The effect of selenium-deficiency on rat fat-cell glucose oxidation

John E. SOUNESS*† and John E. STOUFFER

*Marrs McLean Department of Biochemistry, Baylor College of Medicine, Texas Medical Center,
Houston, TX 77030, U.S.A.*

and Victoria CHAGOYA DE SANCHEZ

*Departamento de Bioenergetica, Centro de Investigaciones en Fisiologia Celular, Universidad Nacional
Autonoma de Mexico, Mexico 20, D.F. Mexico*

(Received 11 March 1983/Accepted 8 April 1983)

When rats are fed a selenium-deficient diet, the glutathione peroxidase activity of epididymal fat-cells decreases to 5–9% of that of control rats fed the same diet supplemented with 0.5 p.p.m. of selenium as sodium selenite. [1-¹⁴C]Glucose oxidation in fat-cells from rats fed a selenium-deficient diet is unresponsive to the action of t-butyl hydroperoxide, which stimulates ¹⁴CO₂ formation from [1-¹⁴C]glucose 4-fold in control rats. Insulin enhances [1-¹⁴C]glucose oxidation and incorporation into lipids in fat-cells from both groups of rats; however, the response elicited is reduced in fat-cells prepared from selenium-deficient animals. The 'C-1/C-6 ratio' (ratio of glucose C-1 to glucose C-6 oxidized) is enhanced by insulin to a similar degree in fat-cells from both groups of animals. The stimulatory action of Zn²⁺ and dithiothreitol on [1-¹⁴C]glucose oxidation observed in fat-cells from selenium-supplemented rats is greatly reduced in fat-cells from selenium-deficient rats. [1-¹⁴C]Glucose oxidation in fat-cells from both groups of animals is highly sensitive to the stimulatory action of adenosine. It is concluded that the enhanced formation and glutathione-linked destruction of H₂O₂ plays, at the most, only a minor role in the stimulation of the flux of glucose through the pentose phosphate pathway elicited by insulin, although elimination of glutathione peroxidase activity may influence the action of insulin on glucose oxidation. Production and subsequent destruction of H₂O₂ may play an important role in the stimulatory action of Zn²⁺ and dithiothreitol on fat-cell [1-¹⁴C]glucose oxidation.

A characteristic feature of insulin action on rat epididymal fat-cells is the preferential stimulation of glucose C-1 oxidation over glucose C-6 oxidation (Katz *et al.*, 1966). A prime determinant of the quantity of glucose passing through the PPP appears to be the capacity of the cell for fatty acid synthesis (Kather *et al.*, 1972; Richardson & Czech, 1978; Fried *et al.*, 1981), the requirement for NADPH of which determines the cytoplasmic [NADPH]/

[NADP⁺] ratio, which, in turn, regulates glucose 6-phosphate dehydrogenase, the probable rate-limiting step of the PPP (Eggleson & Krebs, 1974). However, the ability of insulin to stimulate H₂O₂ formation in fat-cell incubations (Mukherjee & Lynn, 1977; Mukherjee *et al.*, 1978; May & de Haen, 1979), possibly by the stimulation of a plasma-membrane-bound NADPH oxidase (Mukherjee & Lynn, 1977), prompted the proposal that the rate of glucose flux through the PPP could be enhanced in response to insulin by the increased accumulation and catabolism of H₂O₂ through the action of glutathione peroxidase (GSH:H₂O₂ oxidoreductase; EC 1.11.1.9). Glutathione reductase [NAD(P)H:GSSG oxidoreductase; EC 1.6.4.2] regenerates GSH from GSSG at the expense of NADPH. The lowering of the [NADPH]/[NADP⁺]

Abbreviations used: GSH, reduced glutathione; GSSG, oxidized glutathione; PPP, pentose phosphate pathway.

* Present address: Department of Pharmacology, The University of Texas Medical School at Houston, P.O. Box 20708, Houston, TX 77025, U.S.A.

† To whom correspondence and reprint requests should be sent.

ratio, in turn, stimulates passage of glucose through the PPP:

Two kinds of glutathione peroxidase are known. They differ in their requirement for selenium, substrate specificity and subcellular distribution (Lawrence & Burk, 1976, 1978). The selenium-dependent glutathione peroxidase exhibits high activity towards organic hydroperoxides and H_2O_2 as substrates, whereas the selenium-independent enzyme is mainly active towards organic hydroperoxides and has only little activity towards H_2O_2 .

