$ (Presti & Chan, 1982).

We here employed the polarity-uncorrected order parameter expression $S(T_{\perp})$ as an index of the ordering of the steroid long molecular axis about the normal to the membrane surface, by measuring the outer splitting value $2T_{\perp}'$ of the steroid-labelled membrane. Estimates of $2T_{\perp}$ are obtained by correcting for two half-widths of the outer hyperfine lines for the steroid-labelled membranes. Eletr *et al.* (1973) corrected the inner hyperfine splitting measurements, $2T_{\perp}'$, of 5-nitroxide stearate-labelled liver membranes. The need for the correction is exemplified by the small but significant differences in splitting values calculated from oriented versus unoriented lipid dispersions labelled with various nitroxide spin labels (Seelig, 1970). We here arbitrarily correct all $T_{\perp}'$ measurements by 0.16 mT; the approximate peak-to-peak distance (maxima to minima) of single hyperfine lines in oriented hydrated lipid multibilayers containing cholestane spin label, (dipalmitoyl) phosphatidylcholine and cholesterol (molar proportions 1:150:150) were in the range 0.16–0.32 mT (Hsia *et al.*, 1979).

Seelig (1970) earlier calculated order parameters for ordering of the nitroxide $2p\pi$ orbital about the membrane normal for steroid labels dissolved in oriented bilayer structures by measuring the splitting values observed for membranes aligned parallel ($2T_{\perp}$) and perpendicular ($2T_{\parallel}$) to $\vec{H}$. To test the validity of the $S(T_{\perp})$ expression for monitoring the 'wobble' of steroid labels undergoing long-axis-enhanced rotation, the splitting values ($T_{\perp}$) observed in the Seelig (1970) study may be inserted into eqn. (6) of Gordon & Sauerheber (1977). The resultant order parameter $S(T_{\perp})$ compares favourably [$S(T_{\perp}) = -0.281$] with the order parameter S_3 calculated from both the outer and inner hyperfine splitting measurements from the cholestane-labelled oriented layers ($S_3 = -0.28$). The excellent agreement in this case is probably due to the apparent absence of significant polarity differences between the static and time-averaged splitting elements. Here, an order parameter $S(T_{\perp})$ much less than 0 indicates that the probe samples a highly ordered environment, and values increasing to 0 reflect more disordered environments.

Fluorescence measurements

Plasma membranes (100 $\mu\text{g/ml}$) were labelled with DPH as reported previously (Hyslop & York, 1980). Measurements of intensity of polarized fluorescence were performed with an Aminco-Bowman spectrophotofluorimeter, excitation 360 nm, emission 430 nm, with 8 nm bandpass. The cuvette temperature was monitored by a thermistor, and the polarized fluorescence and light-scatter intensities were recorded over the temperature range 2–40°C. Instrumental corrected polarization was calculated from the following parameters:

$$P = \frac{(I_{VV} - L_{VV}) - \left[(I_{VH} - L_{VH}) \left(\frac{I_{HH} - L_{HH}}{I_{HV} - L_{HV}} \right) \right]}{(I_{VV} - L_{VV}) + \left[(I_{VH} - L_{VH}) \left(\frac{I_{HH} - L_{HH}}{I_{HV} - L_{HV}} \right) \right]}$$

where P = steady-state fluorescence polarization, I = fluorescence intensity, L = light-scatter intensity, plus background membrane fluorescence, and the first subscript (H or V) indicates the orientation (horizontal or vertical) of the polarizer, and the second subscript (H or V) indicates the orientation (horizontal or vertical) of the analyser.

Steady-state fluorescence polarization (P) is related to the steady-state fluorescence anisotropy (r_s) of the fluorophore by the relation $r_s = 2P/(3 - P)$ (Shinitzky & Barenholz, 1978). r_s can be resolved into both static (slow-decaying, r_{∞}) and dynamic (fast-decaying, r_f) components (Van Blitterswijk *et al.*, 1981). These authors demonstrated that, for DPH embedded in biological membranes, it is r_{∞} that largely determines the value of r_s . Thus, within limits, $\sqrt{r_s}$ is proportional to a lipid order parameter which describes the wobbling motion of the probe embedded in the membrane matrix.

Results

Temperature profile of basal and insulin-stimulated 2-deoxy-D-glucose uptake in adipocytes

The temperature-dependence of 2-deoxy-D-glucose transport over a wide range for adipocytes incubated without and with insulin (1.7 nM) is indicated in Fig. 1. Increases in temperature from 20° to 35°C cause increases in transport in the presence of insulin, whereas basal transport is only slightly increased. Above 35°C, transport of hexose in the presence of hormone diminishes until it becomes equivalent to basal uptake at approx. 44–45°C. The optimum stimulation temperature of transport in the presence of insulin was defined in each of nine separate temperature-gradient experiments by determining the intersection point of two lines drawn parallel to the straight portion of each side

of the temperature profile. The optimum position averaged $34.6 \pm 2.2^\circ\text{C}$, with a range of $34\text{--}37^\circ\text{C}$.

