Quantification *in vivo* of the effects of insulin on glucose utilization in individual tissues of warm- and cold-acclimated rats

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1. Cold-acclimation of rats (3 weeks, 4 °C) had no effect on basal rates of glucose production or utilization. Under euglycaemic-clamp conditions, in which the circulating insulin concentration was increased by approx. 50 μ units/ml, cold-acclimated rats showed a greater increase in glucose utilization and a greater suppression of endogenous glucose production. 2. Tissue sites of glucose utilization were investigated by using a tracer dose of 2-deoxy-D-[¹⁴C]glucose and a glucose metabolic index determined for each tissue. 3. In 5 h-starved warm-acclimated rats, heart had the highest glucose metabolic index. This was increased further by both cold-acclimation and insulin treatment. The glucose metabolic index of skeletal muscle was 3.5–14-fold lower than that of heart, but, as a result of the large muscle mass, skeletal muscle made the largest contribution to whole-body glucose utilization. 4. White and brown adipose tissue had low glucose metabolic indices in warm-acclimated rats under basal conditions, and the indices were not increased by the insulin treatment. However, cold-acclimation produced a significant increase in the glucose metabolic index of brown adipose tissue, but not of white adipose tissue. In contrast with the warm-acclimated rats, insulin treatment of cold-acclimated rats resulted in a marked increase in the glucose metabolic index of brown adipose tissue. The results provide evidence that cold-acclimation produces a selective alteration in the insulin-sensitivity of brown adipose tissue.

INTRODUCTION

Energy metabolism during acute exposure to and acclimation to cold has been the subject of considerable research. Short-term exposure to cold results in shivering, whereas long-term exposure culminates in an increased rate of non-shivering thermogenesis effected by corresponding physiological changes. The term ‘cold-acclimation’ is restricted to designate the effects of prolonged exposure to chronic cold, where this is the only factor being changed (Hart, 1960). The development of cold-acclimation requires a period of at least 2 weeks (Himms-Hagen, 1972; Chowers *et al.*, 1977; Kennedy *et al.*, 1977). During this time, there is a gradual increase in food consumption and an elevation of basal metabolic rate (Baker & Sellars, 1953). There is a decrease in shivering and an increase in non-shivering heat production. Foster & Frydman (1978) showed that cold-acclimated rats had an increased blood flow to brown adipose tissue, and this tissue is now generally recognized as the major site of non-shivering thermogenesis in the rat.

Over the last decade, considerable attention has been given to the cold-acclimated rat as a model for studying the mechanisms of non-shivering thermogenesis (Foster & Frydman, 1978, 1979; Desautels & Himms-Hagen, 1979, 1980; Young *et al.*, 1982; Cannon & Nedergaard, 1984). In contrast, the effects of cold-acclimation on carbohydrate metabolism have attracted little attention. Baker & Sellars (1953) found that cold-acclimation does not affect either blood glucose concentration or glucose tolerance. Beck *et al.* (1967) noted that rats cold acclimated at 5 °C for 2–7 weeks exhibited significant decreases in both serum glucose and insulin concentrations, thus supporting the notion that cold-acclimation improves insulin-sensitivity. The decrease in insulin, but

not in glucose, was confirmed by Howland & Nowell (1968).

In all of these studies, no measurements of the tissue sites of glucose utilization have been made. It is generally believed that muscle is quantitatively the major site of glucose utilization. However, blood-flow studies using microspheres failed to show any increase in the flow of blood to muscle in cold-acclimated rats (Foster & Frydman, 1978); rather, there was a specific increase in blood flow to brown adipose tissue. In addition, cold-acclimation produces an increase in the maximal activities of hexokinase and 6-phosphofructokinase only in brown adipose tissue (Cooney & Newsholme, 1982). Taken together, these findings have led to the speculation that brown adipose tissue might be of quantitative importance in the utilization of glucose and hence in the control of blood glucose concentration (McCormack, 1982; Cooney & Newsholme, 1982). This speculation is further supported by the findings that chronic treatment of genetically obese (*ob/ob*) and diabetic (*db/db*) mice with a β -adrenoceptor agonist produces a specific increase in the maximal activities of hexokinase and 6-phosphofructokinase in brown adipose tissue, and a normalization of the blood glucose concentrations (Young *et al.*, 1984, 1985). However, alterations in enzyme activity merely provide data on the theoretical maximum flux through a given pathway. To obtain estimates of the actual glucose uptake by individual tissues, several groups (Kraegen *et al.*, 1983; Ferré *et al.*, 1985; Young *et al.*, 1985) have adapted the radio-tracer 2-deoxyglucose technique, which was originally described by Sokoloff *et al.* (1977) for estimating glucose uptake by brain. This method, when coupled with D-[6-³H]glucose turnover and/or the euglycaemic clamp, allows measurements to be made of both whole-body and tissue rates of glucose utilization under steady-state conditions. In

