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The effects of HDV-insulin on carbohydrate metabolism in Type 1 diabetic patients

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Abstract

The aim of this study was to compare the metabolic effects of a single equimolar subcutaneous injection of hepatic directed vesicle-insulin (HDV-insulin) and regular insulin on glucose levels and intermediary metabolism during a 75-g oral glucose tolerance test (OGTT). Nine Type 1 diabetic patients underwent two experiments separated by 4 weeks. Each experimental protocol consisted of an identical evening meal followed by overnight euglycemic control achieved by a continuous low-dose insulin infusion. The next morning a subcutaneous injection (0.1 U/kg) of HDV-insulin or regular insulin was administered 30 min before a 75-g OGTT. The overnight basal insulin infusion was maintained unaltered throughout the 150-min OGTT. Plasma glucose, glucoregulatory hormones (insulin, glucagon, cortisol), and intermediary metabolites (lactate, alanine, glycerol, NEFA, β-hydroxybutyrate) were measured to assess the metabolic effects of the two insulin preparations. Compared to regular insulin, an equivalent subcutaneous dose of HDV-insulin significantly lowered glucose levels during OGTT (mean reduction 2.2 ± 0.4 mmol/l; $P < .005$). Plasma levels of insulin and glucagon were equivalent during both series of experiments. Blood lactate, glycerol and plasma NEFA levels were not different during OGTT indicating similar peripheral action of the insulins. β-Hydroxybutyrate levels were significantly reduced ($P < .05$) following HDV-insulin supporting a preferential hepatic action of the preparation. We conclude that HDV-insulin can significantly lower plasma glucose excursions compared to an equivalent dose of regular insulin during an OGTT in Type 1 diabetic patients. The metabolic profile of equivalent peripheral insulin, glucagon and glycerol levels but reduced β-hydroxybutyrate values support a hepatospecific effect of HDV-insulin.

Keywords: Insulin analog; Glucagon; Glucose; Intermediary metabolites

1. Introduction

Recent large multicenter trials have demonstrated the benefits of tight metabolic control in preventing tissue complications of diabetes in Type 1 and Type 2 diabetic patients (The Diabetes Control and Complications Trial Research Group, 1993; UKPDS Group, 1998). Accordingly, the American Diabetes Association recommends aggressive treatment of diabetes and an ideal therapeutic goal of near euglycemia (American Diabetes Association, 1998). Consequently, during the last decade there has been considerable interest in developing novel treatment options for improving metabolic control in patients with diabetes. All Type 1, and a significant number of Type 2, diabetics have treatment regimens that include insulin. Currently, insulin therapy generally involves subcutaneous injection. This route of delivery reverses the usual portal/peripheral gradient of the hormone and leads to relative hepatic hypoinsulinemia. Previous studies by Canfield, Kaye, and West (1972) have demonstrated that as little as 1% of subcutaneously injected insulin reaches the hepatocytes. The physiologic consequences of relative hepatic hypoinsulinemia can be divided into effects regulating glucose production and uptake. Results from several studies have been used to argue that insulin controls hepatic glucose production through peripheral actions (e.g., reducing the flow of fatty acids and gluconeogenic substrates to the liver) (Ader & Bergman, 1990; Giacca, Fisher, Shi, & Vranic, 1992; Lewis, Zinman, Groenewoud, Vranic, &

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Giacca, 1996). On the other hand, Sindelar, Balcom, Chu, Neal, and Cherrington (1996) have demonstrated the additional importance of a direct action of insulin on reducing hepatic glucose production over and above the indirect action of the hormone on peripheral tissues. Furthermore, a substantial body of work has emphasized the ability of portal insulin to significantly increase hepatic glucose uptake after a glucose load (Adkins, Myers, Hendrick, Williams, & Cherrington, 1987; Myers, McGuinness, Neal, & Cherrington, 1991; Pagliassotti, Moore, Neal, & Cherrington,1992). Thus, it is evident that hepatic actions of insulin play a substantial role in reducing postprandial glycemia by (1) more effectively reducing hepatic glucose output, and (2) increasing glucose uptake by the liver.

