Quantitative Determination of Hepatic Glucose Uptake
Using an Innovative Approach:
Effect of Strict Glycemic Regulation
and Exercise in Diabetic Subjects

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Summary For perfect glycemic control in diabetics, therapeutic modalities which enhance impaired hepatic glucose uptake seen in diabetics should be utilized. In animal experiments, we demonstrated that factors promoting hepatic glucose uptake are the glucose gradient between the central nervous system and the hepato-portal system, portal hyperinsulinemia and normal premeal glycemia. Therefore, we investigated both the effects of intensified insulin therapy and a single bout of exercise on hepatic glucose uptake by euglycemic hyperinsulinemic clamp combined with oral glucose loading. In 56 out of 77 patients, perfect glycemic normalization was established with mean regular insulin doses of 10, 7, 7 U at breakfast, lunch and dinner, respectively. The ratio of splanchnic glucose disposal to the amount of ingested glucose increased significantly from 19.0 to 42.1%. A single bout of exercise enhanced hepatic glucose uptake from 23.4 to 50.5%. Both strict glycemic regulation and exercise enhance hepatic glucose uptake significantly in non-obese diabetic patients.

Key Words diabetes mellitus, insulin sensitivity, insulin secretion, glucose fluxes, hepatic glucose uptake, glucose utilization

There is no doubt that the liver plays an important role in glucose homeostasis. To date, many investigators have utilized tracer dilution techniques to measure glucose fluxes. The development of isotope dilution techniques for determining glucose turnover has permitted a broader analysis of glucoregulation by allowing the investigation of quantitative changes in the rate of hepatic glucose production and whole body glucose utilization. Factors inhibiting hepatic glucose production rate or promoting whole body glucose utilization rate have been intensively investigated.

However, the quantitative determination of hepatic glucose uptake has not yet been undertaken in humans, due to the lack of investigative modalities.

Clinically, deranged hepatic glucose uptake during and after meal intake or exercise, might be the cause of the exaggerated glycemic responses seen in diabetics. Therefore, clarifying the mechanism underlying the regulation of hepatic glucose uptake with regard
to insulin should lead to improvements in therapy, that in turn could normalize physiological hepatic glucose handling.

In this paper, we present a newly-developed, innovative non-invasive method of quantifying hepatic glucose uptake in subjects with diabetes.

**METHODS AND RESULTS**

*Euglycemic hyperinsulinemic clamp combined with oral glucose load*

For the calculation of hepatic glucose uptake in animal studies, tracer methods and AV difference techniques are applied simultaneously. In human studies, however, such invasive techniques are not suitable. The euglycemic hyperinsulinemic clamp technique is widely used for insulin sensitivity measurements in human studies. However, with this method, what we measure is insulin-induced whole body glucose uptake mainly by muscle. Therefore, to measure hepatic glucose uptake quantitatively, we developed an innovative non-invasive method, euglycemic hyperinsulinemic clamp combined with oral glucose loading.

After overnight fasting, a primed constant infusion of insulin was initiated, using an artificial endocrine pancreas, to achieve the desired steady-state plasma insulin concentration and exogenous glucose was infused to keep blood glucose levels within euglycemic range. The rate of glucose infusion required to maintain euglycemia can be considered to reflect glucose disposal by peripheral tissues, since most of the infused glucose is taken up by peripheral tissues, primarily by muscles, under these conditions. A fixed amount of glucose was administered orally after steady-state plasma glucose level was attained. The amount of oral glucose load for each subject was determined, based on the glucose infusion rate prior to oral glucose loading (pre-GIR). After oral glucose loading, the rate of glucose infusion (GIR) begins to decrease, since some fractions of orally administered glucose which are not extracted by splanchnic tissues enter the systemic circulation, and thus reduce the GIR required to maintain euglycemia. Therefore, splanchnic glucose disposal can be calculated from the difference between the summation of GIR decreases after glucose ingestion ($\Sigma$ delta GIR) and the amount of ingested glucose (OGL)(Fig.1). The calculated amount of splanchnic glucose disposal versus the amount of glucose loading was expressed as a ratio (%).