In order to test whether H_2O_2 catabolism plays a role in the increased flux of glucose through the PPP in response to insulin, the effect of the hormone on glucose oxidation has been tested in fat-cells from rats fed a selenium-deficient diet, where the activity of glutathione peroxidase is greatly decreased. Involvement of H_2O_2 has been implicated in the mechanism of action of other hormones and agents known to mimic insulin action on glucose oxidation. The effect of selenium deficiency on the capacity of a selection of these compounds to stimulate glucose oxidation has been examined.

Experimental

Materials

Crude *Clostridium histolyticum* collagenase (EC 3.4.24.3; lot 37C-6860), bovine serum albumin (Cohn fraction V; lot 88C-0252), insulin (bovine), adenosine deaminase, oxytocin, t-butyl hydroperoxide and dithiothreitol were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Radiochemicals were purchased from Amersham Co. (Arlington Heights, IL, U.S.A.). N^6 -(Phenylisopropyl)adenosine was a gift from Dr. Harold Stark of Boehringer-Mannheim G.m.b.H., Mannheim, Germany. All other reagents were of the highest grade available.

Animals and diet

Weanling male Wistar rats were fed on a Torula yeast-based, selenium-deficient diet or on the same diet supplemented with 0.5 p.p.m. of selenium as sodium selenite (controls) for 7–8 weeks (Burk & Masters, 1975; Lotscher *et al.*, 1979). The diet was purchased from ICN Pharmaceuticals Inc., Cleveland, OH, U.S.A., and had the following composi-

tion: 30% Torula yeast, 59% sucrose, 5% tocopherol-stripped lard, 5% salt mix HMW, 100 i.u. of

vitamin E, plus 1% ICN vitamin diet fortification mixture omitting vitamin E. To verify that the diet was selenium-deficient the diet was analysed for selenium by energy-dispersive X-ray fluorescence spectrometry as follows. To 500 mg of the rat feed, $2.5 \mu\text{g}$ of Zr was added as an internal standard. Concentrated Ultrex HNO_3 (10 ml) was added to each sample, and the samples were incubated at 70°C for 4 h and then evaporated. Another portion of conc. HNO_3 (15 ml) was added and the samples were incubated overnight at 150°C . After evaporation, the samples were dissolved in 0.5 ml of HNO_3 . A $20 \mu\text{l}$ portion was applied to a polypropylene sample holder and dried. The samples were then analysed on a Kevex 0600 Ultratrace X-ray fluorescence spectrometer using direct excitation from an Ag-anode X-ray tube filtered with $200 \mu\text{m}$ Ag foil. The selenium content of the diet was below the minimum detection limit of the method employed. In contrast, the technique demonstrated that normal laboratory chow (Formulab Chow 5008) contained $0.42 \mu\text{g/g}$ dry wt. of selenium.

Isolation of fat-cells

Fat-cells were isolated from the epididymal fat-pads by the method of Rodbell (1964). The fat-pads were incubated for 60 min at 37°C in Krebs–Ringer bicarbonate medium (pH 7.4) containing bovine serum albumin (3%) and collagenase (1 mg/ml). After digestion, the cells were filtered through nylon mesh and washed three times with buffer. Fat-cell DNA was measured by the method of Sooranna & Saggerson (1976).

Preparation of fat-cell extracts and assay of glutathione peroxidase

Fat-cell extracts were prepared in 5 mM-potassium phosphate (pH 7.0)/1 mM-EDTA by the method of Sooranna & Saggerson (1976) and the enzyme was assayed by the method of Sies & Moss (1978). The assay medium consisted of 50 mM-potassium phosphate (pH 7.0), 1 mM-EDTA, 1 mM- NaN_3 , 1 mM-GSH, 1 unit of glutathione reductase/ml, $50 \mu\text{M}$ -NADPH and hydroperoxide as indicated.