Postprandial hyperglycemia and subsequent hypoglycemia are major limitations of aggressive subcutaneous insulin therapy. They combine to complicate achievement of the therapeutic goal of euglycemia in Type 1 DM patients. Postprandial hyperglycemia occurs because currently available fast-acting insulin preparations cannot completely reproduce the time action profile achieved by the physiologic, portal delivery of the hormone, nor do they augment hepatic glucose uptake. This latter point is especially significant as physiologic levels of portal insulin can increase hepatic glucose uptake two-fold (Myers et al., 1991). Hypoglycemia in the early or late postabsorptive state occurs because of inappropriate matching of nutrient intake or physical activity and relatively high peripheral insulin levels. This latter situation is exacerbated by increasing the dose of fast-acting insulin before meals in an effort to reduce elevated postprandial glycemia. An ideal preprandial insulin preparation would preferentially target the liver. The advantages of a hepatospecific insulin are two-fold. First, increased insulin action at the liver should limit hepatic glucose output while increasing hepatic glucose uptake. Secondly, improved postprandial glycemic control could be obtained with reduced systemic insulinemia, thereby reducing the risk of subsequent hypoglycemia.

Therefore, this study was undertaken to investigate the metabolic effects of a novel subcutaneous insulin delivery preparation created by the addition of a well-characterized, specific hepatobiliary target molecule (2,6 diisopropylphenylcarbamoyl methyl iminodiacetic acid) (Nadel, 1996; Svensson, Friman, Jacobsson, & Holmberg, 1995) to a soluble insulin liposome preparation. The experiments were designed to test the hypothesis that a single equimolar subcutaneous injection of the new insulin preparation [hepatic directed vesicle-insulin (HDV-insulin)] would, when compared to regular insulin, result in lower plasma glucose levels following a 75-g oral glucose tolerance test (OGTT) in a group of Type 1 diabetic patients. Insulin, glucagon, cortisol, and intermediary metabolite levels were measured at frequent intervals so that the effects of HDV-insulin on peripheral and hepatic metabolic regulation could also be determined and compared with regular insulin.

2. Methods

2.1. Subjects

Nine Type 1 patients with diabetes (six males/three females), age 29 ± 3 years, body mass index 25.4 ± 1.1 kg/m^2, glycosylated hemoglobin (HBA_{1c}) 7.2 ± 0.4% (normal range 4 to 6.5%) were studied. The duration of diabetes was 17 ± 4 years, C-peptide levels were below the lower limit of normal (0.1 ± 0.001 nmol/l) and the usual insulin replacement therapy of the subjects was either multiple daily injections ($n = 4$) or continuous subcutaneous insulin infusion ($n = 5$). With the exception of one patient who was receiving stable maintenance thyroid hormone replacement for hypothyroidism, none of the patients were taking any medication that could affect carbohydrate metabolism. Each patient had a normal electrocardiogram, blood count, plasma electrolytes, liver and renal function. All gave written informed consent. Studies were approved by the Vanderbilt University Human Subjects Institutional Review Board. Patients were asked to avoid any strenuous physical activity for 4 days prior to the experiments. Patients consumed a daily diet of 250 g of carbohydrate and avoided any hypoglycemia for the 3 days before each study.

2.2. Methods and experimental design

2.2.1. Preclinical animal studies

Forty overnight fasted mice were randomly allocated to receive intravenous injections of HDV-insulin labeled with ^{14}C-cholesterol during 240-min experiments. Animals were killed at 30, 60, 120, and 240 min. Peripheral blood, liver and splenic tissue were solubilized and counted on a scintillation counter to determine the amounts of HDV-insulin (proportional to counts of ^{14}C-cholesterol) present in each respective aliquot (Svensson et al., 1995).

