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Pentose Cycle Flux and Fatty Acid Synthesis in Bovine Adipose Tissue Slices Incubated with 6-Aminonicotinamide (41774)

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Abstract. The effects of the purported inhibitor of 6-phosphogluconate dehydrogenase, 6-aminonicotinamide, on lipogenesis from acetate and the metabolism of glucose were investigated in bovine adipose tissue. The incorporation of [U-^{14}C]acetate and tritium from [3-^{3}H]glucose into fatty acids was stimulated by 6-aminonicotinamide proportionately, indicating that the pentose cycle provided the same percentage of NADPH required for fat synthesis in the absence and presence of 6-aminonicotinamide. Tissue samples incubated with 6-aminonicotinamide displayed higher maximal activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase than control samples. The cellular content of 6-phosphogluconate was increased by 6-aminonicotinamide by 40% in samples incubated with 2 m*M* glucose (plus 33 mU/ml insulin) and 10 m*M* acetate; 6-aminonicotinamide stimulated the production of L-lactate in either the absence or presence of acetate. Studies with 1-, 6-, and U-^{14}C-labeled glucose indicated that 6-aminonicotinamide increased the proportion of utilized glucose metabolized by the pentose cycle in the absence, but not in the presence of acetate. Unlike results observed in rat adipose tissue, the primary effect of 6-aminonicotinamide was to increase the proportion of NADPH produced by the pentose cycle that was utilized for fat synthesis secondarily to the stimulation of lipogenesis by an unknown mechanism.

There exist in bovine adipose tissue three pathways that supply NADPH for the biosynthesis of fatty acids: the hexose monophosphate pathway, or pentose cycle, involving the NADP-reductases glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49) and 6-phosphogluconate dehydrogenase (E.C. 1.1.1.44); and pathways involving NADP-isocitrate dehydrogenase (E.C. 1.1.1.42) and NADP-malate dehydrogenase (E.C. 1.1.1.40) (1–3). A recent study by this laboratory (3) attempted to quantify the relative contributions of each pathway to *in vitro* lipogenesis in bovine adipose tissue, based on calculations of pentose cycle flux.

The present study was designed to determine whether the production of NADPH by the pentose cycle was obligatory for fatty acid synthesis from acetate, or whether the other NADP-reductases could compensate for decreases in pentose cycle flux. To accomplish this, adipose tissue slices were incubated with 6-aminonicotinamide, a purported inhibitor of 6-phosphogluconate dehydrogenase (4, 5). Glucose metabolism by the pentose cycle was estimated through the use of 1-, 6-, and U-^{14}C-labeled and 3-^{3}H-labeled glucoses (3, 6, 7). Contrary to results reported for studies of rat adipose tissue (8), 6-aminonicotinamide stimulated lipogenesis in bovine adipose tissue slices.

Materials and Methods. Subcutaneous adipose tissue samples were obtained by biopsy technique (9) from either Hereford or Angus–Hereford crossbred steers (472 ± 5 kg, mean ± SE for five animals). The animals had been adapted previously to a high-concentrate diet designed to maximize weight gain. Adipose tissue was obtained 10–20 cm to the left side of the dorsal midline along the region of the 8th and 12th thoracic vertebrae.

In vitro tissue lipogenesis. Adipose tissue slices (75–125 mg) were incubated in triplicate in 3 ml Krebs–Henseleit Ca^{2+}-free buffer (pH 7.35–7.40) plus the indicated substrates. Each flask received either 10 m*M* acetate (±1 μCi [U-^{14}C]acetate) or 2 m*M* glucose (±1 μCi 1-, 6-, or U-^{14}C-labeled or 3-^{3}H-labeled glu-

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cose). Those flasks which received glucose (with or without radioisotope) also received 33 mU/ml insulin (bovine, Calbiochem,[2] San Diego, Calif.). Flasks were gased for 30 sec with 95% O_2/5% CO_2 and stoppered and incubations were carried out for 3 hr at 37°C at 90 oscillations/min. In addition to the unlabeled and radioactively labeled substrates, some flasks received 5 m*M* 6-aminonicotinamide (6-AN). Amounts of [1-^{14}C]glucose recovered in fatty acids were quite low, and the average variation between triplicate determinations was 20–25% of the total incorporation. This represented the maximum error encountered for the parameters measured from the triplicate determinations.