the present paper, we have used this method to examine the effect of cold-acclimation on both basal and insulin-mediated glucose utilization in muscles and white and brown adipose tissue of the rat.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (180–200 g) (Tuck, Battlebridge, Essex, U.K.) were housed in pairs, under a controlled light cycle (lights on 06:00–18:00 h), at a temperature of either $23 \pm 1^\circ$ or $4 \pm 1^\circ \text{C}$ for 3 weeks before the study. Food (Oxoid rat and mouse breeders diet; H.C. Styles, Bewdley, Worcs., U.K.) and water were provided *ad libitum*.

Euglycaemic-clamp studies and determination of tissue 2-deoxyglucose uptake

Cold-acclimated rats were transferred to ambient room temperature 30 min before commencing the studies. All measurements of whole-body insulin sensitivity and tissue rates of glucose utilization were carried out on anaesthetized (pentobarbitone, 60 mg/kg intraperitoneally) rats in the post-absorptive state (5 h starved). Body temperature was monitored during the study, with a rectal thermocouple, and maintained at 38°C by an electric heating pad.

The right saphenous vein was cannulated and a solution of NaCl (0.9%) containing pig monocomponent insulin (36 munits/ml; Actrapid; Novo, Basingstoke, Hants., U.K.), bovine serum albumin (1%, w/v) and tracer D-[6- ^3H]glucose ($10 \mu\text{Ci/ml}$; 500 mCi/mmol; Amersham International, Amersham, Bucks, U.K.) was infused at the rate of $20 \mu\text{l/min}$. This resulted in an insulin infusion rate of about 0.5 munits/min per 100 g body wt. and was intended to increase the circulating insulin concentration by $50 \mu\text{units/ml}$. In rats in which basal (non-insulin stimulated) rates of tissue glucose utilization were determined, insulin was omitted from the infusate. At the start of the infusion period, all the rats received a priming dose of D-[6- ^3H]glucose ($10 \mu\text{Ci/ml}$ in 0.9% NaCl) intravenously as a bolus (1 ml/kg body wt.) through the catheter in the saphenous vein.

The blood glucose concentration of those rats receiving the insulin infusion was maintained constant at the pre-infusion value by means of a variable-rate infusion of glucose (1 M) delivered by a syringe-type infusion pump attached to the saphenous-vein catheter. At intervals of 5 min after the start of the insulin infusion, samples of blood ($10 \mu\text{l}$) were taken from the cut tip of the tail and haemolysed with 1 ml of maleimide (1.0 mM) containing digitonin (0.04 mM). The glucose content was determined within 2 min of sampling by using a Beckman DU7 spectrophotometer (Schmidt, 1973).

A period of 45 min was allowed to establish a plateau for both normoglycaemia and exogenous glucose infusion rate; the euglycaemic clamp was then continued for a further 60 min. After the 45 min equilibration period, 2-deoxy-D-[1- ^{14}C]glucose ($4 \mu\text{Ci/kg}$ body wt.; 50 mCi/mmol; Amersham International) was injected as a bolus through the catheter in the saphenous vein. The disappearance of the 2-deoxy-D-[1- ^{14}C]glucose from plasma was determined in samples of blood taken from the tail vein at 5, 10, 15, 20, 40 and 60 min after injection.

At the end of the experiment, a further blood sample was taken for the subsequent assay of plasma insulin (Hales & Randle, 1963), with human insulin as standard.

