Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 2965–2969, May 1985
Medical Sciences

Increased activity of the glucose cycle in the liver: Early characteristic of type 2 diabetes

(early diabetes/pathogenesis/futile cycle)

SUAD EFENDIĆ*†, ALEXANDRE WAJNGOT*, AND MLADEN VRANIĆ‡

*Department of Endocrinology, Karolinska Hospital, S-104 01 Stockholm, Sweden; and ‡Department of Physiology and Medicine, University of Toronto, Toronto, Ontario M53 1A8, Canada

Communicated by Rolf Luft, December 14, 1984

ABSTRACT **The aims were to assess in the mild, lean, type 2 diabetics (*i*) the activity of the hepatic futile cycle (glucose cycling) in the basal state and during an infusion of glucose and (*ii*) the overall contribution of futile cycling and the relative contributions of the liver and the periphery to excessive hyperglycemia during a glucose challenge. To determine hepatic futile cycling, we studied seven healthy controls (C) and eight mild, lean, type 2 diabetics with decreased oral glucose tolerance test and blood glucose of 123 ± 4 mg/dl. Experiments included an equilibration period, followed by a 2-hr infusion of glucose at 2 mg/kg of body weight per min. In each subject, two such experiments were performed randomly with infusions of (*i*) [2-^{3}H]glucose or (*ii*) [3-^{3}H]glucose to calculate, respectively, (*i*) total glucose output or total glucose phosphorylation and (*ii*) glucose production or irreversible glucose loss. Futile cycling equals the difference between glucose turnover measured by the two tracers. In controls basal glucose production was 2.0 ± 0.09 mg/kg per min, and it decreased by 75% during glucose infusion; futile cycling could not be detected. Plasma glucose increased by 30% and plasma C-peptide by 88%. In the diabetics total glucose output (2.41 ± 0.17 mg/kg per min) was larger than glucose production (2.12 ± 0.16 mg/kg per min), indicating a glucose cycle. During the glucose infusion, (*i*) glucose production in the diabetics as well as in the controls decreased by 75% (to 0.6 mg/kg per min) despite higher than normal plasma glucose and C-peptide; (*ii*) futile cycling amounted to 0.6 mg/kg per min, which is half of the total glucose output; (*iii*) increase of glucose uptake was essentially only due to phosphorylation of glucose because irreversible uptake increased only marginally; and (*iv*) most glucose taken up by the liver during the glucose challenge reenters the blood stream without being oxidized or polymerized. These findings, when compared to our previous work in which controls were infused with glucose at 4 mg/kg per min, indicate that excessive hyperglycemia in the diabetics during glucose infusion is due to a decrease in irreversible glucose uptake (impaired phosphorylation and futile cycling) and to a decrease in suppression of glucose production. The relative contributions of the liver and periphery to hyperglycemia seem to be almost equivalent. The mechanism behind the increased glucose cycle activity is not clear; it may be due to a relative decrease of glycogen synthase or increase in glucose-6-phosphatase or both.**

It is generally accepted that there are three futile cycles in hepatic glucose metabolism causing ATP hydrolysis without any corresponding change in reactants (1). They operate at the three irreversible stages between glucose and pyruvate: (*i*) the glucose cycle [glucose → glucose 6-phosphate (Glc-6-*P*) → glucose], (*ii*) the fructose 6-phosphate (Fru-6-*P*) cycle [Fru-6-*P* → fructose 1,6-bisphosphate → Fru-6-*P*], and (*iii*) the phospho*enol*pyruvate cycle (pyruvate → phospho*enol*-pyruvate → pyruvate).

