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Estimates of hepatic glyceroneogenesis in type 2 diabetes mellitus in humans

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Abstract

Glyceroneogenesis, that is, formation of triglyceride-glycerol from pyruvate, is a critical component of triglyceride fatty acid cycling in vivo. The quantitative contribution of glyceroneogenesis to triglyceride-glycerol and its hormonal regulation have not been examined in humans. We have quantified the contribution of pyruvate to very low-density lipoprotein (VLDL) triglycerides in subjects with type 2 diabetes mellitus using the deuterium labeling of body water technique. Subjects with type 2 diabetes mellitus were studied before and after a 6-month behavioral intervention therapy, during fasting and during a hyperinsulinemic normoglycemic clamp. Response to glucagon infusion was examined in 5 healthy subjects after an overnight fast. Glyceroneogenesis contributed ~54% to VLDL triglyceride-glycerol in type 2 diabetes mellitus as compared with ~12% contribution of plasma glucose. There was no effect of insulin plus glucose during hyperinsulinemic clamp on glyceroneogenesis even after clinical interventions, when insulin sensitivity had improved. In healthy subjects, the contribution of triosephosphates to plasma VLDL triglycerides was ~45%. Glyceroneogenesis, in contrast to glycolysis, is the predominant source of triglyceride-glycerol carbon for VLDL triglycerides in subjects with type 2 diabetes mellitus. The contribution of glyceroneogenesis to triglyceride-glycerol is not affected by short (4 hours) infusion of insulin in type 2 diabetes mellitus.

1. Background

The failure to regulate the concentration of free fatty acids (FFAs) in the blood is a critical factor in the genesis of insulin resistance in humans [1]. An important element in the control of blood FFA levels is their rate of reesterification in the liver; as much as 65% of the FFAs released from the adipose tissue during fasting are reesterified and redeposited [2]. This *triglyceride/fatty acid cycling* ensures that fatty acids are available to support energy requirements. A central feature of the cycle is *glyceroneogenesis*, an abbreviated version of gluconeogenesis, in which carbon from sources other than glucose or glycerol contributes to the formation of the glycerol-3-phosphate, which is converted to the glyceride-glycerol of triglycerides [2]. The key enzyme in this pathway, the cytosolic form of phosphoenolpyruvate carboxykinase (PEPCK-C), is present in varying activities in tissues that synthesize triglycerides, such as liver, white and brown adipose tissue, and muscle [3]. Alterations in the expression of PEPCK-C in various tissues of transgenic mice have been shown to be associated with changes in triglyceride accumulation or depletion [4-7].

Despite its potential physiological and clinical relevance, there is little information available concerning the rate of glyceroneogenesis and its regulation in humans. The extent of glyceroneogenesis in vivo has been quantified in adipose tissue and liver of rats [8-10] and in the livers of humans [11] using various isotopic tracer techniques. The study with humans demonstrated that hepatic glyceroneogenesis accounted for 30% to 60% of the plasma triglyceride-

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glycerol after an overnight fast. Plasma glycerol provided a low 5% of triglyceride-glycerol. There are no data, however, on the relative contribution of glyceroneogenesis vs glycolysis for glycerol-3-phosphate and their hormonal regulation in humans. In the present study, we have examined the quantitative contribution of glyceroneogenesis to plasma triglyceride-glycerol in subjects with type 2 diabetes mellitus. The response to intravenous glucose and insulin infusion was examined in subjects with diabetes before and after improvement in insulin sensitivity as a result of diet and behavior modification. Because glyceroneogenesis and gluconeogenesis have a common pathway, the impact of stimulation of gluconeogenesis by glucagon was examined in healthy controls. The data underscore the role of glyceroneogenesis in the reesterification of fatty acids in the liver and suggest that the regulation of glucose synthesis in the liver is not linked to the regulation of glyceroneogenesis.

2. Methods

Hepatic glyceroneogenesis was quantified using the deuterium labeling of the body water [12] in subjects with type 2 diabetes mellitus and in healthy controls. The response to a hyperinsulinemic normoglycemic clamp was determined in subjects with type 2 diabetes mellitus. In addition, the response to intravenous glucagon was examined in healthy subjects.