Measurements of whole-body glucose utilization rates

The ^3H label in position 6 of glucose does not recycle in the rat (Katz *et al.*, 1977). Thus whole-body glucose utilization rates were calculated from the specific radioactivities of blood D-[6- ^3H]glucose measured in samples (0.05 ml) taken at 15 min intervals throughout the clamp period. Blood was deproteinized in $\text{Ba}(\text{OH})_2/\text{ZnSO}_4$ (Somogyi, 1945) and the specific radioactivity of D-[6- ^3H]glucose measured as described by Rizza *et al.* (1979). In brief, 0.5 ml samples of deproteinized blood were freeze-dried to remove $^3\text{H}_2\text{O}$. The freeze-dried samples were then reconstituted in 0.2 ml of water and the ^3H contents determined after the addition of 3 ml of scintillation cocktail. Preliminary experiments established that freeze-dried samples contained negligible amounts of [^3H]lactate, [^3H]pyruvate and [^3H]alanine. Since the specific radioactivities of blood glucose were constant over the final 1 h of the euglycaemic clamp, glucose turnover rates were calculated by a steady-state method (Katz, 1979).

Tissue sampling and determination of tissue 2-deoxy[^{14}C]glucose 6-phosphate content

At the end of the study, the rats were killed by cervical dislocation. Samples of 13 different tissues (see Table 3) were taken within 5 min and immediately frozen in liquid N_2 for subsequent determination of 2-deoxyglucose 6-phosphate content.

Weighed samples of frozen tissue (about 0.4 g) were homogenized in 3 ml of HClO_4 (2%, v/v). Precipitated protein was removed by centrifugation (2000 g, 10 min). A 2 ml portion of the supernatant was adjusted to pH 7.4 with 0.5 M-triethanolamine/HCl, pH 7.4, containing 2 M-KOH. Free and phosphorylated 2-deoxy[1- ^{14}C]glucose in 1.0 ml portions of the neutralized tissue extracts were separated by ion-exchange chromatography on Dowex 1-X8 (acetate form) by the method of Hom *et al.* (1984). Preliminary experiments (results not shown) in which tracer amounts of 2-deoxy[^3H]glucose and 2-deoxy[^{14}C]glucose 6-phosphate were added to non-radiolabelled tissue extracts showed that the chromatographic procedure achieved a quantitative separation and recovery of the two sugars.

Calculation of glucose metabolic rate in tissues

Patlak (1981) showed that, subject to several assumptions, the steady-state reaction velocity of a substance (glucose) in an individual tissue can be derived by the use of the bolus administration of a second substance (2-deoxy[^{14}C]glucose) by using eqn. (1):

$$R_g = \frac{C_m^*(\infty)}{\text{L.C.} \int_0^\infty (C_p^*/C_p)} \cdot dt \quad (1)$$

where R_g is the glucose metabolic rate per unit mass of tissue ($\mu\text{mol/min per g}$), $C_m^*(\infty)$ is the tissue content of 2-deoxy[^{14}C]glucose 6-phosphate per unit mass as $t \rightarrow \infty$ (d.p.m./g), L.C. is a dimensionless constant (lumped constant) to account for the discrimination between glucose and 2-deoxy[^{14}C]glucose, C_p^* is the plasma deoxy[^{14}C]glucose concentration (d.p.m./ml) and C_p is

the steady-state plasma glucose concentration ($\mu\text{mol/ml}$).

The accurate determination of glucose metabolic rate in each tissue is dependent on a knowledge of the lumped constant for that tissue. At present, values have only been obtained in the rat for brain (Sokoloff *et al.*, 1977), white adipose tissue and some skeletal muscles (Ferré *et al.*, 1985). Since in the current experiments we were interested in relative changes in glucose metabolic rate in individual tissues after insulin treatment and cold-acclimation, we have omitted the lumped constant and, following the suggestion by Kraegen *et al.* (1985), the term 'tissue glucose metabolic index' has been used for R_g' in eqn. (2). We have also, for experimental reasons connected with maintaining euglycaemia, measured the steady-state concentration of blood glucose (C_b) rather than plasma glucose. This latter change introduces a small underestimate of glucose metabolic index relative to the values obtained by Kraegen *et al.* (1985):

$$R_g' = C_b \cdot C_m^*(T) \int_0^T C_p^* \cdot dt \quad (2)$$

where $C_m^*(T)$ is tissue phosphorylated 2-deoxy[^{14}C]-glucose content/g fresh wt. at $t = 60$ min. Thus the tissue metabolic index ($\mu\text{mol/min per g fresh wt.}$) is equal to the product of tissue 2-deoxy[^{14}C]glucose 6-phosphate content (d.p.m./g) and the steady-state blood glucose concentration ($\mu\text{mol/ml}$) divided by the area under the plasma 2-deoxy[^{14}C]glucose disappearance curve from 0 to 60 min (d.p.m./min per ml). The area under the plasma 2-deoxy[^{14}C]glucose disappearance curve was calculated by means of Simpson's rule, after fitting a third-order polynomial (RS1 software package; BBN Products, Staines, Middx., U.K.) to the experimentally determined plasma 2-deoxy[^{14}C]glucose concentrations.