2.2.2. Type 1 DM patient studies

Intermediate-acting insulin was discontinued 24 h prior to the initiation of each study. Patients were admitted to the Vanderbilt Clinical Research Center at 5:00 p.m. on the evening prior to an experiment. Two intravenous cannulae were inserted under 1% lidocaine local anesthesia. One cannula was placed in a retrograde fashion into a vein on the back of the hand. The other cannula was placed in a wrist vein on the contralateral arm so that insulin could be infused via a variable rate volumetric infusion pump (Imed, San Diego, CA). Each patient received a standardized evening meal containing 1000 kcal (125 g carbohydrate). A low-dose intravenous insulin infusion was used to maintain blood glucose levels between 4.4 and 6.7 mmol/l throughout the night.

On the morning of each experiment, the hand with the retrograde venous cannula was placed in a heated box (55–60°C) so that arterialized blood could be obtained (Abumrad, Rabin, Diamond, & Lacy, 1981). The overnight intravenous

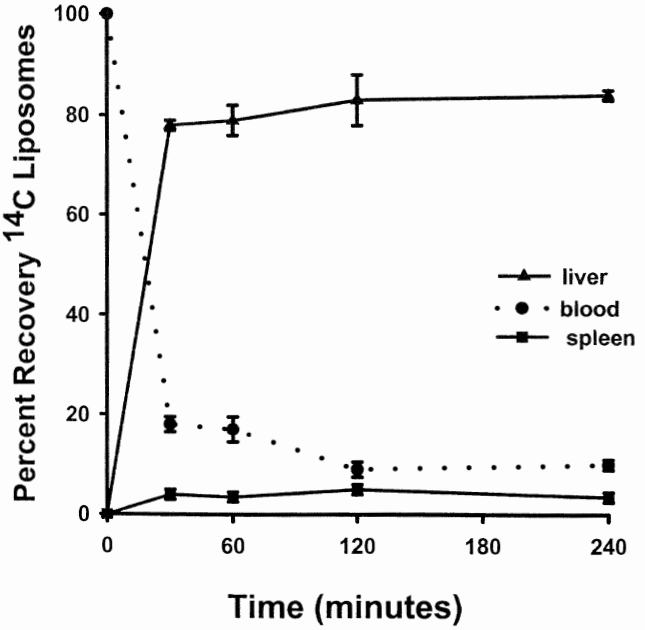

Fig. 1. Effects of intravenous injection of ^{14}C-cholesterol labeled HDV-insulin on recovery of ^{14}C counts in peripheral blood, liver and splenic tissue in overnight fasted mice. Percentage recovery of ^{14}C-cholesterol in significantly greater ($P \leq .01$) in liver as compared to peripheral blood or spleen.

insulin infusion was held constant for at least 1 h before subcutaneous drug administration and throughout the OGTT. This represented the usual "basal" insulin replacement for each patient. Thirty minutes prior to initiating the OGTT, patients received a subcutaneous injection (0.1 U/kg) of either regular insulin or HDV-insulin into the anterior abdominal wall. Each vial of HDV-insulin consisted of 10 ml regular insulin (100 U/ml) to which a mixture of soluble liposomes and hepatic target molecule dissolved in 0.8 ml water for injection was added. Therefore, each subcutaneous injection of HDV-insulin had to be increased by a factor of 10.8/10.0 to give an equivalent molar dose as compared to regular insulin. The 75 g of glucose was dissolved in a flavored drink and all subjects consumed the fluid within 3 min of administration.

Plasma glucose concentrations were measured in triplicate using the glucose oxidase method with a glucose analyzer (Beckman Instruments, Fullerton, CA). Insulin was measured by immunoassay (RIA) using the method of Morgan and Lazarow (1963) with an interassay CV of 11%. HDV-insulin exhibits 100% cross-reactivity with regular insulin in this RIA method. Glucagon was measured as previously described (Morgan & Lazarow, 1963) with an interassay CV of 6%. Cortisol was assayed by using the clinical assays gamma coat radioimmunoassay kit with an interassay CV of 6%. Lactate, glycerol, alanine, and β-hydroxybutyrate were measured in deproteinized whole blood using the method of Lloyd, Burrin, Smythe, and Alberti (1978). Nonesterified fatty acids (NEFA) were measured using the Wako kit (Wako, Dallas, TX) adapted for use on a centrifugal analyzer.