The contribution of glucose and pyruvate carbon to glyceride-glycerol in plasma very low-density lipoprotein (VLDL) triglycerides was quantified in 10 subjects with type 2 diabetes mellitus before and after 6 months of treatment including behavioral intervention alone (n = 8) and combined (n = 2) with the intestinal lipase inhibitor orlistat [13,14]. The data for the 2 subjects on orlistat were not different from those on the placebo; therefore, the entire group of 10 is reported together. The data of weight loss, insulin resistance, regional adiposity, and fatty acids have been reported previously [13,14]. The clinical and metabolic data of study subjects are displayed in Table 1. After enrollment, the volunteers with type 2 diabetes mellitus were asked to withdraw from prior diabetes medication for a 4-week baseline period. A nutritional plan for weight maintenance was provided during this period. After a standardized meal the evening before the study, the subjects fasted overnight. The next morning, an oral dose of deuterated water (~125 g) was administered to achieve a total body water [^{2}H] enrichment ~0.4%. The rate of glucose production was quantified by infusing a prime constant rate infusion of [6,6-$^2H_2$]glucose intravenously. The priming dose of the tracer was adjusted in proportion to the magnitude of fasting hyperglycemia. After 4 hours of tracer infusion for a baseline determination, a hyperinsulinemic normoglycemic clamp was instituted to quantify insulin sensitivity and to examine the effect of glucose and insulin infusion on the incorporation of glucose and pyruvate into triglyceride. A 4-hour continuous infusion of insulin was given at 40 μU/(m^2 min). Plasma glucose was measured every 5 minutes and was allowed to decrease until normoglycemia (100 mg/dL) was achieved, which was then maintained with an adjustable infusion of 20% glucose. Blood samples for the enrichment of [6,6-$^2H_2$]glucose and for the ^{2}H enrichment of VLDL triglyceride-glycerol were obtained at periodic intervals. The glucose infusion was prelabeled with [6,6-$^2H_2$]glucose to maintain stable enrichment of plasma glucose [15].

Five healthy subjects (mean ± SD: age, 40 ± 7.6 years; body weight, 78.6 ± 13.0 kg; body mass index, 26.1 ± 1.5 kg/m^2) were studied after an overnight fast. They were also given oral [$^2H_2$]O 4 g/kg body weight divided into 2 doses given at 4:00 AM and 6:00 AM and [6,6-$^2H_2$]glucose tracer as described above. After a basal period of 3 hours, the response to an unopposed effect of glucagon was examined by infusing octreotide (50 μg/h) along with glucagon (~3 ng/[kg min]) for 3 hours. Blood samples were obtained at frequent intervals through an indwelling cannula.

2.1. Analytical methods

The deuterium enrichment of hydrogens on C-6 of glucose was measured by periodate oxidation followed by conversion of the formaldehyde formed into hexamethylenetetramine (HMT), as described previously [16]. Enrichment of monolabeled m_1 and dilabeled m_2 species of HMT was quantified using gas chromatography–mass spectrometry. The VLDL triglycerides were separated from the plasma by ultracentrifugation [17]. The triglycerides were further extracted using chloroform-methanol (2:1). The

Table 1
Clinical and metabolic data on subjects with type 2 diabetes mellitus

	Age (y)	Weight (kg)	BMI (kg/m^2)	Glucose (mg/dL)	Insulin μU/mL	HOMA-IR[a]	HbA$_{1c}$ (%)	FFA (μmol/L)	TRIG (mg/dL)
Baseline	55.9 (6.2)	95.9 (18.00)	33.3 (6.3)	162.4 (38.0)	17.8 (9.4)	6.6 (3.2)	7.8 (1.3)	650.0 (141.0)	153.3 (60.2)
6 months	56.4 (6.2)	87.1 * (14.3)	30.3 * (5.1)	121.9† (46.5)	12.6† (7.7)	3.6 * (1.9)	6.8 (2.2)	616.1 (90.9)	104.1† (37.3)

Data are mean (±SD). Ten subjects with type 2 diabetes mellitus were studied at baseline and 6 months after an intervention consisting of diet and behavior modification as described in Methods. Blood samples were obtained after an overnight fast. BMI indicates body mass index; HOMA-IR, homeostasis model assessment of insulin resistance; HbA$_{1c}$, glycated hemoglobin; TRIG, plasma triglycerides.