Statistics

Results are expressed as means $\pm$ s.d. Statistical significance of differences between the treatment groups was calculated by non-paired Student's t test.

RESULTS

Body weight, blood glucose and plasma insulin concentrations

The results are given in Table 1. Cold-acclimation at 4°C for 3 weeks produced a small, but significant, decrease ($P < 0.01$) in the body weight of the rats. The blood glucose concentrations of 5 h-starved cold-acclimated rats did not differ from those of warm-acclimated rats, but the plasma insulin concentrations of cold-acclimated rats were significantly decreased ($P < 0.05$).

Whole-body rates of glucose utilization and endogenous glucose production

Whole-body glucose utilization rates and the contribution of endogenous and exogenous glucose to these rates are shown in Table 2. Under basal conditions, blood glucose concentrations remained constant and there was no significant difference in the whole-body rate of glucose utilization of warm- and cold-acclimated rats (Table 2). During the euglycaemic clamp, in which the plasma insulin concentration was increased by approx. $50 \mu\text{units/ml}$, the glucose utilization rate of warm-

Table 1. Body weight, blood glucose, plasma insulin in warm-acclimated and cold-acclimated rats

Male Sprague-Dawley rats (180–200 g) were housed at 23°C (warm) or 4°C (cold) for 3 weeks. All rats were starved for 5 h at room temperature before undergoing the euglycaemic-clamp procedure. Values are means $\pm$ s.d. for six rats: *significantly different from warm-acclimated controls ($P < 0.05$).

Rats	Body wt. (g)	Blood glucose (mM)	Plasma insulin ($\mu\text{units/ml}$)
Warm-acclimated control	342 ± 24	7.49 ± 0.76	129 ± 39
Warm-acclimated insulin-treated	327 ± 20	7.05 ± 0.44	181 ± 61
Cold-acclimated control	304 ± 34	7.13 ± 1.05	$76 \pm 33^*$
Cold-acclimated insulin-treated	302 ± 18	6.78 ± 0.26	123 ± 23

Table 2. Effect of cold-acclimation and insulin on whole-body rates of glucose utilization and endogenous glucose production

Whole-body glucose utilization rates were calculated as described in the Materials and methods section. Endogenous glucose production rates of rats infused with insulin were derived from the difference between the whole-body utilization rate and the exogenous glucose infusion rate. Results are expressed as means $\pm$ s.d. for six rats per group: $\dagger P < 0.05$ versus warm-acclimated insulin-treated rats; $**P < 0.01$ versus cold-acclimated basal-state rats.

	Rate of endogenous glucose production ($\mu\text{mol/min per rat}$)	Rate of glucose utilization ($\mu\text{mol/min per rat}$)
Warm-acclimated basal	16.8 ± 2.5	16.8 ± 2.5
Warm-acclimated + insulin	13.0 ± 2.8	20.0 ± 2.6
Cold-acclimated basal	18.5 ± 2.9	18.5 ± 2.9
Cold-acclimated + insulin	10.5 ± 3.7	$26.7 \pm 4.5^{\dagger**}$

acclimated rats was increased by 19% and endogenous glucose production was decreased by 8%. In the cold-acclimated rats, glucose utilization was increased by 44% and endogenous glucose production decreased by 44%. Thus, even though the cold-acclimated rats had lower plasma insulin concentrations ($123 \pm 23 \mu\text{units/ml}$) than warm-acclimated rats ($181 \pm 61 \mu\text{units/ml}$), insulin-mediated glucose utilization was significantly more increased and endogenous glucose production was significantly more depressed. This indicates that cold-acclimation increases insulin-sensitivity.