Separation of lipid components. Reactions were terminated after 3 hr by injecting 0.5 ml 1 *N* H_2SO_4 into the medium. The production of $^{14}CO_2$ was determined as described previously (3). Lipids were extracted from the adipose tissue slices by the method of Folch *et al.* (10), modified as described by Smith (3). The esterified fatty acids were saponified by the method of Hood *et al.* (11), utilizing sodium methoxide. The recovery of ^{14}C from [U-^{14}C]glucose in lactate was performed as described previously (3).

Enzyme activities. In order to assess enzyme activities under circumstances similar to those employed to measure carbon flux, slices were incubated with 10 m*M* acetate, 2 m*M* glucose, and 33 mU/ml insulin plus or minus 5 m*M* 6-AN, in the absence of radioactive isotope. At 1-hr intervals during the 3-hr incubation period, tissue slices were rinsed with saline and replicates pooled, yielding a total of 450–500 mg tissue. The tissue was homogenized in 3 vol of 0.1 m*M* reduced glutathione (pH 7.4–7.5) and the homogenate was centrifuged at maximum speed for 30 sec in an Eppendorf microcentrifuge. The infranatant fraction, containing the soluble proteins and enzymes, was assayed immediately for the activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase at saturating substrate concentrations by the methods of Bernt and Bergmeyer (12) (for hexokinase). Initial concentrations of glucose-6-phosphate and 6-phosphogluconate were 5 m*M* in a 3-ml cuvette. Preliminary studies indicated that, as expected, 5 m*M* 6-AN had no effect per se on the activities of the pentose cycle reductases when added directly to the assay cuvette.

Metabolite levels. 6-Phosphogluconate was assayed as described previously (4). Lactate was measured by the method of Hohorst (13).

Source of chemicals. [1-^{14}C]Glucose, [6-^{14}C]glucose, [U-^{14}C]glucose, [3-^{3}H]glucose, and [U-^{14}C]acetate were purchased from Amersham Searle (Arlington Heights, Ill.). Biochemicals, including 6-AN, were obtained from Sigma Chemical Company (St. Louis, Mo.); other chemicals were of ACS or equivalent grade from Fisher Chemical Company (Fairlawn, N.J.).

Calculations. The fraction of glucose metabolized by the pentose cycle (PC) was calculated by Method C of Katz *et al.* (14):

$$PC = \frac{G_{1CO_2} - \gamma G_{6CO_2}}{3 - 2(G_{1CO_2} - \gamma G_{6CO_2})}$$

where G_{1CO_2} and G_{6CO_2} equal the fractions of [1-^{14}C]glucose and [6-^{14}C]glucose, respectively, converted to $^{14}CO_2$, and γ is the ratio of ^{14}C in fatty acids from [1-^{14}C]glucose to ^{14}C in fatty acids from [6-^{14}C]glucose.

The proportion of reducing equivalents required for lipogenesis that was supplied by the pentose cycle was determined from the incorporation of label from [3-^{3}H]glucose (3). Tritium from [3-^{3}H]glucose is transferred to NADP at 6-phosphogluconate dehydrogenase, and hence into fatty acids. Assuming recycling of glucose carbon (15), for every mol of NADPH which is tritium-labeled, 5 mole will be unlabeled. Therefore, the fraction of NADPH required for lipogenesis that was derived from the pentose cycle equals:

$$\frac{6 \times (^{3}H \text{ from [3-}^{3}H\text{]glucose in fatty acids})}{(2 \times 7)/8 \times \text{total acetyl units incorporated into fatty acids}}$$

where $(2 \times 7)/8$ is the requirement for NADPH for the synthesis of palmitate.

[2] Mention of a trade name, proprietary product or vendor does not constitute a guarantee or warranty by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may be suitable.