[a] (Fasting plasma glucose × fasting plasma insulin)/22.5.

* Significantly different from the baseline, paired t test: $P < .003$.

† Significantly different from the baseline, paired t test: $P < .01$.

isolated triglycerides were hydrolyzed using potassium hydroxide–ethanol; glycerol was isolated by sequential ion exchange chromatography followed by liquid chromatography. The aqueous layer was passed through an ion exchange column, diameter 0.5 cm, consisting of AG 1X-8 formate and AG 50W-X8 resins (Bio-Rad Laboratories, Hercules, CA). The neutral fraction, eluted with 5 mL water, was dried, reconstituted, and further purified using an Agilent high-performance liquid chromatography system (Agilent Technologies, Wilmington, DE). Aminex HPX-87 column (300 cm × 7.8 mm) was used (Bio-Rad Laboratories). The column temperature was maintained at 80°C, and glycerol was eluted using high-performance liquid chromatography–grade water at 0.6 mL/min. The glycerol peak was evaporated to dryness. An aliquot of glycerol was treated with periodic acid, resulting in the cleavage of both C-1 and C-3 as formaldehyde, which was then converted to HMT as described above. Another aliquot of glycerol was enzymatically converted to glycerol-3-phosphate by incubating for 1 hour at 37°C with 5 mmol/L adenosine triphosphate and 2 U glycerokinase in 0.1 mol/L phosphate buffer (pH 7.4). Glycerol-3-phosphate was isolated by ion exchange chromatography using AG 1X8 resin in 1 N formic acid. The column was sequentially eluted with water, 1 N formic acid, and 4 N formic acid. Glycerol-3-phosphate appeared in the 4-N formic acid fraction. The recovery was ~80%. The isolated glycerol-3-phosphate was treated with periodic acid, resulting in the cleavage of only C-1 of glycerol as formaldehyde, which was then converted to HMT [16]. The deuterium enrichments of hydrogens on C-1 of glycerol were then subtracted from the sum of C-1 and C-3 to estimate the enrichments on hydrogens on C-3.

2.2. Calculation

The fractional contribution of gluconeogenesis to glucose was calculated as follows: percentage of gluconeogenesis from pyruvate = 100 × (0.5 × ^{2}H enrichment of glucose C-6/^{2}H enrichment in body water).

The ^{2}H enrichment of glucose C-6 is halved because 2 hydrogens bound to C-6 glucose are enriched. Because of the extensive exchange between the hydrogens of C-3 of pyruvate and the hydrogens in body water (at alanine aminotransferase and during the oxaloacetate, malate, and fumarate exchange in the tricarboxylic acid cycle), the [^{2}H] enrichment of body water and that of H on C-3 of pyruvate are expected to be the same. Therefore, the ^{2}H enrichment of body water has been used as a measure of ^{2}H enrichment of C-3 of pyruvate—the precursor for both gluconeogenesis and glyceroneogenesis [12,19].

The fractional contribution of pyruvate to VLDL triglyceride was calculated similarly, as follows: percentage of contribution of pyruvate to triglyceride-glycerol = 100 × (0.5 × ^{2}H enrichment of C-3 glycerol/^{2}H enrichment in body water).

The ^{2}H enrichment of C-3 glycerol is halved because of 2 hydrogens bound to C-3 of glycerol. Because the hydrogens on C-1 of glycerol can also be labeled from body water during isomerization of glyceraldehyde-3-phosphate, estimates of glyceroneogenesis using labeling on C-1 should be designated as the contribution of triosephosphate rather than that of pyruvate.

2.3. Contribution of glucose

The fraction of triglyceride-glycerol derived from glucose was estimated by comparing the m_2 enrichment on C-3 of glycerol (derived from 2 deuteriums on C-6 of [6,6-$^2H_2$] glucose) with that on C-6 of glucose. The C-6 of glucose forms C-3 of triglyceride-glycerol. Therefore, [$^2H_2$] tracer from C-6 of glucose will not be expected to appear on C-1 of glycerol. Such was the case both during the basal state and during the hyperinsulinemic clamp in the diabetic subjects (Table 3).

Triglyceride-glycerol from glucose (percentage) = 100 × (2 × m_2 enrichment of C-3 glycerol/m_2 enrichment of C-6 glucose). The results are multiplied by 2 because each molecule of glucose forms 2 molecules of glycerol.