Glucose metabolic indices of individual tissues

Curves for the disappearance of 2-deoxy[^{14}C]glucose from plasma in each experimental condition are given in Fig. 1, together with the calculated areas under these curves. Insulin increased significantly the rate of

Fig. 1. Curves of disappearance from plasma of 2-deoxy[1- 14 C]glucose

Curves of disappearance from plasma of 2-deoxy[14 C]glucose radioactivity were constructed from measurements of the 14 C content of plasma samples taken at intervals after the intravenous administration of a bolus of tracer 2-deoxy[1- 14 C]glucose. Areas under these curves were computed by use of Simpson's rule. Mean curves are displayed for each group (six rats per group): $\circ$ — $\circ$, warm-acclimated, basal; $\circ$ --- $\circ$, warm-acclimated, insulin-treated; $\bullet$ — $\bullet$, cold-acclimated, basal; $\bullet$ --- $\bullet$, cold-acclimated, insulin-treated. The areas under these curves [d.p.m./ml per min ($\times 10^{-3}$); mean $\pm$ s.d.] were: warm-acclimated basal, 676 ± 5 ; warm-acclimated insulin-treated, 584 ± 45 ; cold-acclimated basal, 613 ± 73 ; cold-acclimated insulin-treated, 510 ± 58 .

2-deoxyglucose disappearance from plasma in both the warm- and cold-acclimated rats ($P < 0.05$). In addition, insulin had a significantly greater effect ($P < 0.05$) on the plasma 2-deoxyglucose disappearance rate in cold-acclimated rats than in warm-acclimated rats.

Glucose metabolic indices in various tissues were calculated by using eqn. (2), and are reported in Table 3. In the 5 h starved warm-acclimated rats, heart utilized 2-fold more glucose per g wet wt. than did brain, 3.5–14-fold more than skeletal muscles and 14-fold more than white or brown adipose tissue. There were no significant differences in the glucose metabolic indices of quadriceps muscle and muscles of the back region (quadratus lumborum and longissimus lumborum), but the glucose metabolic index of diaphragm was significantly higher than that for either. Treatment of the warm-acclimated rats under euglycaemic-clamp conditions, to raise the circulating insulin concentration by $50 \mu\text{units/ml}$, did not affect brain glucose utilization. However, rates of glucose utilization in heart increased 2.5-fold, whereas there was an 80% increase in diaphragm and a 50% increase in quadriceps and in muscles of the back region. Treatment of warm-acclimated rats with this low dose of insulin had no significant effect on the glucose metabolic index of either white or brown adipose tissue. Basal glucose metabolic indices of tissues of 5 h-starved cold-acclimated rats did not differ

significantly from those of warm-acclimated rats, except for heart (66% increase), quadriceps (53% increase) and brown adipose tissue (95% increase). In cold-acclimated rats, infusion of insulin resulted in similar-fold increases in the glucose metabolic indices to those obtained in warm-acclimated rats. The exception to this was brown adipose tissue, which showed a 4-fold increase in rate relative to the basal rate in cold-acclimated rats and an 8-fold increase relative to the basal rate in warm-acclimated rats. A number of tissues (spleen, testes, lung, small intestine) were found to have higher glucose metabolic indices than that of skeletal muscle.

The presence of glucose-6-phosphatase activity in small intestine has been demonstrated (Öckerman, 1965; Budohoski *et al.*, 1982). If this activity is demonstrated *in vivo*, it would result in an underestimation of the rate of glucose uptake. Cold-acclimation had no effect on these glucose metabolic indices, and infusion of insulin either produced no effect (spleen and testes) or only a small increase (lung and small intestine).

DISCUSSION

Methodology

The measurement of glucose turnover rate coupled with the glucose clamp technique provides quantitative information on whole-body glucose metabolism and insulin-sensitivity. Originally these techniques were applied to man (De Fronzo *et al.*, 1979; Rizza *et al.*, 1981), but more recently the methods have been adapted for use in small animals (Kraegen *et al.*, 1983; Leturque *et al.*, 1984). These techniques alone, however, do not allow any assessments to be made *in vivo* of rates of glucose utilization by individual tissues. To overcome this deficiency, several groups have used the 2-deoxyglucose tracer technique (Hom *et al.*, 1984; Kraegen *et al.*, 1985; Ferré *et al.*, 1985; Young *et al.*, 1985).