The first cycle may be measured without invasive methods by comparing total hepatic glucose output (or glucose phosphorylation; estimated by use of [2-^{3}H]glucose) with hepatic glucose production (or irreversible glucose loss; assessed by infusing either [^{14}C]glucose, corrected for recycling, or tritiated glucose labeled in position three or six) (2, 3). When tritiated glucose is labeled in position two, it loses its tritium in the hexose isomerase reaction (Glc-6-*P* ⇄ Fru-6-*P*), which is near its equilibrium. Therefore, this tracer measures glucose appearance in plasma irrespective of whether it is newly formed glucose or it merely originates from glucose taken up by the liver and circulated through the glucose cycle. Glucose cycle then equals total hepatic glucose output or glucose phosphorylation minus the rate of glucose production or irreversible glucose loss. The glucose cycling fraction of total glucose output in resting dog amounted to about 20% or more (3–5). This value is similar to that found in rabbit (6) and somewhat lower than that in the rat (2). However, the activity of the glucose cycle was increased in depancreatized dogs and accounted for 48% and 60% of the measured glucose production in animals with good and poor metabolic control, respectively (7).

In addition to diabetes, a number of other conditions associated with increased glucose production also give rise to increased glucose cycling—e.g., infusion of glucagon (4, 7), exercise, infusion of *manno*-heptulose, and chronic treatment with methylprednisolone (4). Glucose infusion *per se* does not affect glucose cycling in control animals, but it does increase it when glucose production is elevated, as is the case in methylprednisolone-treated animals (4). The aims of the present study were to assess in the mild, lean type 2 diabetics: (*i*) the activity of the glucose cycle in the basal state and during a glucose infusion, and (*ii*) the overall contribution of futile cycling and the relative contributions of the liver and the periphery to the excessive hyperglycemia during glucose infusion.

MATERIALS AND METHODS

Subjects. This study of healthy volunteers and patients with type 2 diabetes was approved by the Human Study and the Isotope Committees of the Karolinska Hospital, Stockholm; informed consent was obtained from all subjects. The group of healthy volunteers consisted of six men and one woman with a mean body weight of 96 ± 4.2% (SEM) of the ideal weights (Metropolitan Life Insurance Company Tables) and a mean age of 46.0 ± 4.8 yr (Table 1). They had fasting plasma glucose levels of <5.8 mM in repetitive deter-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: Glc-6-*P*, glucose 6-phosphate; Fru-6-*P*, fructose 6-phosphate.

†To whom reprint requests should be addressed.

Table 1. Clinical data for the control subjects and those with mild diabetes

Group	Patient	Sex	Age, yr	Body weight, % of ideal	Fasting plasma glucose	
					mg/dl	mM
Controls	HA	M	62	94	98	5.5
	TA	M	42	106	100	5.6
	CJ	M	34	74	98	5.5
	BN	M	40	102	104	5.8
	AH	M	32	90	90	5.0
	BE	M	65	104	102	5.7
	LE	F	46	102	90	5.0
Mean ± SEM			46.0 ± 4.8	96.0 ± 4.2	97 ± 2	5.4 ± 0.1
Diabetics	LH	M	46	108	121	6.7
	ID	M	63	99	134	7.4
	LB	M	45	99	111	6.2
	LT	M	47	91	115	6.4
	AS	F	69	86	111	6.2
	RB	M	51	115	134	7.4
	NR	M	52	112	132	7.3
	RB	M	46	112	129	7.2
Mean ± SEM			46.0 ± 4.8	102.8 ± 3.8	123 ± 4	6.9 ± 0.2

minations and normal oral glucose tolerance (OGTT) according to Reaven and Olefsky (8).

Seven of the subjects with mild diabetes were men and one was a woman. Their mean body weight was 102.8 ± 3.8% of ideal weights, and they had a mean age of 46.0 ± 4.8 yr (Table 1). The fasting plasma glucose concentrations varied between 6.2 and 7.4 mM (112–133 mg/dl). Oral glucose tolerance was decreased in all of these subjects. They were outpatients and kept on a controlled diet. The body weights and the ages of the control and the diabetic groups were not different.