Glucose oxidation and incorporation into lipids

Fat-cells were incubated at 37°C for 1 h in bicarbonate-buffered medium (pH 7.4) that contained bovine serum albumin (3%) and [^{14}C]glucose (5 mM; 0.2 μCi). Incubations were terminated by the addition of 0.5 ml of 0.5 M-HClO₄. The $^{14}\text{CO}_2$ was trapped by Hyamine hydroxide (1 M solution in methanol) contained in suspended centre wells, which were subsequently transferred to scintillation vials and counted for ^{14}C -radioactivity in Aquasol (New England Nuclear Corp., Boston, MA, U.S.A.).

The incorporation of [^{14}C]glucose into the glycerol and fatty acid moieties of acylglycerols was determined as follows. After incubation and collection of $^{14}\text{CO}_2$ was complete, the cell suspension was shaken with 10 ml of heptane/propan-2-ol (1:4, v/v) to extract the lipid fraction. The contents of the vial were allowed to stand for 15 min, after which time 4 ml of heptane and 5 ml of water were added. The mixture was shaken vigorously, the phases allowed to separate and the upper organic phase was removed to a clean test tube. The heptane layer was then washed twice with 5 ml of water. Of the extract 4 ml was removed to a clean test tube and the heptane was evaporated off. Freshly prepared ethanolic KOH [2 ml; prepared by adding five parts of aq. 80% (w/v) KOH to 95 parts of ethanol] was added, the tubes stoppered and the lipid saponified by placing the tubes in a water bath at 60°C for 12 h. After cooling the tubes in ice, 0.75 ml of water was added and the sample was extracted twice with 5 ml of light petroleum (b.p. 40–60°C). These light-petroleum fractions were discarded. The remaining ethanolic KOH layer was acidified by adding 0.33 ml of 5 M-H₂SO₄ and extracted three times with 5 ml of light petroleum. The combined light-petroleum fractions were evaporated to dryness in a scintillation vial. Aquasol was added and the radioactivity in the fatty acid moiety of acylglycerols was counted. A portion of the remaining acid aqueous fraction, after light-petroleum extraction of fatty acids, was added directly to Aquasol and counted for radioactivity in the glycerol moiety of acylglycerols.

Statistical comparisons were made by using Student's paired *t* test.

Results*Selenium-independent effects of the diet*

Initial experiments indicated that [^{14}C]glucose oxidation in fat-cells from selenium-deficient and selenium-supplemented rats was only poorly responsive to the action of insulin (3.3 nM) (results not shown). This phenomenon was subsequently explained with the finding that fat-cells from both groups of animals were highly responsive to endogenous adenosine, which masked the action of

insulin. The addition of adenosine deaminase (2 $\mu\text{g}/\text{ml}$) to incubations of fat-cells from selenium-supplemented and selenium-deficient rats greatly reduced the rate of $^{14}\text{CO}_2$ production from [^{14}C]glucose (Table 1) and permitted expression of insulin action. In all subsequent fat-cell incubations endogenous adenosine was eliminated from the incubations by addition of adenosine deaminase.

Glutathione peroxidase activity in fat-cells from selenium-deficient and selenium-supplemented rats

Employing both *t*-butyl hydroperoxide (1 mM) and H₂O₂ (0.35 mM) as substrates, glutathione peroxidase activity was greatly reduced in selenium-deficient rats. In fat-cells from control animals the glutathione peroxidase activity was 1.71 and 1.31 nmol of NADPH oxidized/min per g of DNA using H₂O₂ and *t*-butyl hydroperoxide respectively as substrates. In contrast, the glutathione peroxidase activity in fat-cells from selenium-deficient rats was 0.08 and 0.12 nmol of NADPH oxidized/min per μg of DNA employing H₂O₂ and *t*-butyl hydroperoxide respectively as substrates. The results are means of two experiments performed on different days. Similar results were observed when measuring the glutathione peroxidase activity extracted from whole fat-pads.