Control experiments were performed to determine whether the deleterious effects of high temperature on the insulin stimulation of hexose uptake were due to irreversible thermal denaturation of either the bound insulin or other cellular components. The results of these studies are shown in Table 1. Cells incubated in the presence and absence of insulin at 35°C subsequently exhibited a normal response to the hormone when assayed for hexose uptake at either 35° or 42°C (A and B). When a similar study was performed with cells pre-incubated at 42°C , a depressed insulin stimulation was observed when these cells were assayed for hexose uptake at 42°C (C), but full insulin activation of hexose uptake occurred when the cells were assayed at 35°C . Here, full activation of uptake

Fig. 1. *Temperature-dependence of 2-deoxy-D-glucose transport in the absence and presence of insulin*
Cells were isolated and incubated as described in the text for uptake of 2-deoxy-D-[1- ^{14}C]glucose. Data are averaged from four separate adipocyte preparations, each batch examined over the above range within 1 h of preparation. Basal (●) and insulin-stimulated (○) cells were pre-equilibrated and insulin-treated at each given temperature before addition of hexose substrate. Hormone-stimulation and uptake of radiolabel were conducted for 10 min each.

probably reflects the rapid stimulation (≈ 1 min) at 35°C .

The above reversibility data suggest that degradation of insulin by adipocytes does not cause the inhibition of activity at high temperatures observed in Fig. 1. This is consistent with insulin titrations of adipocytes at 37°C indicating that the membrane transport of hexose is saturated at only 1 ng of hormone/ml. The use of 90 ng of insulin/ml did not further increase hexose transport at any temperature examined (results not shown).

The finding that basal uptake of glucose is not significantly inhibited over a wide range of temperatures up to approx. 45°C suggests that intrinsic transporter activity is not denatured at the temperatures studied. However, treatment of adipocytes for 20 min (but not 10 min) at 43°C does lead to significant irreversible inhibition of transport subsequently measured for 10 min in the presence of insulin at 35°C (results not shown). In additional control studies, 2-deoxy-D-glucose transport assays were conducted for 5 min assay periods over the temperature range $25\text{--}43^\circ\text{C}$, after stimulation by insulin for 5 min at each respective temperature. The general appearance of the temperature profile for transport in the presence and absence of insulin was comparable with that noted in Fig. 1.

We also considered the possibility that the intracellular accumulation of 2-deoxy-D-glucose phosphate, a known metabolic poison at high doses, could affect the observed data. Experiments were conducted in which 2-deoxy-D-[1- ^{14}C]glucose tracer uptake was measured in the presence of 0.1 mM-D-glucose (rather than 2-deoxy-D-glucose carrier). The rate of tracer uptake in the absence and presence of insulin was not significantly affected at 37°C by this manipulation (results not shown).

Temperature profile of adipocytes pre-stimulated with insulin at 35°C

The temperature-dependence of basal and insulin-stimulated 2-deoxy-D-glucose transport was

Table 1. *Temperature-reversal studies on insulin-stimulated hexose uptake*

Adipocytes were isolated and were suspended for uptake assays in physiological Mops buffer as described in the Materials and methods section. Cells were incubated (0.2×10^5 cells/0.25 ml) in both the absence and the presence of insulin (40 ng/ml) for 10 min at the indicated temperature. Immediately before addition of 2-deoxy-D-[1- ^{14}C]glucose substrate, the vials were removed and adjusted to the indicated temperature for hexose-uptake measurements (for an additional 10 min). Data are averaged from four separate batches of cells, and numbers in parentheses represent ± 1 s.d.

	Incubation temp. ($^\circ\text{C}$)	Hexose-uptake assay temp. ($^\circ\text{C}$)	Uptake magnitude (nmol/10 min per 10^5 cells)	
			Basal	+ insulin
A	35	35	0.70 (0.40)	1.78 (0.27)
B	42	42	0.50 (0.15)	0.84 (0.20)
C	35	42	0.45 (0.12)	1.28 (0.25)
D	42	35	0.58 (0.17)	1.26 (0.67)

assessed under conditions where the insulin stimulation was allowed to proceed at 35°C and then hexose-transport measurements were conducted at various temperatures (Fig. 2). Stimulation of cells with insulin in this way yields basal and insulin-stimulated uptakes having minimal temperature sensitivity. Thus pre-stimulation of the cells at 35°C largely protects the insulin-stimulation process from the deleterious effects of high temperature.