All data are presented as mean ± SD. Changes within the group were evaluated by paired *t* test. Linear regression was calculated by the method of least squares.

3. Results

The clinical and metabolic data in the subjects with type 2 diabetes mellitus are displayed in Table 1. The subjects were

Table 2
^{2}H enrichment (percentage), m_1 and m_2, of C-6 of plasma glucose during the basal state and during the hyperinsulinemic normoglycemic clamp

		Basal			Hyperinsulinemic clamp			Body water
		−30 min	−15 min	0 min	+90 min	+210 min	+240 min	
Baseline (10)	m_1	0.37 ± 0.07	0.39 ± 0.05	0.40 ± 0.06	0.44 ± 0.08	0.35 ± 0.13	0.35 ± 0.13	0.34 ± 0.02
	m_2	1.82 ± 0.34	1.93 ± 0.30	1.97 ± 0.27	2.29 ± 0.37	2.46 ± 0.30	2.41 ± 0.27	
6 mo (10)	m_1	0.40 ± 0.08	0.39 ± 0.07	0.40 ± 0.07	0.38 ± 0.09	0.26 ± 0.11	0.23 ± 0.10	0.33 ± 0.03
	m_2	2.03 ± 0.33	2.03 ± 0.31	2.05 ± 0.29	2.35 ± 0.36	2.51 ± 0.29	2.47 ± 0.29	

Data are mean ± SD. Subjects with type 2 diabetes mellitus were studied at baseline and after 6 months of intervention consisting of diet and behavior modification (Methods). After an overnight fast, [6,6-$^2H_2$]glucose was administered as a prime constant rate infusion. The subjects were also given a dose of [$^2H_2$]O to label the body water pool. After a basal period of 4 hours, a hyperinsulinemic normoglycemic clamp was performed. The labeled glucose was also infused during the clamp.

Table 3
M_2 enrichment (percentage) of plasma glucose and VLDL triglyceride-glycerol, and the contribution of plasma glucose to VLDL triglyceride

	m_2 enrichment of glucose		m_2 enrichment of glycerol				% TG from glucose	
	C-6		C-1		C-3		Basal	Clamp
	Basal	Clamp	Basal	Clamp	Basal	Clamp		
Baseline	1.86 ± 0.31	2.39 ± 0.25	0.018 ± 0.03	0.016 ± 0.03	0.144 ± 0.11	0.146 ± 0.07	15.7 ± 11.9	12.3 ± 6.3
6 mo	2.03 ± 0.30	2.45 ± 0.29	0.014 ± 0.01	0.014 ± 0.02	0.126 ± 0.07	0.150 ± 0.07	12.7 ± 7.9	11.8 ± 4.9

Data are mean ± SD. Ten subjects with type 2 diabetes mellitus were studied at baseline and after 6 months of intervention consisting of diet and behavior (Methods). After an overnight fast, [6,6-$^2H_2$]glucose was administered as a prime constant rate infusion. After a basal observation of 4 hours, a hyperinsulinemic euglycemic clamp was performed for 4 hours. [$^2H_2$] enrichment (m_2) of C-6 glucose and those of C-1 and C-3 of glycerol were quantified. TG indicates triglyceride-glycerol.

obese with high body mass index, had fasting hyperglycemia and hyperinsulinemia, and had high plasma FFA and triglyceride levels. In addition, they showed evidence of insulin resistance (homeostasis model assessment) [18]. Six months of intervention resulted in loss of body weight and significant improvement in all clinical and metabolic parameters. Of note, there was a significant reduction in plasma insulin levels and the insulin resistance index (homeostasis model assessment) and a decrease in plasma triglyceride levels.

3.1. Glucose kinetics and gluconeogenesis from pyruvate

The deuterium enrichment of hydrogens on C-6 of glucose is displayed in Table 2 along with the ^2H enrichment of body water. A steady-state enrichment was achieved both for m_1, [^2H from body water], representing gluconeogenesis from pyruvate, and for m_2, representing the dilution of infused [6,6$^2H_2$]glucose. The rate of appearance (Ra) glucose calculated from the dilution of infused tracer was 169.5 ± 32.2 mg/min before intervention and 150.7 ± 27.5 mg/min ($P < .02$) after 6 months of intervention. Because all subjects had a significant loss in body weight ($P < .003$), the glucose Ra, when normalized for body weight (in milligrams per kilogram per minute), was not significantly different. Intervention and weight loss had no impact on the fractional contribution of gluconeogenesis from pyruvate during the basal state (baseline, 60.0% ± 9.4%; 6 months, 60.9% ± 7.8%).