Blood samples for glucose levels were drawn every 10 min following subcutaneous insulin injection. Blood for hormones and intermediary metabolites were drawn every 15 min throughout the 180-min experimental protocol.

2.3. Materials

Human regular insulin was purchased from Eli Lilly (Indianapolis, IN). HDV-insulin was provided by AMDG (Somerville, NJ). The overnight insulin infusion solution was prepared with normal saline and contained 1% (vol/vol) of the subject's own plasma.

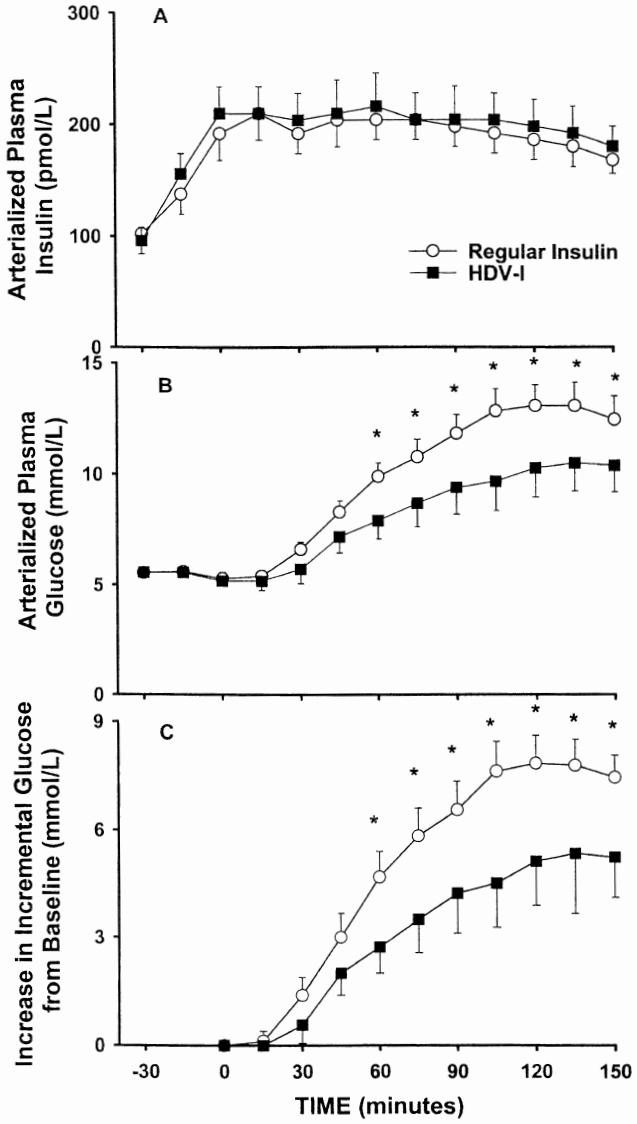

Fig. 2. Effects of subcutaneous injection (0.1 U/kg) of HDV-insulin or regular insulin on arterialized plasma insulin (Panel A), plasma glucose (Panel B), incremental glucose increases from baseline (Panel C) responses following a 75-g OGTT in overnight fasted, euglycemic-controlled Type 1 diabetic patients. * Incremental and absolute plasma glucose levels are significantly reduced ($P < .001$) following HDV-insulin compared to regular insulin.