Glucose oxidation in fat-cells from selenium-deficient and selenium-supplemented rats

t-Butyl hydroperoxide increased, by about 4-fold, [^{14}C]glucose oxidation in fat-cells from control rats, but slightly inhibited $^{14}\text{CO}_2$ formation from [^{14}C]glucose in fat-cells from selenium-deficient rats (Fig. 1). Insulin stimulated [^{14}C]glucose oxidation in fat-cells from both groups of animals; however, the magnitude of the response elicited by insulin was markedly reduced in selenium-deficient rats (Fig. 2). Although the capacity of insulin to

Table 1. [^{14}C]Glucose oxidation in fat-cells from selenium-supplemented and selenium-deficient rats in the presence and absence of adenosine deaminase

Fat-cells were incubated for 1 h at 37°C in Krebs-Ringer bicarbonate buffer containing bovine serum albumin (3%) and [^{14}C]glucose (5 mM, 0.2 μCi). Adenosine deaminase was included at a concentration of 2 $\mu\text{g}/\text{ml}$. Values are means $\pm$ S.E.M. of two experiments performed in duplicate.

Diet mixture Addition	Glucose oxidation (nmol of CO ₂ liberated/h per μg of DNA)	
	Selenium- supplemented	Selenium- deficient
None	18.48 $\pm$ 2.98	18.24 $\pm$ 2.57
Adenosine deaminase	8.23 $\pm$ 2.75	7.11 $\pm$ 1.98

stimulate [$1\text{-}^{14}\text{C}$]glucose oxidation in selenium-deficient rats was reduced, the 'C-1/C-6 ratio' (ratio of glucose C-1 to glucose C-6 oxidized) was not

Fig. 1. Effect of *t*-butyl hydroperoxide on [$1\text{-}^{14}\text{C}$]glucose oxidation in fat-cells from selenium-deficient and selenium-supplemented rats

The conditions employed are the same as those described in the legend to Table 1. The results represent means $\pm$ S.E.M. of three experiments performed in duplicate and are expressed as percentages of the basal rate of [$1\text{-}^{14}\text{C}$]glucose oxidation, which was 13.55 ± 3.00 and 12.58 ± 0.35 nmol of CO_2 liberated/h per μg of fat-cell DNA for incubations of fat-cells from selenium-supplemented and selenium-deficient rats respectively.

appreciably altered by the diet, due to the parallel diminution of the response elicited by insulin on [$6\text{-}^{14}\text{C}$]glucose oxidation (Table 2). Oxidation of [$6\text{-}^{14}\text{C}$]glucose was slightly enhanced by *t*-butyl hydroperoxide in control rats but was strongly inhibited in selenium-deficient animals (Table 2). *t*-Butyl hydroperoxide increased the C-1/C-6 ratio in both groups of animals but the magnitude of the effect was diminished in fat-cells from selenium-deficient rats.

As predicted from previous results (Table 1), N^6 -(phenylisopropyl)adenosine strongly stimulated

Fig. 2. Effect of insulin on [$1\text{-}^{14}\text{C}$]glucose oxidation in fat-cells from selenium-deficient and selenium-supplemented rats

For details, see the legend to Fig. 1.

Table 2. Effect of insulin and *t*-butyl hydroperoxide on [$1\text{-}^{14}\text{C}$]glucose and [$6\text{-}^{14}\text{C}$]glucose oxidation in fat-cells from selenium-supplemented and selenium-deficient rats

The experimental conditions employed are the same as those described in the legend to Table 1. Insulin and *t*-butyl hydroperoxide were included at concentrations of 3.3 nM and 100 μM respectively. Adenosine deaminase (2 $\mu\text{g}/\text{ml}$) was included throughout. Values for glucose oxidation are expressed as nmol of CO_2 liberated/h per μg of DNA and are means $\pm$ S.E.M. of four experiments performed in duplicate. Values in parentheses are percentages of basal values. Significant differences from the basal values are indicated: * $P < 0.01$; † $P < 0.05$.