*The Effect of NIDDM Treatment on Hepatic Glucose Uptake*

There is no doubt that a therapeutic tool which enhances hepatic glucose uptake, especially after food-intake, should be utilized for strict glycemic control in diabetics. Therefore, we investigated both the effects of intensified insulin therapy enabling strict glycemic control and the effects of acute exercise on hepatic glucose uptake.

*Effects of Acute Exercise on Hepatic Glucose Uptake*

Many investigators have demonstrated that exercise improves insulin-stimulated total body glucose uptake. However, the post-exercise dynamics of glucose fluxes in splanchnic and peripheral tissues remain unclear. The acute effect of exercise on glucose handling by

both splanchnic and peripheral tissues was investigated in subjects with non-insulin-dependent diabetes mellitus (NIDDM).

Six non-insulin-dependent diabetics without diabetic complications participated in this study. Four patients had been treated with sulfonylureas and 2 patients had been treated with diet alone. A euglycemic hyperinsulinemic clamp combined with oral glucose loading was used to determine glucose disposal by splanchnic and peripheral tissues, respectively.

Assessment of glucose handling by the clamp method was performed twice, at 3-day
Effects of exercise on glucose uptake by peripheral and splanchnic tissues in NIDDM patients (MEAN±SEM). •, before exercise; △, after exercise

intervals, while patients were sedentary, and after exercise. Each subject had a single bout of exercise on a cycle ergometer. The intensity and energy expenditure of the load were set at 90% of measured anaerobic threshold and 200 kcal, respectively.

During the euglycemic clamp study, the mean plasma concentrations of glucose and insulin (IRI) were 93±13 mg/dl and 169±39 µU/ml, respectively. Plasma concentrations of glucose and IRI did not change significantly after glucose ingestion. Glucose disposal by peripheral tissues was 8.6 ± 1.7 mg/kg/min in the sedentary state and 9.6 ± 1.7 mg/kg/min at 3 hours after exercise, demonstrating that acute exercise enhances peripheral glucose uptake; however, this change was not statistically significant. The ratio of splanchnic glucose disposal to the amount of ingested glucose, on the other hand, increased significantly, to 50.5±6.1%, from 23.4±6.5% (Fig.2).

In conclusion, a single bout of exercise appears to improve glucose handling by splanchnic tissues in NIDDM patients.

Effects of Strict Glycemic Regulation with Prandial Regular Insulin Injections on Hepatic Glucose Uptake in Non-obese NIDDM Patients with Secondary Failure on Sulfonylureas

The authors have previously reported that NIDDM is characterized by decreased meal-related insulin secretion(1). Recently, we also demonstrated that sufficient insulin supplementation before each meal in NIDDM patients prevented beta cell exhaustion and was followed by restoration of endogenous basal insulin secretion(2).

Taking all these results into consideration, it would appear that the substitution of prandial insulin by injections of regular insulin before each meal is essential.

The following working hypothesis, as the basis for a treatment regimen for non-obese NIDDM patients with secondary failure on sulfonylureas, has been proposed (Fig.3). The raised portal insulin concentration obtained by sufficient regular insulin supplementation before each meal, and the subsequently obtained normalization of pre-meal glycemia, which establishes the hepato-portal-arterial glucose gradient prandially, might together augment hepatic glucose uptake. Through these mechanisms, normalization of post-meal glycemia would be realized. Sufficient insulin supplementation before each meal prevents

Fig. 3. Working hypothesis for the treatment of non-obese NIDDM patients with secondary failure on sulfonylureas.

beta-cell exhaustion and is followed by restoration of endogenous basal insulin secretion. If secreted endogenous insulin is sufficient to normalize midnight and early morning glycemia, this therapy is the most suitable one for NIDDM patients.