Table 1
Effects of a single subcutaneous dose (0.1 U/kg) of HDV-insulin (HDV-I) or regular insulin (Reg) on plasma glucagon and intermediary metabolite levels during a 75-g OGTT in overnight euglycemic-controlled patients with Type 1 diabetes

	Baseline period			Duration of OGTT (min)									
	−30	−15	0	15	30	45	60	75	90	105	120	135	150
Plasma cortisol (nmol/l)													
Reg	339±77	326±74	317±58	372±74	433±83	353±61	342±58	356±72	328±61	303±55	268±41	270±44	268±47
HDV-I	268±25	268±22	259±25	326±33	353±41	358±33	323±33	273±30	273±22	243±22	223±17	207±14	190±14
Plasma glucagon (ng/l)													
Reg	38±3	38±3	36±3	38±3	39±3	39±3	37±3	36±3	36±2	35±3	35±3	34±3	35±3
HDV-I	37±3	36±3	37±3	38±3	39±4	39±4	39±4	38±4	37±3	35±3	35±3	34±3	36±3
Blood lactate (μmol/l)													
Reg	750±120	710±70	730±70	820±130	840±140	870±110	950±130*	960±120*	930±100*	980±120*	960±100*	910±90*	890±80*
HDV-I	690±50	690±30	730±40	780±40	830±40*	840±50*	920±50*	910±80*	940±50*	950±50*	970±60*	920±50*	890±40*
Blood glycerol (μmol/l)													
Reg	37±8	41±9	27±5	22±3†	22±4†	25±4†	24±3†	24±4†	23±4†	22±3†	24±5†	24±5†	21±5†
HDV-I	43±9	40±8	29±4	23±3†	26±4†	26±5†	26±4†	24±4†	23±4†	21±3†	23±4†	21±4†	20±3†
Plasma NEFA (μmol/l)													
Reg	383±54	377±89	291±69	217±38†	191±47†	196±74†	184±66†	169±63†	167±75†	152±66†	150±63†	150±72†	151±65†
HDV-I	387±54	419±76	292±57	205±28†	176±29†	167±33†	150±31†	132±27†	127±34†	108±21†	107±24†	125±34†	143±49†
Blood β-hydroxybutyrate (μmol/l)													
Reg	104±40	134±54	156±73	77±29	52±19†	45±24†	46±29†	36±21†	36±24†	33±19†	32±20†	26±17†	25±15†
HDV-I	157±56	174±56	157±57	96±37	54±24†	38±16†	28±12†	20±8†,‡	17±5†,‡	15±4†,‡	13±3†,‡	12±3†,‡	11±3†,‡

Values are means±S.E.M.
* Values are significantly increased ($P<.05$) relative to baseline period.
† Values are significantly decreased ($P<.05$) relative to baseline period.
‡ Values are significantly reduced ($P<.05$) during HDV compared to regular insulin.

2.4. Statistical analysis

Data are expressed as the mean ± S.E.M. unless otherwise stated. Incremental area under the curve (AUC) was calculated using the trapezoidal rule. Data were analyzed using standard parametric, two-way ANOVA with a repeated measured design. Incremental AUC values were analyzed by paired Student's t tests. A value of $P < .05$ indicated significant difference.

3. Results

3.1. Preclinical animal studies

By the end of the 240-min tissue labeling experiments, 85 ± 5% of ^{14}C-cholesterol counts were obtained from liver tissue, 10 ± 5% counts were obtained in peripheral blood, and 5 ± 2% of total counts were recovered from spleen samples. These data demonstrate the specific hepatic targeting of the HDV-insulin molecule for the liver.

3.2. Glucose challenge studies in Type 1 diabetic patients

3.2.1. Insulin levels

The plasma insulin values after overnight infusion to achieve glycemic control were equivalent at the time of HDV and regular insulin injection (102 ± 15 vs. 108 ± 18 pmol/l, respectively). Insulin values increased by similar amounts during the OGTT so that peak, mean, and incremental AUC values were indistinguishable between the groups (Fig. 2, Panel A).

3.2.2. Glucose levels

Plasma glycemia was maintained until ingestion of glucose loads (Fig. 1, Panel B) equivalent in both groups (5.4 ± 0.1 mmol) by a low-dose 'basal' intravenous infusion of insulin. During the OGTT, plasma glycemia was significantly lower overall ($P < .005$) following HDV-insulin compared to regular insulin (Fig. 2, Panels B,C). In terms of peak levels (10.5 ± 1.1 vs. 13.1 ± 1.0 mmol/l), incremental increases from baseline (2.6 ± 0.6 vs. 4.8 ± 0.5 mmol/l) and incremental AUC (462 ± 122 vs. 572 ± 79 mmol/l) glucose values were significantly reduced ($P < .005$) following HDV-insulin compared to regular insulin.