Intervention resulted in an improvement in insulin sensitivity, as evidenced by higher glucose uptake during the clamp (baseline, 246 ± 122 mg/min; 6 months, 335 ± 153 mg/min; $P = .07$). There was a significant dilution of the m_1 enrichment on C-6 of glucose during normoglycemic hyperinsulinemia (clamp) after clinical intervention, from 0.40% ± 0.7% to 0.23% ± 0.1% (Table 2). However, the precise contribution of gluconeogenesis during the hyperinsulinemic clamp could not be calculated because of the large contribution of m_1 from the infused glucose and because of a lack of isotopic tracer equilibrium during the infusion of glucose and insulin.

3.2. Source of VLDL triglyceride-glycerol

3.2.1. Contribution of glucose

As shown in Table 3, m_2 enrichment of C-1 of glycerol was minimal or insignificant; and the m_2 enrichment of glycerol as a result of [6,6-$^2H_2$]glucose infusion was only evident on C-3 of glycerol. Approximately 16% of triglyceride-glycerol was derived from glucose. The contribution of glucose to triglyceride-glycerol did not change as a result of 6 months of clinical intervention or acutely during insulin plus glucose infusion (hyperinsulinemic normoglycemic clamp).

3.2.2. Contribution of pyruvate (glyceroneogenesis)

The contribution of pyruvate to VLDL triglyceride-glycerol was estimated from the [^2H] enrichment of C-3 (m_1) of glycerol. The m_1 enrichment on C-1 was significantly

Table 4
Hepatic glyceroneogenesis in vivo

	Deuterium enrichment (m_1) in body water	Deuterium enrichment (m_1) on TG						TG from pyruvate	
	%	C-1 + C-3		C-1		C-3		%	
		Basal	Clamp	Basal	Clamp	Basal	Clamp	Basal	Clamp
Baseline	0.34 (0.02)	0.32 (0.07)	0.35 (0.06)	0.29 * (0.06)	0.30 † (0.05)	0.36 * (0.09)	0.36 † (0.08)	53.0 (11.6)	53.4 (9.6)
6 mo	0.33 (0.03)	0.33 (0.07)	0.34 (0.07)	0.31 (0.06)	0.29 (0.08)	0.36 (0.13)	0.37 (0.12)	53.9 (18.0)	56.3 (16.9)

Data are mean (±SD).Ten subjects with type 2 diabetes mellitus were studied before and after 6 months of diet and behavior modification. After an overnight fast, [^2H] enrichment (m_1) of body water and those of hydrogens on C-1 and C-3 of triglyceride were determined.

* Significantly different, paired t test: $P < .015$.

† Significantly different, paired t test: $P < .01$.

less than that on C-3 in the baseline study, both during the basal state and during the hyperinsulinemic clamp (Table 4). These differences were present subsequent to interventions in 9 of 10 subjects. The estimated contribution of glyceroneogenesis (pyruvate) to triglyceride-glycerol was ~53% in the basal state and remained unchanged during the hyperinsulinemic normoglycemic clamp. In addition, dietary and physical activity intervention and associated improvement in insulin sensitivity had no effect on the contribution of pyruvate to triglyceride-glycerol (Table 4).

In the healthy control subjects, the contribution of pyruvate to glucose was 38.4% ± 10.8%. The contribution of triosephosphate to plasma triglyceride-glycerol, measured by ^{2}H enrichment of C-1 of triglyceride-glycerol, was 44.9% ± 9.3%. Intravenous glucagon for 3 hours did not affect gluconeogenesis, whereas it caused a small but significant increase ($P < .01$) in the contribution of trioses to plasma triglyceride-glycerol.

3.3. Correlations

A positive correlation was observed between gluconeogenesis and glyceroneogenesis in subjects with type 2 diabetes mellitus after interventions when their insulin sensitivity had improved ($r^2 = 0.564$).