Infusions. All experiments were performed when the subjects were in the postabsorptive state at 8 a.m. after a 12-hr overnight fast. An indwelling catheter was placed in a cubital vein of each arm, one for infusion of unlabeled or isotopically labeled glucose and the other for blood sampling. The subjects were recumbent during the experiments. On each subject two experiments were performed in random order—i.e., after cannulation-primed constant infusions of sterile and nonpyrogenic glucose, either [3-^{3}H]- or [2-^{3}H]glucose was started. The infusions were continued throughout the experiment at a rate of 0.1 ml/min (0.083–0.150 μCi; 1 Ci = 3.7 GBq). The priming dose of labeled glucose was the same as the amount infused during 120 min. After an equilibration period of 120 min in which the labeled glucose was infused, an infusion of unlabeled 15% glucose was started and continued for 120 min with the help of a Tecmar Pump. The concentration of glucose in the infusing solution was measured in each experiment by the same assay used for the plasma samples.

During the equilibration period, blood samples were taken at 0, 90, 100, 110, and 120 min. At the start of the infusion of unlabeled glucose the clock was reset to zero, and samples were drawn every 10 min during the experiment.

Analytical Procedures. Heparinized blood was kept on ice until centrifuged at 4°C. A plasma sample was used for determination of blood glucose in triplicate, and the rest was frozen and kept at −20°C for later analyses of C-peptide and specific activity of plasma glucose. Glucose was determined by the glucose oxidase method (9). C-peptide was determined by RIA with a commercially available kit (Novo Research, Bagsvaerd, Denmark). Blood samples for glucagon were collected in prechilled tubes containing Trasylol and EDTA; the RIA was based on the method of Faloona and Unger using the "30K" antibody (10).

Tracer Methods and Calculations. Measurements of specific activity of glucose were performed on deproteinized plasma. Under reduced pressure, the supernatant was evaporated to dryness at 40°C and, after addition of 1 ml of water and liquid scintillation solution, its radioactivity was measured in a β scintillation counter with the use of an external standard for quenching. All samples and standards were assayed for 50 min, and the radioactivities of the samples were at least 4 times higher than that of background samples. Tritium counting efficiency averaged 34%. The rates of glucose production (the rate of appearance of glucose) and of glucose uptake (the rate of disappearance of glucose) were determined by the method of primed constant tracer infusion (11). This method is based on a modified single-compartment analysis of glucose turnover, in which it is assumed that rapid changes in the specific activity and concentration of glucose do not occur uniformly within the entire glucose pool. To compensate for this nonuniform mixing, a term of the non-steady-state equation was multiplied by a correction factor (pool fraction) of 0.65 (12, 13). A sliding-fit technique using three consecutive values of glucose concentration and specific activity also was used in the calculations as described (13). This approach for calculating non-steady-state kinetics of glucose turnover was validated *in vivo* and confirmed (13). During glucose infusions the endogenous rate of glucose production was calculated by subtracting the rate of infusion of exogenous glucose from the tracer-determined total rate of glucose appearance.

Statistical Methods. Results are expressed as mean ± SEM, and Student's *t* test was performed for nonpaired and paired data.

RESULTS

Fig. 1 shows the concentration of glucose, C-peptide, and glucagon on the two different days when tracer experiments utilized glucose labeled either in position three or two. The patterns of these parameters were essentially identical on the two experimental days. During glucose infusion in healthy subjects, plasma glucose concentration plateaued at 130 mg/dl, whereas in diabetics glycemia of about 160 mg/dl was eventually achieved. C-peptide release increased to approximately the same extent in both groups, although basal C-peptide levels were higher in the diabetics. However, these concentrations of C-peptide at 160 mg/dl of glucose were much smaller than those observed in a previous study in normal subjects where such high glucose levels were obtained with a larger glucose infusion (14). Hyperglycemia sup-