3.2.3. Glucagon and cortisol levels

Glucagon values were similar at the time of injection of HDV-insulin or regular insulin (37 ± 3 vs. 38 ± 3 ng/l, respectively). During OGTT, glucagon values remained similar to baseline and were indistinguishable between groups (Table 1). Plasma cortisol levels were also similar at the time of baseline injection of HDV-insulin or regular insulin (280 ± 30 vs. 347 ± 72 nmol/l, respectively). During OGTT, plasma cortisol levels exhibited a similar response in both groups. Peak cortisol levels were obtained during the first 30 min of the OGTT, then declined by identical amounts in both groups and were ≃70 ± 25 nmol/l below the baseline value at the conclusion of the study.

3.2.4. Intermediary metabolites

Baseline blood lactate levels were similar and increased ($P < .01$) equivalently following HDV-insulin or regular insulin (Table 1). Blood alanine levels were identical at baseline (0.3 ± 0.03 mmol/l) and did not change during the OGTT in either group. Blood glycerol and plasma NEFA values were not different between groups at baseline and fell by similar significant amounts ($P < .01$) following the administration of HDV-insulin or regular insulin. β-Hydroxybutyrate levels, however, fell to significantly lower levels ($P < .05$) following HDV-insulin as compared to regular insulin (Table 1).

4. Discussion

This present study compared the metabolic effects of a novel hepatic targeted insulin preparation (HDV-insulin) with regular insulin during a 75-g OGTT in a group of Type 1 diabetic patients. Our results clearly demonstrate that, despite identical insulin and glucagon levels, plasma glucose levels during the OGTT were significantly reduced following the subcutaneous injection of HDV-insulin as compared to regular insulin.

Recent large multicenter trials have emphasized the importance of good glucose control in reducing the risk of tissue complications of diabetes (The Diabetes Control and Complications Trial Research Group, 1993; UKPDS Group, 1998). Good glycemic control over time requires stringent regulation of both pre- and postprandial glucose levels. Elevated postprandial glycemia is an independent risk factor for tissue complications of diabetes (Jarrett, McCartney, & Keen, 1982). Consequently, two recent therapeutic options (lispro insulin and α-glucosidase inhibitors) have been introduced in an attempt to control postprandial glucose excursions. These agents are effective therapeutic tools in reducing prandial glycemia, and do so by different mechanisms. Lispro insulin does not associate into hexamers and thus can be absorbed more rapidly than regular insulin from a subcutaneous depot (Howey, Bowsher, Brunelle, & Woodworth, 1994). This provides a quicker onset of action and limits postprandial glucose excursions when the insulin is injected prior to a meal. α-Glucosidase inhibitors block small intestinal breakdown of complex carbohydrates into simple sugars, which in turn blunts glucose elevations following a meal. The mechanism of HDV-insulin's action in reducing glycemic excursions after a glucose load is distinct from currently available therapeutic agents. A hepatic targeted molecule that is incorporated within the liposomes specifically directs insulin to hepatocytes and increases uptake of the hormone by the liver. We therefore hypothesized that the increased hepatic insulinemia allowed greater suppression of

glucose production and importantly, significantly elevated postprandial glucose uptake by the liver.

Previous studies by Myers et al. (1991) have demonstrated that physiologic increases of portal vein insulin result in a doubling of glucose uptake by the liver (9.4 ± 2.2 to 19.8 ± 4.1 μmol/kg/min). It should be noted that this insulin-enhanced hepatic glucose uptake is quantitatively significant, as it is equivalent in magnitude to values of hepatic glucose production following an overnight fast (Myers et al., 1991). In the present study, the glycemia that occurred during an OGTT was reduced by 2.2 ± 0.4 mmol/l/h following HDV-insulin. If this level of glycemic improvement was obtained following three daily meals, an overall reduction in HBA_{1c} could conservatively be expected in the region of 0.6% to 0.8%. This is very similar to HBA_{1c} reductions obtained in the intensive group of the UKPDS and translates into a significant risk reduction for microvascular complications (The Diabetes Control and Complications Trial Research Group, 1993; UKPDS Group, 1998).