4. Discussion

In the present study, we have examined the relative contribution of glucose and pyruvate to triglyceride-glycerol and the impact of insulin in type 2 diabetes mellitus. The data show that pyruvate via triosephosphates, that is, glyceroneogenesis, and not glucose via glycolysis, is the major carbon source for the VLDL triglyceride-glycerol in humans. The contribution of glucose to triglyceride was low compared with that of pyruvate. Short-term infusion of insulin and glucose during a hyperinsulinemic clamp had no impact on the contribution of glucose or pyruvate to triglyceride-glycerol in subjects with type 2 diabetes mellitus before or after improvement in insulin sensitivity. A short-term infusion of glucagon caused an increase in the triglyceride-glycerol from triosephosphate in healthy controls. These data suggest that the primary source for VLDL triglyceride-glycerol carbon in humans is pyruvate and that plasma glucose makes only a small contribution to triglyceride-glycerol, even when glucose plus insulin are administered acutely.

We used the total body water labeling method, using [$^2H_2$]O to quantify the contribution of pyruvate to triglyceride-glycerol. As discussed previously [12,19], the methyl hydrogens (C-3) of pyruvate that form C-6 of glucose and C-3 of glyceraldehyde-3-P exchange with hydrogens in the body water so that [^{2}H] enrichment of the hydrogens bound to the C-3 of pyruvate or phosphoenolpyruvate is similar to that of the water. One hydrogen on the C-1 of dihydroxyacetone phosphate (DHAP), the immediate precursor of glycerol-3-phosphate, is obtained from body water during the conversion of phosphoenolpyruvate to glyceraldehyde-3-P. The second hydrogen is also obtained from body water during the isomerization of DHAP and glyceraldehyde-3-P so that the [^{2}H] enrichment of both the hydrogens on C-1 of glycerol-3-phosphate is the same as that of body water. Thus, the [^{2}H] enrichment of hydrogens on the C-1 and C-3 of triglyceride-glycerol formed from pyruvate will be the same as that of body water. The hydrogens on C-3 truly represent the contribution of pyruvate, whereas those on C-1 represent the contribution of triosephosphate. As shown in Table 4, the ^{2}H enrichment of C-1 and C-3 of triglyceride-glycerol in subjects with type 2 diabetes mellitus was the same after the intervention study, both during the basal state and during insulin-glucose infusion. The ^{2}H enrichment on C-1 was significantly less than that on C-3 during the baseline study, when the magnitude of resistance to insulin was high. The reason(s) for the difference is not readily apparent.

4.1. Sources of carbon for triglyceride

As shown in Tables 3 and 4, pyruvate was the predominant source of triglyceride-glycerol carbon; and glucose C was a minor contributor. The latter is of particular interest because glucose-insulin infusion during hyperinsulinemic clamp had no effect on the contribution of glucose in the subjects with type 2 diabetes mellitus. The insulin clamp study was performed for 4 hours, when glucose uptake by the liver should have increased, particularly in the studies performed after the intervention, when insulin sensitivity had improved. Whether the observed contribution of glucose to triglyceride is a net contribution or simply represents an appearance of label due to futile cycling cannot be ascertained from the present data.

Glucose carbon recycling via pyruvate, either through the periphery (muscle) or as a result of hepatic zonation, cannot be estimated from these studies because hydrogens on C-3 of pyruvate and C-1 trioses, irrespective of the source of carbon and labeling pattern of hydrogen, will rapidly equilibrate with body water and become enriched to the same magnitude as body water.

Our data show that glyceroneogenesis and glucose contributed ~50% and ~15% to glyceride-glycerol, respectively. Our previous data have shown that the contribution of plasma glycerol was ~5% to triglyceride-glycerol [11]. The discrepant ~30% represents the unlabeled triglyceride pool in the circulation because of the slow turnover rate of this pool. We speculate that had we continued the studies for a prolonged period, the relative contributions of glyceroneogenesis, glucose, and glycerol, although unchanged, would have been ~70%, 23%, and 7% (total = 100%), respectively.