E.s.r. measurements in isolated adipocyte plasma membranes

The effects of temperature alterations on the order parameters calculated from I(12,3)-labelled adipocyte plasma membranes from 10 to 43°C were next examined (Figs. 3a and 3b). A divergence of $S(T_{\perp})$ and S from the $S(T_{\parallel})$ curve occurs at temperatures below approx. 33°C. Increases in temperature cause progressive decreases in the order parameters, and the magnitudes of the slopes of the order-parameter-versus- $1/T$ curves increase in the order $S(T_{\perp}) < S < S(T_{\parallel})$. These observed differences in slopes are much more pronounced in the membrane labelled with the high probe/membrane ratio (Fig. 3b), where $S(T_{\perp})$ is only minimally affected by changes in temperature. A difference order-parameter plot (Fig. 3c) was constructed as described by Gordon *et al.* (1978) from the data presented in Figs. 3(a) and 3(b). The data in Fig. 3(c) indicate that $S(T_{\parallel})$ is essentially independent of the concentration of probe over the range 33–20°C, where the $\Delta S(T_{\perp})$ curve decreases and diverges considerably from that for $\Delta S(T_{\parallel})$.

Fig. 2. Temperature-dependence of basal and insulin-stimulated 2-deoxy-D-glucose uptake

Adipocytes were prepared and incubated as indicated in the text. Cells were pretreated either without (●) or with (○) insulin at 35°C (40 ng/ml) for 10 min, before the hexose uptake assays were conducted, at the indicated temperatures, for 10 min (see the Materials and methods section). The data are from a representative of four separate experiments.

Fig. 3. Temperature effects on order parameters calculated from I(12,3)-labelled adipocyte plasma membranes

Membranes were purified from epididymal adipose tissue by the Jarett (1974) procedure with modifications described in the text. Membranes were labelled with 'low' (a) and 'high' (b) I(12,3)-probe/membrane-protein ratios (4 and 15 nmol/350 μ g respectively) (Sauerheber *et al.*, 1977). S , $S(T_{\parallel})$ and $S(T_{\perp})$ were calculated from e.s.r. spectra of the labelled membranes as described in the Materials and methods section. The temperature-dependence of ΔS , $\Delta S(T_{\parallel})$ and $\Delta S(T_{\perp})$ is presented in (c), where $\Delta(\text{order parameter})$ was calculated from rat adipocyte plasma membranes at a given temperature as the percentage difference between values measured at high and low I(12,3)/membrane-protein ratios.

Fig. 4. Effects of temperature on adipocyte plasma membranes labelled with cholestane

Membranes were prepared as in the legend to Fig. 3, and were labelled as described in the Materials and methods section. The order parameter $S(T_{\perp})$ was calculated, as indicated in the text, from spectra recorded with the 'magnified wings' procedure, where the outer hyperfine peaks were recorded at a high gain setting. Data are from one of three separate experiments.

E.s.r. spectra of adipocyte plasma membranes, labelled with the cholesterol-like spin label cholestane, were similar to those reported for steroid-labelled membrane dispersions by Presti & Chan (1982), and indicate that the label undergoes rapid enhanced rotation about the long steroid axis. A representative study of the effects of temperature on adipocyte plasma membranes labelled with cholestane is shown in Fig. 4. Increases in temperature lead to progressive increases in $S(T_{\perp})$ (decreases in the magnitude of the outer hyperfine splitting) over the range 10–40°C in our experiments. A sharp 'break' in the plot of $-S(T_{\perp})$ versus $1/T$ was noted at approx. 23°C. The slope of the plot of $-S(T_{\perp})$ versus $1/T$ sharply increases in absolute magnitude as the temperature is increased above 23°C.

Fluorescence measurements in isolated adipocyte plasma membranes

The temperature-dependence of adipocyte plasma membranes labelled with DPH is shown in Fig. 5. Increases in temperature from 10° to 40°C cause a decrease in the polarization of DPH fluorescence in the labelled membranes. A divergence in the plot of $\log P$ versus $1/T$ is evident at approx. 19°C.