The accurate determination of tissue glucose metabolic rate by using 2-deoxyglucose uptake is dependent on knowledge of the lumped constant for that tissue. This is a measure of the discrimination between glucose and 2-deoxyglucose at the glucose transport and phosphorylation steps. Its accurate determination must either be made *in vivo* or by using a preparation *in vitro* that exactly mirrors the condition *in vivo*. For those tissues such as brown adipose tissue for which there is no satisfactory preparation *in vitro*, lumped constants are not available. Moreover, Pardridge (1983) has noted that the lumped constant may vary with the metabolic situation, and thus, even in tissues such as muscle and white adipose tissue for which values have been determined, these values may not be exactly representative. As a result of the lack of knowledge of the physiological validity of lumped constants, we have followed the precedent of Kraegen *et al.* (1985) and have expressed our results as tissue glucose metabolic indices. Furthermore, our data show that the sum of tissue glucose metabolic indices accounts for approx. 50% of the whole-body glucose utilization rate, irrespective of treatment. These findings suggest that our values for glucose metabolic indices closely reflect actual rates of tissue glucose utilization. (Liver and kidney were excluded from the study, because they have a high activity of glucose-6-phosphatase (Walker, 1966) and the

Table 3. Glucose metabolic indices *in vivo* in various tissues of warm- and cold-acclimated rats

Glucose metabolic indices were calculated as described in the Materials and methods section, according to eqn. (2). Results are presented as means $\pm$ S.D. for six determinations. Statistical significance of comparisons: different from control (basal) values from rats kept at the same acclimation temperature * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; different from similarly treated warm-acclimated rats † $P < 0.05$, †† $P < 0.01$, ††† $P < 0.001$.

Tissue	Glucose metabolic index (nmol/min per g wet wt.)			
	Warm-acclimated		Cold-acclimated	
	Basal	Insulin-treated	Basal	Insulin-treated
Brain	133 $\pm$ 3	133 $\pm$ 12	120 $\pm$ 33	115 $\pm$ 14
Heart	284 $\pm$ 132	705 $\pm$ 157***	474 $\pm$ 158†	863 $\pm$ 42**
Diaphragm	85 $\pm$ 33	156 $\pm$ 32**	107 $\pm$ 18	201 $\pm$ 65**
Back muscle (quadratus lumborum + longissimus lumborum)	20 $\pm$ 5	29 $\pm$ 6*	23 $\pm$ 5	37 $\pm$ 10*
Quadriceps muscle	17 $\pm$ 5	30 $\pm$ 11	26 $\pm$ 6†	48 $\pm$ 31
Spleen	112 $\pm$ 16	107 $\pm$ 26	110 $\pm$ 17	102 $\pm$ 20
Pancreas	30 $\pm$ 12	28 $\pm$ 7	21 $\pm$ 9	23 $\pm$ 1
Small intestine	64 $\pm$ 15	87 $\pm$ 9**	68 $\pm$ 9	77 $\pm$ 17
Testes	75 $\pm$ 19	76 $\pm$ 10	84 $\pm$ 8	79 $\pm$ 8
Lung	60 $\pm$ 17	89 $\pm$ 18*	69 $\pm$ 10	80 $\pm$ 18
Skin	18 $\pm$ 5	23 $\pm$ 7	20 $\pm$ 7	20 $\pm$ 8
White adipose	20 $\pm$ 8	25 $\pm$ 9	14 $\pm$ 8	19 $\pm$ 6
Interscapular brown adipose	21 $\pm$ 6	34 $\pm$ 18	41 $\pm$ 14††	162 $\pm$ 48***†††

consequent dephosphorylation of 2-deoxy[^{14}C]glucose 6-phosphate invalidates the methodology for these tissues.}

2-Deoxyglucose is not a substrate for the glucose transport system of the renal brush border (Turner & Silverman, 1977). In the present experiments, the contents of the bladder were aspirated at the end of the experiment and the radiochemical content of 2-deoxy-[^{14}C]glucose-derived material was determined. Between 38 and 47% of the injected 2-deoxy[^{14}C]glucose was recovered from the urine.

Chromatographic procedures established that over 95% of the radiolabelled material was not phosphorylated. Urinary excretion of radiolabelled material was independent of treatment in the present experiments, but, in situations in which there are several-fold changes in the whole-body glucose utilization, it is likely that there will be significant differences in the amount of urinary excretion. However, this loss of 2-deoxyglucose from the system does not affect the determination of tissue glucose metabolic indices, but it does prevent the use of plasma disappearance of 2-deoxy[^{14}C]glucose for the calculation of whole-body rates of glucose utilization.