The reduced glucose excursion caused by HDV-insulin during the OGTTs is consistent with enhanced hepatic effects of the preparation. The finding that HDV-insulin had a more rapid effect (i.e., 30 min) than regular insulin in reducing glycemia during OGTT suggests that the preparation has a direct effect on hepatic glucose metabolism, as opposed to an indirect effect via lowering NEFA levels, which usually takes 1–2 h to develop (Yki-Jarvinen, Pahakainen, & Koivisto, 1991). Furthermore, data obtained from preclinical animal studies clearly demonstrate that the overwhelming majority of HDV-insulin ($\simeq 85 \pm 5\%$) is specifically targeted to the liver. Analysis of the data concerning glucoregulatory hormone and intermediary metabolites from the present study also suggests a hepatospecific effect of HDV-insulin. All patients received identical evening meals and a low-dose overnight infusion of insulin was used to achieve equivalent levels of plasma glucose and insulin. Thus, at the start of both OGTT tests, plasma glucose, insulin, glucagon, intermediary metabolites, and hepatic glycogen levels were equivalent. In view of this it is difficult to argue that metabolic events prior to the administration of the two insulin preparations could have independently influenced results. Plasma insulin and glucagon levels responded equivalently during both series of OGTTs. These data are, of course, critical to the interpretation of the study. Increased insulinemia following HDV-insulin administration could have explained the reduced glucose excursions following OGTT. Additionally, a disparity in glucagon levels (either increased following regular insulin or relatively decreased following HDV-insulin administration) could also have been the primary mechanism for the observed difference in the glucose profiles.

An indication that HDV-insulin had a greater effect on hepatic glucose metabolism relative to regular insulin can be obtained from the analysis of the intermediary metabolite results. If HDV-insulin reduced glycemia through a predominantly greater peripheral effect (i.e., increased glucose uptake by muscle and adipose tissue), lipolysis should also have been suppressed by a greater amount. However, plasma glycerol levels (the best indicator of lipolysis as NEFA levels can be subject to reesterification and therefore relatively overestimate the lipolytic rate) were similar between groups. This refutes a preferential peripheral effect of HDV-insulin. The β-hydroxybutyrate levels also strongly support a preferential hepatic effect of HDV-insulin. Acute changes of ketone production by the liver are primarily dependent upon the influx of NEFA, and the hepatic concentrations of insulin and glucagon. β-Hydroxybutyrate levels were reduced ≃two-fold during the final 90 min of the OGTT. Thus, as peripheral levels of insulin, glucagon, and NEFA were similar in both groups, any reduction of ketone body metabolism must have been due to a preferential action of HDV-insulin on the liver.

In summary, this study demonstrates that an equimolar single-dose (0.1 U/kg) subcutaneous injection of HDV-insulin is significantly more effective at reducing glycemic excursions during a 75-g OGTT in Type 1 DM patients than regular insulin. Analysis of intermediary metabolites, in the presence of equivalent plasma insulin and glucagon levels, indicates that HDV-insulin lowers plasma glucose levels by a preferential hepatic effect. We conclude that HDV-insulin may represent a novel and effective therapeutic agent for reducing postprandial glycemia in insulin-deficient diabetic patients and that this might be achieved without increasing the risk of subsequent hypoglycemia.

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References

Abumrad, N., Rabin, D., Diamond, M., & Lacy, W. (1981). Use of a heated superficial hand vein as an alternative site for measurement of amino acid concentration and for the study of glucose and alanine kinetics in man. *Metabolism*, *30*, 936–940.