This hypothesis was tested in 77 NIDDM patients with secondary failure on sulfonylureas who were admitted to hospital to switch to insulin therapy. In these patients, the insulin plasma profile remained at levels lower than 20 μU/ml, even though endogenous insulin secretion was stimulated with a maximal amount of glibenclamide and by hyperglycemia. Patients were given regular insulin 30 min preprandially at 3 meals. Insulin injections were initiated at doses of 10U, 8U, and 6U at breakfast, lunch, and dinner, respectively. Then insulin doses were adjusted in accordance with the daily plasma glucose profile, taken every 3–4 days, to obtain normal pre- and 2-h postprandial glycemia. At 4 to 5 weeks after the initiation of insulin therapy, perfect normalization of glycemia was established in 56 of the 77 patients, with mean doses of 10, 7, and 7U at breakfast, lunch and dinner, respectively (Fig.4). Mean urinary excretion of C-peptide immunoreactivity (CPR) at night, from 11 p.m. to 6 a.m. was 1.85 μg/h at 4 weeks, and urinary CPR divided by plasma glucose was increased two-fold. These amounts seemed to be sufficient to suppress hepatic glucose production and to increase peripheral glucose uptake. Thus, it was clearly shown that in NIDDM, insulin secretory ability, especially basal insulin secretion, is dynamic and reversible. HbA1c decreased markedly, even though insulin doses were reduced. This phenomenon may be caused by increased hepatic glucose uptake after each meal, showing that the insulin sensitivity of the patients increased gradually.

The effects of strict glycemic control on glucose handling by both splanchnic and
Fig. 4. Mean plasma glucose profile in response to prandial regular insulin injections in 56 non-obese NIDDM patients with secondary failure on sulfonylureas.

Fig. 5. Effects of strict glycemic regulation with premeal regular insulin injections on glucose uptake by peripheral and splanchnic tissues in non-obese NIDDM patients with secondary failure on sulfonylureas (MEAN ± SEM). ●, before strict glycemic control; ▲, after strict glycemic control.

Peripheral tissues was investigated in six of these NIDDM patients. In the present study, we also used the euglycemic hyperinsulinemic clamp combined with oral glucose loading to determine glucose disposal by splanchnic and peripheral tissues separately. Assessment of glucose handling by this method was performed before and after 3 to 4 weeks of strict glycemic control with pre-meal regular insulin injections.

In these 6 patients who received intensified insulin therapy, near normal glycemic excursions were established within 2 weeks after initiation of the insulin treatment, and fasting plasma glucose levels improved significantly, to a level of 111 ± 8 mg/dl, from 177 ± 12 mg/dl. HbA1c levels were rapidly and significantly reduced, to 8.3 ± 0.3 from 11.2 ± 0.8%. Glucose disposal by peripheral tissues tended to improve (7.9 ± 1.1 mg/kg/min from 7.3 ± 0.8 mg/kg/min), but this change was not statistically significant.

The ratio of splanchnic glucose disposal to the amount of ingested glucose, on the
other hand, increased significantly, to 42.1 ± 11.7 % from 19.0 ± 5.9 % (Fig.5).

Thus, enabling of the regulation of prandial glycemia with smaller doses of regular insulin before each meal with an increase in hepatic glucose uptake after meal-intake, was clearly demonstrated. In other words, hepatic insulin sensitivity was remarkably augmented.

DISCUSSION

Regarding the factors that regulate hepatic glucose uptake, we have, firstly, reported that portally-administered insulin enhanced hepatic glucose uptake to a much greater degree than the same amount of insulin administered peripherally(3).