Results and Discussion. Contrary to expected results, 6-AN increased lipogenesis from [U-^{14}C]acetate, both in the absence and presence of glucose (Table I). The incorporation of label from [3-^{3}H]glucose into fatty acids was also increased by 6-AN. However, the pentose cycle provided the same proportion of reducing equivalents in the presence of 6-AN, as indicated by the constant ratio of [3-^{3}H]glucose:[U-^{14}C]acetate incorporated into fatty acids (Table I). The stimulatory effect of 6-AN on lipogenesis from acetate was observed in adipose tissue samples from every animal. Due to the large variability of [U-^{14}C]acetate incorporation into fatty acids observed among animals (Table I), this effect was significant only at the 0.10 level (paired *t* test). However, the reproducibility of the effect of 6-AN on lipogenesis is indicated by the significant percentage increase in lipogenesis (Table I). Large variability among lipogenic parameters is a common characteristic of adipose tissue from steers, even in animals of the same sex and breed on identical diets (2, 9). Katz and Rognstad (6) reported large variations in total glucose utilization by rat adipose tissue; however, the specific yields of 1-, 6-, and U-^{14}C-labeled glucoses in the major end-products were relatively constant, making it possible to accurately calculate the contribution of the pentose cycle to glucose metabolism (6).

Samples incubated with 6-AN maintained higher maximal activities of glucose-6-phos-

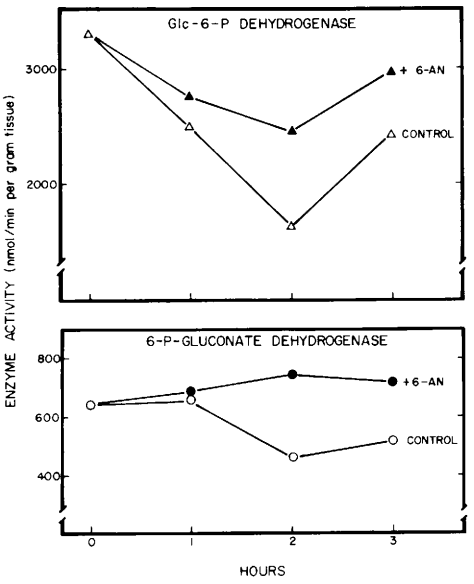

FIG. 1. Effect of 6-aminonicotinamide on the maximal activities of glucose-6-phosphate (Glc-6-P) dehydrogenase and 6-phosphogluconate (6-P-gluconate) dehydrogenase. Slices of subcutaneous adipose tissue were incubated with 10 m*M* acetate plus 2 m*M* glucose and 33 mU/ml insulin for the indicated periods of time in the absence (open symbols) or presence (filled symbols) of 5 m*M* 6-aminonicotinamide (6-AN). After each time interval, slices were pooled, rinsed with saline, and extracts of the samples were processed as described in the text. Activities of Glc-6-P dehydrogenase (△, ▲) and 6-P-gluconate dehydrogenase (○, ●) were assayed within 10 min after removal of the slices from the incubation medium. Initial concentrations of Glc-6-P and 6-P-gluconate were 5 m*M*. Data points are means of samples from three animals.

TABLE I. INCORPORATION OF LABEL FROM [U-^{14}C]ACETATE AND [3-^{3}H]GLUCOSE INTO GLYCERIDE FATTY ACIDS IN THE ABSENCE AND PRESENCE OF 6-AMINONICOTINAMIDE

		Radioisotope				
		[U-^{14}C]Acetate		[3-^{3}H]Glucose		
Substrates	6-AN	Incorporation (nmole/3 hr/100 mg tissue)	Percentage control	Incorporation (nmole/3 hr/100 mg tissue)	Percentage control	^{3}H:^{14}C Ratio
Acetate	−	43.3 ± 12.3	—	—	—	
	+	59.8 ± 19.7[a]	135 ± 9[b]	—	—	
Acetate + glucose	−	81.6 ± 27.0	—	11.7 ± 4.0	—	0.15 ± 0.01
	+	106.2 ± 39.4[a]	128 ± 8[b]	15.7 ± 6.1[a]	135 ± 12[b]	0.16 ± 0.02

Note. Results are expressed as means ± SEM for five animals. Samples were incubated with 10 m*M* acetate, 2 m*M* glucose (plus 33 mU/ml insulin), and 5 m*M* 6-aminonicotinamide (6-AN) for 3 hr.