4.2. Effect of insulin administration

Normoglycemic hyperinsulinemic clamp studies showed that insulin sensitivity had significantly improved in the subjects with type 2 diabetes mellitus after 6 months of

dietary and behavioral intervention. However, it had no impact on the contribution of glucose or pyruvate to triglyceride-glycerol (Tables 3 and 4). Although improved sensitivity to insulin appeared to result in lower contribution of gluconeogenesis from pyruvate, accurate estimates of gluconeogenesis during the hyperinsulinemic clamp could not be made because of a lack of equilibrium in the glucose pools. Data from several studies suggest that the short-term effect of insulin on glucose production is primarily via suppression of glycogenolysis and not gluconeogenesis [20-23].

The lack of any effect of insulin on contribution of glucose to triglyceride-glycerol is of interest. During normoglycemic hyperinsulinemic clamp, the hepatic glucose output decreased. A lack of change in the contribution of glucose to triglyceride-glycerol suggests that hyperinsulinemia during the clamp did not increase hepatic glucose uptake nor did it effect the intrahepatic regulation of the synthesis of triglyceride-glycerol from glucose. In addition, insulin infusion did not impact the conversion of pyruvate to triglyceride-glycerol before and after clinical intervention. These data are consistent with the observation on the short-term effects of insulin on gluconeogenesis, which shows a minimal effect both in humans and in animals [20-23]. Short-term administration of insulin in healthy subjects has been shown to suppress VLDL secretion [24,25]. Such an effect of insulin on VLDL secretion would not impact the fractional contributions of pyruvate (or triosephosphate) to VLDL triglyceride, as seen in the present study, although the total quantitative contribution (fraction × VLDL secretion rate) would decrease.

These data suggest that the major effect of short-term infusion of insulin on gluconeogenesis is at a site in the pathway that is above the triosephosphate branch point between gluconeogenesis and glyceroneogenesis, most likely at fructose-1,6-biphosphatase (FBPase). This enzyme is highly regulated and controls the potential "futile cycling" of carbon between fructose-6-phosphate and fructose-1,6-biphosphate [26,27]. The relative activity of both FBPase and phosphofructokinase is controlled by a change in fructose-2,6-biphosphate and adenosine monophosphate and triphosphate; the levels of both of these compounds would be expected to be altered by the metabolic events initiated by infusion of insulin. In addition, glyceroneogenesis and gluconeogenesis will not be affected acutely by insulin infusion because PEPCK-C is not allosterically controlled and the flux over this enzyme would not be altered by a 4-hour infusion of insulin [3]. Insulin effects PEPCK-C by inhibiting the transcription of the gene; and hence, the effects of insulin are not apparent acutely, as in the present study.

4.3. Correlation between gluconeogenesis and glyceroneogenesis

The positive correlation between gluconeogenesis and glyceroneogenesis in subjects with type 2 diabetes mellitus after the improvement in insulin sensitivity