Addition	Glucose oxidation		C-1/C-6 ratio
	[$1\text{-}^{14}\text{C}$]Glucose $\rightarrow$ $^{14}\text{CO}_2$	[$6\text{-}^{14}\text{C}$]Glucose $\rightarrow$ $^{14}\text{CO}_2$	
Selenium-supplemented			
None	11.87 ± 2.86 (100)	6.59 ± 1.54 (100)	1.80
Insulin	37.18 ± 7.74 (313)*	11.09 ± 3.81 (168)*	3.35
<i>t</i> -Butyl hydroperoxide	56.63 ± 8.01 (477)*	10.39 ± 5.18 (158)*	5.45
Selenium-deficient			
None	13.29 ± 2.93 (100)	7.54 ± 1.14 (100)	1.76
Insulin	29.64 ± 4.44 (223)*	8.49 ± 0.79 (113)*	3.49
<i>t</i> -Butyl hydroperoxide	10.96 ± 1.87 (83)*	3.91 ± 0.70 (52)*	2.80

Table 3. *Effect of insulin-like agents on [1-¹⁴C]glucose oxidation in fat-cells from selenium-supplemented and selenium-deficient rats*

The conditions employed were the same as those described in the legend to Table 1. Oxytocin (0.1 μ M), Zn²⁺ (added as ZnCl₂) (0.5 mM), N⁶-(phenylisopropyl)adenosine (PIA) (0.1 μ M) and dithiothreitol (DTT) (0.175 mM) were included where indicated. Adenosine deaminase (2 μ g/ml) was present throughout. Values are means $\pm$ S.E.M. of three experiments performed in duplicate. Values in parentheses are percentages of the basal value. Significant differences from the basal values are indicated: * P < 0.01; † P < 0.05.

Addition	Diet mixture	...	Glucose oxidation (nmol of CO ₂ /h per μ g of DNA)	
			Selenium-supplemented	Selenium-deficient
None			10.20 $\pm$ 1.33 (100)	14.00 $\pm$ 4.01 (100)
Oxytocin			12.55 $\pm$ 1.17* (123)	18.03 $\pm$ 4.44† (129)
Zn ²⁺			14.78 $\pm$ 1.07* (144)	13.40 $\pm$ 3.07 (96)
PIA			29.03 $\pm$ 1.89* (285)	26.78 $\pm$ 3.79* (191)
DTT			23.63 $\pm$ 1.85* (232)	16.45 $\pm$ 3.65 (117)

Table 4. *Effects of insulin on [1-¹⁴C]- and [6-¹⁴C]-glucose incorporation into the glycerol and fatty acid moieties of acylglycerols in the fat-cells from selenium-deficient and selenium-supplemented rats*

The experimental conditions employed are the same as those described in the legend to Table 1. Insulin was included at a concentration of 3.3 nM. Adenosine deaminase (2 μ g/ml) was included throughout. Values are means $\pm$ S.E.M. of four experiments performed in duplicate. Values in parentheses are the percentages of basal rates of [¹⁴C]glucose conversion into lipids.

Addition	Glucose conversion (nmol of CO ₂ liberated/h per μ g of DNA)			
	[¹⁴ C]Glucose conversion into fatty acids moiety		[¹⁴ C]Glucose conversion into glycerol moiety	
	C-1	C-6	C-1	C-6
Selenium-supplemented				
None	2.94 $\pm$ 0.85	4.48 $\pm$ 1.44	15.87 $\pm$ 3.35	15.09 $\pm$ 2.32
Insulin	18.98 $\pm$ 2.85 (635)	24.95 $\pm$ 3.98 (557)	51.94 $\pm$ 10.80 (327)	46.87 $\pm$ 10.50 (311)
Selenium-deficient				
None	4.29 $\pm$ 1.97	4.86 $\pm$ 2.10	22.15 $\pm$ 3.37	17.70 $\pm$ 3.37
Insulin	15.77 $\pm$ 1.79 (368)	19.17 $\pm$ 3.01 (394)	45.58 $\pm$ 8.09 (206)	37.74 $\pm$ 5.76 (213)

[1-¹⁴C]glucose oxidation in fat-cells from both groups of rats; however, like insulin, the response elicited by N⁶-(phenylisopropyl)adenosine was smaller in fat-cells from selenium-deficient rats. Zn²⁺ significantly (P < 0.01) stimulated [1-¹⁴C]glucose oxidation by about 40% in fat-cells from control rats but had no effect on fat-cells from selenium-deficient rats. Dithiothreitol elicited a highly significant stimulation (P < 0.01) of ¹⁴CO₂ production from [1-¹⁴C]glucose in fat-cells from selenium-supplemented rats; however, no significant effect of dithiothreitol was observed in fat-cells from selenium-deficient rats. Oxytocin weakly stimulated [1-¹⁴C]glucose conversion into ¹⁴CO₂ in fat-cells from both groups of animals. These results are summarized in Table 3.