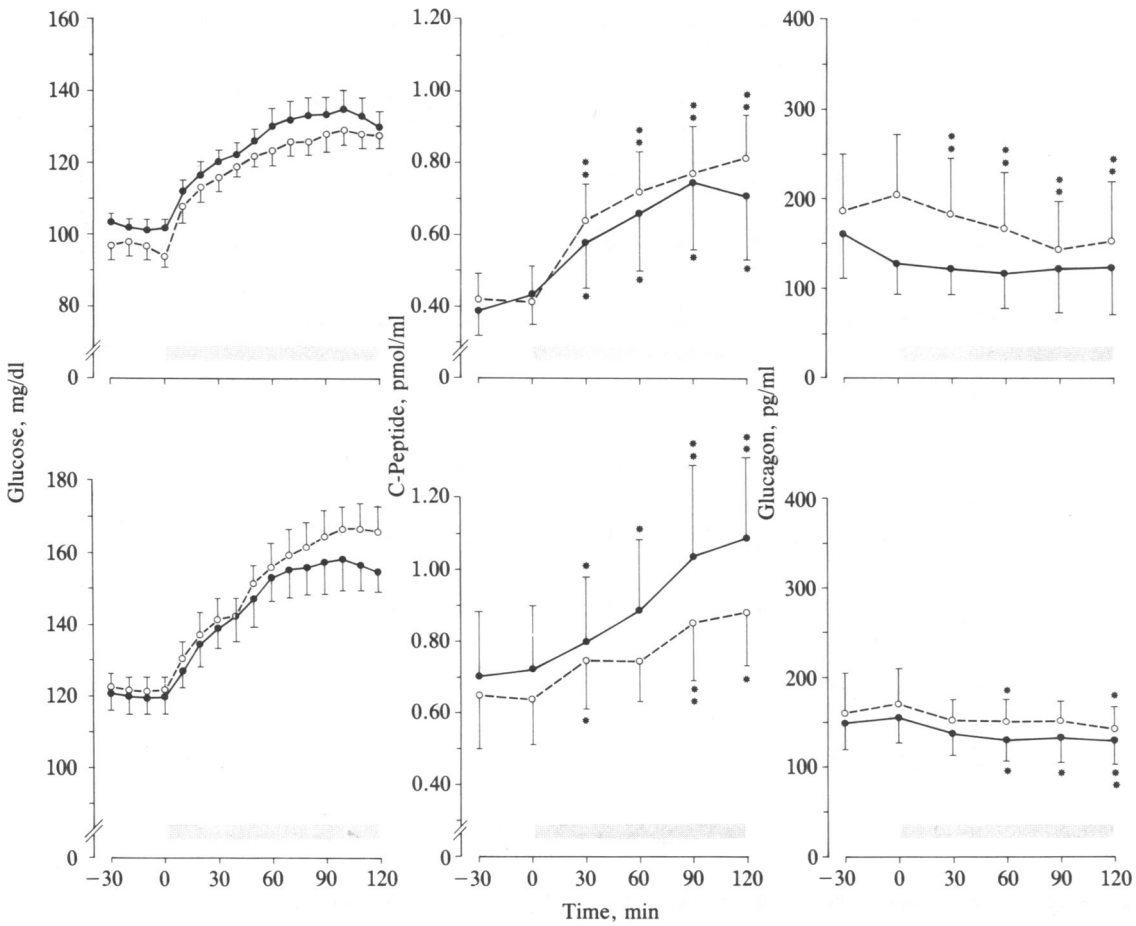

FIG. 1. Effect of glucose infusion on plasma concentration of glucose, C-peptide, and glucagon in controls ($n = 7$) (*Upper*) and subjects with mild type 2 diabetes ($n = 8$) (*Lower*). Glucose was infused at a rate of 2 mg/kg per min (shaded horizontal bar starting at time 0). Experiments with two tracers were performed in random order. ○, [2-^{3}H]glucose; ●, [3-^{3}H]glucose. Data are shown as means ± SEM. Probabilities, compared with basal concentrations for that group: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

pressed marginally glucagon levels in the controls and the diabetics.