[a] $P < 0.10$ compared with control.

[b] $P < 0.025$ compared with control (100%).

phate dehydrogenase and 6-phosphogluconate dehydrogenase during the 3-hr incubation period (Fig. 1), which was consistent with the increased incorporation of tritium from [3-^{3}H]glucose into fatty acids observed under these conditions. However, it is unlikely that pentose cycle flux was influenced by the increase in maximal activities of the reductases, since the activity of 6-phosphogluconate dehydrogenase was 40- to 50-fold greater than that required to provide NADPH at rates commensurate with the rates of lipogenesis from acetate listed in Table I. The activity of glucose-6-phosphate dehydrogenase was four to 5-fold greater than that of 6-phosphogluconate dehydrogenase; greater activity of glucose-6-phosphate dehydrogenase relative to that of 6-phosphogluconate dehydrogenase has been demonstrated previously in cattle fed a high-energy ration (16).

The mechanism by which 6-AN maintained higher activities of the pentose cycle reductases is unknown. The inhibitory metabolite of 6-AN, 6-ANADP, is a competitive inhibitor of both dehydrogenases (5), and as such was not expected to affect their maximal activities. The observation that tissue slices incubated with 6-AN displayed higher activities of the pentose cycle reductases, in spite of the fact that 6-AN had no effect on reductase maximal activities per se, provided evidence that 6-AN entered the cells of the tissue slices and was metabolized to some other form, probably 6-ANADP.

Unlike results observed in rat adipose tissue (8), 6-AN had no effect on the cellular content of 6-phosphogluconate when glucose was the sole substrate (Table II). When acetate was also present, 6-AN caused a 40% increase ($P < 0.05$; paired t test) over levels observed in the absence of the inhibitor. This response was not nearly as great as that observed in rat adipose tissue in which greater than 10-fold increases of 6-phosphogluconate have been demonstrated (8), and could have been due to the increase in glucose-6-phosphate dehydrogenase maximal activity observed under these conditions (Fig. 1), rather than a decreased affinity of 6-phosphogluconate dehydrogenase for 6-phosphogluconate. Krieglstein and Stock (17), utilizing an isolated perfused rat brain preparation from animals pretreated with 6-AN, demonstrated that 6-AN decreased lactate production in their system (17). Although 6-AN did not alter markedly the cellular content of 6-phosphogluconate in the present study, it consistently increased the production of lactate in samples incubated with either glucose or glucose plus acetate (Table II). A high initial lactate level was observed, and only in samples incubated with 6-AN was lactate increased over this value. This suggests that the flux of glucose carbon to lactate was increased by 6-AN or its metabolites.

Kohler *et al.* (5) demonstrated that injections of 6-AN into pregnant rats resulted in embryos that exhibited depressed conversion

TABLE II. EFFECT OF ACETATE AND 6-AMINONICOTINAMIDE ON LEVELS OF 6-PHOSPHOGLUCONATE AND LACTATE IN BOVINE ADIPOSE TISSUE

Conditions	6-AN	Metabolite levels (nmole/100 mg tissue)	
		6-Phosphogluconate	Lactate
Initial	–	4.0 ± 1.0	67.1 ± 7.8
Glucose	–	5.9 ± 1.1	75.8 ± 6.4
	+	5.3 ± 1.0	103.2 ± 2.6[a,b]
Glucose + acetate	–	4.2 ± 0.3	62.9 ± 7.5
	+	5.9 ± 0.6[b]	92.8 ± 9.6[a,b]

Note. Samples were incubated with glucose (2 mM, plus 33 mU/ml insulin), ±10 mM acetate and 5 mM 6-aminonicotinamide (6-AN) for 3 hr. At the end of the incubation period, samples were extracted as described under Materials and Methods, and the first aqueous rinse assayed for metabolite levels. Initial levels of metabolites were those observed in samples in which 1 ml 1 N H_2SO_4 had been added at the start of the incubation period. Data expressed as means ± SE for five animals.

[a] $P < 0.05$ compared to initial value.