Glucose conversion into fatty acid and glycerol moieties of acylglycerols in fat-cells from selenium-deficient and selenium-supplemented rats

The magnitude of the insulin-elicited stimulation of both [1-¹⁴C]glucose and [6-¹⁴C]glucose incor-

poration into the glycerol and fatty acid moieties of acylglycerols was reduced by a similar degree in fat-cells from selenium-deficient rats in comparison with fat-cells from control animals (Table 4).

Discussion

Glucose oxidation in fat-cells from rats fed the selenium-deficient and selenium-supplemented diets exhibited a responsiveness to adenosine much greater than previously noted (Schwabe *et al.*, 1974; Fain & Wieser, 1975; Wieser & Fain, 1975; see Table 1). The high responsiveness to endogenous adenosine almost completely masked the action of insulin on ¹⁴CO₂ formation from [1-¹⁴C]glucose (results not shown). Increased sensitivity to the antilipolytic actions of adenosine and its *R*-site analogues has been reported in fat-cells from hypothyroid (Ohisalo & Stouffer, 1978) and adrenalectomized rats (Fernandez & Saggerson, 1978; Saggerson, 1980). Interestingly, insulin elicits only a weak stimulatory effect on glucose oxidation

in fat-cells from hypothyroid rats (Czech *et al.*, 1980). The factor(s) in the diet responsible for increasing the sensitivity of the fat-cells to adenosine has not been identified. The high (59%) sucrose content of the diet has been ruled out as the element responsible, since a high carbohydrate diet (68% sucrose) (ICN Pharmaceuticals Inc., Cleveland, OH, U.S.A.) failed to increase similarly the responsiveness of the cells to the action of adenosine (J. E. Souness, J. E. Stouffer & V. Chagoya de Sanchez, unpublished work).

By maintaining rats on a diet deficient in selenium the activity of fat-cell glutathione peroxidase can be decreased to 9% and 4–5% of controls, using *t*-butyl hydroperoxide and H_2O_2 respectively as substrates (see the Results section).

t-Butyl hydroperoxide, which, unlike H_2O_2 , is not degraded by catalase (Srivastava *et al.*, 1974), strongly stimulated $[1-^{14}C]$ glucose oxidation in control rats (Fig. 1); however, it had no effect on $^{14}CO_2$ formation from $[1-^{14}C]$ glucose in fat-cells from selenium-deficient rats (Fig. 1). This is consistent with the idea that glutathione metabolism, either directly or via coupled changes in the $[NADPH]/[NADP^+]$ ratio, may play a significant role in the short-term control of the PPP (May, 1981). In control rats, *t*-butyl hydroperoxide is inactivated with the concomitant oxidation of GSH and generation of GSSG. GSH is regenerated at the expense of NADPH. The resulting lowering of the $[NADPH]/[NADP^+]$ ratio de-inhibits glucose 6-phosphate dehydrogenase (Eggleston & Krebs, 1974) resulting in increased flux of glucose carbon atoms through the PPP as reflected by the enhanced $[1-^{14}C]$ glucose oxidation. In fat-cells from selenium-deficient rats, the almost complete absence of glutathione peroxidase activity is associated with an inability of *t*-butyl hydroperoxide to stimulate $[1-^{14}C]$ glucose oxidation, suggesting that the effect of this compound is mediated by its degradation by glutathione peroxidase. The strong inhibition of $[6-^{14}C]$ glucose conversion into $^{14}CO_2$ elicited by *t*-butyl hydroperoxide in fat-cells from selenium-deficient rats (Table 2) may be associated with the destructive properties of a reactive oxygen species such as *t*-butyl hydroperoxide exhibited under conditions where an important mechanism for its destruction is absent.