Whole-body glucose utilization rates

In the present experiments, no significant difference was found between the rates of glucose utilization in warm- and cold-acclimated rats. However, plasma insulin concentrations were lower in cold-acclimated rats, suggesting that they were more insulin-sensitive. This is in agreement with the earlier conclusions of Baker & Sellars (1953) and Beck *et al.* (1967), which were made as a result of non-steady-state studies using an exogenous insulin injection and glucose tolerance tests

Table 4. Contribution of selected tissues to whole-body glucose turnover rate

Glucose metabolic indices (Table 2) were multiplied by total tissue mass and then expressed as a percentage of the whole-body glucose turnover rate (Fig. 1).

Tissue	Contribution (%)			
	Warm-acclimated		Cold-acclimated	
	Basal	Insulin-treated	Basal	Insulin-treated
Brain	1.4	1.2	1.3	0.9
Heart	2.2	4.7	3.4	4.3
Muscle	16	22	20	23
Skin	5	6	5	4
White adipose tissue	3	3	0.9	0.9
Brown adipose tissue	0.5	0.7	0.9	2.3
Total determined	44	54	46	47

respectively. Infusions of insulin, under euglycaemic-clamp conditions to raise the circulating insulin concentration by 50 $\mu\text{units/ml}$, produced a significant increase in whole-body glucose utilization in cold-acclimated rats (Table 2). The plasma insulin concentrations of the insulin-treated cold-acclimated rats ($123 \pm 23 \mu\text{units/ml}$) and warm-acclimated rats in the basal state ($129 \pm 39 \mu\text{units/ml}$) were similar, whereas the whole-body glucose utilization rate was increased by 60% (warm-acclimated, basal, $16.8 \pm 2.5 \mu\text{mol/min per rat}$; cold-acclimated, insulin-treated, $26.7 \pm 4.5 \mu\text{mol/min}$

per rat; $P < 0.001$). Since the blood glucose concentrations of the two groups of rats were the same, these results show unequivocally that cold-acclimated rats are more insulin-sensitive than warm-acclimated rats.

The combined use of the euglycaemic clamp and [^3H]glucose-turnover technique allows an assessment to be made of the reactive contributions of endogenous and exogenous glucose to the overall glucose utilization rate. In the cold-acclimated rats, insulin infusion produced a greater depression of endogenous glucose production than in warm-acclimated rats. The mechanism by which cold-acclimation causes enhanced whole-body insulin sensitivity is not known. Cold-acclimation caused a small decrease in body weight in the present experiments, and body weight has been shown to be an important determinant of insulin sensitivity. However, James *et al.* (1984) showed that decrease in body weight by energy restriction, to a similar degree to that produced by cold-acclimation in the current experiment, did not alter insulin-mediated glucose utilization. Thus it is unlikely that the small decrease in body weight is a major factor in the improvement in insulin-sensitivity in cold-acclimated rats.

Tissue glucose metabolic indices

In the present study, the glucose metabolic index of whole brain was not altered by either cold-acclimation or insulin treatment. The latter result agrees with previous studies in whole brain (reviewed by Pardridge, 1983), although insulin has been shown to decrease the glucose utilization rate of certain brain regions (Grunstein *et al.*, 1985). The contribution of brain glucose metabolic index to whole-body glucose utilization ranged from 0.9% in the insulin-treated cold-acclimated rats to 1.4% in control warm-acclimated rats. Assuming a lumped constant of 0.51, as determined *in vivo* by Sokoloff *et al.* (1977) for anaesthetized rats, these values are similar to those found by other workers. Sokoloff *et al.* (1977) showed that anaesthesia decreases brain glucose utilization by about 30–40%, and it is likely that anaesthesia will also decrease glucose utilization in other tissues, particularly postural muscles. In our experiments the glucose metabolic indices of quadriceps and postural muscles of the back (quadratus lumborum and longissimus lumborum) were only about 20% of those of the brain, and insulin and cold-acclimation produced an increase of less than 2-fold (Table 3). In contrast, the glucose metabolic index of diaphragm was 4–5-fold higher than in quadriceps and postural muscles of the back region. Furthermore, insulin increased the rate in warm-acclimated rats by 80%. It is not known whether diaphragm has a relatively greater intrinsic glucose metabolic index or whether the higher index merely relates to the fact that diaphragm is still functional during anaesthesia. By taking a mean of the metabolic indices for back muscle and quadriceps and multiplying this by the total muscle weight, which was 48% of body weight in both cold- and warm-acclimated rats, it is calculated that the contribution of muscle to the overall glucose utilization rate ranges from 16 to 23% (see Table 4). Even in the anaesthetized rat, muscle makes the greatest contribution to the insulin-induced increase in glucose utilization in the cold-acclimated rat.