[b] $P < 0.05$ compared to level observed in the absence of 6-AN.

of [1-^{14}C]glucose to $^{14}CO_2$, indicating inhibition of pentose cycle flux. The increased recovery of label from [3-^{3}H]glucose in fatty acids (Tables I and III) suggests that in bovine adipose tissue, 6-AN actually stimulated flux through the pentose cycle. This is supported by the increased conversion of [1-^{14}C]glucose to $^{14}CO_2$ observed in adipose tissue slices incubated with 6-AN (Table III). It is possible that 6-AN increased pentose cycle flux indirectly, by stimulating *de novo* fatty acid synthesis; the recovery of all ^{14}C-labeled glucoses in fatty acids was nearly doubled by 6-AN (Table III), indicating a generalized stimulation of fatty acid synthesis. This could in part explain the increased yield of label from [3-^{3}H]glucose in fatty acids elicited by 6-AN in the absence of acetate (Table III).

Acetate increased the conversion of all labeled glucoses into CO_2, and decreased lactate production from [U-^{14}C]glucose; 6-AN had little effect on CO_2 production under these conditions (Table III). Although 6-AN elicited increases in fatty acid synthesis from glucose, it had no significant effect ($P > 0.10$) on lactate production or total glucose utilization. Acetate caused a 2- to 3-fold increase in the incorporation of tritium from [3-^{3}H]glucose into fatty acids, which was consistent with the increased *de novo* fatty acid synthesis which occurred in the presence of acetate.

Some label from [3-^{3}H]glucose was detected in glyceride-glycerol, indicating that triose phosphate isomerase (E.C. 5.3.1.1) did not completely labilize the tritium on dihydroxyacetone-phosphate (14). The presence of acetate significantly decreased the amount of tritium recovered in glyceride–glycerol ($P < 0.05$), possibly due to the increased flux of glucose through the pentose cycle under these conditions. However, there was no effect of 6-AN on the incorporation of [3-^{3}H]glucose into glyceride-glycerol in either the absence or presence of acetate.

The ratios of labeled to unlabeled lactate can be calculated from the data in Tables II

TABLE III. EFFECTS OF ACETATE AND 6-AMINONICOTINAMIDE ON GLUCOSE UTILIZATION

Substrates	6-AN	Products	Labeled substrate: [1-^{14}C]Glucose	[6-^{14}C]Glucose	[U-^{14}C]Glucose	[3-^{3}H]Glucose	Glucose utilization
			Nanomoles substrate incorporated/3 hr/100 mg tissue				
Glucose	–	CO_2	18.7 ± 4.3	6.1 ± 1.2	10.3 ± 1.9	—	
		G-G	5.3 ± 1.5	18.9 ± 4.3	10.5 ± 2.5	1.6 ± 0.6	75.4
		FA	2.0 ± 1.0	4.6 ± 1.8	2.0 ± 0.8	5.5 ± 1.9	± 8.0
		Lactate	—	—	52.6 ± 6.9	ND	
	+	CO_2	23.2 ± 6.6[a]	6.2 ± 1.2	11.9 ± 2.8[a]	—	
		G-G	5.9 ± 1.5[b]	20.1 ± 4.7[b]	11.0 ± 2.5	1.4 ± 0.3	61.2
		FA	3.6 ± 1.5[a]	8.4 ± 3.3[b]	3.8 ± 1.6[a]	8.7 ± 3.1[b]	± 10.7
		Lactate	—	—	34.4 ± 4.3	ND	
Glucose + acetate	–	CO_2	24.8 ± 7.6	8.6 ± 2.1	14.6 ± 3.7	—	
		G-G	1.5 ± 0.4	16.2 ± 3.2	9.4 ± 1.9	0.6 ± 0.2	57.2
		FA	0.3 ± 0.1	1.5 ± 0.5	0.6 ± 0.2	13.9 ± 3.7	± 9.9
		Lactate	—	—	32.7 ± 4.8	ND	
	+	CO_2	27.9 ± 8.7[a]	9.6 ± 2.0[a]	14.1 ± 3.2	—	
		G-G	2.0 ± 0.6[a]	18.5 ± 4.1[a]	8.9 ± 1.9[a]	1.0 ± 0.5	79.3
		FA	0.5 ± 0.3[a]	2.5 ± 1.0[a]	0.7 ± 0.3[a]	19.1 ± 6.1[a]	± 17.3
		Lactate	—	—	55.6 ± 17.1	ND	