Insulin stimulates $^{14}CO_2$ formation from $[1-^{14}C]$ glucose in selenium-supplemented and selenium-deficient rats (Fig. 2), indicating that any fluctuation in the $[GSH]/[GSSG]$ ratio, elicited in response to hormonal stimulation of H_2O_2 , is not the prime mechanism by which the $[NADPH]/[NADP^+]$ ratio is lowered in order to facilitate increased flux of glucose through the PPP. However, the size of the response elicited by insulin in selenium-deficient rats is significantly reduced. Whether this is related to the

decreased glutathione peroxidase activity and the resulting disappearance of PPP-linked H_2O_2 catabolism is uncertain, since the response elicited by insulin on $[6-^{14}C]$ glucose oxidation is also depressed in selenium-deficient rats and the enhancement of the C-1/C-6 ratio by insulin is almost identical in fat-cells from both groups of animals (Table 2). The enhancement elicited by insulin on the incorporation of $[1-^{14}C]$ glucose and $[6-^{14}C]$ glucose into the glycerol and fatty acid moieties of acylglycerols is similarly diminished in selenium-deficient rats (Table 4).

The lowered response elicited by insulin on $[1-^{14}C]$ glucose oxidation in fat-cells from selenium-deficient rats may be indicative of an effect on hexose transport. The rate of degradation of reactive oxygen species such as H_2O_2 in cells with greatly lowered glutathione peroxidase may produce alterations in the properties of cell membranes. Peroxidation can modify the fluidity state of membranes (Dobretsov *et al.*, 1977), which has been shown to influence hexose-transport rates in fat-cells (Czech, 1980). The redox state of mitochondrial nicotinamide nucleotides may be controlled by glutathione peroxidase and glutathione reductase and is a factor in the balance of Ca^{2+} between mitochondria and medium (Lotscher *et al.*, 1979). Exposure of fat-cells to insulin alters the stable mitochondrial calcium pool (McDonald *et al.*, 1976) and it is feasible that enhanced H_2O_2 production and destruction by glutathione peroxidase may serve a role in the action of insulin on mitochondrial Ca^{2+} . The low glutathione peroxidase activity in fat-cells from selenium-deficient rats may thus alter the ability of insulin to elicit changes in the calcium balance, which may, in turn, affect the hormone's actions on glucose oxidation.

N^6 -(Phenylisopropyl)adenosine, oxytocin, dithiothreitol and Zn^{2+} all stimulate glucose oxidation in rat fat-cells (Muchmore *et al.*, 1981, 1982; Goko *et al.*, 1981; May & Contoreggi, 1982). N^6 -(Phenylisopropyl)adenosine, oxytocin and Zn^{2+} are known to stimulate formate oxidation (Muchmore *et al.*, 1981, 1982; May & Contoreggi, 1982), a process believed to be indicative of intracellular H_2O_2 production (Mukherjee *et al.*, 1978; May & de Haen, 1979) and it has been suggested that H_2O_2 may be implicated in the mechanism of action of dithiothreitol (Goko *et al.*, 1981). The absence of glutathione peroxidase in fat-cells from selenium-deficient rats had no effect on the small enhancement of glucose oxidation elicited by oxytocin (Table 3). N^6 -(Phenylisopropyl)adenosine strongly stimulated $^{14}CO_2$ production from $[1-^{14}C]$ glucose in fat-cells from both groups of animals; however, as with insulin, the size of the response elicited was depressed in fat-cells from selenium-deficient rats. The results suggest that catabolism of H_2O_2 by

glutathione peroxidase may be an important factor in the stimulatory action of Zn^{2+} and dithiothreitol on fat-cell $[1-^{14}\text{C}]$ glucose oxidation, since selenium-deficiency eliminated the weak (44%) enhancement of $^{14}\text{CO}_2$ formation in response to the bivalent metal and greatly reduced the magnitude of the response elicited by dithiothreitol (Table 3).

In conclusion, the low glutathione peroxidase activity in rats fed a selenium-deficient diet is associated with a loss of the stimulatory effect of t-butyl hydroperoxide on fat-cell $[1-^{14}\text{C}]$ glucose oxidation, indicating that the PPP-linked degradation of the hydroperoxide is the single most important factor in its stimulatory action on $[1-^{14}\text{C}]$ glucose oxidation. Formation and subsequent glutathione-linked degradation of H_2O_2 appears to be, at most, of only minor importance in the stimulation of the PPP by insulin; however, results suggest that selenium-deficiency decreases the responsiveness of fat-cells to the action(s) of insulin on glucose oxidation and incorporation into lipids. Moreover, glutathione peroxidase may play an important role in the mechanism of action of Zn^{2+} and dithiothreitol on fat-cell glucose oxidation.