The tissue with the highest glucose metabolic index (per g wet wt.) is heart. In the control, warm-acclimated rats, the index was 284 ± 132 nmol/min per g wet wt.

(Table 3). This is of a similar order to the maximum rate of glucose utilization (insulin conc. 7800 $\mu\text{units/ml}$) in skeletal muscle (Ferré *et al.*, 1985). The metabolic index of heart was increased significantly by both insulin and cold-acclimation (Table 3). However, its contribution to whole-body glucose utilization was small, ranging from 2.2 to 4.7% (Table 4).

In epididymal white adipose tissue, the glucose metabolic index was similar to that of quadriceps muscle (Table 3), but, unlike in muscle, glucose utilization by this tissue was not influenced by insulin treatment or cold-acclimation. Assuming that all white-adipose-tissue sites have the same glucose metabolic index as epididymal fat, then the contribution of adipose tissue to overall glucose utilization ranged from 3% in warm-acclimated rats to less than 1% in the cold-acclimated rats (Table 4). This decrease was mainly the result of a decrease in adiposity of the cold-acclimated rats (4.2% of body weight, as opposed to 6.6% in warm-acclimated rats).

In control warm-acclimated rats, the glucose metabolic index of interscapular brown adipose tissue was not significantly different from that of the epididymal white adipose tissue. The basal glucose metabolic index was increased 2-fold in the cold-acclimated rats (Table 3), although these rats had a lower plasma insulin concentration (Table 1). Insulin treatment of cold-acclimated rats produced a further 4-fold increase. Thus, in comparison with the control warm-acclimated rats, which had similar plasma insulin concentrations, the glucose metabolic index of the interscapular brown adipose tissue of the cold-acclimated insulin-treated rats was increased 8-fold. Under the latter conditions, the contribution of total brown adipose tissue (assuming that it all behaves like interscapular brown adipose and that total brown adipose tissue is 4 times the mass of interscapular brown adipose tissue; Foster & Frydman, 1978) to the whole-body glucose utilization rate was 2.3%. This contribution is significantly more than in brain and about half of that of heart (Table 4). Its contribution to the insulin-stimulated increase in glucose utilization in cold-acclimated rats was equivalent to that of the heart.

Cooney & Newsholme (1982) showed that cold-acclimation of rats resulted in an increase from 6 to 12 $\mu\text{mol/min per g}$ fresh wt. in the maximal activity of hexokinase in brown adipose tissue. Comparison of these values with those in Table 3 shows that the glucose metabolic index was only 1–2% of the theoretical maximal capacity of hexokinase. Although it is probable that further stimulation with insulin and/or noradrenaline (Gibbins *et al.*, 1985) will increase glucose utilization by brown adipose tissue, it seems unlikely that the glucose metabolic index of brown adipose tissue will ever approach the theoretical maximum capacity of hexokinase. Indeed, it is noteworthy that the hexokinase activity of brain (Cooney & Newsholme, 1982) is at least 20-fold greater than measured rates of glucose utilization (Ferré *et al.*, 1985; Pardridge, 1983).

In energy terms, glucose utilization by brown adipose tissue is unlikely to make a significant contribution to basal or insulin-stimulated metabolic rate. The basal metabolic rate of rats of this size is about 10 kJ/h, and the measured brown-adipose-tissue glucose metabolic index, if the glucose is totally oxidized to CO_2 , is equivalent to about 0.1 kJ/h. However, these data do

show that cold-acclimation results in a selective increase in insulin-sensitivity of brown adipose tissue. This may indicate that increased glucose uptake is important in maintaining the increased activity of non-shivering thermogenesis in brown adipose tissue, possibly by increasing the availability of glycolytic and citric acid cycle intermediates.

We gratefully acknowledge the skilled technical assistance of Laura King and Sarah Hook. We are grateful to Mr. N. Stewart-Long for his help with the analysis of plasma 2-deoxyglucose disappearance curves, and to Mrs. J. S. Hodder and Miss L. Collis for their help in preparing the manuscript.

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Received 28 October 1985/2 April 1986; accepted 8 April 1986