In control subjects basal rate of appearance of glucose was 2.0 ± 0.09 mg/kg per min as measured with [3-^{3}H]glucose. An almost identical value was obtained with the [2-^{3}H]glucose tracer (Fig. 2). During the glucose infusion, the rate of appearance of glucose was suppressed by 75%, irrespective of the tracer used. Thus, in basal state and during glucose infusion, a glucose cycle could not be detected. There were also no significant differences registered in the increase of the rate of disappearance of glucose during the glucose infusion, implying that the total rate of glucose phosphorylation as measured by [2-^{3}H]glucose was the same as the irreversible glucose loss measured by [3-^{3}H]glucose.

In the diabetics basal total glucose output (2.41 ± 0.17 mg/kg per min) was larger ($P < 0.05$) than glucose production by the liver (2.12 ± 0.16 mg/kg per min), indicating a glucose cycle. This difference between total glucose output and glucose production became more pronounced during glucose infusion and was as high as 0.6 mg/kg per min ($P < 0.01$) at 80–110 min. At the end of the glucose infusion period, glucose production was about 0.60 mg/kg per min. Hence, approximately half of the total glucose output was due to glucose released throughout the glucose cycle. The figure for the rate of disappearance of glucose reveals that, in basal state, total glucose phosphorylation but not irreversible glucose uptake was elevated in diabetics when compared to controls. During glucose infusion, hyperglycemia in the diabetics resulted in a markedly increased total glucose phosphorylation, whereas the irreversible loss of glucose increased only slightly above the basal value. It is also noteworthy that, in the diabetics, the glucose infusion was initially accompanied by suppression of glucose uptake.

DISCUSSION

Present studies confirm our previous observations suggesting that in lean, mild type 2 diabetics, two defects are responsible for carbohydrate intolerance: a decreased insulin responsiveness to a glucose challenge and a decreased insulin sensitivity (14, 15). The basal rate of the irreversible loss of glucose from plasma (measured with [3-^{3}H]glucose) in diabetics was not elevated and increased only marginally during glucose infusion despite the fact that hyperglycemia and C-peptide levels were higher than in the controls (Fig. 2). This indicates insulin resistance. A part of insulin resistance was apparently due to incomplete suppression of glucose production despite hyperglycemia of 160 mg/dl. Glucose production was suppressed to the same extent in both the controls and the diabetics when the i.v. glucose was administered, although the latter group had considerably higher glucose and C-peptide levels. It is amply documented that hyperglycemia *per se* can suppress glucose production (16).

To shed some light on the mechanisms behind insulin resistance that is combined with less insulin response to hyperglycemia, we also studied glucose turnover with [2-^{3}H]-glucose, which measures total glucose output from the liver and total glucose phosphorylation in the body. The difference between the total glucose output and the glucose production reflects activity of the glucose cycle. In healthy sub-