Note. Substrate concentrations as in Table II. Abbreviations: 6-AN, 6-aminonicotinamide; G-G, glyceride-glycerol; FA, fatty acids; ND, not detectable. Data expressed as means ± SE for five animals. Data for 1- and 6-^{14}C-labeled glucoses expressed as if the amount of radioactivity measured was derived from [U-^{14}C]glucose.

[a] $P < 0.10$ compared with control.

[b] $P < 0.05$ compared with control.

and III. Values of 0.74 ± 0.17 and 0.56 ± 0.10 were observed in samples incubated in the absence and presence of acetate, respectively. Since 1 mole of [U-^{14}C]glucose produces 2 mole of lactate, only one of which is ^{14}C-labeled, ratios of approximately 0.5 indicate that virtually all of the lactate present in the tissue slices and media at the end of the incubation was derived from exogenous glucose. Considering the high initial levels of lactate (Table II), a rapid equilibration between lactate derived from exogenous glucose and that initially present must have occurred.

When glucose was the sole substrate, 6-AN substantially lowered the ratio of [^{14}C]lactate:total lactate (to 0.33 ± 0.03), by increasing total lactate production while simultaneously decreasing the ^{14}C yield in lactate ($P < 0.05$; paired t test). This indicates glucose carbon pool dilution, probably by glycogen; therefore, the rates of incorporation of glucose into specific products were underestimated by approximately 30% under these conditions.

Glucose pool dilution by endogenous precursors could have also materially affected the calculation of the proportion of glucose metabolized by the pentose cycle, or PC (14) (Table IV). However, the values of PC listed in Table IV were estimated by Method C of Katz *et al.* (14); the parameters of this method should be insensitive to glucose pool dilution (cf. Materials and Methods). Acetate approximately doubled the percentage of glucose metabolized by the pentose cycle (Table IV). The value of PC was increased significantly by 6-AN, in spite of potential glucose pool dilution, when glucose was the sole substrate. In the presence of acetate, flux through the pentose cycle was limited by some other factor, so that even though fatty acid synthesis from acetate was stimulated by 6-AN, pentose cycle flux was not.

The production of $^{14}CO_2$ from [1-^{14}C]glucose occurs at the same step in the pentose cycle at which tritium from [3-^{3}H]glucose is transferred to NADP. Consequently, the ratio of tritium recovery in fatty acids from [3-^{3}H]glucose to pentose cycle-derived $^{14}CO_2$ from [1-^{14}C]glucose indicates the proportion of NADPH produced by the pentose cycle that was utilized for lipogenesis. These calculations are listed in Table V. The product γG_{6CO_2} is equal to the amount of $^{14}CO_2$ produced from [1-^{14}C]glucose by pathways other than the pentose cycle (14).

The net effect 6-AN had on pentose cycle metabolism was to increase the proportion of pentose cycle-generated NADPH utilized for fat synthesis (Table V). When glucose was the sole substrate, this was increased from 34 to 42% by the addition of 6-AN. Acetate increased this proportion to 59%, which was elevated further to 73% by 6-AN (all increases significant at $P < 0.25$; paired t test). Since the pentose cycle produced NADPH in quantities that exceeded utilization for lipogenesis from acetate it is unlikely that NADPH availability limited acetate incorporation into fatty acids. Furthermore, since the metabolism of glucose by the pentose cycle was not pro-

TABLE IV. PERCENTAGE GLUCOSE METABOLIZED BY THE PENTOSE CYCLE

Substrates	6-AN	G_{1CO_2}	G_{6CO_2}	γ	Percentage metabolized by the pentose cycle
Glucose	−	0.25 ± 0.04	0.08 ± 0.01	0.41 ± 0.08	8 ± 2
	+	0.36 ± 0.05[a]	0.10 ± 0.01	0.40 ± 0.03	14 ± 2[a]
Glucose + acetate	−	0.41 ± 0.06	0.15 ± 0.02	0.14 ± 0.04	18 ± 4
	+	0.37 ± 0.11	0.13 ± 0.03	0.18 ± 0.03	18 ± 8