Discussion

Adipocyte plasma membranes labelled with low and high $I(12,3)$ /membrane-protein ratios suggest that at approx. 33°C a lipid phase change occurs that is distinct from, and concomitant with, changes in the flexibility of the lipid. The diverg-

Fig. 5. Dependence of fluorescence polarization of DPH-labelled adipocyte plasma membrane on temperature. Polarization values (P) were calculated from membranes purified and labelled with DPH as indicated in the text. Data are from one of three representative experiments.

ence of S and $S(T_{\perp})$ from the curve for $S(T_{\parallel})$ versus $1/T$ probably reflects enhanced probe-probe interactions in the membrane. For example, the observed effect is most dramatic for membranes labelled with the high probe/membrane ratio. Further, the difference order-parameter plot (Fig. 3c) suggests that the flexibility and polarity of the environment of the label are independent of the probe concentration over the range 20–33°C and that the observed decreases in $\Delta S(T_{\perp})$ are due to the clustering of probe molecules as the temperature is lowered. These results and additional difference plots of membranes labelled with intermediate probe concentrations (results not shown) are consistent with the above assignment of approximate onset temperature.

It is possible that the onset of clustering of $I(12,3)$ below 33°C and the changes in motional properties of the CSL and DPH probes reflect a thermotropic lipid phase separation in the membrane similar to that detected previously in rat liver plasma membrane. This was identified in comparable studies with DPH and $I(12,3)$ (Livingstone & Schachter, 1980), and was confirmed in light-scattering studies between 19° and 28°C (Dipple & Houslay, 1978).

Thermotropic lipid structural alterations may play a part in mediating the temperature-induced effects on the glucose transport system in adipocytes. Transport in the presence of maximal insulin concentrations changes dramatically over the temperature range (23–33°C) at which 'ordered'

and 'bulk fluid' lipid (Gordon *et al.*, 1978) would coexist in the membrane bilayer. The segregation of membrane lipid components may thus affect the responsiveness or activity of the hexose-transport system in the intact adipocyte. Also, it is possible that the low-temperature end of the phase separation is involved in the near-cessation of the insulin stimulation of transport above basal at low temperatures (Fig. 1).

Previous studies correlated thermotropic lipid structural changes detected in physical-biochemical studies with transitions in the transport of hexose into intact adipocytes (Amatruda & Finch, 1979; Sauerheber *et al.*, 1982) and in the adipocyte glucose transporter reconstituted into artificial lipid bilayers (Czech, 1980). In another study, a 'break' in the temperature-dependence of specific D-glucose transport was noted at 33°C in plasma membranes prepared from control and insulin-treated rat adipocytes (Ludvigsen & Jarett, 1979). Here, the temperature-induced loss in uptake activity in the presence of insulin is manifested initially as a decrease in the slope of the curve for rate of uptake versus temperature (Fig. 1) at approx. 30°C. We cannot rule out the possibility that the temperature optimum of insulin-stimulated hexose uptake reflects reversible thermal protein-conformation changes.

The change in magnitude of the basal uptake of 2-deoxy-D-glucose with temperature in intact adipocytes is not as dramatic as transport in the presence of insulin when hormone is added at the uptake assay temperature. Uptake is completely dissociated from insulin stimulation at approx. 45°C, and perhaps also (by extrapolation only) at temperatures below 20°C (see Fig. 1). We thus suggest that the stimulation process caused by insulin is itself highly temperature-sensitive, for the most part giving rise to the observed optimum at 35°C.

Consistent with the above suggestion, pre-stimulation of intact adipocytes with insulin at 35°C (Table 1 and Fig. 2) yields results distinct from studies where hormone activation occurs at different temperatures (Fig. 1). The temperature studies of specific D-glucose uptake into purified plasma membranes prepared from control and insulin-treated adipocytes noted that parallel Arrhenius plots occurred between 22° and 43°C for the basal and insulin-activated membranes (Ludvigsen & Jarett, 1979). The magnitude of stimulation of uptake by insulin was 3–4-fold over the entire range, and the percentage increase in uptake caused by temperature increases from 25° to 35°C for basal and insulin-stimulated membrane transport were approx. 65% and 55% respectively. Our data (Fig. 2) suggest that, after stimulation by hormone, insulin-sensitive transporters exhibit properties very similar to those of basal transporters (over the

range 30–43°C). The results (Figs. 1 and 2) may also partly explain the observance of parallel basal and insulin-stimulated hexose transport/uptake plots as a function of temperature in some studies, but not in others (Crofford & Renold, 1965; Czech, 1976; Kono *et al.*, 1977; Amatruda & Finch, 1979; Sauerheber *et al.*, 1982).

It is possible that the insulin-stimulation process involves or resides in membrane domains that are relatively temperature-sensitive. Alternatively, an intracellular component involved in the insulin stimulation of glucose transport is sharply activated from 25° to 35°C and inhibited from 35° to 43°C. Indeed, the insulin-stimulation process requires the presence of intact cells and may involve the recruitment of intracellular hexose transporters to the surface membrane (Cushman & Wardzala, 1980; Suzuki & Kono, 1980).

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