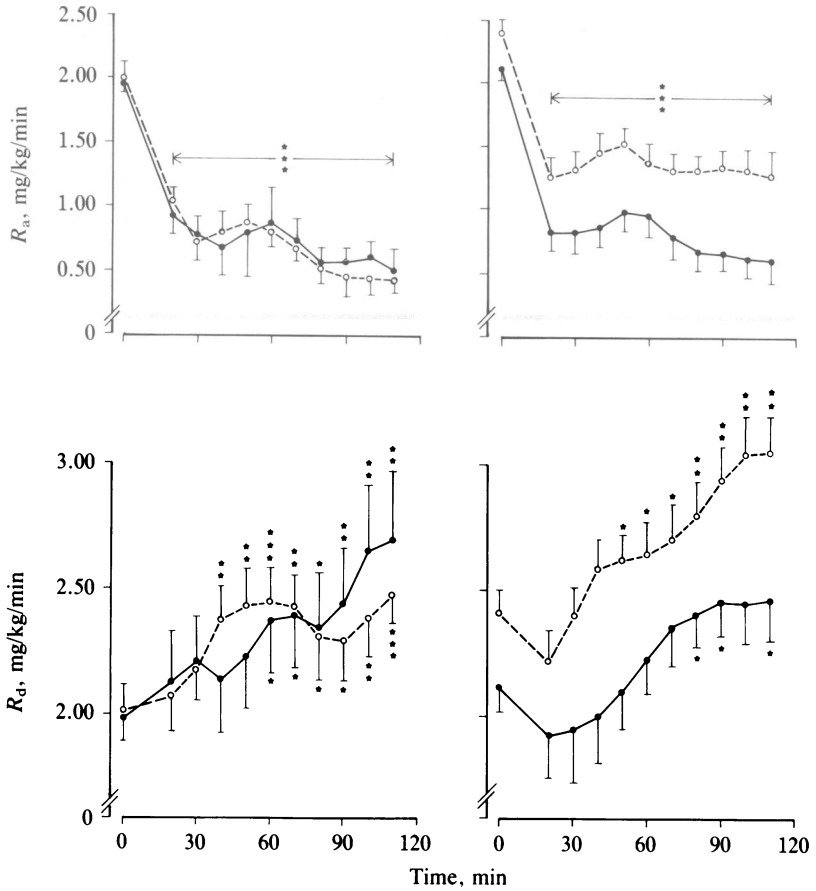

FIG. 2. Effect of glucose infusion on the rate of appearance of glucose (R_a) and the rate of disappearance of glucose (R_d) in controls (*Left*) and in subjects with mild type 2 diabetes (*Right*). Glucose was infused at a rate of 2 mg/kg per min (shaded horizontal bar starting at time 0). Data are shown as means ± SEM. See the legend of Fig. 1 for *n* and *P* values. ○, [2-^{3}H]glucose; ●, [3-^{3}H]glucose.

jects the glucose cycle could not be detected, implying that essentially all phosphorylated glucose was further metabolized both during normo- and hyperglycemia. In contrast, in the diabetics basal glucose output was significantly larger than glucose production, indicating a glucose cycle. During glucose infusion, the difference between total glucose output and glucose production increased and became as high as 0.6 mg/kg per min at 80 min. Since glucose production at the end of glucose infusion was also about 0.6 mg/kg per min, approximately half of the total glucose output was due to glucose released through the glucose cycle. Thus, in the diabetics with a basal glucose production of 2.12 mg/kg per min, an additional amount of glucose (0.29 mg/kg per min) is taken up by the liver, phosphorylated, and then promptly dephosphorylated. We do not know how much glucose is taken up by the liver, but the amount of glucose flowing through the futile cycle seems quite significant. This becomes even more noticeable during glucose infusion, when as much as 0.6 mg of glucose per kg/min was metabolized through futile cycle. Hyperglycemia in the diabetics resulted in a markedly increased glucose phosphorylation, whereas the irreversible loss of glucose increased only marginally. By the end of the glucose infusion period, the irreversible glucose uptake was about 2.44 mg/kg per min, and the total phosphorylation amounted to 3.05 mg/kg per min.

To assess the relative contributions of liver and periphery to the excessive hyperglycemia in the glucose-infused diabetics, we compare in Table 2 some data from the present experiments with those from a previous study (14) in which healthy subjects were infused with glucose at 4 mg/kg/min. In both studies during glucose infusion, the glucose levels reached were about 160 mg/dl. However, whereas in healthy subjects irreversible glucose uptake was 4 mg/kg per min, in diabetics it was only 2.4 mg/kg per min. If one assumes that in healthy subjects glucose cycle does not operate at this level of glycemia (4), the rate of glucose phosphorylation also would amount to 4 mg/kg per min. It appears that, in the diabetics at this level of glycemia (160 mg/dl), irreversible glucose uptake was decreased by 1.6 mg/kg per min (Table 2). From this amount 1.0 mg was due to defective phospho-

Table 2. Comparison of glucose production and uptake in healthy subjects and lean type 2 diabetics

Subjects	Glucose infusion, mg/kg/min	Plasma glucose mg/dl	Glucose production, mg/kg/min	Futile cycle, mg/kg/min	Glucose uptake, mg/kg/min	
					Irreversible (T-3)	Total phosphorylation (T-2)
Controls*	4	160	0	0 (assumed)	4.0	4.0 (assumed)
Diabetics†	2	160	0.6	0.6	2.4	3.0
Difference				0.6	1.6	1.0