Ader, M., & Bergman, R. (1990). Peripheral effects of insulin dominate suppression of fasting hyperglycemia. *American Journal of Physiology*, *258*, E1029–E1032.

Adkins, B., Myers, S. R., Hendrick, G. K., Williams, P., & Cherrington, A. (1987). Importance of the route of intravenous glucose delivery on hepatic glucose balance in the conscious dog. *Journal of Clinical Investigation*, *79*, 557–565.

American Diabetes Association (1998). Clinical practice recommendations 1998. *Diabetes Care*, *1*, S1 - S98 (Supplement).

Canfield, R. E., Kaye, G. I., & West, S. B. (1972). The preparation and evaluation of tritiated polyalanyl insulin derivatives. *Endocrinology*, *90*, 112 – 122.

Giacca, A., Fisher, S., Shi, Z. O., & Vranic, M. (1992). Importance of peripheral insulin levels for insulin induced suppression of glucose production in depancreatized dogs. *Journal of Clinical Investigation*, *90*, 1769 – 1777.

Howey, D., Bowsher, R., Brunelle, R., & Woodworth, J. (1994). [Lys(b28), pro(B29)]-human insulin. A rapidly adsorbed analog of human insulin. *Diabetes*, *43*, 396 – 402.

Jarrett, R., McCartney, P., & Keen, H. (1982). The Bedford Survey. Ten year mortality rates in newly diagnosed diabetics, borderline diabetics and normoglycemic controls and risk indices for coronary heart disease in borderline diabetics. *Diabetologia*, *22*, 79 – 84.

Lewis, G. F., Zinman, B., Groenewoud, Y., Vranic, M., & Giacca, A. (1996). Hepatic glucose production is regulated both by direct hepatic and extrahepatic effects of insulin in humans. *Diabetes*, *45*, 454 – 462.

Lloyd, B., Burrin, J., Smythe, P., & Alberti, K.G.M.M. (1978). Enzymatic fluorometric continuous flow assays for blood glucose, lactate, pyruvate, alanine, glycerol and β-hydroxybutyrate. *Clinical Chemistry*, *24*, 1724 – 1729.

Morgan, C., & Lazarow, A. (1963). Immunoassay of insulin two antibody system of plasma levels in normal, subdiabetic and diabetic rats. *Diabetes*, *12*, 115 – 121.

Myers, S. R., McGuinness, O., Neal, D., & Cherrington, A. (1991). Intraportal glucose delivery alters the relationship between net hepatic glucose uptake and the insulin concentration. *Journal of Clinical Investigation*, *87*, 930 – 939.

Nadel, H. (1996). Hepatobiliary scintigraphy in children. *Seminars in Nuclear Medicine*, *26*, 25 – 42.

Pagliassotti, M., Moore, M., Neal, D., & Cherrington, A. (1992). Insulin is required for the liver to respond to intraportal glucose delivery in the conscious dog. *Diabetes*, *41*, 1247 – 1256.

Sindelar, D. K., Balcom, J. H., Chu, C., Neal, D., & Cherrington, A. (1996). A comparison of the effects of selective increases in peripheral or portal insulin on hepatic glucose production in the conscious dog. *Diabetes*, *45*, 1594 – 1604.

Svensson, G., Friman, S., Jacobsson, L., & Holmberg, S. (1995). Hepatocyte and Kupffer cell function after liver transplantation in the rat-in-vivo evaluation with dynamic scintigraphy. *Liver*, *15*, 189 – 195.

The Diabetes Control and Complications Trial Research Group (1993). The effect of intensive treatment of diabetes on the development and progression of long term complications in insulin-dependent diabetes mellitus. *New England Journal of Medicine*, *329*, 977 – 986.

UKPDS Group (1998). Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet*, *352*, 837 – 853.

Yki-Jarvinen, H., Pahakainen, I., & Koivisto, V. (1991). Effect of free fatty acids on glucose uptake and nonoxidative glycolysis across human forearm tissues in the basal state and during insulin stimulation. *Journal of Clinical Endocrinology and Metabolism*, *34*, 205 – 211.