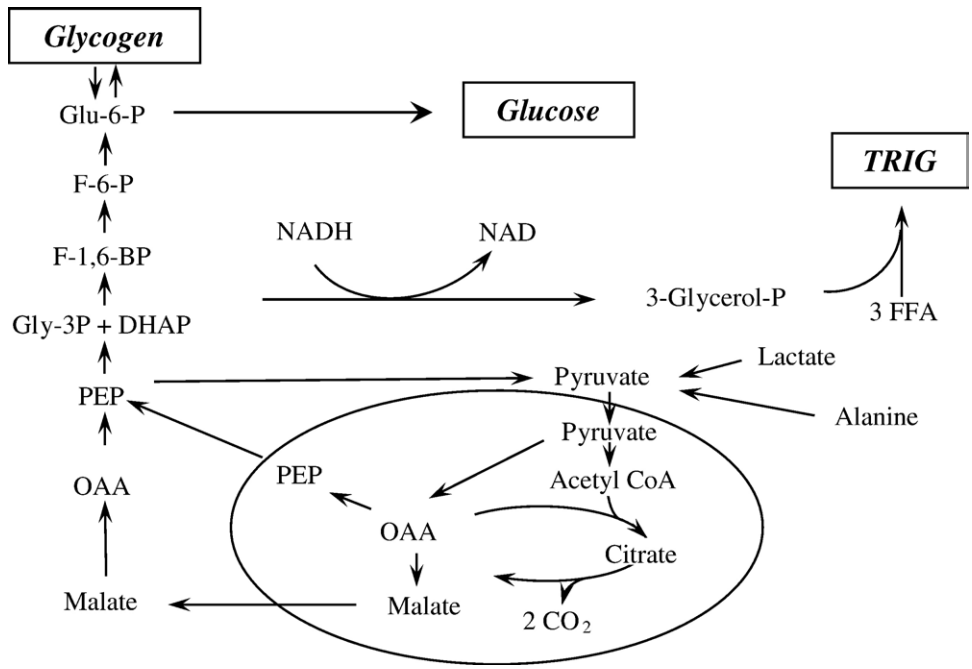

Fig. 1. The common pathway of glyceroneogenesis and gluconeogenesis in the mammalian liver. The pathway for the conversion of precursors such as alanine, lactate, and pyruvate to either triglyceride or glucose is shown. The major branch point in the pathway is the reduction of DHAP to 3-glycerol phosphate by 3-glycerol phosphate dehydrogenase. This reaction is redox sensitive, and its equilibrium favors the formation of 3-glycerol phosphate. Our data demonstrate that insulin controls glyceroneogenesis independently of gluconeogenesis, perhaps by an alteration in flux through the FBPase reaction.

suggests substrate regulation of gluconeogenesis and glyceroneogenesis; that is, the carbon flows from pyruvate to glucose and to glycerol-3-phosphate are parallel and are determined by the uptake and delivery of pyruvate carbon to the liver.

In response to glucagon, there was a minimal effect on the fractional contribution of pyruvate to glucose in healthy controls and a small increase in the contribution of triosephosphate to VLDL triglyceride-glycerol. These data are consistent with the observation by others [28-30] that the short-term effect of glucagon on glucose Ra is by increasing glycogenolysis rather than gluconeogenesis. We assume that the increase in the contribution of triosephosphate to triglyceride-glycerol was the consequence of an increase in cyclic adenosine monophosphate, which inhibits futile cycling at pyruvate kinase, resulting in increased flux of pyruvate carbon to glyceraldehyde-3-phosphate [31,32]. Whether glucagon has any effect on VLDL triglyceride transport is not known.

Data from subjects with type 2 diabetes mellitus show that pyruvate remained the predominant source of triglyceride-glycerol. The infusion of insulin for 4 hours had no effect on the fractional contribution in subjects with type 2 diabetes mellitus before or after the dietary and behavioral intervention. These data suggest that the primary regulator of the quantitative appearance of trioses in the plasma VLDL triglyceride pool may be the rate of release of VLDL triglycerides.

4.4. Glyceroneogenesis and gluconeogenesis share a common pathway

The metabolic pathways for the synthesis of triglyceride-glycerol and glucose share a common set of reactions. Substrates such as lactate or alanine provide carbon for both pathways. The branch point is at the conversion of DHAP to glycerol-3-phosphate via glycerol-3-phosphate dehydrogenase (Fig. 1); the latter compound is the precursor of triglyceride-glycerol. Glycerol-3-phosphate dehydrogenase is a redox-sensitive reaction, in which NADH is oxidized to NAD. The rate of flux of triose phosphate to glucose via this pathway is rapid, and the diversion of DHAP to glycerol-3-phosphate from this pool is dependent on the redox state of the liver. In addition, the equilibrium of glycerol-3-phosphate dehydrogenase strongly favors the formation of glycerol-3-phosphate. During periods of glyceroneogenesis/gluconeogenesis, the oxidation of both fatty acids and lactate would provide the reducing equivalents needed to drive the synthesis of glycerol-3-phosphate. The quantitatively higher flux of pyruvate to triose phosphate to glucose (compared with pyruvate to glycerol-3-phosphate) explains our observation that glycerol accounts for only 6% of the triglyceride found in the VLDL triglyceride of overnight-fasted humans [11]; the glycerol-3-phosphate formed from glycerol via glycerol kinase is diluted by the greater flow of triose phosphate from gluconeogenic precursors, such as lactate and alanine, through the triose phosphate pool. The important role of cytosolic redox state in the regulation of the diversion of triose phosphate from gluconeogenesis to glyceroneogenesis is supported by the studies of Siler et al [33], which demonstrated that ethanol greatly enhanced the rate of synthesis of triglyceride glycerol via glyceroneogenesis in the perfused rat liver. This was attributed to a shift in the NAD/NADH ratio induced by the metabolism of ethanol.

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