*Infused with glucose at 4 mg/kg per min.
†Infused with glucose at 2 mg/kg per min.

rylation, whereas 0.6 mg/kg per min would correspond to activity of the glucose cycle in the liver. These calculations also can provide some indication on the participation of the liver and the periphery in the rate of glucose utilization in the diabetics. Namely, one could assume that phosphorylation of glucose in the liver is not rate-limiting, so that the main defect relates to futile cycling (0.6 mg/kg per min). On the other hand, since glucose cycle does not operate in the periphery, the decrease in the rate of total phosphorylation would reflect the peripheral deficiency (1 mg/kg per min). Thus, of the total defect of irreversible glucose uptake in the diabetics, two-thirds would reside in the periphery and one-third in the liver. Furthermore, because at hyperglycemia of 160 mg/dl glucose production was completely suppressed in the normal subjects but in the diabetics was maintained at 0.7 mg/kg per min, it could be that the relative contribution of the liver (glucose production in spite of the hyperglycemia plus the glucose cycle) and the periphery (decreased phosphorylation) to hyperglycemia of diabetics is almost equal.

The reason for the increased futile cycling in diabetes is not yet known. In healthy subjects hyperglycemia inhibits glucose phosphatase and activates glycogen synthase (17, 18). The activity of the glucose cycle depends on the rate of glucose phosphorylation in the liver, its polymerization to glycogen, oxidation, and dephosphorylation. Since the hexose isomerase reaction is considered to be non-rate-limiting, it could be that the increased glucose cycle could be due to decreased activity of glycogen synthase or to increased activity of glucose-6-phosphatase or to both of these defects. In alloxan-induced diabetic rats, an increased activity of glucose-6-phosphatase was found (19–21), implying that this enzyme may play a key role in augmentation of the glucose cycle also in patients with type 2 diabetes.

It is not yet clear how glucose administration affects metabolism of Glc-6-*P*. Hers and co-workers (17, 18) proposed that glucose binds to glycogen phosphorylase *a*, which in turn initiates the following processes: conversion of phosphorylase *a* into less-active phosphorylase *b* → deinhibition of glycogen-synthase phosphatase → conversion of glycogen synthase *b* into the more-active glycogen synthase *a* → accelerated glycogen synthesis with concomitant reduction in the tissue concentration of UDP-glucose and Glc-6-*P*. The declining Glc-6-*P* is seen as the main factor responsible for the diminished output of free glucose from the liver (Pull hypothesis). An opposite approach was advocated by El-Refai and Bergman (22) and Nordlie *et al.* (23)—i.e., glucose causes an increase in hepatic Glc-6-*P* by inhibiting glucose-6-phosphatase, which in turn serves to force glucose carbon into glycogen (Push hypothesis). Thus, suppression of glucose phosphatase would be the primary event. Finally, it has been suggested recently that glucose initially stimulates glycogen synthase and later on directly inhibits glucose-6-phosphatase (24). Thus, this unified concept of Newgard *et al.* (24) suggests that the pull and push mechanisms are important, since activation of synthase and inhibition of phosphatase are both primary events. The latter authors also propose that the membrane translocase that transports Glc-6-*P* from the extramicrosomal to intramicrosomal compartment may be an important site for metabolic regulation. If indeed the translocase plays such an important role, one could question whether, in patients with diabetes, an altered recognition of hyperglycemia by this enzyme leads to increased activity of glucose-6-phosphatase, which in turn augments activity of the glucose cycle.

We conclude that glucose intolerance in mild, lean, type 2 diabetics is not only due to a decreased peripheral glucose phosphorylation but also to a defect in the liver. As a result of this, a significant part of glucose phosphorylated in the liver cannot be further utilized because it is dephosphorylated through a glucose cycle. In addition, glucose production in liver is inadequately suppressed by hyperglycemia. We speculate that contributions of liver and periphery to glucose intolerance could be of similar magnitudes. We believe that these observations in mild, lean, type 2 diabetics have implications also in some other types of diabetes, since we have demonstrated recently that futile cycling is even more marked in obese type 2 diabetics and that it could account in part for the diabetogenic effect of growth hormone in the acromegalics (25).