The research work was supported by Grant no. Q-275 from the Robert A. Welch Foundation. We thank Dr. W. J. Thompson, Dr. S. J. Strada and Dr. V. Lavis for helpful comments and Dr. W. E. Seifert for performing the selenium analysis of the rat diet.

References

- Burk, R. F. & Masters, B. S. (1975) *Arch. Biochem. Biophys.* **170**, 124–131
- Czech, M. P. (1980) *Diabetes* **29**, 399–409
- Czech, M. P., Malbon, C. C., Kerman, K., Gitomer, W. & Pilch, P. F. (1980) *J. Clin. Invest.* **66**, 574–582
- Dobretsov, G. E., Borschevskaya, T. A., Petrov, V. A. & Vladimirov, Yu. A. (1977) *FEBS Lett.* **84**, 125–128
- Eggleston, L. V. & Krebs, H. A. (1974) *Biochem. J.* **138**, 425–435
- Fain, J. N. & Wieser, P. B. (1975) *J. Biol. Chem.* **250**, 1027–1034
- Fernandez, B. M. & Saggerson, E. D. (1978) *Biochem. J.* **174**, 111–118
- Fried, S. K., Lavau, M. & Pi-Sunyer, F. X. (1981) *J. Lipid Res.* **22**, 753–762
- Goko, H., Takashima, S., Kawamuro, A. & Matsuoka, A. (1981) *Biochem. J.* **200**, 425–428
- Kather, H., Rivera, M. & Brand, K. (1972) *Biochem. J.* **128**, 1089–1096
- Katz, J., Landau, B. R. & Bartsch, G. E. (1966) *J. Biol. Chem.* **241**, 727–740
- Lawrence, R. A. & Burk, R. F. (1976) *Biochem. Biophys. Res. Commun.* **71**, 952–958
- Lawrence, R. A. & Burk, R. F. (1978) *J. Nutr.* **108**, 211–215
- Lotscher, H. R., Winterhalter, K. H., Carafoli, E. & Richter, C. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4340–4344
- May, J. M. (1981) *Arch. Biochem. Biophys.* **207**, 117–127
- May, J. M. & Contoreggi, C. S. (1982) *J. Biol. Chem.* **257**, 4362–4368
- May, J. M. & de Haen, C. (1979) *J. Biol. Chem.* **254**, 2214–2220
- McDonald, J. M., Bruns, D. E. & Jarett, L. (1976) *Biochem. Biophys. Res. Commun.* **71**, 114–121
- Muchmore, D. B., Little, S. A. & de Haen, C. (1981) *J. Biol. Chem.* **256**, 365–372
- Muchmore, D. B., Little, S. A. & de Haen, C. (1982) *Biochemistry* **21**, 3886–3892
- Mukherjee, S. P. & Lynn, W. S. (1977) *Arch. Biochem. Biophys.* **184**, 69–76
- Mukherjee, S. P., Lane, R. H. & Lynn, W. S. (1978) *Biochem. Pharmacol.* **27**, 2589–2594
- Ohisalo, B. M. & Stouffer, J. E. (1978) *Biochem. J.* **179**, 249–251
- Richardson, D. K. & Czech, M. P. (1978) *Am. J. Physiol.* **234**, E182–E189
- Rodbell, M. (1964) *J. Biol. Chem.* **239**, 375–380
- Saggerson, E. D. (1980) *FEBS Lett.* **115**, 127–128
- Schwabe, U., Schonhofer, P. S. & Ebert, R. (1974) *Eur. J. Biochem.* **46**, 537–545
- Sies, H. & Moss, K. M. (1978) *Eur. J. Biochem.* **84**, 377–383
- Sooranna, S. R. & Saggerson, E. D. (1976) *FEBS Lett.* **64**, 36–39
- Srivastava, S. K., Awasthi, Y. C. & Beutler, E. (1974) *Biochem. J.* **139**, 289–295
- Wieser, P. B. & Fain, J. N. (1975) *Endocrinology* **96**, 1221–1225