Note. Substrate concentrations as in Table II. Data are means ± SEM for five animals. 6-AN, 6-aminonicotinamide. G_{1CO_2} is the fraction of metabolized [1-^{14}C]glucose converted to $^{14}CO_2$, and G_{6CO_2} is the fraction of metabolized [6-^{14}C]glucose converted to $^{14}CO_2$. Gamma (γ) is the ratio of fractions of [1-^{14}C]glucose:[6-^{14}C]glucose converted to fatty acids. The percentage glucose metabolized by the pentose cycle calculated by Method C of Katz *et al.* (18) as described under Materials and Methods.

[a] $P < 0.05$ compared with controls.

TABLE V. REDUCING EQUIVALENT GENERATION BY THE PENTOSE CYCLE

Substrates	6-AN	Acetyl units incorporated	NADPH required	Tritium from [3-^{3}H]glucose incorporated	PC-CO_2	$\frac{^3H}{PC\text{-}CO_2}$	NADPH provided by the pentose cycle
		(nmole/3 hr/100 mg tissue)				Percentage	
Glucose	–	4.0	7.0	5.5	16.1	34	>100
	+	7.6	13.3	8.7	20.6	42[a]	>100
Glucose + acetate	–	82.8	144.9	13.9	23.4	59	35
	+	107.8	188.7	19.1	26.0	73[a]	33

Note. Values are sums from Table I for acetyl units from acetate and Table III for acetyl units from glucose. One mole of [U-^{14}C]glucose incorporated into fatty acids was assumed equivalent to 2 mole acetyl units. Data expressed as means ± SE for five animals. NADPH requirement calculated as (2 × 7)/8 times the number of acetyl units incorporated into fatty acids, based on the requirement for the synthesis of palmitate. PC-CO_2 represents the generation of $^{14}CO_2$ from [1-^{14}C]glucose by the pentose cycle. Calculated as $^{14}CO_{2G1} - \gamma^{14}CO_{2G6}$, where $^{14}CO_{2G1}$ and $^{14}CO_{2G6}$ are $^{14}CO_2$ production from [1-^{14}C]glucose and [6-^{14}C]glucose, respectively, and γ is as defined under Materials and Methods. The ratio of ^3H/PC-CO_2 is equal to the percentage of NADPH generated by the pentose cycle that was utilized for fatty acid synthesis. Provision of NADPH by the pentose cycle calculated as 6 × (^{3}H yield from [3-^{3}H]glucose in fatty acids)/total NADPH required (3). Endogenous yields of tritium incorporation in fatty acids (observed when glucose ± 6-AN was the sole substrate) were subtracted prior to the calculations.

[a] $P < 0.025$ compared with control.

portional to acetyl unit incorporation into fatty acids, it is doubtful that glucose stimulated lipogenesis from acetate (Table I) by providing NADPH via the pentose cycle.

Calculations of NADPH requirements for lipogenesis and provision by the pentose cycle are also listed in Table V. Since the incorporation of label from [3-^{3}H]glucose into fatty acids in the absence of exogenous acetate represented endogenous incorporation (Table III), it was substracted from the incorporation observed in samples incubated with glucose plus acetate (±6-AN) (3); the calculations included in Table V were based on these corrected values. Based on the yield of [3-^{3}H]glucose in fatty acids, the pentose cycle provided 35% of the reducing equivalents for lipogenesis from acetate (Table V). This percentage was not altered by 6-AN, even though the incorporation of acetyl units into fatty acids increased markedly. Therefore, the increase in the incorporation of tritium from [3-^{3}H]glucose into fatty acids elicited by 6-AN occurred secondarily to the stimulation of lipogenesis from acetate by an as yet unknown mechanism.

In summary, it was not possible to block glucose metabolism by the pentose cycle with 6-AN. However, the stimulatory effect of 6-AN on lipogenesis from acetate provided information that indicated that NADPH availability does not limit *in vitro* lipogenesis in bovine adipose tissue.

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