Secondly, we showed that the amount of glucose taken up by the liver after glucose loading depends on the route of glucose administration and that hepatic removal of glucose is greater after portal glucose infusion than after peripheral glucose delivery(4). Even though the exact mechanism of this phenomenon is not clearly understood, the arterial-portal venous glucose gradient has been speculated to be an important signal for augmenting hepatic glucose disposal. We also demonstrated that this phenomenon(4) cannot be explained by portal and hepatic arterial glucose gradients. Therefore, to elucidate this phenomenon, we investigated the significance of the glucose gradient between the central nervous and hepatoportal systems in hepatic glucose uptake. This investigation was carried out in normal dogs with pulsed Doppler flow probes chronically implanted on the portal vein (PV) and hepatic artery (HA) and cannulae in the hepatic vein (HV), superior mesenteric vein (SMV), and PV. Intravenous glucose loading was performed according to the following 4 protocols; 1) PE: peripheral infusion (7 mg/kg/min), 2) PO: intraportal infusion via SMV (same amount as PE), 3) PO+CNS: portal infusion (same as PO) plus additional glucose infusion into unilateral carotid (CA) and vertebral arteries (VA) to abolish the glucose gradient between the CNS and the hepatoportal system (amounts of glucose infused into the CA and VA were calculated based on the plasma flow ratio of PV to CA and VA), and 4) PO+PE: the same amount of additional glucose was infused into the peripheral vein instead of into the CNS. Net hepatic glucose balance (NHGB) and hepatic extraction of glucose (HERG) were calculated from plasma flows and plasma glucose levels in the PV, HA, and HV.

In PE and PO+CNS, the hepatoportal-CNS glucose gradients had negative and almost zero values, respectively. In PO and PO+PE, on the other hand, the hepatoportal-CNS glucose gradients had positive values. Glucose infusion via the portal route caused a significant increase in both NHGB and HERG compared to PE under comparable levels of plasma glucose and IRI. This increase in hepatic glucose uptake significantly decreased during PO+CNS, but returned to a value similar to that of PO during PO+PE.

These results suggest that the glucose gradient between the CNS and the hepatoportal system appears to play a crucial role in regulating hepatic glucose disposal.

Then, to examine the effects of prevailing plasma glucose levels on hepatic glucose handling, hepatic glucose uptake during intraportal glucose infusion (7 mg/kg/min) was measured under euglycemic (EUGL) and hyperglycemic (HYPGL) clamp conditions in
normal dogs. Insulin was administered into the portal vein, at a rate of 20x\text{B} (\text{B}=225 \mu \text{U/kg\cdot min}), and somatostatin (0.5 \mu \text{U/kg\cdot min}) was infused to suppress endogenous insulin secretion. Pulsed Doppler flow probes were chronically implanted on the portal vein (PV) and the hepatic artery (HA), and three indwelling cannulae were placed in the superior mesenteric vein, left common hepatic vein (HV), and PV. Net hepatic glucose balance (NHGB) and hepatic extraction rate of glucose (HERG) were calculated from the plasma flow data and plasma glucose levels in the PV, HA, and HV.

In this study, the hepatoportal-CNS glucose gradient, which was postulated to be one of the important modulators of hepatic glucose handling, was kept comparable in both EUGL and HYPGL, since the plasma glucose level during portal glucose loading was maintained by peripheral glucose infusion. Arterial plasma glucose level in HYPGL was about twice as high as that in EUGL, but there were no significant differences in insulin concentrations between the two groups. Although NHGB in HYPGL was higher than in EUGL, HERG was significantly reduced in HYPGL compared to EUGL.

From the clinical point of view, these data suggest that strict glycemic control to establish normal premeal glycemia is essential for the promotion of hepatic glucose disposal, even in diabetic subjects.

As the results presented here show, hepatic glucose uptake has been quantitatively evaluated by a non-invasive method. In addition, it was clearly demonstrated that normalization of glycemic excursions with exogenous insulin corrected hepatic insulin sensitivity, even in non-obese NIDDM.

It was also demonstrated that the liver acts as a glucose uptake organ after a single bout of exercise, probably to replenish previously consumed glycogen. This finding implied that insulin-treated patients who undertake heavy exercise should not take carbohydrate before, but should take during and after exercise.

REFERENCES