We are grateful to Mr. Arved Roovete and Mrs. Kerstin Waldelöf for excellent technical assistance. This work was supported by the Swedish Medical Research Council (grant 19X-00034), the Nordic Insulin Foundation (Gentofte, Denmark), and the Swedish Diabetic Association.

1. Katz, J. & Rognstadt, R. (1976) *Curr. Top. Cell. Regul.* **64,** 237–289.
2. Katz, J. & Dunn, A. (1967) *Biochemistry* **6,** 1–5.
3. Altszuler, N., Barkai, A., Bjerknes, C., Gottlieb, B. & Steele, R. (1975) *Am. J. Physiol.* **229,** 1662–1667.
4. Issekutz, B., Jr. (1977) *Metabolism* **26,** 157–170.
5. Lickley, H. L., Ross, G. G. & Vranić, M. (1979) *Am. J. Physiol.* **236,** 255–262.
6. Dunn, A., Katz, J., Golden, S. & Chenoweth, M. (1976) *Am. J. Physiol.* **230,** 1159–1162.
7. Vranić, M., Lickley, H. L. A., Kemmer, F. W., Perez, G., Hetenyi, G., Hatton, T. W. & Kovacevic, N. (1981) in *Etiology and Pathogenesis of Insulin-Dependent Diabetes Mellitus*, eds. Martin, J. M., Ehrlich, R. M. & Holland, F. J. (Raven, New York), pp. 153–178.
8. Reaven, G. M. & Olefsky, J. M. (1978) in *Advances in Metabolic Disorders*, eds. Levine, R. & Luft, R. (Academic, New York), Vol. 9, pp. 313–331.
9. Hugget, A. S. G. & Nixon, D. A. (1957) *Lancet* **ii,** 368–370.
10. Faloona, G. R. & Unger, R. H. (1974) in *Glucagon*, eds. Jaffe, B. M. & Behrman, H. R. (Academic, New York), pp. 317–330.
11. De Bodo, R. C., Steele, R., Altszuler, N., Dunn, A. & Bishop, J. S. (1963) *Recent Prog. Horm. Res.* **19,** 445–488.
12. Cowan, J. S. & Hetenyi, C. (1971) *Metabolism* **20,** 360–373.
13. Radziuk, J., Norwich, K. H. & Vranić, M. (1978) *Am. J. Physiol.* **234,** 84–93.
14. Wajngot, A., Roovete, A., Vranić, M., Luft, R. & Efendić, S. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 4432–4436.
15. Efendić, S., Wajngot, A., Cerasi, E. & Luft, R. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 7425–7429.
16. Bergman, R. N. (1977) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **36,** 265–270.
17. Hers, H. G. (1976) *Annu. Rev. Biochem.* **45,** 167–189.
18. Hers, H. G. & Hue, L. *Annu. Rev. Biochem.* **52,** 617–653.
19. Fisher, C. J. & Stetten, M. R. (1966) *Biochim. Biophys. Acta* **121,** 102–109.
20. Jakobsson, S. V. & Dallner, G. (1968) *Biochim. Biophys. Acta* **165,** 380–392.
21. Hanson, T. L. & Nordlie, R. C. (1970) *Biochim. Biophys. Acta* **198,** 66–75.
22. El-Refai, M. & Bergman, R. N. (1976) *Am. J. Physiol.* **231,** 1608–1619.
23. Nordlie, R. C., Sukalski, K. A. & Alvares, F. L. (1980) *J. Biol. Chem.* **255,** 1834–1838.
24. Newgard, C. B., Foster, D. W. & McGarry, J. D. (1984) *Diabetes* **33,** 192–195.
25. Karlander, S., Roovete, A., Wajngot, A., Vranić, M. & Efendić, S. (1984) *Diabetologia* **27,** 294A (abstr.).